Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Altered fecal microbiota composition in patients with major depressive disorder

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ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 29 March 2015
Accepted 29 March 2015
Available online xxxx

Keywords:
Depression
Gut bacteria
Inflammation
Gut–brain
Antidepressant

ABSTRACT

Studies using animal models have shown that depression affects the stability of the microbiota, but the actual structure and composition in patients with major depressive disorder (MDD) are not well understood. Here, we analyzed fecal samples from 46 patients with depression (29 active-MDD and 17 responded-MDD) and 30 healthy controls (HCs). High-throughput pyrosequencing showed that, according to the Shannon index, increased fecal bacterial α-diversity was found in the active-MDD (A-MDD) vs. the HC group but not in the responded-MDD (R-MDD) vs. the HC group. Bacteroidetes, Proteobacteria, and Actinobacteria strongly increased in level, whereas that of Firmicutes was significantly reduced in the A-MDD and R-MDD groups compared with the HC group. Despite profound interindividual variability, levels of several predominant genera were significantly different between the MDD and HC groups. Most notably, the MDD groups had increased levels of Enterobacteriaceae and *Alistipes* but reduced levels of *Faecalibacterium*. A negative correlation was observed between *Faecalibacterium* and the severity of depressive symptoms. These findings enable a better understanding of changes in the fecal microbiota composition in such patients, showing either a predominance of some potentially harmful bacterial groups or a reduction in beneficial bacterial genera. Further studies are warranted to elucidate the temporal and causal relationships between gut microbiota and depression and to evaluate the suitability of the microbiome as a biomarker.

1. Introduction

Depression is a common, life-disrupting and highly recurrent illness and a leading source of disability worldwide (Moussavi et al., 2007). Despite the fact that antidepressant medication is widely used to treat depressive symptoms, 30–40% of patients do not respond to current drug strategies (Rush et al., 2006). Studies on depression have focused mainly on the genetic, behavioral, and neurological aspects of the disease, although the contributions of environmental risk factors and immune dysregulation to the etiology of depression have gained significant attention.

Accumulating evidence from animal studies supports the hypothesis that gut microbiota play an important role in central nervous system function, namely through inflammation, and the hypothalamic–pituitary–adrenal (HPA) axis, and by affecting neurotransmission (Bangsgaard Bendtsen et al., 2012; Collins et al., 2012; Cryan and Dinan, 2012; Dinan and Cryan, 2013; Dinan et al., 2013; Wang and Kasper, 2014). Although the pathways linking gut bacteria with the brain are incompletely understood, "leaky gut", induced by stress, could play a role (Rook and

Abbreviations: BDNF, brain-derived neurotrophic factor; DSM-IV, the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition; HCs, healthy controls; HPA, hypothalamic–pituitary–adrenal; IL-6, interleukin-6; IL-1β, interleukin-1 beta; HAMDS, Hamilton's Depression Scale; MADRS, Montgomery–Asberg Depression Rating Scale; MDD, major depression disorder; TNF-α, tumor necrosis alpha.

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http://dx.doi.org/10.1016/j.bbi.2015.03.016
0889-1591/

Lowry, 2008; Rook et al., 2014). Specifically, increased translocation of bacterial products, due to a compromised gut barrier has been linked to activation of the immune system and HPA axis (Maes et al., 2012, 2013). These effects can be reversed in mice by oral administration of probiotics (Ait-Belgnaoui et al., 2012, 2014; Savignac et al., 2014). In line with these findings, human studies have demonstrated increased bacterial translocation in mood disorders such as depression (Maes et al., 2008, 2012). Indeed, recent reports of trials using probiotics in healthy subjects demonstrated improvements in depression or anxiety outcome measures (Messaoudi et al., 2011).

These results indicate that gut bacteria could possess therapeutic potential for mental illnesses. Within the past year, accumulating experimental data has strongly supported the view that depression could also influence microbiota composition. O'Mahony et al. (2009) reported that the fecal microbiome composition of adult rats subjected to maternal separation was significantly altered compared with that of non-separated controls. Bangsgaard Bendtsen et al. (2012) demonstrated that the community microbiota structure of mice exposed to a prolonged restraint stressor differed significantly from that of non-stressed controls. Bailey et al. (2011) observed that social stressors significantly altered the relative abundance of bacteria, particularly when microbiota were assessed immediately following stressor exposure. Despite such animal studies indicating an association between gut microbiota and depression, this relationship remains poorly understood in humans. Therefore, detailed assessment of the fecal microbiota of depression patients should be undertaken before firm conclusions are drawn.

Here, we investigated whether gut microbiota are altered during major depressive episodes or in response to antidepressant treatment. We also identified microbiota signatures specific for depression and their relationships with clinical patterns and physiological measures using massively parallel barcoded 454 pyrosequencing on 76 fecal samples taken from 46 patients diagnosed with depression and 30 matched healthy controls (HCs).

2. Methods

2.1. Subject selection

This study protocol was approved by the Ethics Committee of The Seventh People's Hospital of Hangzhou (Zhejiang, China). After receiving a written description of the aim of this study, all participants gave written informed consent prior to enrollment. The recruitment of participants and the process of sample collection are depicted in Fig. 1.

Forty-six patients (age, 18–40 years) were ultimately recruited from the Seventh People's Hospital of Hangzhou in Hangzhou, Zhejiang, from May 2013 to December 2013 (Table 2). One experienced psychiatrist performed the screening examinations. The Mini-International Neuropsychiatric Interview was used as a systematic psychiatric screening tool to detect preexisting psychiatric disorders (Sheehan et al., 1998). The Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition was used to verify major depressive disorder (First MB). Participants completed the Hamilton's Depression Scale (HAMDS), a 24-item clinician-administered measure of depression, to assess the severity of depressive symptoms (Hamilton, 1960). The HAMDS generates scores from 0 to 63; scores $\geqslant$20 are indicative of clinically significant depression. Depressive symptoms were also assessed using the Montgomery–Asberg Depression Rating Scale (MADRS), a clinician-related depression scale and one of the most commonly used symptom severity scales for depression. It consists of 10 items scored from 0 to 6 (Montgomery and Asberg, 1979). HC subjects (n = 30) from the same cohort were screened using a semi-structured clinical interview to exclude those with psychiatric or physical illnesses.

All subjects were examined clinically before sampling and were subsequently divided into three groups: active-MDD (A-MDD) group (n = 29), responding-MDD (R-MDD) group (n = 17), and HCs (n = 30). The A-MDD group was defined as having an HAMDS score $\geqslant$20. The patients in the R-MDD group were defined as those with a baseline HAMDS scores $\geqslant$20 upon admission to the hospital. Fecal and serum samples were collected at the time of their HAMDS scores showed a 50% reduction after 4 weeks treatment.

The following exclusion criteria were established: hypertension; cardiovascular disease; diabetes mellitus; obesity; liver cirrhosis; fatty liver disease; irritable bowel syndrome; inflammatory bowel disease; drug or alcohol abuse in the last year; use of antibiotics, probiotics, prebiotics, or synbiotics in the month before collection of the fecal sample; and known active bacterial, fungal, or viral infections.

2.2. Fecal sample collection and DNA extraction

Fecal samples were collected in a sterile plastic cup after the participants completed the HAMDS and MADRS assessments and were kept in an icebox. Samples for bacterial genomic DNA extraction were delivered to the laboratory within 15 min and stored at −80 °C. Fecal microbial DNA was extracted from 200-mg feces using the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with the additional glass-bead beating steps on a Mini-beadbeater (FastPrep; Thermo Electron Corp., Boston, MA, USA). DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Electron); integrity and size were assessed by 1.0% agarose gel electrophoresis on gels containing 0.5 mg/mL ethidium bromide. DNA was stored at −20 °C before analysis.

2.3. Serum cytokine and BDNF detection

Blood samples were collected immediately after the HAMDS and MADRS assessment, transferred to the laboratory immediately in an icebox and stored at −80 °C within 15 min after preparation for further analysis. Serum tumor necrosis factor-α (TNF-α), interleukin-1β (IL)-1β, IL-6, and brain-derived neurotrophic factor (BDNF) levels were determined using commercially available enzyme-linked immunosorbent assay kits (RayBiotech, Norcross, GA, USA).

2.4. Polymerase chain reaction (PCR) and pyrosequencing

Triplicate PCR reactions were performed on each sample. The bacterial genomic DNA was amplified with the 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) primers specific for the V1–V3 hypervariable regions of the 16S rRNA gene. Each forward primer incorporated FLX Titanium adapters and a sample barcode at the 5′ end of the reverse primer to allow all samples to be included in a single 454 FLX sequencing run (Table S1). All PCRs were performed in 50-μL triplicates and combined after PCR. The products were extracted with the QIAquick Gel Extraction kit (Qiagen) and quantified on a NanoDrop ND-1000 spectrophotometer, QuantiFluor-ST fluorometer (Promega, Madison, WI, USA), and an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Equimolar concentrations of the 76 samples were pooled and sequenced on a 454 Life Sciences genome sequencer FLX system (Roche, Basel, Switzerland) according to the manufacturer's recommendations.

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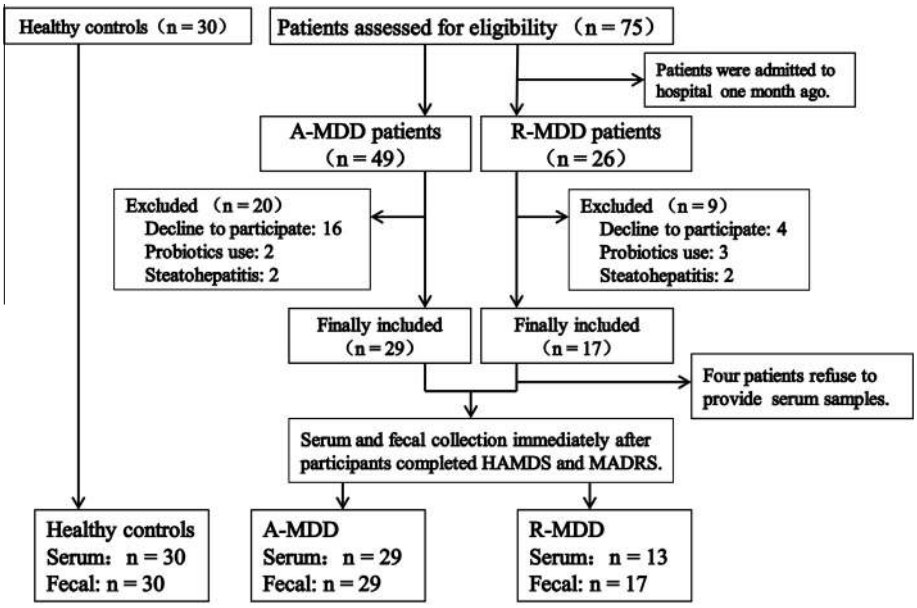

Fig. 1. The recruitment of participants and the process of sample collection.

Table 1
Comparison of phylotype coverage and diversity estimation of the 16S rRNA gene libraries at 97% similarity from the pyrosequencing analysis.

Group	No. of reads	No. of OTUs[a]	Good's (%)[b]	Richness estimator				Diversity index		
				ACE	95%CI	Chao 1	95%CI	Shannon	Simpson	Evenness[c]
HCs	229,038	8271	97.83	28,714	27,970–29,486	18,712	17,892–19,602	5.038359	0.023243	0.005202
A-MDD	244,508	10,365	97.55	31,911	31,173–32,675	21,584	20,796–22,431	5.344609*	0.019268	0.005007
R-MDD	135,029	6737	97.04	21,848	21,224–22,498	14,982	14,266–15,766	4.987910	0.027811	0.005337

[a] The operational taxonomic units (OTUs) were defined with 97% similarity level.
[b] The coverage percentage (Good's), richness estimators (ACE and Chao1) and diversity indices (Shannon and Simpson) were calculated using Good's method and the mothur program, respectively.
[c] The Shannon index of evenness was calculated with the formula $E = H/\ln(S)$, where H is the Shannon diversity index and S is the total number of sequences in that group.
* Compared with HCs, $P < 0.05$.

Table 2
Descriptive data of included adults in the study.

Parameter	Value for groups		
	Healthy controls ($n = 30$)	Active-MDD ($n = 29$)	Responsed-MDD ($n = 17$)
Sociodemographics			
Proportion of Females, No. (%)*	15 (50%)	11 (38%)	8 (47%)
Age (years; means ± SD) (range)¶	26.8 (5.4) (18–38)	25.3 (5.4) (18–40)	27.1 (5.4) (19–40)
BMI (means ± SD) (range)¶	19.6 (3.4) (18.6–23.5)	20.3 (3.4) (19.6–22.5)	21.8 (3.4) (21.6–23.6)
Employed, No. (%)*	26 (87%)	21 (72%)	14 (82%)
High school or less, No. (%)*	12 (43%)	9 (31%)	4 (24%)
Smoking, No. (%)*	2 (7%)	3 (10%)	2 (12%)
Antidepressant treatment			
SSRIs or SNRIs treatment, No. (%)	0	21 (72%)	17 (100%)#
Atypical antipsychotic, No. (%)	0	7 (24%)	5 (29%)
Benzodiazepines, No. (%)	0	24 (83%)	10 (58.9%)
Blood markers			
BDNF (ng/ml), mean (SD)¶	12.77 (4.60)	6.38 (4.37)‡	9.00 (4.54)‡#
IL-6 (pg/ml), mean (SD)¶	30.66 (2.99)	27.83 (8.56)	27.36 (6.03)
IL-1β (pg/ml), mean (SD)¶	0.10 (0.16)	0.13 (0.36)	0.10 (0.07)
TNF-α (pg/ml), mean (SD)¶	73.09 (322.34)	102.88 (515.05)	27.53 (37.64)
Severity of depressive symptoms			
HAMDS§	NA	29.8 (7.6)	8.3 (4.6)#
MADRS§	NA	27.4 (8.5)	6.9 (4.3)#

* Chi-square test; ¶ One-way ANOVA; § Student's t test; ‡ compared with HCs, $P < 0.05$; # compared with A-MDD, $P < 0.05$; Abbreviations: MDD, major depressive disorder; SD, standard deviation; BMI, body mass index; SSRIs, selective serotonin reuptake inhibitors; SNRIs, selective norepinephrine reuptake inhibitors; BDNF, brain-derived neurotrophic factor; IL-6, interleukin-6; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis alpha; HAMDS, Hamilton's Depression Scale; MADRS, Montgomery–Asberg Depression Rating Scale; NA, not available.

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2.5. Bioinformatics and statistical analysis

The raw pyrosequencing reads obtained from the sequencer were denoised using Titanium PyroNoise software. According to the barcode and primer sequences, the resulting pyrosequencing reads were filtered using a combination of tools from Mothur (ver. 1.25.0; http://www.mothur.org) and custom Perl scripts. Preliminary quality control steps were performed according to a previous study. Using ChimeraSlayer, chimera sequences arising from the PCR amplification were detected and excluded from the denoised sequences. The high-quality sequences were assigned to samples according to the barcodes. The high-quality reads were clustered into operational taxonomic units (OTUs) using Mothur. OTUs that reached a 97% nucleotide similarity level were used for alpha diversity (Shannon, Simpson, and evenness indices), richness (ACE and Chao1), Good's coverage, Venn diagram, and rarefaction curve analyses using Mothur according to our previous study (Ling et al., 2014). Phylogenetic beta diversity measures, such as unweighted UniFrac distance metrics analysis and principal coordinate analysis, were performed using OTUs for each sample and the Mothur program.

Taxonomy-based analyses were performed by classifying each sequence using the Naïve Bayesian Classifier program of the Michigan State University Center for Microbial Ecology Ribosomal Database Project database (http://rdp.cme.msu.edu/) with a 50% bootstrap score. The metastats program from Mothur was used to identify significantly different phylotypes among the groups. Microorganism features distinguishing fecal microbiota specific to MDD were identified using the linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/) for biomarker discovery, which emphasizes both statistical significance and biological relevance (metagenomic biomarker discovery and explanation). LEfSe uses the Kruskal–Wallis rank-sum test with a normalized relative abundance matrix to detect features with significantly different abundances between assigned taxa and performs LDA to estimate the effect size of each feature. An alpha significance level of 0.05 and an effect-size threshold of 2 were used for all biomarkers. Correlations between variables were calculated using Spearman's rank-correlation analysis. Statistical analyses were performed using the SPSS ver. 16.0 data analysis software (SPSS Inc., Chicago, IL, USA). All tests for significance were two sided, and a $P < 0.05$ was considered to indicate significance.

2.6. Accession numbers

The sequence data from this study have been deposited in the GenBank Sequence Read Archive with the accession number SRP042956.

3. Results

3.1. Collection of 16S data

We obtained 608,575 high-quality sequences, accounting for 64.6% of the valid sequences (941,616 reads in total) from 76 participant fecal samples. According to barcode and primer sequence filtering, an average of 8007 (range, 4983–12,776) sequences per barcoded sample was recovered for downstream analysis. Thus, 229,038 sequences were obtained from control subjects for the phylogenetic analysis, whereas 244,508 sequences were obtained from the MDD group and 135,029 from the HC group. The total number of unique sequences from the three groups was 18,022, and all phylotypes were represented. In particular, 8271 species-level OTUs in control subjects, 10,365 OTUs in the A-MDD group, and 6737 OTUs in R-MDD group were obtained. Detailed characteristics of each sample are shown in Table S1. Coverage approached 97.0% for all sequences in the three groups, indicating good sequencing depth for investigation of the depression-associated fecal microbiota.

3.2. Overall structure of the fecal bacterial communities

We evaluated the ecological features of the fecal bacterial communities in the A-MDD, R-MDD, and HC groups using a variety of indices based on the OTU level (Table 1). Species richness is the number of bacterial species assigned by OTUs detected in the samples. Richness estimates were obtained from the observed number of species by extrapolation using estimators such as the ACE and Chao1 indices. Evenness is the degree of homogeneity of abundance of the species detected in the samples. Diversity estimates were obtained from species richness and evenness was obtained by using several different indices to confirm our results. Bacterial diversity was significantly higher in the A-MDD group than that in the controls, as indicated by the Shannon index (Table 1). No other significant differences were found among the three groups (Suppl. Fig. S1). The trend in species richness in patients with depression was similar to that of the HCs, based on the rarefaction analysis estimates (Suppl. Fig. S2). The OTU analysis showed a long tail in the rank abundance curves, indicating that the majority of OTUs were present at low abundance (Suppl. Fig. S3). A beta diversity analysis indicates the extent of similarity between microbial communities by measuring the degree to which membership or structure is shared between communities. Due to significant interindividual variation, the fecal microbiotas of the three groups could not be divided into clusters according to community composition using unweighted UniFrac metrics and could not be separated clearly by principal coordinates analysis (Suppl. Fig. S4). However, the clustering was complemented by an analysis of bacterial richness using the number of shared and unique OTUs in the three groups by a Venn diagram, which was generated to compare OTUs among the three groups (Suppl. Fig. S5).

3.3. Altered microbiota composition in A-MDD

We used Metastats to investigate the associations between fecal microbiota composition and A-MDD. The A-MDD and HC groups exhibited statistically significant differences with regard to the three dominant phyla, Firmicutes, Bacteroidetes, and Proteobacteria; the A-MDD and HC groups also showed statistically significant differences with regard to Fusobacteria and Actinobacteria (Fig. 2a). We found 11 statistically significant differences between A-MDD and HC groups at the family level. The relative proportions of Acidaminococcaceae, Enterobacteriaceae, Fusobacteriaceae, Porphyromonadaceae, and Rikenellaceae were significantly higher in the A-MDD group compared with the HC group; we also found significantly lower levels of Bacteroidaceae, Erysipelotrichaceae, Lachnospiraceae, Prevotellaceae, Ruminococcaceae, and Veillonellaceae in the A-MDD than in the HC group (Fig. 2b).

Bacterial communities were also compared at the genus level. The abundances of 72 genera differed between the A-MDD and HCs groups, including 15 predominant (>1% of the total sequences in either group) and 57 less-predominant genera. Among the different predominant genera, *Alistipes*, *Blautia*, *Clostridium XIX*, *Lachnospiracea incertae sedis*, *Megamonas*, *Parabacteroides*, *Parasutterella*, *Phascolarctobacterium*, *Oscillibacter* and *Roseburia* were relatively more abundant in the A-MDD vs. HC group; However, *Bacteroides*, *Dialister*, *Faecalibacterium*, *Prevotella*, and *Ruminococcus* were relatively more abundant in the HC vs. A-MDD group (Fig. 2c).

The metagenome analysis LEfSe approach was applied to identify the key phylotypes responsible for the difference between the

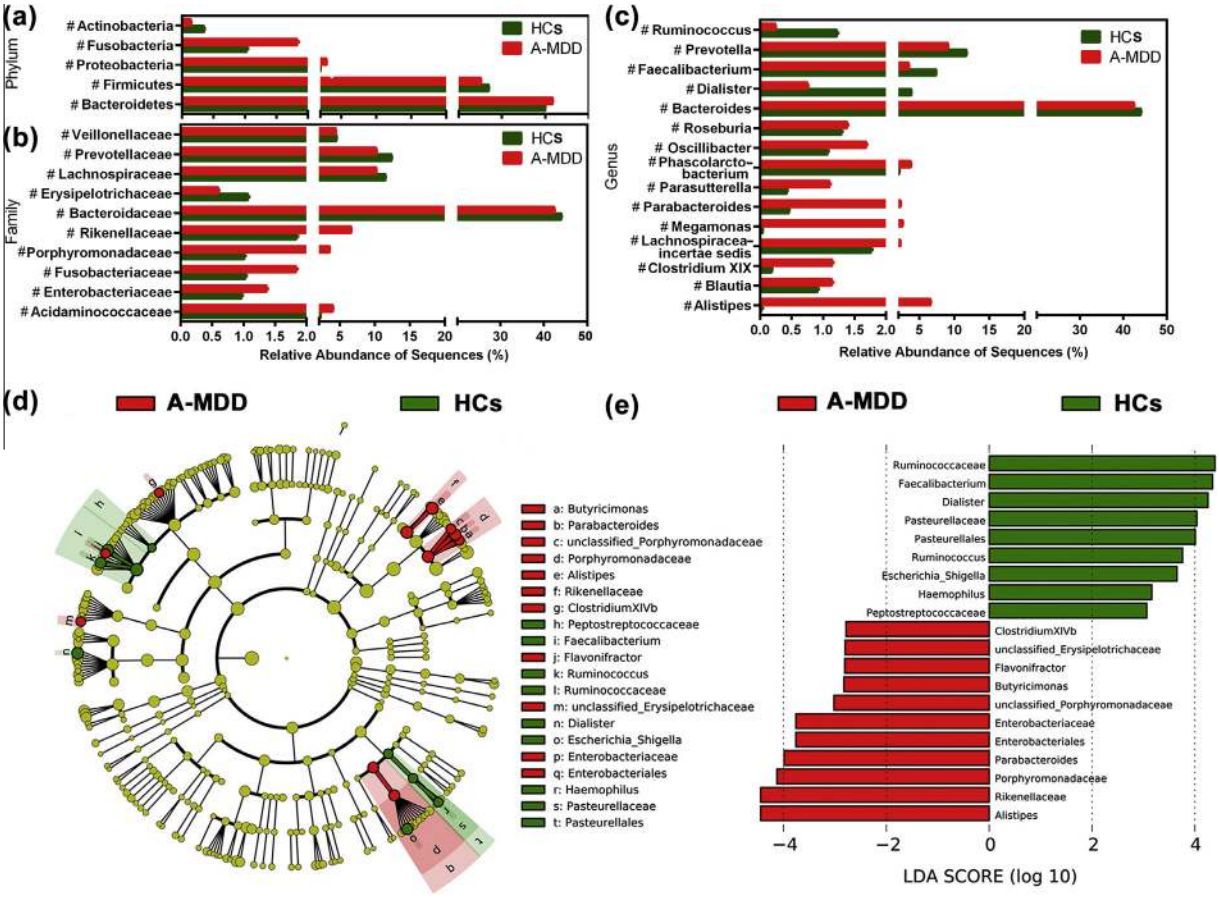

Fig. 2. Taxonomic differences of fecal microbiota between HC and A-MDD groups. Comparison of relative abundance at the bacterial phylum (a), family (b) and genus (c) levels between HC and A-MDD groups. # indicates $P < 0.05$. LEfSe identified the most differentially abundant taxons between HC and A-MDD groups. Taxonomic cladogram obtained from LEfSe analysis of 16S sequences (relative abundance $\geqslant 0.5\%$). (Red) A-MDD taxa; (Green) taxa enriched in HCs. The brightness of each dot is proportional to its effect size (d). HC-enriched taxa are indicated with a positive LDA score (green), and taxa enriched in A-MDD have a negative score (red). Only taxa meeting an LDA significant threshold >2 are shown (e). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A-MDD and HC groups. *Alistipes*, *Flavonifractor*, *Butyricimonas*, and *Clostridium* XlVb, which were most abundant in the A-MDD, and *Faecalibacterium*, *Dialister*, *Ruminococcus*, *Escherichia/Shigella*, *Haemophilus*, which were most abundant in the HCs, were the dominant phylotypes that contributed to the difference between the intestinal microbiota of A-MDD and HCs (Fig. 2d and e).

3.4. Altered microbiota composition in R-MDD

A taxonomy-based comparison was performed to determine the differences between the microbiota of R-MDD and HC groups. At the phylum level, Firmicutes, Fusobacteria, and Actinobacteria were significantly more abundant in the fecal microbiota of the HC than the R-MDD group, whereas Bacteroidetes and Proteobacteria were significantly more abundant in the fecal microbiota of the R-MDD group (Fig. 3a). Compared with the HC group, the abundances of the more prevalent families (Acidaminococcaceae, Bacteroidaceae, Enterobacteriaceae, Porphyromonadaceae, and Rikenellaceae) were increased in the R-MDD group, whereas those of the Lachnospiraceae, Ruminococcaceae, and Veillonellaceae families were decreased (Fig. 3b). Several significant differences between the HC and R-MDD groups were observed at the genus level. Among the abundant genera, five (*Alistipes*, *Bacteroides*, *Parabacteroides*, *Phascolarctobacterium*, and *Roseburia*) were increased in the R-MDD group, whereas another six (*Escherichia/Shigella*, *Oscillibacter*, *Dialister*, *Faecalibacterium*, *Prevotella*, and *Ruminococcus*) were decreased significantly in this group (Fig. 3c).

The metagenome analysis LEfSe approach was also applied to identify the key phylotypes responsible for the difference between the R-MDD and HC groups. *Clostridium sensu stricto*, *Ruminococcus*, and *Haemophilus*, which were abundant in the HCs, were the key phylotypes that contributed to the difference in the intestinal microbiota composition between R-MDD patients and the HCs (Fig. 3d and e).

3.5. Associations among fecal microbiota and clinical indicators

We evaluated the inflammatory state and BDNF levels in the serum of the A-MDD and R-MDD patients compared with that of the HCs. The analysis was performed by measuring IL-6, TNF-α, IL-1β, and BDNF levels in 29 A-MDD, 13 R-MDD, and 30 HC individuals (Table 2). No significant difference in serum inflammatory biomarker concentrations was observed in the A-MDD and R-MDD groups compared with the HCs. However, the BDNF levels in the A-MDD and R-MDD groups were lower than those in the HCs ($P < 0.01$).

We also evaluated correlations among the relative abundances of bacterial genera, the BDNF, serum inflammatory biomarkers, and the severity of depressive symptoms. With significantly interindividual variability, we identify that only one genus, *Clostridium* XlVb, negatively correlated with the sereum BDNF level ($P < 0.05$) (Fig. 4a), while other key phylotypes showed no strong correlation with serum BDNF level. In addition, there were negative relationships between the relative abundance of *Faecalibacterium*

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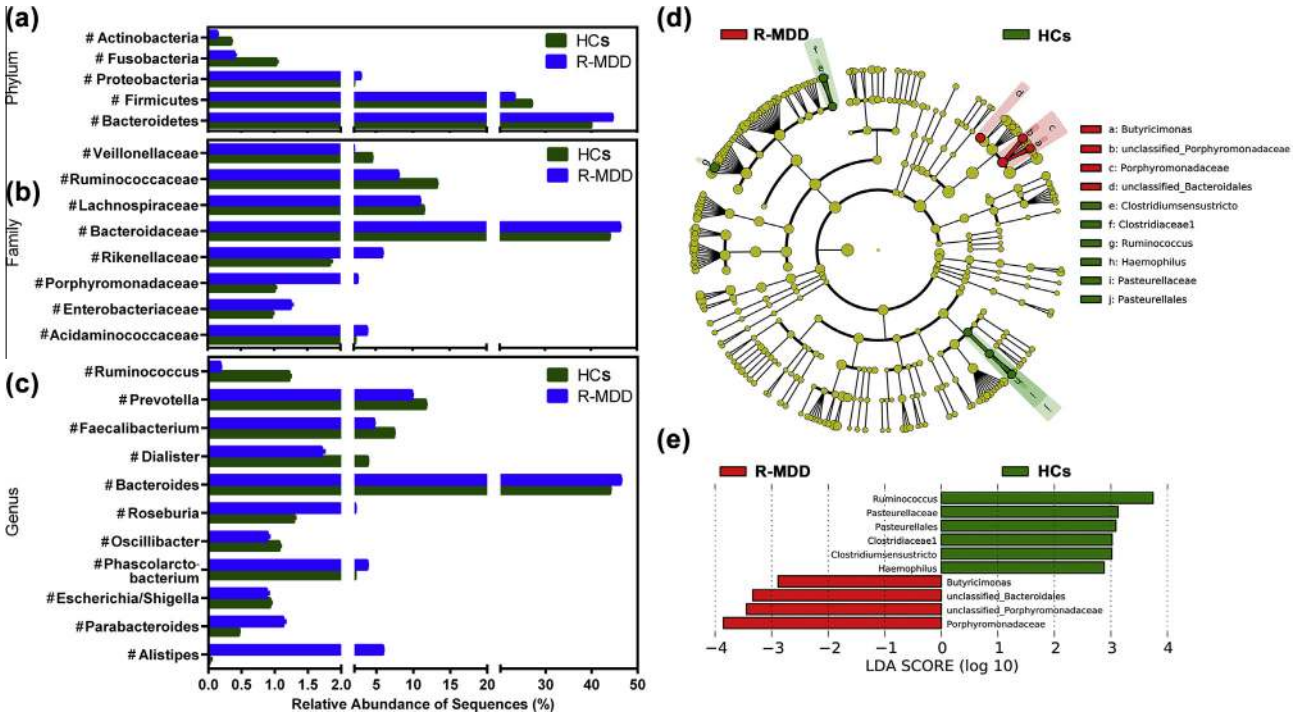

Fig. 3. Taxonomic differences of fecal microbiota between HC and R-MDD groups. Comparison of relative abundance at the bacterial phylum (a), family (b) and genus (c) levels between HC and R-MDD groups. # indicates $P < 0.05$. Taxonomic cladogram obtained from LEfSe analysis of 16S sequences (relative abundance $\geqslant 0.5\%$). (Red) R-MDD taxa; (Green) taxa enriched in HCs (d). HC-enriched taxa are indicated with a positive LDA score (green), and taxa enriched in R-MDD have a negative score (red) (e). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

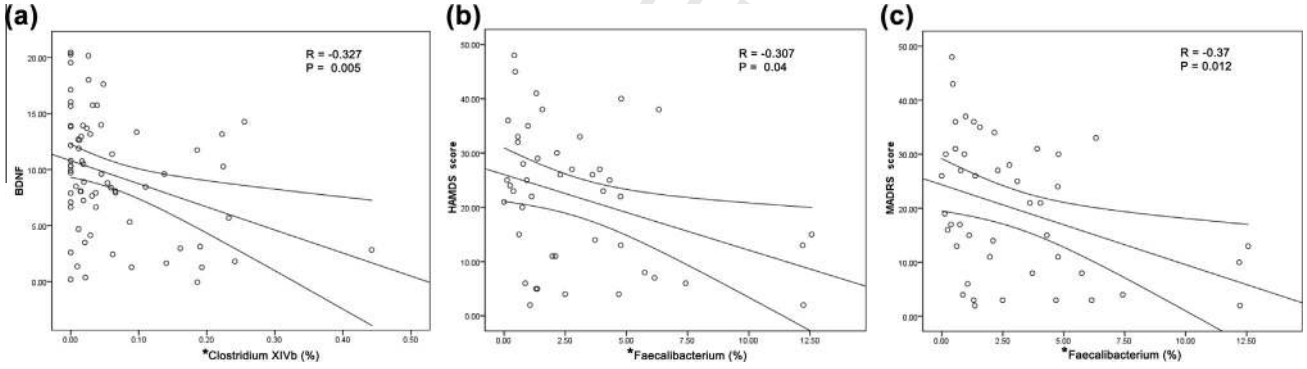

Fig. 4. (a) Correlation between serum BDNF level and the relative abundance of the genera *Clostridium* XIVb. (b) and (c) Correlation between the relative abundance of the genera *Faecalibacterium* and severity of depressive symptoms. The spearman rank correlation (R) and probability (P) were used to evaluate statistical importance. *One outlier was excluded for better representation of the data.

and severity of depressive symptoms ($P < 0.05$) (Fig. 4b and c). If we remove the one outlier from correlation analysis, the results were the same.

4. Discussion

This study found differences in alpha diversity, and in the levels of specific bacterial taxa in gut microbiomes associated with MDD, particularly among patients with clinically significant depressive symptoms. To date, only one study by Naseribafrouei et al. (2014) had compared the fecal microbiota of depressed ($n = 37$) and non-depressed ($n = 18$) individuals, with no significant group differences reported in microbiota diversity. In the present study, fecal microbial diversity (estimated using the Shannon Index) was unexpectedly somewhat greater in A-MMD patients. Although greater bacterial diversity is potentially beneficial to human health, its role in CNS function remains subject to debate. A previous study demonstrated greater gut microbe diversity in formula- vs. breast-fed infants (Fan et al., 2014; Roger et al., 2010). However, breast-fed infants were characterized by superior neurodevelopmental outcomes and higher intelligence test scores (Kramer et al., 2008). Furthermore, increased gut microbe diversity and richness was also detected in a sample of autistic children (Finegold et al., 2010); therefore, the precise consequences of increased bacterial diversity for A-MDD remains unclear. It should be noted that the diversity of gut bacteria is influenced by several factors, including health status, age, diet, and antibiotic treatment (Lozupone et al., 2012). In contrast with our study, –Naseribafrouei et al. (2014) recruited a control group from an outpatient neurological unit. Although no disorders were evident in these patients, their bacterial diversities may have differed from ours because we used healthy subjects as a control group. Furthermore, as bacterial diversity changes with

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age, the inconsistent results may be explained by differences in age between the samples of the two studies. In addition, dietary differences between Western and Eastern countries may have also contributed to differences in bacterial diversity. However, both studies lack data on the dietary habits of subjects; this is a limitation of both extant studies investigating the association between gut microbiota and depression. However, no significant difference was found in between the body mass indices of the study groups, which argues against the existence of major dietary differences.

In our study, intestinal dysbiosis was characterized by significant taxonomical differences between three of the major phyla in A-MDD vs. control subjects. Bacteroidetes and Proteobacteria were significantly more abundant in A-MDD subjects, whereas the proportion of Firmicutes was markedly lower. *Parabacteroides* and *Alistipes*, of the Porphyromonadaceae and Rikenellaceae families, respectively, principally contributed to the increase in Bacteroidetes. Bailey et al. (2011) also reported greater genus *Parabacteroides* abundance in mice exposed to social stressors vs. non-stressed controls. Naseribafrouei et al. (2014) reported enrichment of the *Alistipes* in the depressed subjects. *Alistipes* species are indole-positive and may thus influence tryptophan availability (Song et al., 2006). As tryptophan is also the precursor of serotonin, increased abundance of *Alistipes* might therefore disrupt the balance in the intestinal serotonergic system. Saulnier et al. (2011) found that higher levels of *Alistipes* were associated with a greater frequency of abdominal pain in patients with irritable bowel syndrome, and speculated that *Alistipes* is associated with gut inflammation; thus, further research is needed to clarify the role of this genus in gut inflammation and the serotonergic system in depression. A decrease in *Bacteroides* is assumed to be associated with metabolic diseases such as obesity and diabetes (Bervoets et al., 2013; Zhang et al., 2013); depression and metabolic disease comorbidity is also common (Butnoriene et al., 2014). Consistent with previous animal and human studies (Bailey et al., 2011; Naseribafrouei et al., 2014), *Bacteroides* expression was significantly decreased in A-MDD vs. control patients. Although obesity and diabetes were exclusionary criteria, an association between *Bacteroides* and metabolism cannot be ruled out in our depressed patients.

Expression of the Lachnospiraceae and Ruminococcaceae families, within the phylum Firmicutes, was decreased in the A-MDD vs. the control group. Bangsgaard Bendtsen et al. (2012) also reported that the expression of various species of Lachnospiraceae and Ruminococcaceae in mice correlated with behavioral changes induced by stress. The Lachnospiraceae family, which includes the *Roseburia*, *Blautia*, and *Lachnospiraceae incertae sedis* genera, is known to participate in the breakdown of carbohydrates into short-chain fatty acids (SCFAs) (Duncan et al., 2007); a decrease in these fermentation-related bacteria precipitates a decline in SCFA production, which in turn causes intestinal barrier dysfunction (Vince et al., 1990; Wong et al., 2006). The genus *Faecalibacterium*, affiliated with the Ruminococcaceae family, was highly enriched in healthy controls. An extensive body of data demonstrates that depression is associated with a chronic low-grade inflammatory response (Berk et al., 2013). *Faecalibacterium* was characterized by anti-inflammatory activity within the gut (Sokol et al., 2008); we speculated that the relative abundance of this genus in depressed subjects at least partly mediated the degree of inflammation. However, *Phascolarctobacterium* (Acidaminococcaceae family) and *Clostridium* XIX (Clostridiaceae family) were significantly increased in patients with A-MDD. Acidaminococcaceae enrichment in the A-MDD group also accorded with previous research using pyrosequencing fecal samples, in which patients with both irritable bowel syndrome and depression exhibited significant fecal overgrowth of Acidaminococcaceae (Jeffery et al., 2012). In another study, in which depression in rats was induced by stress, Clostridiaceae was prevalent in the cecum (Bailey et al., 2011). *Oscillibacter*, of the family Oscillospiraceae, was also significantly increased in A-MDD patients. Naseribafrouei et al. (2014) similarly reported greater *Oscillibacter* enrichment in depressed vs. non-depressed subjects.

We also detected overgrowth of the phylum Proteobacteria, specifically of the Gammaproteobacteria class (including Enterobacteriales and Enterobacteriaceae) in our A-MDD group. The Enterobacteriaceae family includes inflammogenic enteric pathogens such as *Hafnia alvei*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas putida*, *Citrobacter koseri* and *Klebsielle pneumonia* (Maes et al., 2008; Maes et al., 2012, 2013). All of these gram-negative bacteria are observed in normal gut flora. Increased permeability of the gut wall in depressed patients may allow invasive gram negative bacteria to translocate into mesenteric lymph nodes or the systemic circulation (Berg and Garlington, 1979; O'Malley et al., 2010). Clinical depression is accompanied by increased plasma immunoglobulin (Ig)A and/or IgM, which are directed against these bacteria (Maes et al., 2012). Moreover, previous studies have demonstrated that the presence of these pathogenic bacteria in the gastrointestinal tract can induce behavioral and psychological changes in animals and humans (Goehler et al., 2008; Lowe et al., 2014; Lyte et al., 1998, 2006).

We also observed that the levels of several genera in the R-MDD group were differed from those in the HC group. It should be noted that atypical antipsychotic use was widespread among our patients. A previous animal study demonstrated that chronic antipsychotic administration increased and decreased the relative abundance of Firmicutes and Bacteroidetes, respectively, in the caecum (Davey et al., 2012, 2013). Although our study had very strict inclusion criteria to control potential confounders, we cannot completely rule out the possibility that atypical antipsychotics may have affected our results. Therefore, it remains unclear whether these changes were caused by atypical antipsychotics or depressive symptoms. This is a major limitation of this study, and further research involving patients who are not receiving antipsychotics is needed to elucidate this issue.

Depression is associated with biomarkers of inflammation, such as elevated IL-6, TNF-α, and IL-1β levels (Lopresti et al., 2014). Alterations in the gut microbiota of patients with depression may modulate the inflammatory response. It was unexpected that no difference in serum inflammatory agents was detected among the three groups, probably due to the high prevalence of antidepressant use among the included patients (Hiles et al., 2012). But we found the serum level of BDNF differed significantly among these three groups. Previous studies have reported that changes in hippocampal BDNF mRNA and protein levels are related to the gut microbiota composition (Bercik et al., 2010, 2011; Neufeld et al., 2011). Our present results demonstrate that the prevalence of *Clostridium* XIVb was negatively associated with the serum BDNF level. We used the HAMD and MARDS scales to evaluate depressive symptoms in patients with MDD. Although a negative correlation was found between the abundance of *Faecalibacterium* and disease severity, we did not use HAMDS and MADRS to measure the depressive symptoms of the HCs. Hence, these findings need to be treated cautiously due to the risk of overestimation in small cohorts. Furthermore, the absence of HCs' scores may also explain the limited statistically significant correlations between gut microbiota and depressive severity.

In summary, this study contributes to previous reports on the involvement of gut microbiota in psychiatric disorders by demonstrating that microbial populations are associated with depression. Investigations of whether the *Faecalibacterium* protects against depression or whether Enterobacteriaceae and *Alistipes* are indicators of increased permeability of the gut wall are needed. Future

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studies involving larger cohorts and incorporating metagenomics, or even metabolomics, may elucidate the temporal and causal relationships between gut microbiota and depression.

Conflicts of interest

The authors declare no competing interest.

Funding

This study was supported by the National Key Stone Basic Research Program (973 Program) of China (2013CB531401), the Major Projects of Science Research for the 12th Five-Year Plan of China (2013ZX10004904, 2011ZX10004901), and the Fundamental Research Funds for the Central Universities, China (2014XZZX008, 2013XZZX00).

Uncited reference

First et al. (1996).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2015.03.016.

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