Gut microbiome, a potential indicator for differential diagnosis of major depressive disorder and general anxiety disorder

Zaiquan Dong
Sichuan University West China Hospital
Xiaoling Shen
Sichuan University West China Hospital
Yanni Hao
Sichuan University West China Hospital
Jin Li
Sichuan University West China Hospital
Haoran Li
Sichuan University West China Hospital
Haizheng Xu
Sichuan University West China Hospital
Weihong Kuang (kwhhlj@163.com)
Sichuan University West China Hospital

Research article

Keywords: gut microbiome, anxiety, depression, 16S ribosomal RNA, differential diagnosis

Posted Date: August 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-53518/v1

Version of Record: A version of this preprint was published at Frontiers in Psychiatry on September 13th, 2021. See the published version at https://doi.org/10.3389/fpsyt.2021.651536.

Abstract

Background Major depressive disorder (MDD) and general anxiety disorder (GAD) share many common features, leading to many challenges in their differential diagnosis. Given the importance of the microbiota-gut-brain axis, we aimed to investigate the differences in gut microbiota between representative cases of these two diseases and sought to develop a microbiome-based approach for their differential diagnosis.

Methods We enrolled 23 patients with MDD, 21 patients with GAD, and 10 healthy subjects (health control, HC) in the present study. We used 16S rRNA gene sequencing analysis to determine the microbial compositions of gut microbiome based on Illumina Miseq according to Illumina's standard protocol.

Results We found that patients with MDD or GAD exhibited significant differences in the relative abundance of gut microbiota. We identified the microbial signatures of subjects with MDD and GAD relative to HC and found differences in levels of *Faecalibacterium* and *LachnospiraceaeND3007group*. Moreover, we also found correlations between the bacteria and neuroendocrine and clinical symptoms, namely, a significant negative correlation between *Fusicatenibacter* and free thyroxine(FT4) in MDD, significant negative correlation between *Fusicatenibacter* and cortisol (PTC) in GAD, positive correlation between *LachnospiraceaeND3007group* and sleep disturbance factor of Hamilton Depression Rating Scale, and negative correlation between *LachnospiraceaeND3007group* and PTC (all $P<0.05$).

Conclusions The present study elucidated a unique gut microbiome signature associated with MDD and GAD that could facilitate differential diagnosis and targeted therapy.

Background

Anxiety and depression are two common disorders that show high comorbidity[1-3]. Although they share a number of causal and descriptive features, there are some differences in the clinical features and etiological factors associated with each disorder [4]. The separation of anxiety and depression disorders is extremely important for the elucidation of underlying mechanisms and the development of specific pharmacological and psychological treatments. Although a large number of studies have distinguished anxiety and depression from the perspective of symptomatology and psychological, social, and physiological etiology[5-8], there is still no convincing evidence to distinguish between the two.

A growing body of evidence indicates that gut microbiota play a crucial role in modulating both brain function and human behavior[9]. Furthermore, differences in gut microbiota have been identified in a variety of psychiatric diseases, including depression, bipolar disorder, and schizophrenia[10, 11], and several animal models of psychiatric diseases[12-14]. There is evidence for altered microbiota composition in depressed individuals[15-17], with levels of *Faecalibacterium* negatively correlating with symptom severity[18]. Studies have also shown that probiotic administration of *Bifidobacterium longum* and *Lactobacillus helveticus* could decrease anxiety[19-21]. Recent studies have suggested that the change in intestinal microflora may be used as a biomarker for the diagnosis and monitoring of depression[22, 23]. Zheng et al. recently identified distinct gut microbial compositions in major depressive disorder (MDD) compared to bipolar disorder (BD) and provided a novel marker panel to distinguish MDD from BD based on gut microbiome signatures[24].

To date, there is no information about the differences in intestinal flora as a biological marker for the identification of anxiety and depression. The purpose of this study was to compare the differences in intestinal flora of patients with diagnoses of anxiety to those with diagnoses of depression to determine whether intestinal flora can help to

distinguish between these two groups. To achieve this, we examined MDD without obvious anxiety symptoms and GAD without obvious depressive symptoms and used 16S rRNA gene sequencing analysis to maintain the purity of clinical representation and help distinguish between the differences in intestinal flora in these two diseases. Additionally, we analyzed the effects of different bacteria on clinical symptoms and the neuroendocrine system to further explore the function of these bacteria.

Methods

Subjects

Patients with MDD and GAD and normal control subjects participated in the present study. Both the MDD patients and GAD patients consisted of a series of outpatients who received treatment at the West China Hospital from January 2019 to June 2019. All samples are from Chengdu, Sichuan, China, which is a relatively geographically closed area and people residing in it have similar eating habits. The patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (*DSM-5*) [25]at the first clinical examination; the diagnoses were confirmed by two psychiatrists. Patients younger than 18 years or older than 45 years, with organic etiology for their psychiatric symptoms, or psychotic features, or mental retardation were excluded. The normal control subjects were 10 worker volunteers, aged between 18 and 45 years, and without current or past major psychiatric disorders. Subjects with the following conditions were also excluded: 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 six months before collection of the fecal sample; known active bacterial, fungal, or viral infections; and obvious dietary preferences (e.g., vegetarians). To minimize the impact of comorbidities, we also excluded GAD patients with a lifetime history of major depressive episodes and/or with a comorbid diagnosis of depression or 24-items Hamilton Depression Rating Scale (HAD) $\geq$ 20 and MDD patients with a lifetime history of anxiety episodes and/or with a comorbid diagnosis of any type of anxiety disorder or 14-items Hamilton Anxiety Rating Scale (HAMA) $\geq$ 14 from the study.

Neuroendocrine hormone analysis

The hypothalamic-pituitary-thyroid (HPT) axis test indicators include thyroid-stimulating hormone (TSH, normal value: 0.27–4.2 mU/L), triiodothyronine (T3, normal value: 1.3–3.1 mmol/L), thyroxine (T4, normal values: 62.0–164.0 mmol/L), free triiodothyronine (FT3, normal value: 3.6–7.5 pmol/L), and free thyroxine (FT4, normal value: 12.0–22.0 pmol/L). The hypothalamic-pituitary-adrenal (HPA) axis test indicators include Corticotropin (ACTH, normal value: 5.0–78.0 ng/L) and 8:00 a.m. cortisol (PTC, normal value: 147.3–609.3 mmol/L). Fasting venous blood was taken by drawing 4 mL of cubital venous blood at 8 a.m. after overnight fasting. All of the analyses were performed the Roche Cobas e601 via an electrochemiluminescence method. All reagents and calibrations were from Roche's original kit.

Sample collections and DNA extraction

Fecal samples were immediately frozen on collection in a sterile plastic cup and stored at −80 °C before analysis. Microbial genomic DNA was extracted using the QIAamp DNA Stool Mini Kit according to the instructions (Qiagen, Hilden, Germany). The 16S rRNA V3 amplicons were generated using the NIH Human Microbiome Project protocols (16S 454 Sequencing Protocol HMP Consortium ,https://www.hmpdacc.org).

16S rRNA gene sequencing analysis

Libraries were prepared and subjected to paired-end sequencing with Illumina Miseq by following Illumina's standard protocol [26]. These QIIME2 16S rRNA sequencing protocols were used to pick and analyze operational taxonomic units (OTUs) [27].

Bioinformatics and statistical analysis

The sequence.index file was used to identify and extract the sample data saved in FASTQ format. Barcodes and the primers in the beginning and the end were used to identify and select sequence reads. The sequence number of each sample was normalized, and OTUs with 97% identity thresholds were used by the UPARSE (version 7.1 http://drive5.com/uparse/) software program. Chimeric sequences were identified and removed using UCHIME (version 4.1 http://drive5.com/uchime/). The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) using the SILVA (SSU 115) 16S rRNA database at a confidence threshold of 70% [28].

Gut-microbiota-specific microbial characteristics (genes, pathways, or taxa) were analyzed using the linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/galaxy/), which emphasizes both statistical significance and biological relevance.The Kruskal–Wallis test was used to compare the relative abundance of microbes identified by 16S rRNA sequencing.

Statistical analyses were performed using SPSS version 21(SPSS, Chicago, IL, USA). One-way ANOVA was used to compare the continuous variables including age, BMI, and clinical scales. Fisher's exact test was used for the analysis of contingency tables.Chi-Square method was used to compare the variables of all three groups.

Results

Demographic features

We collected 54 fecal samples from the study participants, including 10 subjects in the healthy crowd (HC) group, 23 in the MDD group, and 21 in the GAD group. The mean age at assessment was 30.04±5.90 years for the MDD group, 30.43±7.59 years for the GAD group, and 30.22±6.50 years for the HC group; no significant difference was found between the groups by one-way analysis of variance (ANOVA). The BMI, sex ratio, marital status, and family history were not significantly different among the three groups (Table 1).

Table 1 Clinical and demographic characteristics of MDD and GAD patients and HC individuals

GROUP	MDD（n=23）	GAD (n=21)	HC (n=10)	*P*
Age (years, Mean±SD)	30.04±5.90	30.43±7.95	30.22±6.50	0.982
BMI (Mean±SD)	21.87±3.00	21.19±2.89	21.45±2.80	0.743
HAMD-24 (Mean±SD)	29.26±7.51	12.10±5.25	*NA*	< 0.001
HAMA (Mean±SD)	8.00±3.55	23.71±7.30	*NA*	< 0.001
Sex, n (%)				0.929†
Male	7 (30.43)	7 (33.33)	4 (40.00)	
Female	16 (69.57)	14 (66.67)	6 (60.00)	
Marital status, n (%)				0.935†
Never married	9 (39.13)	8 (38.10)	3 (30.00)	
Married	14 (60.87)	13 (61.90)	7 (70.00)	
Family history, n (%)			*NA*	0.481‡
Yes	4 (17.39)	6 (28.57)		
No	19 (82.61)	15 (71.43)		

† Fisher's exact probability method was used.

‡ Chi-square test was used.

Analysis of 16S data

Accounting for 70% of the valid sequences, we obtained 1,620,000 high-quality sequences from 54 fecal samples of all participants: the HC group contains 630,000 sequences, the MDD group contains 690,000 sequences, and the GAD group contains 300,000 sequences. In particular, we obtained 10,996 species-level OTUs from the HC group, 14,406 OTUs from the MDD group, and 15,010 OTUs from the GAD group (Table 2). Venn analysis results show that 5069 OTUs were common to all three groups (Fig. 1).

Table 2 Comparison of phylotype coverage and diversity estimation of 16S rRNA gene libraries at 97% similarity from sequencing analysis

Group	Number of reads	Number of OTUs†	Coverage (%)‡	Richness estimator				Diversity index		
				ACE	95% CI	Chao	95% CI	Shannon	Simpson	Evenness§
HC	630000	10996	97.19	4257.84	4031.74–4506.03	3090.89	2840.24–3395.07	4.733331714	0.032098	0.354556683
MDD	690000	14406	97.47	3762.74	3557.59–3989.13	2790.49	2562.74–3069.66	4.647938217	0.036133	0.34454694
GAD	300000	15010	96.15	5978.93	5689.91–6291.70	4170.2	3857.99–4538.97	5.017976	0.025108	0.397936241

† The operational taxonomic units (OTUs) were defined with 97% similarity level.

‡ The coverage percentage, richness estimators (ACE and Chao), and diversity indices (Shannon and Simpson) were calculated using Good’s method and the mothur package, respectively.

§ 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.

The richness of gut bacterial communities in all three groups was estimated by ACE and Chao, and the diversity was estimated by using the Shannon diversity index and Simpson diversity index. ACE and Chao analysis showed that most of the gut microbial diversity in each sample had been captured with the current sequencing depth. After rarefying the sequencing depth among all of the samples using a bootstrap method (30,000 reads per sample), Shannon diversity index and Simpson diversity index estimates were calculated. There was no significant difference in richness and diversity between HC and MDD in this study. However, GAD showed significant difference in richness and diversity of the microbiota compared to HC (Fig. 2).

Analysis of fecal bacterial community

We analyzed the gut bacterial composition and relative abundance at the phylum level. Compared with the HC group, we found a considerable increase in the relative abundance of *Proteobacteria* and *Actinobacteria* in MDD and a considerable decrease in the relative abundance of *Bacteroidetes*. Compared with the HC group, the relative abundance of *Fusobacteria*, *Tenericutes,* and *Verrucomicrobia* increased and that of *Firmicutes* decreased in GAD; there was no obvious change in phylum levels. Compared with the MDD group, we found an increase in the relative abundance of *Fusobacteria*, *Tenericutes*, *Verrucomicrobia,* and *Bacteroidetes*, but a decrease in the relative abundance of *Proteobacteria*, *Actinobacteria,* and *Firmicutes* in the GAD group (Fig. 3a). At the family level, compared with the HC group, we found that the counts of some bacteria, such as *Desulfovibrionaceae,* were considerably reduced in the GAD and MDD patients. However, levels of bacteria, such as *Enterobacteriaceae,* were considerably higher in MDD patients, but showed no change in GAD patients. The levels of nine species, including *Fusobacteriaceae*, did not change in MDD patients, but were higher in GAD patients (Fig. 3b). At the genus level, compared with the HC group, we found that the levels of *Megamonas* were remarkably reduced in GAD and MDD patients. The counts of bacteria, such as *Faecalibacterium,* were higher in MDD patients, but showed no change in GAD patients. Compared with the HC group, the levels of *Bacteroides* did not change in MDD patients, but increased in GAD patients (Fig. 3c).

In summary, we found that patients with MDD or GAD showed considerable changes in the gut microbiota, and there were differences in the relative abundance of gut microbiota in patients with MDD and GAD.

Analysis of the differences in gut microbiota

Analysis of the differences among the three groups at the genus level revealed significant differences in the gut microbiota ($P<0.05$) (Fig. 4a). Compared with HC individuals, we found that levels of *Butyricimonas, Megamonas*, *Fusicatenibacter*, *Sutterella*, *Coprococcus_3*, *Bergeyell* , *Bilophila, Acinetobacter*, *Ruminococcus_2,* and *Rothia* were significantly different in MDD patients. To identify key significant differences between MDD and HC individuals, we analyzed the metagenome data by using the LEfSe method (Fig. 4b). We found that *Butyricimonas*, *Megamonas,* *Fusicatenibacter*, *Sutterella*, *Coprococcus_3*, *Bergeyella,* and *Bilophila* were abundant in HC, and *Acinetobacter*, *Ruminococcus_2,* and *Rothia* were abundant in MDD. Additionally, we found that *Butyricimonas*, *Christensenellaceae_R_7_group, Megamonas, Sutterella, Fusicatenibacter, Haemophilus, Coprococcus_3,* and *Lachnospira* were abundant in the HC, and *Lactobacillus, Hungatella, Coprobacter* and *Flavonifractor* were abundant in the GAD (Fig. 4c). Of note, we found that levels of *Faecalibacterium*, *Sutterella*, some *Bacteroides,* and some *Carnobacterium* showed clear differences between MDD and GAD patients. To identify key significant differences between MDD and GAD, we used the LEfSe method to analyze the metagenome data (Fig. 4d). We found that

Sutterella, *Burkholderiaceae,* and *betaproteobacteriales* were abundant in GAD patients, whereas *Faecalibacterium*, *Klebsiella*, *Ruminococcus, Rothia,* and *Subdoligranulum* were abundant in MDD patients. These were the dominant phylotypes that contributed to the differences between the intestinal microbiota of GAD and MDD patients.

Relationship between gut microbiota and clinical parameters

We evaluated correlations among the relative abundance of bacterial genera, the hormones (including PTC, ACTH, FT3, FT4, TT3, TT4, and TSH), the total and factor scores of HAM-D (Hopelessness, Sleep disturbance, Block, Diurnal/variation, Cognitive impairment, Weight and Anxiety/somatic) in the MDD group and the total and factor scores of HAM-A (Psychic anxiety and Somatic anxiety) in the GAD group. Compared with the GAD group, we found significant differences at the genus level for*Fusicatenibacter, LachnospiraceaeND3007group, Coprococcus3, Faecalibacterium, norank_Firmicutes, ChristensenellaceaeR7group, Gutmetagenome,* and *norank_Proteobacteria* in MDD patients (Fig .5a). We observed that *ChristensenellaceaeR7group* negatively correlated with the total score and Hopelessness factor of HAM-D and ACTH (P<0.05, Fig. 5b, c, d), *Coprococcus3* and *LachnospiraceaeND3007group* negatively correlated with PTC (P<0.05, Fig. 5e g), *LachnospiraceaeND3007group* positively correlated with Sleep disturbance factor of HAM-D (P<0.05, Fig. 5f), *Fusicatenibacter* negatively correlated with FT4 (P<0.05, Fig. 5h), and other key phylotypes showed no strong correlation. *Fusicatenibacter, LachnospiraceaeND3007group, Coprococcus3, Faecalibacterium, norank_Firmicutes, ChristensenellaceaeR7group, Gutmetagenome,* and *norank_Proteobacteria* showed significant interindividual variability in GAD (Fig. 5i). In the GAD group, we found that *ChristensenellaceaeR7group, LachnospiraceaeND3007group,* and *Fusicatenibacter* negatively correlated with PTC (P<0.05, Fig. 5j, k, l), whereas other key phylotypes showed no strong correlation.

Discussion

Human intestinal flora interact with the central nervous system via immune, biochemical, and neuroendocrine pathways, leading to neuropsychiatric diseases such as anxiety and depression[29]. This study is the first to characterize the gut microbial composition of MDD and GAD in comparison to each other and to healthy individuals (HC). We identified unique microbial signatures of subjects with MDD and GAD relative to HC, which can help to distinguish MDD from GAD, as well as to help identify novel therapeutic targets for MDD and GAD.

Bacterial abundance has been recently linked to disease. *Parabacteroides* is an important species in the human body whose abundance has been negatively correlated with the status of obesity and other diseases, suggesting that it may play a positive regulatory role in glucose and lipid metabolism[30].

In this study, we found that most of the bacteria were similar in abundance and composition with only a few changes that may be important in causing depression or anxiety. Both MDD and GAD patient samples were rich in *Parabacteroides*, *Prevotella*, *Acetitomaculum*, *Escherichia-Shigella*, and *Dialister*, and there were no significant differences in these bacteria compared with the HC samples (Suppl.S.) We believe that this is because these bacteria help in maintaining normal physiological functions..

Furthermore, we found that intestinal flora of patients with depression is similar to that of normal people, whereas intestinal flora of patients with anxiety disorders is different in terms of the genus (ACE, Chao, Shannon, and Simpson). The composition of intestinal microflora is complex and is subject to various factors such as gender, genetics, age, region, drugs, and diet[31]. The content of microbes such as archaea, fungi, and especially of bacteria is critical for the development of disease. The dominant flora are *Firmicutes* and *Bacteroides*, which account for over 98% of the intestinal microbiota[32]. We identified unique microbial signatures of subjects with MDD and GAD

relative to HC, which can help to distinguish MDD from GAD as well as to help identify novel therapeutic targets for MDD and GAD. We found that the order of dominant flora in patients with depression or anxiety was *Bacteroidetes* > *Firmicutes* > *Proteobacteria* > *Actinobacteria*. But the relative abundance of these bacteria is different: *Bacteroidetes* (HC=GAD>MDD), *Firmicutes* (MDD>HC>GAD), *Proteobacteria* (MDD>GAD>HC), and *Actinobacteria* (MDD>GAD>HC). Similar differences were observed in relative abundance at the family and genus levels. Altogether, nine species of bacteria showed significant variation between MDD and GAD patients at the genus level: *Fusicatenibacter*, *LachnospiraceaeND3007group*, *Coprococcus3*, *Sutterella, Faecalibacterium*, *norank_Firmicutes*, *ChristensenellaceaeR7group*, *Gutmetagenome,* and *norank_Proteobacteria*.

We also found significant correlation between some bacteria and the clinical symptoms such as a negative correlation between *ChristensenellaceaeR7group* and the factor score of hopelessness or the total HAM-D score negative correlation between *LachnospiraceaeND3007*group and the factor score of sleep disturbance of HAM-D in MDD patients. Jiang et al. found that the abundance of *Faecalibacterium* negatively correlated with symptom severity[18]; our results further validate this association.

In addition, we found a correlation between the bacteria and the HPA axis in the form of significant negative correlations between *ChristensenellaceaeR7group* and ACTH, *Coprococcus3* and PTC, *LachnospiraceaeND3007group* and PTC in MDD and between PTC and *ChristensenellaceaeR7group*, *LachnospiraceaeND3007group*, or *Fusicatenibacter* in GAD. Numerous studies have verified the association between the HPT or HPA systems and the onset of depression and anxiety[33, 34]. Sudo et al. found that intestinal flora played an important role in maintaining the normal activity of the HPA axis[35]; hence, absence of the gut microbiota exacerbates the neuroendocrine and behavioral responses to acute stress[36-38]. Abnormal HPT axis function is another contributor to the possible pathogenesis of depression. Brownlie et al. proposed that a dynamic decrease in thyroid hormone levels, especially of FT3 and FT4[39], could be related to the occurrence of depression. In this study, we also found a correlation between microbiota and the hormones of the HPT axis in the form of a significant negative correlation between *Fusicatenibacter* and FT4 in MDD. These observations suggest that abnormal intestinal microbiota may affect the HPA and HPT axes, which may be why individuals have different clinical phenotypes including anxiety and depression and which warrants further study to elucidate the mechanism underlying the correlation.

This study has some limitations. First, the sample size of this study is relatively small because of which there may be sampling bias. Second, the 16S rRNA gene sequencing used in this study has limited functional information; whole-genome sequencing and whole-macrotranscriptome sequencing need to be performed in future studies.

Conclusions

In summary, we found that patients with MDD or GAD exhibited significant changes in the gut microbiota, and there were differences in the relative abundance of gut microbiota in these patients with MDD and GAD. We identified the microbial signatures of subjects with MDD and GAD relative to HC. We found differences in *Faecalibacterium* and *LachnospiraceaeND3007group* between MDD and GAD. Moreover, we also found correlation between the bacteria and neuroendocrine and clinical symptoms such as a significant negative correlation between *Fusicatenibacter* and FT4 in MDD, a significant negative correlation between *Fusicatenibacter* and PTC in GAD, a positive correlation between *LachnospiraceaeND3007group* and Sleep disturbance factor of HAM-D, and a negative correlation between *LachnospiraceaeND3007group* and PTC. We concluded that intestinal microflora might serve as molecular markers that distinguish MDD from GAD.

Abbreviations

MDD: major depressive disorder; GAD: general anxiety disorder; HC: health control; BD: bipolar disorder; *DSM:* Diagnostic and Statistical Manual of Mental Disorders ; HAMD: Hamilton Depression Rating Scale; HAMA: Hamilton Anxiety Rating Scale; HPT: hypothalamic-pituitary-thyroid; TSH: thyroid-stimulating hormone; T3: triiodothyronine; T4: thyroxine; FT3: free triiodothyronine; FT4: free thyroxine; HPA: hypothalamic-pituitary-adrenal; ACTH: Corticotropin; PTC: cortisol; LDA: linear discriminant analysis ; LEfSe: linear discriminant analysis effect size; OTUs: operational taxonomic units.

Declarations

Acknowledgements

This work was supported by the Asbios (Tianjin) Biotechnology Co., LTD. We would like to thank Editage for English language editing.

Authors' contributions

ZQD was critically involved in the study design and wrote the manuscript. XLS, YNH, JL, HRL and HZX were involved in subject recruitment. WHK guided this research and supervised the entire project. All authors read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China [Grants No.81621003]; The National Natural Science Foundation of China (Grant No. 81801350); and the Key R&D projects of science and Technology Department of Sichuan Province (Grant No. 2019YFS0217).

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures contributing to this work comply with the ethical standards of national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects and patients were approved by the Ethics Committee of West China Hospital (WCH) of Sichuan University (approval number:2019-268). Written informed consent was obtained from all study subjects.

Consent for publication

Not Applicable.

Competing interests

All researchers declare that they have no other conflicts of interest.\

References

1. Costello EJ, Mustillo S, Erkanli A, Keeler G, Angold A. Prevalence and development of psychiatric disorders in childhood and adolescence. Arch Gen Psychiatry. 2003; 60(8):837-844.
2. Godfrey K, Yung A, Killackey E, Cosgrave E, Yuen HP, Stanford C, Buckby J, McGorry P. Patterns of current comorbidity in young help-seekers: implications for service planning and delivery. Australas Psychiatry. 2005; 13(4):379-383.
3. Reiter S, Emodi-Perlman A, Goldsmith C, Friedman-Rubin P, Winocur E. Comorbidity between depression and anxiety in patients with temporomandibular disorders according to the research diagnostic criteria for temporomandibular disorders. J Oral Facial Pain Headache. 2015; 29(2):135-143.
4. Goldberg D. Towards DSM-5: the relationship between generalized anxiety disorder and major depressive episode. Psychol Med. 2008; 38(11):1671-1675.
5. Clark LA, Watson D. Tripartite model of anxiety and depression: psychometric evidence and taxonomic implications. J Abnorm Psychol. 1991; 100(3):316-336.
6. Cohen JR, Young JF, Gibb BE, Hankin BL, Abela JR. Why are anxiety and depressive symptoms comorbid in youth? A multi-wave, longitudinal examination of competing etiological models. J Affect Disord. 2014;, 161:21-29.
7. Krueger RF. The structure of common mental disorders. Arch Gen Psychiatry. 1999; 56(10):921-926.
8. Krueger RF, Caspi A, Moffitt TE, Silva PA. The structure and stability of common mental disorders (DSM-III-R): a longitudinal-epidemiological study. J Abnorm Psychol. 1998; 107(2):216-227.
9. Mayer EA, Knight R, Mazmanian SK, Cryan JF, Tillisch K. Gut microbes and the brain: paradigm shift in neuroscience. J Neurosci. 2014; 34(46):15490-15496.
10. Dickerson F, Severance E, Yolken R. The microbiome, immunity, and schizophrenia and bipolar disorder. Brain Behav Immun. 2017; 62:46-52.
11. Rogers GB, Keating DJ, Young RL, Wong ML, Licinio J, Wesselingh S. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. Mol Psychiatry. 2016; 21(6):738-748.
12. Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell. 2019; 177(6):1600-1618 e1617.
13. Wong ML, Inserra A, Lewis MD, Mastronardi CA, Leong L, Choo J, et al. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. Mol Psychiatry. 2016; 21(6):797-805.
14. Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. Sci Adv. 2019; 5(2):eaau8317.
15. Chen Z, Li J, Gui S, Zhou C, Chen J, Yang C, et al. Comparative metaproteomics analysis shows altered fecal microbiota signatures in patients with major depressive disorder. Neuroreport. 2018; 29(5):417-425.
16. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol. 2019; 4(4):623-632.
17. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, et al: Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol Psychiatry. 2016; 21(6):786-796.
18. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, et al: Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav Immun. 2015; 48:186-194.
19. Jang HM, Lee HJ, Jang SE, Han MJ, Kim DH. Evidence for interplay among antibacterial-induced gut microbiota disturbance, neuro-inflammation, and anxiety in mice. Mucosal Immunol. 2018; 11(5):1386-1397.

20. Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A, et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br J Nutr. 2011; 105(5):755-764.
21. Mohammadi AA, Jazayeri S, Khosravi-Darani K, Solati Z, Mohammadpour N, Asemi Z, et al: The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers. Nutr Neurosci. 2016; 19(9):387-395.
22. Chen JJ, Zheng P, Liu YY, Zhong XG, Wang HY, Guo YJ, et al. Sex differences in gut microbiota in patients with major depressive disorder. Neuropsychiatr Dis Treat. 2018; 14:647-655.
23. Lin P, Ding B, Feng C, Yin S, Zhang T, Qi X, et al: Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. J Affect Disord. 2017; 207:300-304.
24. Zheng Q, Wang Y, Lu J, Lin W, Chen F, Jiao N.: Metagenomic and Metaproteomic Insights into Photoautotrophic and Heterotrophic Interactions in a Synechococcus Culture. mBio. 2020; 11(1).
25. Francesmonneris A, Pincus H, First M. Diagnostic and Statistical Manual of Mental Disorders: DSM-5: American Psychiatric Association; 2013.
26. Bartram AK, Lynch MD, Stearns JC, Moreno-Hagelsieb G, Neufeld JD.: Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end illumina reads. Appl Environ Microbiol. 2011; 77(11):3846-3852.
27. Kuczynski J, Stombaugh J, Walters WA, Gonzalez A, Caporaso JG, Knight R. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Curr Protoc Microbiol. 2012; Chapter 1:Unit 1E 5.
28. Amato, KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, et al. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. The ISME Journal. 2013; 7:1344–1353.
29. Kupfer DJ, Frank E, Phillips ML. Major Depressive Disorder: New Clinical, Neurobiological, and Treatment Perspectives. Focus (Am Psychiatr Publ). 2016; 14(2):266-276.
30. Wang K, Liao M, Zhou N, Bao L, Ma K, Zheng Z, et al. Parabacteroides distasonis Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. Cell Rep. 2019; 26(1):222-235 e225.
31. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human genetics shape the gut microbiome. Cell. 2014; 159(4):789-799.
32. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI; Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol; 2008; 6(10):776-788.
33. Foster JA, McVey Neufeld KA; Gut-brain axis: how the microbiome influences anxiety and depression. Trends Neurosci; 2013; 36(5):305-312.
34. Holsboer F; The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology; 2000; 23(5):477-501.
35. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol. 2004; 558(Pt 1):263-275.
36. Crumeyrolle-Arias M, Jaglin M, Bruneau A, Vancassel S, Cardona A, Dauge V, et al. Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. Psychoneuroendocrinology. 2014; 42:207-217.
37. Hryhorczuk C, Sharma S, Fulton SE. Metabolic disturbances connecting obesity and depression. Front Neurosci. 2013; 7:177.

38. Pellegrini C, Antonioli L, Calderone V, Colucci R, Fornai M, Blandizzi C. Microbiota-gut-brain axis in health and disease: Is NLRP3 inflammasome at the crossroads of microbiota-gut-brain communications? Prog Neurobiol. 2020; 191:101806.
39. Brownlie BE, Rae AM, Walshe JW, Wells JE. Psychoses associated with thyrotoxicosis-'thyrotoxic psychosis.' A report of 18 cases, with statistical analysis of incidence. Eur J Endocrinol. 2000; 142(5):438-444.

Supplementary Information

Supplementary information accompanies this paper in the NCBI Short Read Archive under BioProject ID PRJNA647236.

Figures

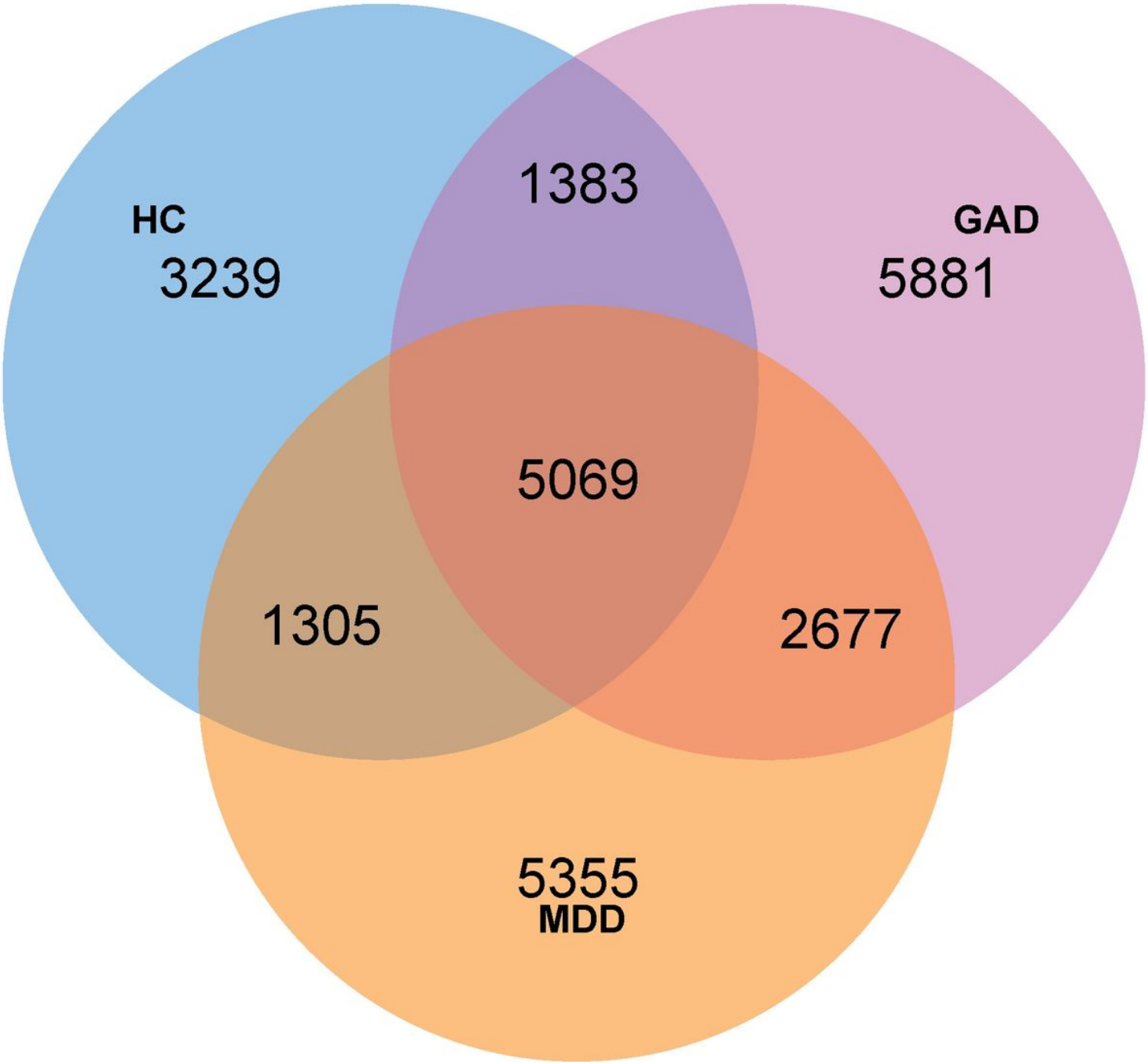

Figure 1

The Venn diagram shows common and unique OTUs. Overlap indicates the common OTUs in multiple groups; nonoverlapping areas indicate unique OTUs

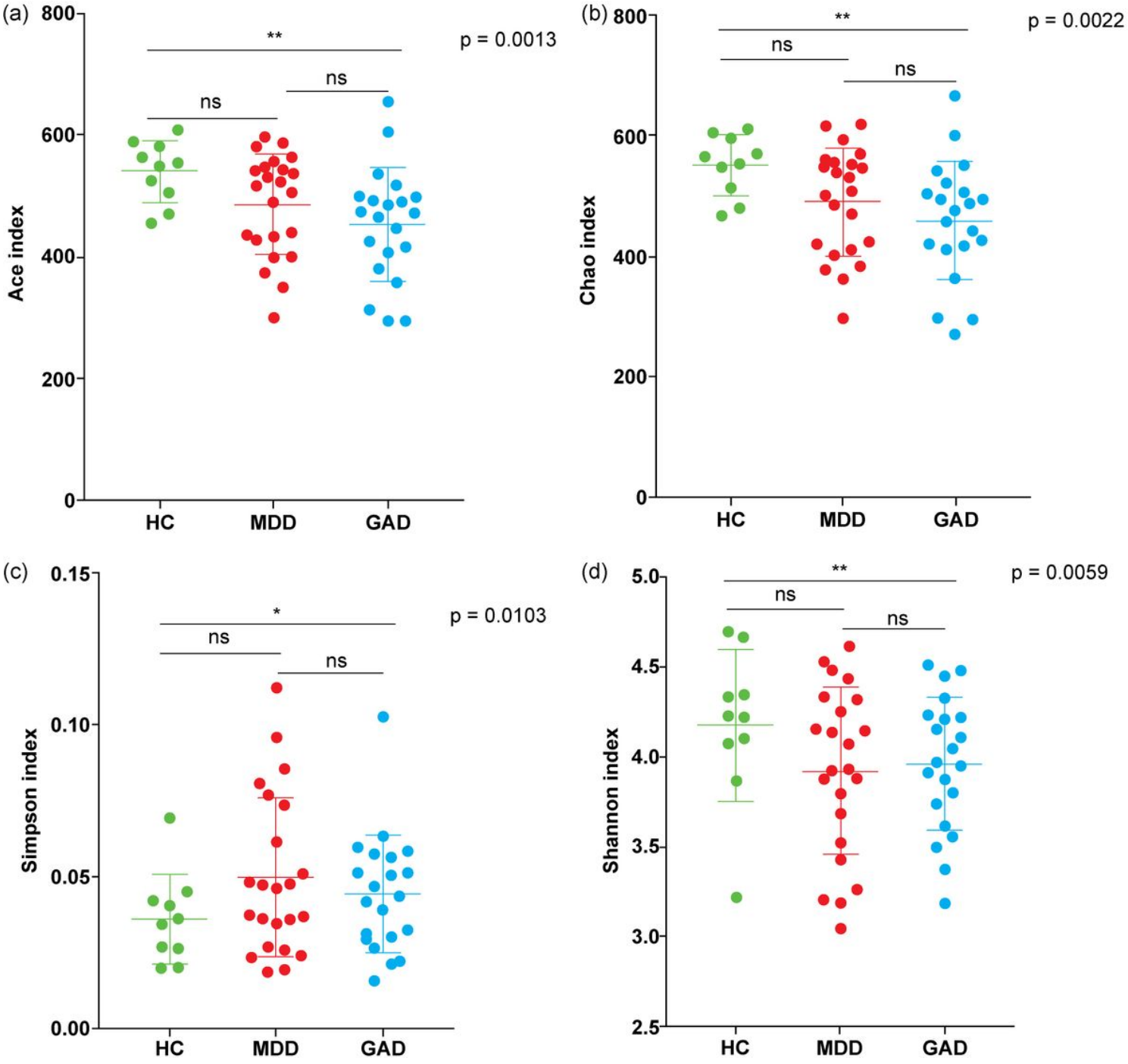

Figure 2

Analysis of variation in richness (ACE and Chao indices) and diversity (Simpson and Shannon indices). MDD compared with HC: no significant difference in the richness and diversity; GAD compared with HC: significant difference in richness and diversity, * indicates $P < 0.05$, ** indicates $P < 0.01$. MDD compared with GAD: no significant difference in richness and diversity.

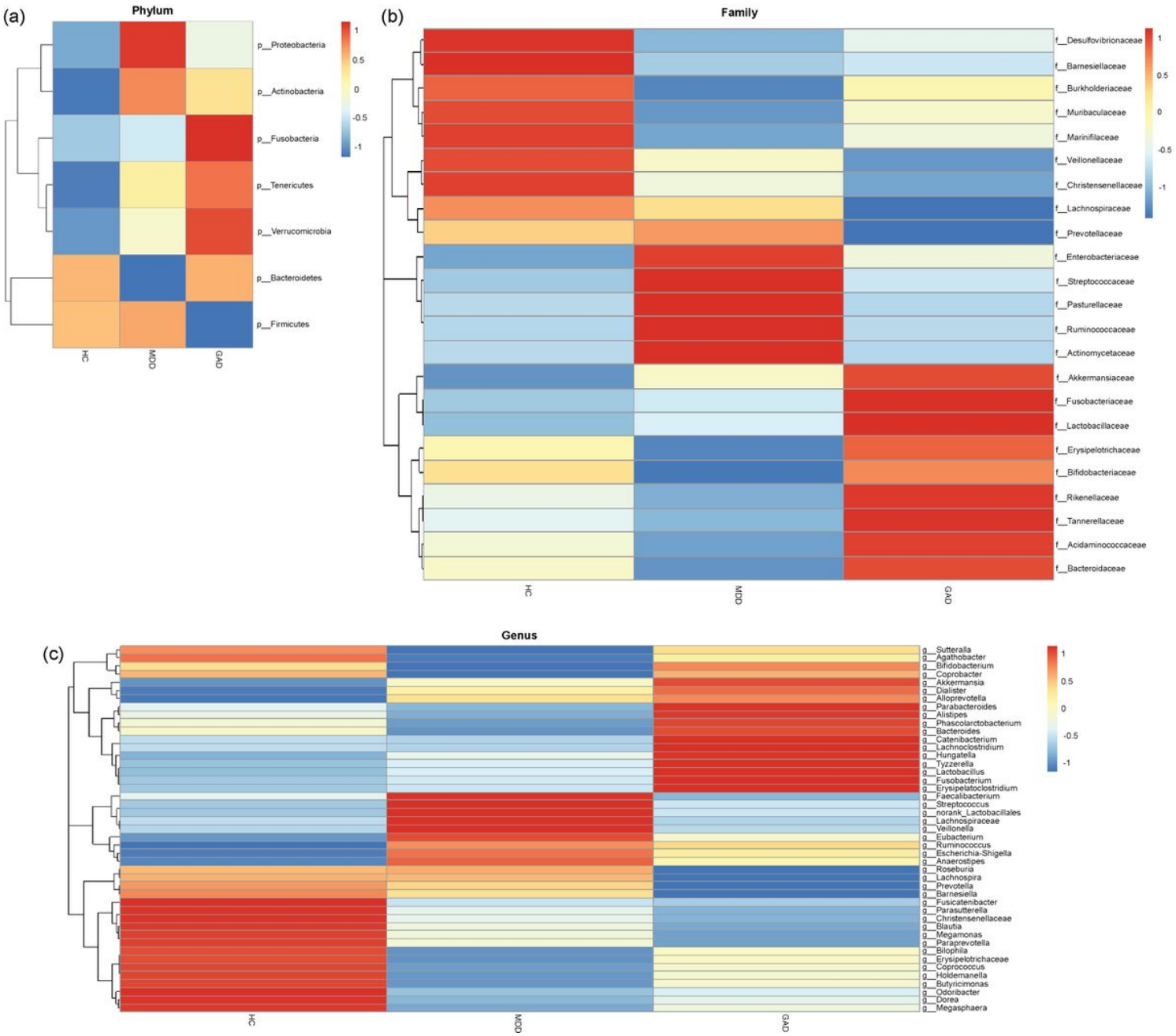

Figure 3

Differences in abundance of flora were analyzed with heat maps. In the heatmap legends, red indicates high content, and blue indicates low content. a) Phylum level; b) Family level; c) Genus levels.

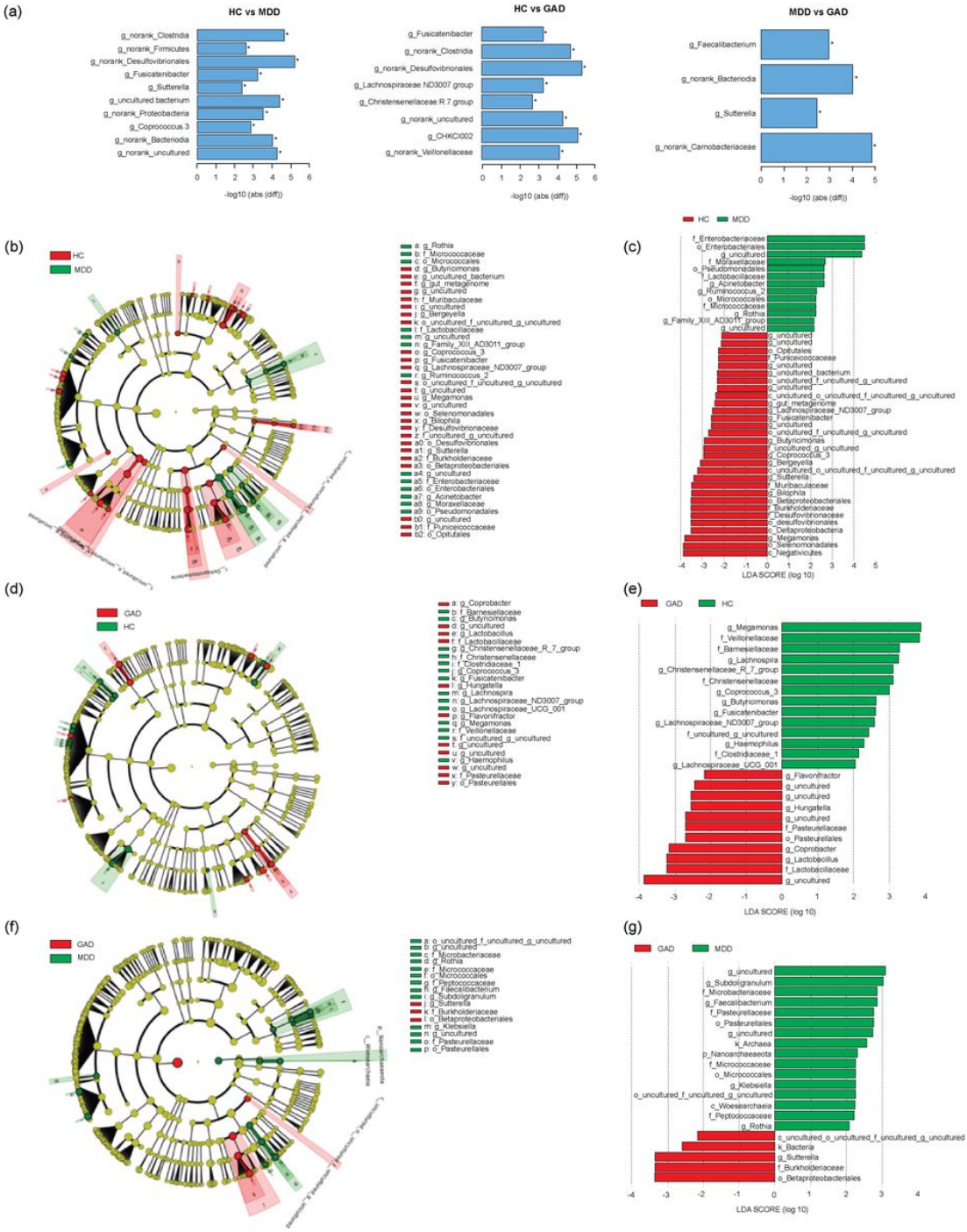

Figure 4

Taxonomic differences in fecal microbiota between HC, MDD, and GAD groups. (a) Comparison of relative abundance at bacterial genus levels between two groups. * indicates $P < 0.05$. (b–d) LEfSe identified the most differentially abundant taxons between two groups. Taxonomic cladogram obtained from LEfSe analysis of 16S sequences.

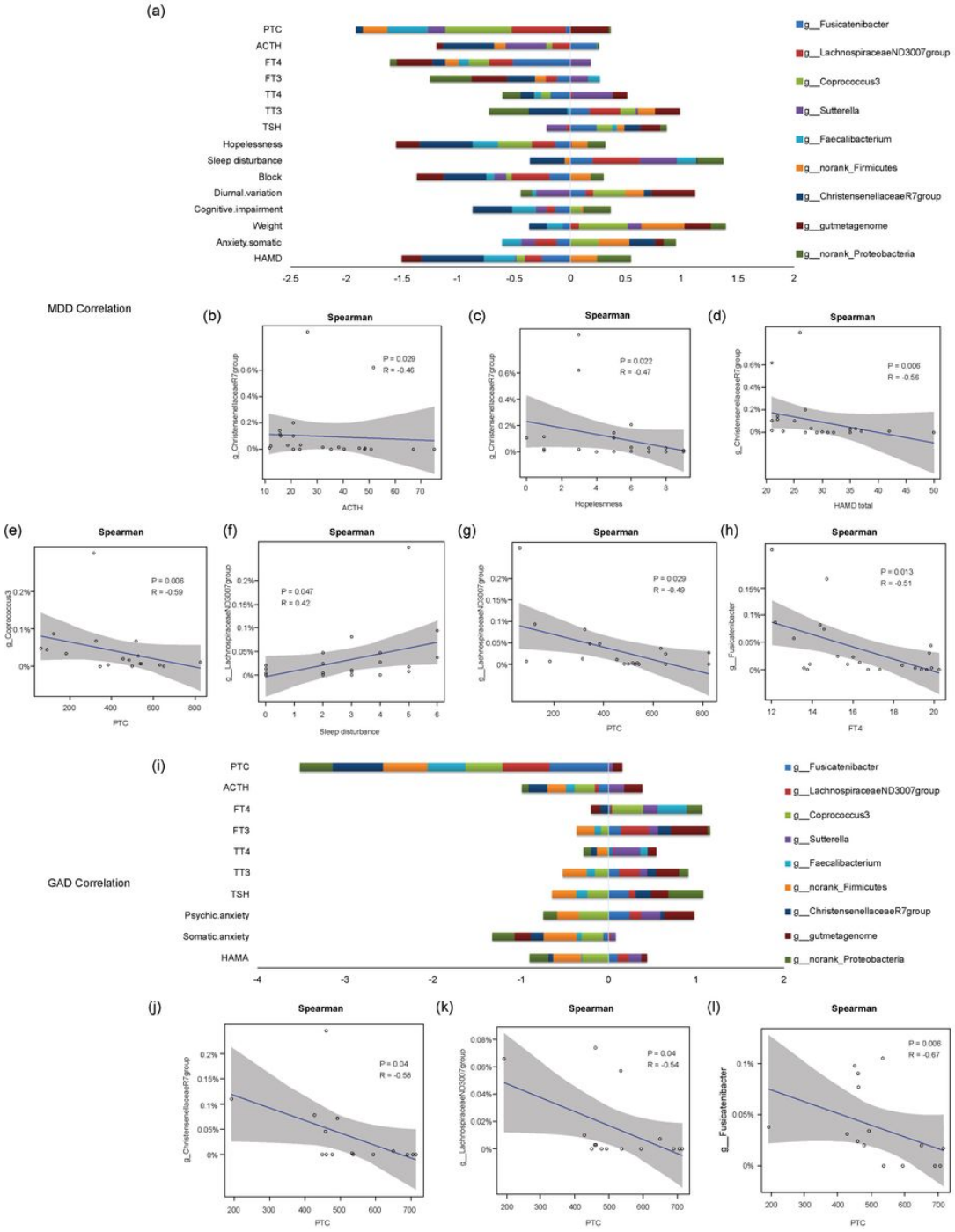

Figure 5

Relationship between gut microbiota and clinical parameters. (a) Correlation between gut microbiota and clinical parameters in MDD patients, positive (+) indicates positive correlation, negative (-) indicates negative correlation; (b–h) Linear correlation of gut microbiota with clinical parameters of the MDD group by Spearman method. (i) Correlation of gut microbiota with clinical parameters in GAD patients, positive (+) indicates positive correlation, negative (-) indicates negative correlation; (j–l) Linear correlation of gut microbiota with clinical parameters of GAD patients by Spearman method.