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# Disruption of the gut microbiota-inflammation-brain axis in unmedicated bipolar disorder II depression

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The relationships of the gut microbiota-inflammation-brain axis in depressive bipolar disorder (BD) remains under-elaborated. Sixty-five unmedicated depressive patients with BD II and 58 controls (HCs) were prospectively enrolled. Resting-state functional MRI data of static and dynamic amplitude of low-frequency fluctuation (ALFF) was measured, and abnormal ALFF masks were subsequently set as regions of interest to calculate whole-brain static functional connectivity (sFC) and dynamic functional connectivity (dFC). Fecal samples were collected to assess gut diversity and enterotypes using 16S amplicon sequencing. Blood samples were also collected, serum was assayed for levels of cytokines (interleukin [IL]-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor [TNF]-α). Patients with BD II exhibited decreased static ALFF values in the left cerebellum Crus II, and decreased cerebellar sFC and dFC to the right inferior parietal lobule and right superior frontal gyrus, respectively. Moreover, higher pro-inflammatory and anti-inflammatory cytokines levels, and increased proinflammatory bacteria and glutamate and gamma-aminobutyric acid metabolism related bacteria were identified in BD II. The interaction of *Parabacteroides* levels × IL-8 levels was an independent contributor to static ALFF in the left cerebellar Crus II. The findings bridged a gap in the underlying pathophysiological mechanism of the gut microbiota-inflammation-brain axis in BD II depression.

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## INTRODUCTION

Bipolar disorder (BD) is characterized by alternating episodes of depression and mania (bipolar disorder type I [BD I]/hypomania (bipolar disorder type II [BD II]), and is one of the leading contributors to disability worldwide [1]. The global prevalence of BD II is estimated to be 1.57% [2], with approximately 75% of the symptomatic period being characterized by depressive episodes or symptoms [1]. BD II depression is capable of affecting interpersonal connections, finances, and physical health [1, 2]. Timely identification and intervention are linked to a more favorable prognosis [2, 3], but early and accurate diagnosis of BD II depression is still challenging in clinical practice [3, 4], resulting in suboptimal treatment, escalated healthcare expenses, and unfavorable clinical outcomes [3]. Actually, the pathophysiological basis of BD II depression remains under-elaborated.

Resting-state functional magnetic resonance imaging (rs-fMRI) contributes to functional characterization of neuronal activity that enhances our comprehension in the fields of the neuropathophysiological mechanisms underlying BD [5, 6]. Static amplitude of low-frequency fluctuation (sALFF) reflects regional intensity of spontaneous fluctuations [7, 8], static functional connectivity (sFC) calculates the interaction across the whole brain [9]. Numerous rs-fMRI studies have reported regional sALFF and sFC alterations in the cerebellum, default mode network (DMN), anterior cingulate cortex, and medial frontal gyrus in patients with BD [10–13]. More

recently, dynamic ALFF (dALFF) and dynamic FC (dFC) have emerged as important complements to traditional static measures that could quantify the spatial dynamic organization variability and capture time-varying characteristics throughout the entire scan period [14, 15]. Previous rs-fMRI studies from dynamic perspectives had showed that patients with BD displayed reduced dALFF and dFC variability in the DMN and central executive network relative to healthy controls (HCs) [16–18]. However, the findings were inconsistent or conflicting, which could attribute to mixed samples with BD I and BD II, distinct mood states, medical comorbidity, or medication effect. Investigations with a much purer sample may minimize the potentially confounding effects.

The reciprocal interaction between the gut microbiota and the brain, referred to as the “gut-brain axis” (GBA), has been supposed to be regarded as a key branch to understand BD pathogenesis [19–22]. The dynamic bidirectional influence of the GBA is primarily thought to involve immunomodulatory mechanisms (intestinal, systemic and brain inflammation) [23–25], thereby influencing host emotion, behavior, and brain function [26–28]. A prior study had found a positive correlation between *Clostridium bartlettii* levels and hippocampal ALFF in patients with BD [29]. BD-dysfunctional connectomes were identified to associated with multiple microbial abundance in depressive patients with BD [30]. The composition of gut microbiota community exhibited lower alpha-diversity and significant altered beta-diversity in BD

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compared to HCs [30–32]. According to prior systematic reviews and meta-analyses, proinflammatory bacteria (*Ruminococcus*, *Eggerthella*, *Enterobacteriaceae*, *Proteobacteria*) and glutamate (Glu) and gamma-aminobutyric acid (GABA) metabolism related bacteria (*Bifidobacterium*, *Escherichia*, *Bacteroides*, *Parabacteroides*, *Eggerthella*) were commonly reported to increase in relative microbial abundance in BD compared to HCs [32–34]. However, the composition of gut microbiota varies across different clinical manifestations (types and phases) of BD [23], and pharmacological treatment can have an important impact on gut microbiota [35, 36]. So far, alterations in gut microbiota in individuals with unmedicated BD II depression are not well documented.

Cytokines serve as key signaling molecules involved in the mediation of inflammatory responses [37, 38], they could access the central nervous system to influencing the neuroendocrine system, neurogenesis, and the metabolism of neurotransmitters relevant to mood [39, 40]. Previous systematic reviews and meta-analyses had reported elevated concentrations of pro-inflammatory cytokines (e.g., interleukin [IL]-1- $\beta$ , IL-2, IL-6, IL-8, tumor necrosis factor [TNF]- $\alpha$ , interferon [INF]- $\gamma$ ) [38, 41–44] and anti-inflammatory cytokines (e.g., IL-4, IL-10) [38, 42, 43] in patients with BD relative to HCs. A prior work of our team found an association in BD II between increased IL-6 levels and decreased sFC of the insula, a region crucial for emotional regulation [45]. Another study had found significant associations among DMN network measures, gut microbiota, and inflammatory cytokines in end-stage renal disease [46]. The study had also identified a mediation effect of *Roseburia* levels on sFC in the DMN through IL-6 in patients with end-stage renal disease [46]. However, the gut microbiota-inflammation-brain axis in BD II depression was not further investigated, and the mechanisms underlying the collaborative interaction among gut microbiota changes, inflammation and immune dysregulation, and brain dysfunction in BD II depression remain to be elucidated.

To date, the investigation of patients with BD II has been sporadic. Patients with BD II suffer from more depressive episodes and longer depressive state compared to those with BD I [47]. Distinct symptoms and severity between BD I and BD II could be attributed to diverse pathophysiological and neurobiological mechanism [48], the distinction between BD I and BD II has genetic validity [49]. Therefore, it is essential to conduct investigations utilizing a homogeneous sample of BD II to enhance comprehension of the disorder.

Given above considerations, we thus aimed to explore interaction and association among gut microbiota composition, inflammation, and spontaneous neural functional alterations in unmedicated patients with BD II depression. In this study, mood scales were obtained; rs-fMRI data were collected for sALFF and dALFF analysis, with subsequent sFC and dFC being further performed based on ALFF findings; stool samples and blood samples were collected for the purpose of analyzing the composition of gut microbiota and cytokines levels (IL-2, IL-4, IL-6, IL-8, IL-10, TNF- $\alpha$ ), respectively. In additions, the classification of patients with BD II depression and HCs was performed using the support vector machine (SVM) method, utilizing the aforementioned separate and fusion features. On the basis of previous studies, we hypothesized that patients with BD II depression relative to HCs showed altered spontaneous neural function in the cerebellum and the DMN, dysbiosis of proinflammatory and Glu and GABA metabolism related bacteria, as well as altered proinflammatory cytokines levels. Moreover, the fusion features of altered gut microbiota composition, cytokines and rs-fMRI measures had a good performance in the classification.

## METHODS AND MATERIALS

### Participants

From March 2022 to March 2023, a total of 65 patients with BD II depression and 65 healthy controls were recruited from the psychiatry

department, First Affiliated Hospital of Jinan University, Guangzhou, China. The inclusion criteria for patients with BD II depression were as follows: (1) met the diagnostic criteria of BD II without other Axis-I psychiatric disorders, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-V, the Structured Clinical Interview for DSM-V Patient Edition (SCID-P) was conducted by two experienced psychiatrists (Y.J. and S.Z., with 23 and 8 years of experience, respectively); (2) the 24-item Hamilton Depression Rating Scale (HDRS-24) score >21 and the Young Mania Rating Scale (YMRS) score <7, HDRS-24 and YMRS were used to assess the clinical state of patients; (3) The age ranged from 18 to 55 years; (4) At the time of testing, all patients were either medication-naïve, or had not been medicated for at least 6 months; (5) no history of neurological diseases, alcohol/substance abuse, cardiovascular diseases, or major physical illness; (6) right-handed; (7) the absence of contraindication to MRI; (8) did not take any probiotics, antibiotics, immunosuppressive drugs, or catharsis drugs for at least 1 month before the recruitment. The inclusion criteria for HCs were as follows: (1) no history of any psychiatric disorders in individuals, nor in their first-degree relatives; (2) no history of neurological diseases, alcohol/substance abuse, cardiovascular diseases, or major physical illness; (3) the absence of contraindication to MRI; (4) did not take any probiotics, antibiotics, immunosuppressive drugs, or catharsis drugs for at least 1 month before the recruitment.

The ethics committee of the First Affiliated Hospital of Jinan University (Guangzhou, China) conducted a thorough review and granted approval for this study. Prior to their participation, all individuals involved provided written informed consent after receiving comprehensive written and verbal explanations regarding the study. Additionally, two experienced clinical psychiatrists assessed and confirmed the participants' capacity to provide consent for their involvement in the examination.

### MRI data acquisition and preprocessing

All brain imaging data were performed on the GE Discovery MR750 3.0 T System with an 8-channel phased array head coil. During the scanning procedure, participants were required to maintain a state of relaxation and keep their eyes closed without falling asleep. Subsequently, each participant was confirmed to stay awake after the experiment. Detailed parameters of the rs-fMRI data were reported in supplementary materials.

### Functional image preprocessing

The preprocessing was carried out using Data Processing Assistant for Resting-State fMRI (DPABI\_V3.0, <http://restfmri.net/forum/DPABI>) [50] which is based on Statistical Parametric Mapping (SPM12, <http://www.fil.ion.ucl.ac.uk/spm/>) (see the supplementary materials).

### Static ALFF and FC analysis

For sALFF analysis, the time series was first converted to the frequency domain using a fast Fourier transform for a given voxel after functional data preprocessing. Subsequently, the square root of the power spectrum was computed and then averaged across a predefined frequency interval (0.01–0.1 Hz) for routine ALFF calculation. This averaged square root was termed ALFF at the given voxel [51]. The ALFF maps were smoothed with a 6 mm full width at half maximum (FWHM) Gaussian kernel.

For sFC analysis, we selected the identified result clusters in the sALFF and dALFF analysis as regions of interest (ROIs) to calculate the sFC to the whole brain voxels. The Pearson's correlation coefficients were calculated between the mean time series of the ROIs and the time series of each voxel in the whole brain to generate individual rs-FC maps. The subject-level correlation maps were converted into z-value maps by Fisher's r-to-z transform to improve the normality. The sFC maps were smoothed with a 6 mm FWHM Gaussian kernel.

### Dynamic ALFF and FC analysis

Using the sliding-window method based on the Temporal Dynamic Analysis (TDA) toolkits integrated in the DPABI software (<http://rfmri.org/DPABI>), the Hamming sliding window was selected for the whole-brain BOLD signal time series; a window length of 50 TRs and a step width of 1 TRs were chosen for the dALFF and dFC analysis (see the supplementary materials).

Additionally, two supplementary window lengths (30 TRs and 70 TRs) were conducted to validate the main results of dALFF and dFC with the window length of 50 TRs.

### Fecal samples collection, DNA extraction, intestinal flora 16S ribosomal DNA gene sequencing and analysis

Fecal samples were collected from the subjects within a three-day window before or after the MR examination. No instances of apparent diarrhea or constipation were observed among all subjects. Participants were instructed to provide a stool sample by depositing it into a collection bowl, which was then handed over to a clinical assistant. The sample was subsequently aliquoted using a scoop into a tube, snap-frozen in dry ice, and promptly stored at  $-80^{\circ}\text{C}$  within 30 min of collection. The extraction of microbial community DNA was conducted in accordance with the manufacturer's instructions, utilizing the MagPure Stool DNA KF kit B (Magen, China). For PCR amplification, the variable regions V4 of the 16S rRNA gene were chosen, following the Illumina protocol, and performed on the Illumina MiSeq platform. Clean reads were generated after removing adaptor sequences, short length, ambiguous bases and low complexity [52]. Vsearch was employed joining of paired-end reads, quality control and dereplication. Following this, the sequences that underwent quality filtering were mapped to the chimera-free amplicon sequence variants (ASVs) using Usearch [53]. ASVs were acquired as a reference for quantification via alignment using Vsearch. The taxonomy of ASVs was classified with the Ribosomal Database Project (RDP, Version 16) reference database with a minimum confidence threshold of 0.8. Analysis of alpha diversity, including the abundance-based coverage estimator, Shannon index, and Simpson index, as well as beta diversity (principal coordinate analysis [PCoA] based on Bray-Curtis distances) was performed based on output normalized data within RStudio software (version 4.1). Moreover, we employed the Linear discriminant analysis Effect Size (LEfSe) analysis to discern the variations in the abundance of bacterial taxa between patients with BD II depression and HCs. Taxa that obtained a log linear discriminant analysis (LDA) score  $>2.0$ , and  $p < 0.05$  in the Wilcoxon test were considered significant differences in group comparison.

### Cytokines measures

Blood samples were acquired from individuals diagnosed with BD II depression and HCs in the morning, following a fasting period and abstinence from alcoholic beverages for at least 24 hours prior to testing. The samples were then processed and frozen by trained technicians. Fasting serum samples were collected in serum tubes, allowed to clot for a duration of 30 min, and subsequently stored at a temperature of  $-80^{\circ}\text{C}$  until further utilization. A panel of 6 types immune cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, TNF- $\alpha$ ) were measured using the Bio-Plex Pro Human Cytokine Assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Samples were assayed in duplicate. The data acquisition was performed using Bio-Plex Manager Software, version 6.1, on a Bio-Plex 200 array reader (Bio-Rad), following the manufacturer's instructions.

### Statistical Analysis

Independent-sample t-test (normal variable) and Mann-Whitney U test (skewed variables) were used to compare demographic data (except for gender), and mood scale (HDRS-24, Hamilton Anxiety Scale [HAMA], YMRS) between patients with BD II depression and HCs. A chi-square test was conducted to compare the distribution of sex. All tests were two-tailed, and a significant level of  $p < 0.05$  was considered statistical significance. The statistical software IBM SPSS Statistics 22 (Armonk, NY, USA) was utilized for data analysis.

For sALFF and dALFF, a two-sample t-test was conducted to identify significant differences between patients with BD II depression and HCs. Age, sex, education years and FD were included as nuisance covariates. Cluster-level multiple comparisons correction based on Gaussian random field (GRF) theory (voxel  $p$ -value  $< 0.001$ ; cluster  $p$ -value  $< 0.05$ , GRF corrected) was applied. For sFC and dFC, a one-sample t-test was performed on z-score maps for each ROI to demonstrate within-group FC spatial distribution for patients and HCs within a brain mask ( $p < 0.05$ , uncorrected). Then, a two-sample t-test was performed to assess the significant differences of the whole brain FC in each region between patients and HCs within the union mask of one-sample t-test results of both groups, by also controlling for age, sex, years of education and mean frame-wise displacement. The cluster-level multiple comparison correction was conducted using GRF theory correction (voxel  $p$ -value  $< 0.001$ ; cluster  $p$ -value  $< 0.05/n$ , GRF corrected,  $n$  was defined as the number of identified clusters in the ALFF analysis). Appropriate statistics methods for group comparison of the gut microbiota were mentioned above.

For cytokines analysis, deviations from normality were tested using the Shapiro-Wilk test. Independent-sample t-test (normal variable) and Mann-

Whitney U test (skewed variables) were used to compare levels of cytokines between patients with BD II depression and HCs.

For an exploratory analysis, significant altered brain functional measures (sALFF, dALFF, sFC, and dFC values), gut microbiota (relative abundance), cytokines, and clinical variables (onset age of illness, number of episodes, duration of illness, HDRS-24 scores, HAMA scores, and YMRS scores) in the group comparison were further used to explore the relationship among these factors in partial correlation analyses. Age, sex and years of education were included as nuisance covariates. Further, multiple linear regression was employed to model the relationship between psychopathological variables, rs-fMRI measures, gut microbiota, and cytokines levels. The significant level was set as  $p < 0.05$ , two tailed.

### SVM classification

SVM was executed with the LIBSVM software (<https://www.csie.ntu.edu.tw/~cjlin/libsvm/>) to determine whether the separate or fusion features of the altered relative abundance of gut microbiota, levels of cytokines, and rs-fMRI measures could be used to discriminate patients with BD II depression from HCs. Briefly, a Gaussian radial basis function (GRBF) SVM model is a hyperplane separating distinct classes of features (i.e., data from rs-fMRI measures, gut microbiota, and cytokines) in the most optimal manner. The grid search method was performed to seek the best  $c$  (penalty coefficient) and width parameter  $g$  (gamma). Furthermore, the "10-fold" cross-validation technique was employed to achieve optimal accuracy, sensitivity, and specificity. The classification performance of each established model was evaluated by applying the area under the curve (AUC) of the receiver-operating characteristic (ROC). The SVM results were also verified by a permutation test (10,000 times).

## RESULTS

### Demographic and clinical characteristics

The demographic and clinical data of all participants in this study were presented in Table 1. Seven HCs were excluded from subsequent analyses due to excessive head motion. A total of 65 patients with BD II depression and 58 HCs were included in the analysis. No significant differences were observed in age, sex, years of education, and FD parameters between patients with BD II depression and HCs ( $p > 0.05$ ). In the present study, stool samples were obtained from a total of 42 patients and 46 HCs, while blood samples were collected from 34 patients and 45 HCs. The demographic and clinical data of participants whose stool and blood samples collected were presented in Tables S2 and S3, no significant differences were observed in age, sex, years of education, and FD parameters in the group comparisons either ( $p > 0.05$ ).

### Static and dynamic ALFF

As shown in Table 2 and Fig. 1A, compared to HCs, patients with BD II depression displayed decreased sALFF values in the left posterior lobe of cerebellum (mainly in cerebellar Crus II) (voxel  $p < 0.001$ ; cluster  $p < 0.05$ , GRF corrected). No significant altered dALFF cluster was identified in the group comparison ( $p > 0.05$ ).

### Static and dynamic FC

The one-sample t test revealed the sFC patterns for the left cerebellar Crus II in two groups (Figure. S1), respectively. Compared to HCs, patients with BD II depression displayed decreased sFC between the left cerebellar Crus II and the right inferior parietal lobe (IPL) (voxel  $p < 0.001$ ; cluster  $p < 0.05$ , GRF corrected) (Table 2, Fig. 1B).

The one-sample t test revealed the dFC variability patterns for the left cerebellar Crus II in two groups (Figure. S1), respectively. Compared to HCs, patients with BD II depression displayed decreased dFC between the left cerebellar Crus II and the right medial prefrontal cortex (mPFC) (voxel  $p < 0.001$ ; cluster  $p < 0.05$ , GRF corrected) (Table 2, Fig. 1B). The dFC results of 30 TRs and 70 TRs sliding-window length validated the main dFC results (50 TRs) (Figure S2, and Figure S3).



**Table 1.** Demographic and clinical data for patients with BD II depression and HCs.

	BD II depression	HCs	<i>p</i> -value
<b>Demographic</b>			
Number of participants	65	58	N/A
Sex (male/female)	29/36	24/34	0.718†
Age (years)	29.12 (8.88)	30.07 (11.22)	0.603 <sup>a</sup>
Education (years)	14.18 (2.88)	14.53 (4.19)	0.587 <sup>a</sup>
Age at onset (years)	23.85 (2.02)	N/A	N/A
Number of episodes	2.78 (1.80)	N/A	N/A
Duration of illness (months)	36.78 (53.12)	N/A	N/A
FD (mm)	0.05 (0.02)	0.05 (0.03)	0.783 <sup>a</sup>
<b>Mood scales</b>			
HDRS-24 score	27.30 (3.94)	N/A	N/A
HAMA score	13.14 (7.05)	N/A	N/A
YMRS score	3.81 (1.45)	N/A	N/A
<b>Biomarkers of inflammation</b>			
IL-2 (pg/ml)	4.71 (4.09, 5.33)	4.50 (4.09, 4.92)	0.149 <sup>b</sup>
IL-4 (pg/ml)	0.97 (0.82, 0.97)	0.97 (0.82, 0.97)	0.192 <sup>b</sup>
IL-6 (pg/ml)	2.57 (2.18, 2.83)	2.31 (2.05, 2.57)	0.034 <sup>b*</sup>
IL-8 (pg/ml)	11.00 (7.85, 21.21)	7.85 (6.72, 11.00)	0.007 <sup>b*</sup>
IL-10 (pg/ml)	7.50 (6.54, 9.10)	6.54 (5.69, 7.39)	0.001 <sup>b*</sup>
TNF- $\alpha$ (pg/ml)	22.90 (16.73, 25.01)	18.13 (16.73, 20.90)	0.039 <sup>b*</sup>

Mean (S.D.) or Median (LQ, UQ) are reported. *BD* Bipolar disorder, *HCs* healthy controls, *HDRS* Hamilton Depression Rating Scale, *HAMA* Hamilton Anxiety Scale, *YMRS* Young Mania Rating Scale, *FD* framewise displacement for in-scanner head motion, *IL-2* interleukin 2, *IL-4* interleukin 4, *IL-6* interleukin 6, *IL-8* interleukin 8, *IL-10* interleukin 10, *TNF- $\alpha$*  tumor necrosis factor alpha. †The *p*-value for gender distribution was obtained by chi-square test. <sup>a</sup>The *p*-values were obtained by independent-sample *t*-tests. <sup>b</sup>The *p* values were obtained by Mann-Whitney *U* tests. \**p* < 0.05.

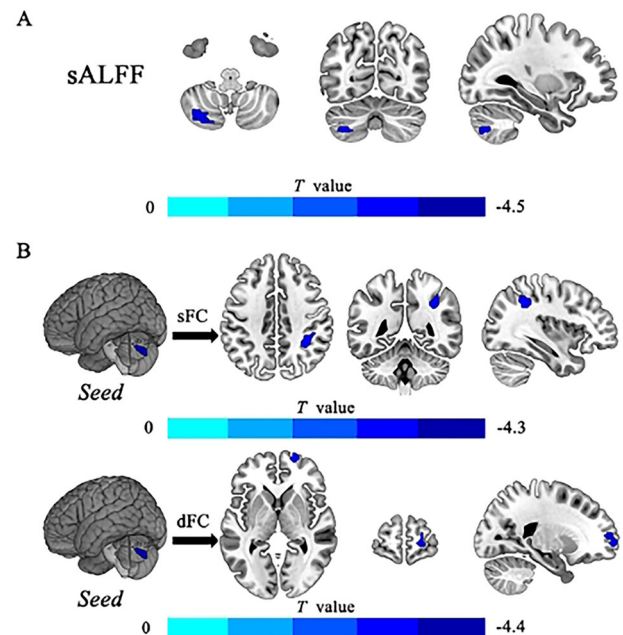
### Gut microbiota analysis

The alpha-diversity analysis (Fig. 2A) included the calculation of ACE, Shannon and Simpson indices, however, no significant difference was found between patients with BD II depression and HCs (all *p* > 0.05). In addition, the beta-diversity analysis based on Bray-Curtis further showed a distinct clustering of the microbiota structural composition between patients with BD II depression and HCs (*p* < 0.001) (Fig. 2B). The histogram of the microbial composition of the genus level showed that sequences from the BD II group were mainly assigned to *Phocaeicola* and *Bacteroides*, followed by *Prevotella* and *Faecalibacterium*; and sequences from HCs group were mainly assigned to *Phocaeicola* and *Prevotella*, followed by *Bacteroides* and *Faecalibacterium* (Fig. 2C). By using the LEfSe differential analysis based on the all levels of species abundance, it was found that the BD II group was mainly enriched in the *Proteobacteria*, *Actinobacteria*, *Bifidobacteriaceae*, *Porphyromonadaceae*, *Enterobacteriaceae*, *Bifidobacterium*, *Bacteroides*, *Parabacteroides*, *Lachnospiraceae* and *Pseudodescherichia*; while HCs

**Table 2.** The areas of altered sALFF and its corresponding FC between patients with BD II depression and HCs.

Brain region	Cluster size (voxels)	MNI			Peak <i>t</i> value
		x	y	z	
sALFF					
L Cerebellum Crus II	52	−33	−69	−45	−4.5154
sFC					
R inferior Parietal Lobule	136	36	−45	39	−4.3277
dFC					
R Medial Frontal Cortex	43	21	57	9	−4.4424

sALFF static amplitude of low-frequency fluctuation, FC functional connectivity, *BD* Bipolar disorder, *HCs* healthy controls, sFC Static FC, dFC dynamic FC, MNI Montreal Neurological Institute, L left, R Right.



**Fig. 1** Resting-state functional alterations between BD II depression and HCs. **A** The sALFF value differences. **B** The whole brain sFC and dFC differences for cerebellar Crus II seed. *BD* bipolar disorder, *HCs* healthy controls, sALFF static amplitude of low-frequency fluctuation, sFC static functional connectivity, dFC dynamic functional connectivity.

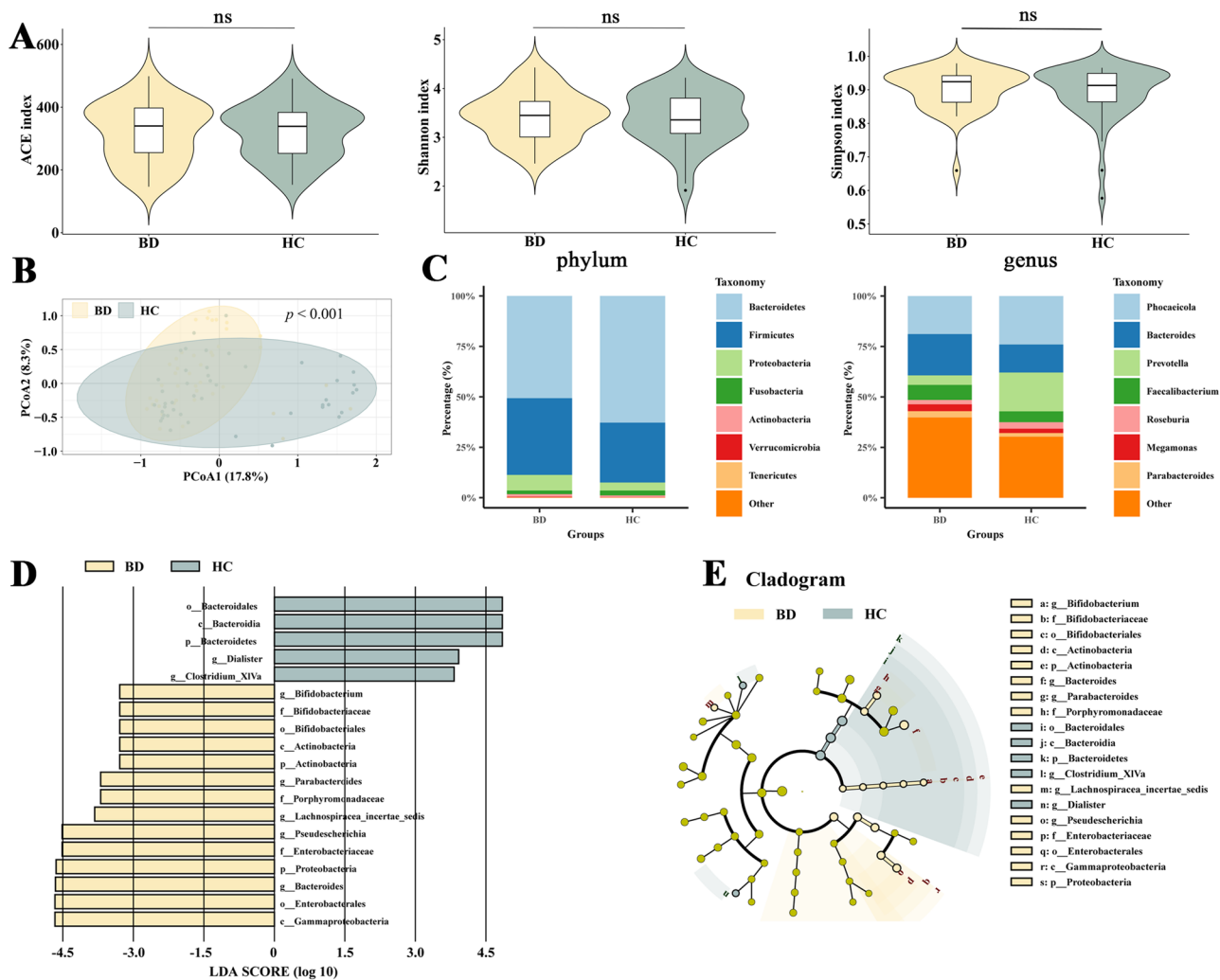
group was mainly enriched in the *Bacteroidetes*, *Clostridium* and *Dialister* (Fig. 2D–F).

### Cytokines measures

The levels of IL-2, IL-4, IL-6, IL-8, IL-10 and TNF- $\alpha$  of the two groups are shown in Table 1. Compared to HCs, patients with BD II depression displayed increased levels of IL-6 (*z* = −2.122, *p* = 0.034), IL-8 (*z* = −2.691, *p* = 0.007), IL-10 (*z* = −3.276, *p* = 0.001), and TNF- $\alpha$  (*z* = −2.319, *p* = 0.023). No significant difference was found in the levels of IL-2 and IL-4 in group comparison (Table 1).

### Correlation analyses

The relative abundance of genus *Bacteroides* was positively correlated with HDRS-24 score (*r* = 0.411, *p* = 0.022) in patients



**Fig. 2** Gut microbiota analysis between BD II depression and HCs. **A** Microbial alpha-diversity indices (ACE, Shannon and Simpson). **B** principal coordinate analysis (PCoA) based on Bray-Curtis distances comparing the sample distribution. The red dots represent BD II, and the green dots represent HCs. **C** Microbial composition at the phylum and genus levels. **D** Linear discriminant analysis (LDA) scores derived from LEfSe analysis, showing the biomarker taxa (LDA scores (log<sub>10</sub>) > 2.0 and a significance of  $p < 0.05$  determined by the Wilcoxon signed-rank test. Red and green colors represent an increase of abundance in BD II and HCs. **E** Cladogram generated from LEfSe analysis showing the relationship between taxon (the levels represent, from the inner to outer rings, phylum, class, order, family, and genus). BD bipolar disorder, HCs healthy controls.

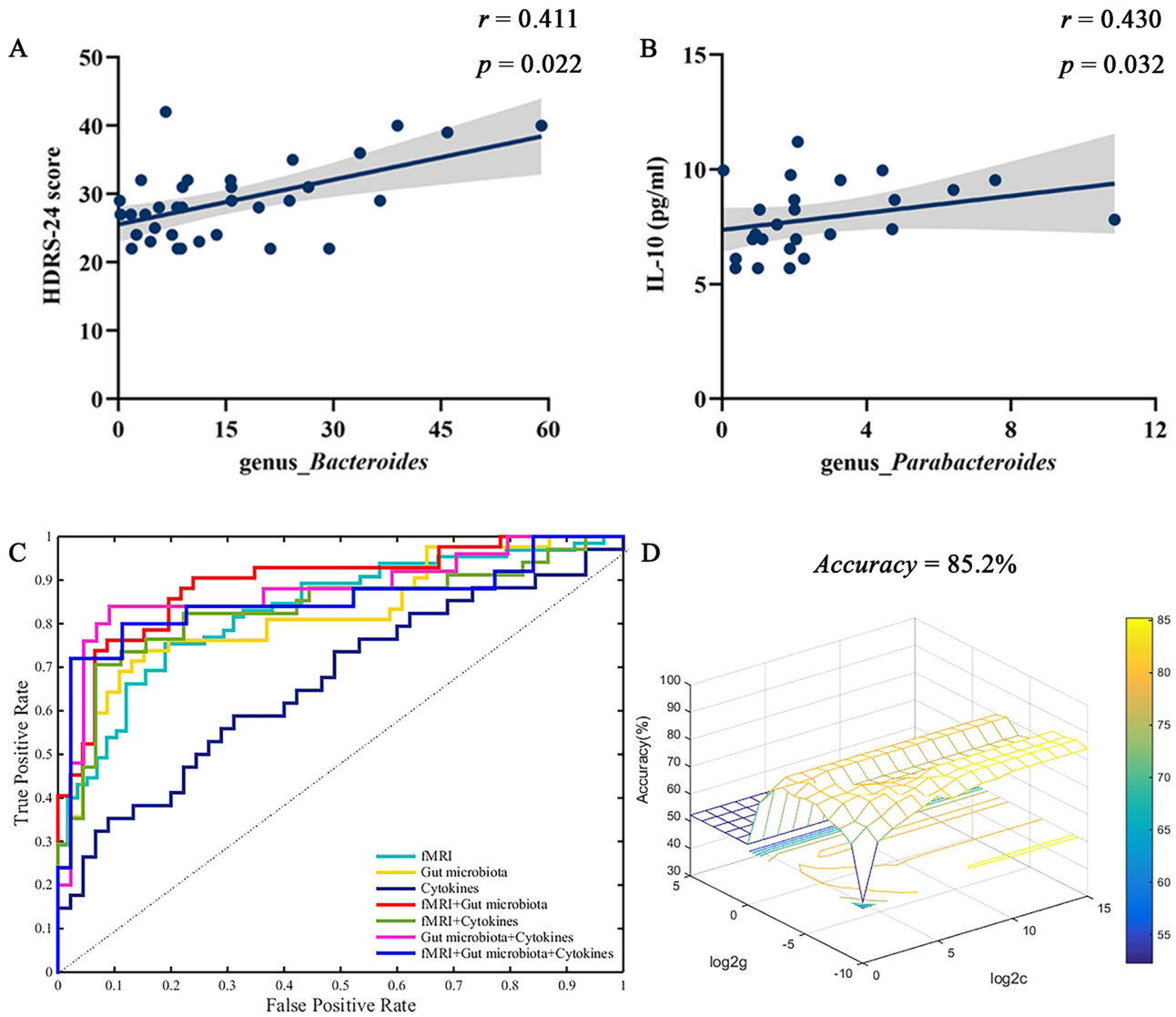
with BD II depression (Fig. 3A). The relative abundance of genus *Parabacteroides* was positively correlated with the IL-10 levels ( $r = 0.430$ ,  $p = 0.032$ ) in patients with BD II depression (Fig. 3B). No significant correlation was observed between clinical variables, the relative abundance of gut microbiota, the cytokine levels, and rs-fMRI measures in HCs. Multiple regression analysis indicated that interaction effect of relative abundance of genus *Parabacteroides* levels and IL-8 levels (genus *Parabacteroides* levels  $\times$  IL-8 levels) was associated with the sALFF values in the left cerebellar Crus II ( $\beta = 0.567$ ,  $t = 2.22$ ,  $p = 0.038$ ) in patients with BD II depression (Table S3).

#### SVM classification

Both separate and fusion features of the altered relative abundance of gut microbiota, levels of cytokines, and rs-fMRI measures had well performance in the SVM models to distinguish patients from HCs (Table S4). The fusion features of altered rs-fMRI measures and gut microbiota had the best performance in classification (with a 0.892 AUC, an 80.4% accuracy, an 80.4% specificity, and an 81.0% sensitivity) (Fig. 3C, D, Table S4).

#### DISCUSSION

To the best of our knowledge, this is the first study to explore the mechanisms underlying the collaborative interaction among brain dysfunction, gut microbiota changes, and inflammation and immune dysregulation in BD II depression. The main findings are the following: (i) compared to HCs, patients with BD II depression displayed decreased sALFF values in the left cerebellar Crus II, as well as decreased cerebellar sFC to the right IPL, and decreased cerebellar dFC to the right mPFC; (ii) significant differences were found in beta-diversity between BD II depression and HCs; LEfSe analysis identified increased relative abundance of proinflammatory bacteria (i.e., *Proteobacteria*, *Enterobacteriaceae*, *Porphyromonadaceae*, *Pseudoschlerichia*), and Glu and GABA metabolism related bacteria (i.e., *Bifidobacterium*, *Bacteroides*, *Parabacteroides*, *Pseudoschlerichia*); (iii) patients with BD II depression showed increased IL-6, IL-8, IL-10, and TNF- $\alpha$  levels compared to HCs; (iv) multiple regression analysis indicated that genus *Parabacteroides* levels  $\times$  IL-8 levels was associated with the sALFF values in the left cerebellar Crus II in BD II depression; the relative abundance of genus *Bacteroides* and genus *Parabacteroides* was positively correlated with depressive symptoms (HDRS-24 score)



**Fig. 3** Correlation analyses and SVM classification performance in BD II depression. **A** The correlation between the genus *Bacteroides* levels and HDRS-24 score. **B** The correlation between the genus *Parabacteroides* levels and IL-10 levels. **C** The ROC curves for separate and fusion features classifiers. **D** 3D view of the classified accuracy of fusion features of altered fMRI measures and gut microbiota with the best parameters (best  $C = 128$ ,  $g = 0.008$ ). SVM support vector machine, BD bipolar disorder, ALFF amplitude of low-frequency fluctuation, HDRS-24 24-item Hamilton Depression Rating Scale, IL-10 interleukin 10, AUC area under the curve.

and IL-10 levels in BD II depression, respectively. Moreover, a combination of altered fMRI measures and relative abundance of gut microbiota had achieved satisfied classification performance in distinguish BD II depression from HCs. An overview of our findings is presented in abstract figure.

In the current study, we found decreased sALFF values in the left cerebellum (cerebellar Crus II) in patients with BD II depression, suggesting disrupt spontaneous neural function in the cerebellum in BD II. Consistent with our finding, decreased sALFF values in the left cerebellum were evidenced in patients with BD in a prior meta-analysis of 51 studies [5]. Numerous rs-fMRI studies had found that patients with BD in a depressive state displayed cerebellar alterations of function [10, 54, 55] and structure [56, 57]. It was reported that cerebellar impairments was directly associated with the onset of BD [12]. Actually, the cerebellum directly or indirectly receives input from cortical association areas and the midbrain, as well as generate output to the limbic system and hypothalamic and thalamic nuclei [58, 59]. These regions are crucial in the field of mood disorders due to their roles in emotional processing [12]. Moreover, the cerebellum

also establishes communication with the monoamine-producing brainstem nuclei, which provide the limbic system and the brain with essential neurotransmitters such as dopamine, serotonin, Glu, and GABA [12, 60]. Notably, an association between *Parabacteroides* levels  $\times$  IL-8 levels and the left cerebellar Crus II ALFF values was identified in the study. *Parabacteroides* have also been linked to the production of GABA in human, and microbial-derived GABA may have the potential to impact the host by modulating circulating concentrations of GABA [61]. It was reported that BD is associated with GABAergic deficit [61–63]. More recently, a study on patients with depressive BD identified that altered neuroactive microbes (including *Parabacteroides*) and microbiota-derived neuroactive metabolites (GABA) were associated with brain intrinsic function [22]. The study proposed that gut microbiota may contribute to BD by affecting the metabolism of certain neuroactive metabolites (GABA), which might, in turn, regulate function of the bipolar disorder brain [22]. Dysbiosis of gut microbiota would result in dysfunctions in intestinal permeability, translocation of commensal microbes, and chronic proinflammatory states [64]. Inflammation in the gut may lead to an

upregulation of proinflammatory cytokines (e.g., IL-8), which could pass the blood–brain barrier and enter the central nervous system (CNS) [40]. Neuroinflammation may consequently occur, concomitant with neurotoxic effects and an interruption of brain homeostasis, thereby resulting in a dysfunctional brain in BD [65, 66]. In short, the interaction effect of microbiota-derived neuroactive metabolites (specifically GABA) disturbances and gut dysbiosis-derived up-regulation of systemic inflammation would affect the cerebellar intrinsic function.

Cortico-cerebellar connections are crucial part of the mood network of BD [12]. We had also identified the disrupted static and dynamic cerebellar-DMN FC in BD II depression in the study. Specifically, patients with BD II depression showed decreased sFC between the left cerebellar Crus II and right IPL, as well as decreased dFC between the left cerebellar Crus II and right mPFC. The IPL and mPFC were major hub nodes of the DMN [67, 68], dysfunction of which was proposed to be broadly involved in the neuropathological mechanisms of BD [13]. Recent meta-analytic evidence from 23 rs-fMRI studies identified that patients with BD displayed reduced sFC in the IPL relative to HCs [69]. Another cognitive task-based meta-analysis of 69 studies also reported abnormal activation in the IPL in patients with BD relative to HCs [70]. DFC serves as a potent complement to sFC [14, 15], and may more sensitively detect aberrant connectivity patterns [71]. Previous rs-fMRI studies had also found reduced dFC variability [72, 73] and dALFF [74] value in the mPFC in patients with BD relative to HCs. Therefore, the current investigation provided supplementary evidence in favor of disrupted cerebellar-DMN connectivity in BD II depression from both static and dynamic perspectives.

This is the first study to investigate the gut microbiota of BD II depression with pure samples. The beta-diversity analysis was employed to assess whether gut microbiota community composition differed in the group comparison [32], and our finding of altered beta-diversity in BD II depression was consistent with previous systematic reviews on BD [31, 32]. In line with prior systematic reviews and meta-analyses [32, 33], enrichment of proinflammatory bacteria and Glu and GABA metabolism related bacteria were also identified in BD. BD is suggested to be a whole-body disease accompanied with up-regulation systemic inflammatory [23, 75] and changed GABAergic deficit profile [61]. Enriched proinflammatory bacteria can will disrupt the intestinal barrier, and induce the following peripheral and neuro-inflammation in BD [23, 76]. Moreover, we also identified a positive correlation between *Bacteroides* levels and depressive symptoms (HDRS-24 score) in patients with BD II depression. Enrichment of *Bacteroides* may promote utilization of Glu and synthesis of GABA [32]. GABA is the major inhibitory neurotransmitter in the human brain that enhances GABAergic inhibition of nearby dopaminergic neurons, thereby contributing to core depressive symptoms [61, 77]. In line with our results, a prior systematic review had reported that fecal genus *Bacteroides* was consistently positively correlated with depressive symptoms in BD and depression in most study [21]. Actually, *Bacteroides* produce short-chain fatty acids (SCFAs) to maintain intestinal homeostasis and reduce inflammation that are generally considered beneficial to the host [78, 79]. The enrichment of *Bacteroides* may potentially also serve as a compensatory mechanism, rather than exerting a negative impact on the host's mood [21].

Cytokines are key signaling molecules in the regulation of the inflammatory response, serving as a bridge for the “crosstalk” between the immune system and all organ systems [37, 40]. In the current study, increased IL-6, IL-8, IL-10, and TNF- $\alpha$  levels were found in BD II depression relative to HCs, and increased IL-10 levels were positively correlated with genus *Parabacteroides* in BD II depression. In line with our findings, elevated concentrations of serum pro-inflammatory cytokines (i.e., IL-6, IL-8, and TNF- $\alpha$ ) were reported in previous meta-analyses [41–43], supporting inflammatory hypothesis with higher immune activation in BD

[41, 44, 80]. The findings of circulating IL-10 levels in previous studies were inconsistent [42, 81]. IL-10 is an anti-inflammatory cytokine that can suppress immune responses [42]. It was reported that pro-inflammatory cytokines (like IL-6) would induce an increase in circulating IL-10 levels in the context of pro-inflammatory conditions [82]. An elevation in IL-10 may contribute to compensate or counterbalance the effects of an activated inflammatory response in BD [42, 81, 83]. As with aforementioned *Bacteroides*, *Parabacteroides* also produce SCFAs to reduce inflammatory effects and increase anti-inflammatory mediators in the blood [79, 84]. Therefore, the observed positive correlation between IL-10 levels and *Parabacteroides* could be further interpreted as a compensatory mechanism to the pro-inflammatory state in BD II depression.

Notably, fusion features of altered fMRI measures and gut microbiota had achieved the best performance for distinguishing BD II depression from HCs. Compared to previous studies, the current classifier exhibited a more satisfactory classified effect than previous classified models with a single feature of gut microbiota [20, 29], neuroimaging [85], or cytokines [86] in the identification of BD from HCs. Intriguingly, a combination of altered fMRI measures, gut microbiota and cytokines did not achieve better performance in the classification. A possible explanation could be attributed to only four specific types of cytokines included in the classifier. Nevertheless, it could be somehow suggested that incorporating more miscellaneous but inefficient information does not necessarily improve classification performance. Collectively, the integration of gut microbiota and static and dynamic imaging measures would provide a more accurate and useful diagnostic value for BD II depression.

There were several limitations in the study. First, the sample size was relatively small, and the sample recruitment was from a single center. Moreover, the training set and the test set in the SVM analysis were not completely separated, as feature selection was based on the results of inter-group comparisons of all subjects, which may lead to overfitting. In the future, studies with a larger multi-center sample are required for further validation. Second, results of correlation analysis in this study should be considered exploratory, as they did not survive after multiple comparison corrections. Thirdly, the causal relationship among gut microbiota, cytokines and fMRI alterations cannot be studied in the current cross-sectional designed study. Moreover, dietary is considered vital to the composition of gut microbiota community. Given the diversity of dietary patterns observed globally, our findings must be interpreted with caution. Finally, cytokines (i.e., IL-2, IL-4, IL-6, IL-8, IL-10, and TNF- $\alpha$ ) examined in this study were hypothesis-driven based on previous literature findings.

## CONCLUSIONS

In conclusion, the current study identified disrupted spontaneous activity and connectivity in cerebellar-DMN in patients with unmedicated BD II depression. Increased intestinal proinflammatory bacteria, and Glu and GABA metabolism related bacteria, as well as elevated serum pro- and anti-inflammatory cytokines were also found in BD II depression. Moreover, the combination of gut microbiota and functional alterations in the cerebellar-DMN provided a more accurate and complementary diagnostic value for depressive BD II. The study provided valuable insights into the underlying pathophysiological mechanism of the gut microbiota-inflammation-brain axis, and bridged a gap on this topic among patients with the BD II depression.

## DATA AVAILABILITY

The sequencing data supporting the conclusions of this article are available in the NCBI Bioproject repository, PRJNA1068750 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1068750>).



## REFERENCES

- Anderson IM, Haddad PM, Scott J. Bipolar disorder. *BMJ*. 2012;345:e8508 <https://doi.org/10.1136/bmj.e8508>
- Nierenberg AA, Agutini B, Kohler-Forsberg O, Cusin C, Katz D, Sylvia LG, et al. Diagnosis and Treatment of Bipolar Disorder: A Review. *JAMA*. 2023;330:1370–80. <https://doi.org/10.1001/jama.2023.18588>
- Sasayama D, Hori H, Teraishi T, Hattori K, Ota M, Matsuo J, et al. Difference in Temperament and Character Inventory scores between depressed patients with bipolar II and unipolar major depressive disorders. *J Affect Disord*. 2011;132:319–24. <https://doi.org/10.1016/j.jad.2011.03.009>
- Gong J, Chen G, Chen F, Zhong S, Chen P, Zhong H, et al. Association between resting-state functional connectivity of amygdala subregions and peripheral pro-inflammation cytokines levels in bipolar disorder. *Brain Imaging Behav*. 2022;16:1614–26. <https://doi.org/10.1007/s11682-022-00636-7>
- Chen G, Wang J, Gong J, Qi Z, Fu S, Tang G, et al. Functional and structural brain differences in bipolar disorder: a multimodal meta-analysis of neuroimaging studies. *Psychol Med*. 2022;52:2861–73. <https://doi.org/10.1017/S0033291722002392>
- Chen CH, Suckling J, Lennox BR, Ooi C, Bullmore ET. A quantitative meta-analysis of fMRI studies in bipolar disorder. *Bipolar Disord*. 2011;13:1–15. <https://doi.org/10.1111/j.1399-5618.2011.00893.x>
- Zuo XN, Di Martino A, Kelly C, Shehzad ZE, Gee DG, Klein DF, et al. The oscillating brain: complex and reliable. *NeuroImage*. 2010;49:1432–45. <https://doi.org/10.1016/j.neuroimage.2009.09.037>
- Tang X, Guo Z, Chen G, Sun S, Xiao S, Chen P, et al. A Multimodal Meta-Analytical Evidence of Functional and Structural Brain Abnormalities Across Alzheimer's Disease Spectrum. *Ageing Res Rev*. 2024;95:102240. <https://doi.org/10.1016/j.arr.2024.102240>
- Zhang J, Kucyi A, Raya J, Nielsen AN, Nomi JS, Damoiseaux JS, et al. What have we really learned from functional connectivity in clinical populations? *NeuroImage*. 2021;242:118466. <https://doi.org/10.1016/j.neuroimage.2021.118466>
- Wang Y, Zhong S, Jia Y, Sun Y, Wang B, Liu T, et al. Disrupted Resting-State Functional Connectivity in Nonmedicated Bipolar Disorder. *Radiology*. 2016;280:529–36. <https://doi.org/10.1148/radiol.2016151641>
- Chen G, Zhao L, Jia Y, Zhong S, Chen F, Luo X, et al. Abnormal cerebellum-DMN regions connectivity in unmedicated bipolar II disorder. *J Affect Disord*. 2019;243:441–7. <https://doi.org/10.1016/j.jad.2018.09.076>
- Lupo M, Siciliano L, Leggio M. From cerebellar alterations to mood disorders: A systematic review. *Neurosci Biobehav Rev*. 2019;103:21–28. <https://doi.org/10.1016/j.neubiorev.2019.06.008>
- Zovetti N, Rossetti MG, Perlini C, Maggioni E, Bontempi P, Bellani M, et al. Default mode network activity in bipolar disorder. *Epidemiol Psychiatr Sci*. 2020;29:e166. <https://doi.org/10.1017/S2045796020000803>
- Wang Q, Chen B, Zhong X, Hou L, Zhang M, Yang M, et al. Static and dynamic functional connectivity variability of the anterior-posterior hippocampus with subjective cognitive decline. *Alzheimer's Res Ther*. 2022;14:122. <https://doi.org/10.1186/s13195-022-01066-9>
- Fu Z, Caprihan A, Chen J, Du Y, Adair JC, Sui J, et al. Altered static and dynamic functional network connectivity in Alzheimer's disease and subcortical ischemic vascular disease: shared and specific brain connectivity abnormalities. *Hum Brain Mapp*. 2019;40:3203–21. <https://doi.org/10.1002/hbm.24591>
- Wang J, Wang Y, Huang H, Jia Y, Zheng S, Zhong S, et al. Abnormal intrinsic brain functional network dynamics in unmedicated depressed bipolar II disorder. *J Affect Disord*. 2019;253:402–9. <https://doi.org/10.1016/j.jad.2019.04.103>
- Luo Z, Chen G, Jia Y, Zhong S, Gong J, Chen F, et al. Shared and specific dynamics of brain segregation and integration in bipolar disorder and major depressive disorder: A resting-state functional magnetic resonance imaging study. *J Affect Disord*. 2021;280:279–86. <https://doi.org/10.1016/j.jad.2020.11.012>
- Wang J, Wang Y, Huang H, Jia Y, Zheng S, Zhong S, et al. Abnormal dynamic functional network connectivity in unmedicated bipolar and major depressive disorders based on the triple-network model. *Psychol Med*. 2020;50:465–74. <https://doi.org/10.1017/S003329171900028X>
- Lucidi L, Pettorruso M, Vellante F, Di Carlo F, Ceci F, Santovito MC, et al. Gut Microbiota and Bipolar Disorder: An Overview on a Novel Biomarker for Diagnosis and Treatment. *Int J Mol Sci*. 2021;22. <https://doi.org/10.3390/ijms22073723>
- Hu S, Li A, Huang T, Lai J, Li J, Sublette ME, et al. Gut Microbiota Changes in Patients with Bipolar Depression. *Adv Sci (Weinh)*. 2019;6:1900752. <https://doi.org/10.1002/adv.201900752>
- Knuesel T, Mohajeri MH. The Role of the Gut Microbiota in the Development and Progression of Major Depressive and Bipolar Disorder. *Nutrients*. 2021;14. <https://doi.org/10.3390/nu14010037>
- Li Z, Lai J, Zhang P, Ding J, Jiang J, Liu C, et al. Multi-omics analyses of serum metabolome, gut microbiome and brain function reveal dysregulated microbiota-gut-brain axis in bipolar depression. *Mol Psychiatry*. 2022;27:4123–35. <https://doi.org/10.1038/s41380-022-01569-9>
- Ortega MA, Alvarez-Mon MA, Garcia-Montero C, Fraile-Martinez O, Monserrat J, Martinez-Rozas L, et al. Microbiota-gut-brain axis mechanisms in the complex network of bipolar disorders: potential clinical implications and translational opportunities. *Mol Psychiatry*. 2023;28:2645–73. <https://doi.org/10.1038/s41380-023-01964-w>
- Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. *Nat Rev Microbiol*. 2012;10:735–42. <https://doi.org/10.1038/nrmicro2876>
- Starling S. Bacterial Pathogenesis: Microbial manipulation of the gut-brain axis. *Nat Rev Microbiol*. 2017;15:131. <https://doi.org/10.1038/nrmicro.2017.9>
- Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci*. 2012;13:701–12. <https://doi.org/10.1038/nrn3346>
- Morais LH, Schreiber HLT, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. *Nat Rev Microbiol*. 2021;19:241–55. <https://doi.org/10.1038/s41579-020-00460-0>
- Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA*. 2011;108:3047–52. <https://doi.org/10.1073/pnas.1010529108>
- Lai J, Li A, Jiang J, Yuan X, Zhang P, Xi C, et al. Metagenomic analysis reveals gut bacterial signatures for diagnosis and treatment outcome prediction in bipolar depression. *Psychiatry Res*. 2022;307:114326. <https://doi.org/10.1016/j.psychres.2021.114326>
- Xi C, Li A, Lai J, Huang X, Zhang P, Yan S, et al. Brain-gut microbiota multimodal predictive model in patients with bipolar depression. *J Affect Disord*. 2023;323:140–52. <https://doi.org/10.1016/j.jad.2022.11.026>
- Nguyen TT, Hathaway H, Kosciolk T, Knight R, Jeste DV. Gut microbiome in serious mental illnesses: A systematic review and critical evaluation. *Schizophr Res*. 2021;234:24–40. <https://doi.org/10.1016/j.schres.2019.08.026>
- McGuinness AJ, Davis JA, Dawson SL, Loughman A, Collier F, O'Hely M, et al. A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Mol Psychiatry*. 2022;27:1920–35. <https://doi.org/10.1038/s41380-022-01456-3>
- Nikolova VL, Smith MRB, Hall LJ, Cleare AJ, Stone JM, Young AH. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. *JAMA psychiatry*. 2021;78:1343–54. <https://doi.org/10.1001/jamapsychiatry.2021.2573>
- Zang Y, Lai X, Li C, Ding D, Wang Y, Zhu Y. The Role of Gut Microbiota in Various Neurological and Psychiatric Disorders-An Evidence Mapping Based on Quantified Evidence. *Mediators Inflamm*. 2023;2023:5127157. <https://doi.org/10.1155/2023/5127157>
- Pascale A, Marchesi N, Govoni S, Barbieri A. Targeting the microbiota in pharmacology of psychiatric disorders. *Pharm Res*. 2020;157:104856. <https://doi.org/10.1016/j.phrs.2020.104856>
- Doestzada M, Vila AV, Zernakova A, Koonen DPY, Weersma RK, Touw DJ, et al. Pharmacomicrobiomics: a novel route towards personalized medicine? *Protein Cell*. 2018;9:432–45. <https://doi.org/10.1007/s13238-018-0547-2>
- Sakrajda K, Szczepankiewicz A. Inflammation-Related Changes in Mood Disorders and the Immunomodulatory Role of Lithium. *Int J Mol Sci*. 2021;22. <https://doi.org/10.3390/ijms22041532>
- Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry*. 2016;21:1696–709. <https://doi.org/10.1038/mp.2016.3>
- Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry*. 2009;65:732–41. <https://doi.org/10.1016/j.biopsych.2008.11.029>
- Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol*. 2016;16:22–34. <https://doi.org/10.1038/nri.2015.5>
- Solmi M, Suresh Sharma M, Osimo EF, Fornaro M, Bortolato B, Croatto G, et al. Peripheral levels of C-reactive protein, tumor necrosis factor-alpha, interleukin-6, and interleukin-1beta across the mood spectrum in bipolar disorder: A meta-analysis of mean differences and variability. *Brain, Behav, Immun*. 2021;97:193–203. <https://doi.org/10.1016/j.bbi.2021.07.014>
- Modabbernia A, Taslimi S, Brietzke E, Ashrafi M. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. *Biol Psychiatry*. 2013;74:15–25. <https://doi.org/10.1016/j.biopsych.2013.01.007>
- Rowland T, Perry BL, Upthegrove R, Barnes N, Chatterjee J, Gallacher D, et al. Neurotrophins, cytokines, oxidative stress mediators and mood state in bipolar disorder: systematic review and meta-analyses. *Br J psychiatry: J Ment Sci*. 2018;213:514–25. <https://doi.org/10.1192/bjp.2018.144>
- Huang MH, Chan YE, Chen MH, Hsu JW, Huang KL, Li CT, et al. A longitudinal study of the association between pro-inflammatory cytokines and mood symptoms in bipolar disorder. *Acta Psychiatr Scand*. 2023;147:81–91. <https://doi.org/10.1111/acps.13508>



45. Chen P, Chen F, Chen G, Zhong S, Gong J, Zhong H, et al. Inflammation is associated with decreased functional connectivity of insula in unmedicated bipolar disorder. *Brain, Behav, Immun.* 2020;89:615–22. <https://doi.org/10.1016/j.bbi.2020.07.004>
46. Wang YF, Zheng LJ, Liu Y, Ye YB, Luo S, Lu GM, et al. The gut microbiota-inflammation-brain axis in end-stage renal disease: perspectives from default mode network. *Theranostics.* 2019;9:8171–81. <https://doi.org/10.7150/thno.35387>
47. Judd LL, Akiskal HS, Schettler PJ, Coryell W, Maser J, Rice JA, et al. The comparative clinical phenotype and long term longitudinal episode course of bipolar I and II: a clinical spectrum or distinct disorders? *J Affect Disord.* 2003;73:19–32. [https://doi.org/10.1016/s0165-0327\(02\)00324-5](https://doi.org/10.1016/s0165-0327(02)00324-5)
48. Baek JH, Park DY, Choi J, Kim JS, Choi JS, Ha K, et al. Differences between bipolar I and bipolar II disorders in clinical features, comorbidity, and family history. *J Affect Disord.* 2011;131:59–67. <https://doi.org/10.1016/j.jad.2010.11.020>
49. Lewis KJS, Richards A, Karlsson R, Leonenko G, Jones SE, Jones HJ, et al. Comparison of Genetic Liability for Sleep Traits Among Individuals With Bipolar Disorder I or II and Control Participants. *JAMA psychiatry.* 2020;77:303–10. <https://doi.org/10.1001/jamapsychiatry.2019.4079>
50. Yan CG, Wang XD, Zuo XN, Zang YF. DPABI: Data Processing & Analysis for (Resting-State) Brain Imaging. *Neuroinformatics.* 2016;14:339–51. <https://doi.org/10.1007/s12021-016-9299-4>
51. Zang YF, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev.* 2007;29:83–91. <https://doi.org/10.1016/j.braindev.2006.07.002>
52. Fadrosh DW, Ma B, Gajer P, Sengamalai N, Ott S, Brotman RM, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome.* 2014;2:6. <https://doi.org/10.1186/2049-2618-2-6>
53. Prodan A, Tremaroli V, Brolin H, Zwiderman AH, Nieuwdorp M, Levin E. Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *PloS one.* 2020;15:e0227434. <https://doi.org/10.1371/journal.pone.0227434>
54. Liu CH, Li F, Li SF, Wang YJ, Tie CL, Wu HY, et al. Abnormal baseline brain activity in bipolar depression: a resting state functional magnetic resonance imaging study. *Psychiatry Res.* 2012;203:175–9. <https://doi.org/10.1016/j.psychres.2012.02.007>
55. Li M, Huang C, Deng W, Ma X, Han Y, Wang Q, et al. Contrasting and convergent patterns of amygdala connectivity in mania and depression: a resting-state study. *J Affect Disord.* 2015;173:53–58. <https://doi.org/10.1016/j.jad.2014.10.044>
56. Zhao L, Wang Y, Jia Y, Zhong S, Sun Y, Zhou Z, et al. Cerebellar microstructural abnormalities in bipolar depression and unipolar depression: A diffusion kurtosis and perfusion imaging study. *J Affect Disord.* 2016;195:21–31. <https://doi.org/10.1016/j.jad.2016.01.042>
57. Watson DR, Anderson JM, Bai F, Barrett SL, McGinnity TM, Mulholland CC, et al. A voxel based morphometry study investigating brain structural changes in first episode psychosis. *Behav Brain Res.* 2012;227:91–99. <https://doi.org/10.1016/j.bbr.2011.10.034>
58. Schmahmann JD. The role of the cerebellum in affect and psychosis. *J Neuro-linguist.* 2000;13:189–214. [https://doi.org/10.1016/S0911-6044\(00\)00011-7](https://doi.org/10.1016/S0911-6044(00)00011-7)
59. Guo Z, Tang X, Xiao S, Yan H, Sun S, Yang Z, et al. Systematic review and meta-analysis: multimodal functional and anatomical neural alterations in autism spectrum disorder. *Mol autism.* 2024;15:16. <https://doi.org/10.1186/s13229-024-00593-6>
60. Chaves T, Fazekas CL, Horvath K, Correia P, Szabo A, Torok B, et al. Stress Adaptation and the Brainstem with Focus on Corticotropin-Releasing Hormone. *Int J Mol Sci.* 2021;22. <https://doi.org/10.3390/ijms22169090>
61. Strandwitz P, Kim KH, Terekhova D, Liu JK, Sharma A, Levering J, et al. GABA-modulating bacteria of the human gut microbiota. *Nat Microbiol.* 2019;4:396–403. <https://doi.org/10.1038/s41564-018-0307-3>
62. Benes FM, Lim B, Matzilevich D, Walsh JP, Subburaju S, Minns M. Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. *Proc Natl Acad Sci USA.* 2007;104:10164–9. <https://doi.org/10.1073/pnas.0703806104>
63. Cheryl SY, Woon PS, Liu JJ, Ong WY, Tsai GC, Sim K. Genetic association studies of glutamate, GABA and related genes in schizophrenia and bipolar disorder: a decade of advance. *Neurosci Biobehav Rev.* 2010;34:958–77. <https://doi.org/10.1016/j.neubiorev.2010.01.002>
64. Li XJ, You XY, Wang CY, Li XL, Sheng YY, Zhuang PW, et al. Bidirectional Brain-gut-microbiota Axis in increased intestinal permeability induced by central nervous system injury. *CNS Neurosci Therapeutics.* 2020;26:783–90. <https://doi.org/10.1111/cns.13401>
65. Rantala MJ, Luoto S, Borraz-Leon JI, Krams I. Bipolar disorder: An evolutionary psychoneuroimmunological approach. *Neurosci Biobehav Rev.* 2021;122:28–37. <https://doi.org/10.1016/j.neubiorev.2020.12.031>
66. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. *J Neuroinflammation.* 2013;10:43. <https://doi.org/10.1186/1742-2094-10-43>
67. Raichle ME. The brain's default mode network. *Annu Rev Neurosci.* 2015;38:433–47. <https://doi.org/10.1146/annurev-neuro-071013-014030>
68. Halko MA, Farzan F, Eldaief MC, Schmahmann JD, Pascual-Leone A. Intermittent theta-burst stimulation of the lateral cerebellum increases functional connectivity of the default network. *J Neurosci : Off J Soc Neurosci.* 2014;34:12049–56. <https://doi.org/10.1523/JNEUROSCI.1776-14.2014>
69. Xue C, Zhang X, Cao P, Yuan Q, Liang X, Zhang D, et al. Evidence of functional abnormalities in the default mode network in bipolar depression: A coordinate-based activation likelihood estimation meta-analysis. *J Affect Disord.* 2023;326:96–104. <https://doi.org/10.1016/j.jad.2023.01.088>
70. Schumacher MC, Chase HW, Rozovsky R, Eickhoff SB, Phillips ML. Prefrontal, parietal, and limbic condition-dependent differences in bipolar disorder: a large-scale meta-analysis of functional neuroimaging studies. *Mol Psychiatry.* 2023;28:2826–38. <https://doi.org/10.1038/s41380-023-01974-8>
71. Moguilner S, Garcia AM, Perl YS, Tagliazucchi E, Piquet O, Kumfor F, et al. Dynamic brain fluctuations outperform connectivity measures and mirror pathophysiological profiles across dementia subtypes: A multicenter study. *NeuroImage.* 2021;225:117522. <https://doi.org/10.1016/j.neuroimage.2020.117522>
72. Tang Q, Cui Q, Chen Y, Deng J, Sheng W, Yang Y, et al. Shared and distinct changes in local dynamic functional connectivity patterns in major depressive and bipolar depressive disorders. *J Affect Disord.* 2022;298:43–50. <https://doi.org/10.1016/j.jad.2021.10.109>
73. Nguyen TT, Kovacevic S, Dev SI, Lu K, Liu TT, Eyler LT. Dynamic functional connectivity in bipolar disorder is associated with executive function and processing speed: A preliminary study. *Neuropsychology.* 2017;31:73–83. <https://doi.org/10.1037/neu0000317>
74. Liang Y, Jiang X, Zhu W, Shen Y, Xue F, Li Y, et al. Disturbances of Dynamic Function in Patients With Bipolar Disorder I and Its Relationship With Executive-Function Deficit. *Front psychiatry.* 2020;11:537981. <https://doi.org/10.3389/fpsy.2020.537981>
75. Pinto JV, Moulin TC, Amaral OB. On the transdiagnostic nature of peripheral biomarkers in major psychiatric disorders: A systematic review. *Neurosci Biobehav Rev.* 2017;83:97–108. <https://doi.org/10.1016/j.neubiorev.2017.10.001>
76. Hiippala K, Jouhten H, Ronkainen A, Hartikainen A, Kainulainen V, Jalanka J, et al. The Potential of Gut Commensals in Reinforcing Intestinal Barrier Function and Alleviating Inflammation. *Nutrients.* 2018;10. <https://doi.org/10.3390/nu10080988>
77. Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. *Mol Psychiatry.* 2011;16:383–406. <https://doi.org/10.1038/mp.2010.120>
78. Zafar H, Saier MH Jr. Gut Bacteroides species in health and disease. *Gut Microbes.* 2021;13:1–20. <https://doi.org/10.1080/19490976.2020.1848158>
79. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci USA.* 2014;111:2247–52. <https://doi.org/10.1073/pnas.1322269111>
80. Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. *Neurosci Biobehav Rev.* 2011;35:804–17. <https://doi.org/10.1016/j.neubiorev.2010.10.001>
81. Karthikeyan S, Dimick MK, Fiksenbaum L, Jeong H, Birmaher B, Kennedy JL, et al. Inflammatory markers, brain-derived neurotrophic factor, and the symptomatic course of adolescent bipolar disorder: A prospective repeated-measures study. *Brain, Behav, Immun.* 2022;100:278–86. <https://doi.org/10.1016/j.bbi.2021.11.020>
82. Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab.* 2003;285:E433–437. <https://doi.org/10.1152/ajpendo.00074.2003>
83. Hsu JW, Lirng JF, Wang SJ, Lin CL, Yang KC, Liao MH, et al. Association of thalamic serotonin transporter and interleukin-10 in bipolar I disorder: a SPECT study. *Bipolar Disord.* 2014;16:241–8. <https://doi.org/10.1111/bdi.12164>
84. Lei Y, Tang L, Liu S, Hu S, Wu L, Liu Y, et al. Parabacteroides produces acetate to alleviate heparanase-exacerbated acute pancreatitis through reducing neutrophil infiltration. *Microbiome.* 2021;9:115. <https://doi.org/10.1186/s40168-021-01065-2>
85. Li H, Cui L, Cao L, Zhang Y, Liu Y, Deng W, et al. Identification of bipolar disorder using a combination of multimodality magnetic resonance imaging and machine learning techniques. *BMC psychiatry.* 2020;20:488. <https://doi.org/10.1186/s12888-020-02886-5>
86. Wollenhaupt-Aguiar B, Librenza-Garcia D, Bristot G, Przybylski L, Stertz L, Kubiachi Burke R, et al. Differential biomarker signatures in unipolar and bipolar depression: A machine learning approach. *Aust N Z J Psychiatry.* 2020;54:393–401. <https://doi.org/10.1177/0004867419888027>

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### AUTHOR CONTRIBUTIONS

ZXG, SX, and YW conceived and designed the experiments. ZXG, SX, GMC, SMZ, HZ, SLS, PC, and XYT performed the experiments and analyzed the data. All authors contributed to the interpretation of the results. HWY, YBJ, ZNY, LH, and YW drafted the first version of the manuscript. ZXG and YW reviewed and edited the manuscript. All authors provided critical comments and approved the final version of the manuscript.

### COMPETING INTERESTS

The authors declare no competing interests.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All samples were collected from for participates whom full written consent in accordance with the World Medical Associations Declaration of Helsinki.

### ADDITIONAL INFORMATION

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