Integrated Multi-Omics Analysis of Gut Microbiome and Serum Metabolome in Unipolar and Bipolar Depression

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Abstract

This study aims to differentiate between unipolar and bipolar depressive episodes through an integrated analysis of gut microbiome and serum metabolome. The study involved 82 patients experiencing depressive episodes, with 38 diagnosed with Major Depressive Disorder (MDD) and 44 with Bipolar Disorder (BD). The gut microbiome and serum metabolome were analyzed using 16S rRNA sequencing and ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS), respectively. The results revealed distinct microbial compositions and metabolic pathways between the two groups. Seventeen microbial groups and fifty serum metabolites were found to be significantly different between the two groups. Four genera and eight serum metabolites demonstrated strong diagnostic potential for differentiating BD from MDD. The study also found correlations between certain differential genera and metabolites and the severity of clinical symptoms. This integrated multi-omics approach provides a promising direction for the differential diagnosis of unipolar and bipolar depression.

1. Introduction

Major Depressive Disorder (MDD) and Bipolar Disorder (BD) are distinct mood disorders that often present with overlapping symptoms, particularly during depressive episodes[1, 2]. This similarity leads to diagnostic challenges, and studies have shown that up to 69% of BD patients are initially misdiagnosed, often with unipolar depression [3]. Such misdiagnoses result in inadequate treatment plans that can worsen the patient's condition and create a financial burden for families and society [4–6]. Such misdiagnoses result in incorrect treatment, worsening conditions, and financial strain on families and healthcare systems [7]. Given the differing treatment strategies for MDD and BD, there is an urgent need for early and accurate diagnosis [8–10].

Current diagnostic methods are mostly subjective, relying on clinical evaluations by psychiatrists [11]. The absence of reliable biological markers for differentiating MDD and BD underscores the need for more objective diagnostic tools [4–6].

The gut microbiome, interacting with the central nervous system via the Microbiota-Gut-Brain Axis, has become a focal point in mental health research [12–15]. While some studies suggest changes in gut microbial composition in both disorders, the data is inconsistent, necessitating further investigation [16–19], the results are far from conclusive. One previous study has identified a microbiota marker comprising 26 Operational Taxonomic Units (OTUs) that could potentially distinguish between MDD and BD [20]. However, due to methodological and reporting heterogeneity across studies, a uniform conclusion has not yet been reached, underlining the need for further research in this area.

Metabolomics, as a scientific tool, provides valuable insights into metabolic changes that may be associated with these disorders [21–24]. Yet, there's a gap in studies directly comparing metabolic profiles in MDD and BD during depressive episodes [25–27]. Blood acts as a transporter for metabolites produced by the gut microbiota, connecting the brain and intestinal flora [28, 29]. Increasing evidence
points to both MDD and BD as systemic diseases affecting not just mental state but also gut flora and metabolic processes [30, 31].

To better understand these interconnected systems, our study aims to perform an integrated analysis of the gut microbiome and serum metabolome in patients with unipolar and bipolar depressive episodes. The ultimate goal is to identify potential biomarkers that could aid in more accurate differential diagnosis.

2. Participants and methods

2.1 Participants

The study sample consisted of 82 patients experiencing depressive episodes between July 2021 and October 2022 at the First Hospital of Hebei Medical University. Of these, 38 were diagnosed with MDD (MDD group), and 44 were diagnosed with BD (BD group).

For inclusion in the BD group, participants had to meet the following criteria: (1) a diagnosis of bipolar disorder as per the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5); (2) age 16–65 years; (3) no specific dietary restrictions such as food allergies, intolerances, or vegetarianism; (4) The Hamilton Depression Scale-24 (HAMD-24) score ≥ 20, Young Mania Rating Scale (YMRS) score < 6; (5) provision of written informed consent to participate in the study [32, 33].

For inclusion in the MDD group, participants had to meet similar criteria: (1) a DSM-5 diagnosis of major depressive disorder; (2)~(5) were the same as that of (2)~(5) in the BP group.

Exclusion criteria for both groups were: (1) comorbidity with other psychiatric disorders; (2) presence of severe cardiovascular diseases or other organic brain diseases such as epilepsy or tumors; (3) acute or chronic inflammatory conditions, hepatobiliary diseases, rheumatic diseases, immune system diseases, or gastrointestinal diseases; (4) a history of severe alcohol or drug abuse; and (5) use of antibiotics, probiotics, or prebiotics within 4 weeks prior to screening.

The study was approved by the Ethics Committee of the First Hospital of Hebei Medical University (ethical approval number: 20210719). All procedures were conducted in alignment with the principles outlined in the Declaration of Helsinki. Both patients and their parent and/or legal guardian were informed of the potential risks and benefits associated with participation in the study, and had informed consent to participate in this study.

2.2 Methods

2.2.1 General demographic information collection

On the day of enrollment, all subjects underwent both physical and psychiatric examinations. General demographic information, including age, sex, Body Mass Index (BMI), years of education, age of onset,
duration of illness, smoking habits, alcohol consumption, family history, marital status, and dietary habits, was collected from each participant.

### 2.2.2 Scale Assessment

Psychiatric assessments were conducted by the same psychiatrist for consistency. The HAMD-24 was used to evaluate the severity of depression, while the Hamilton Anxiety Scale (HAMA)\(^{34}\) was employed to assess anxiety levels. Additional tests, including the Trail Making Test A (TMT-A), the Digit Symbol Substitution Test (DSST), and the Digital Span Test (DST), were used to assess cognitive functioning.

### 2.2.3 Microbiome

#### 2.2.3.1 Fecal sample collection

Subjects provided approximately 5 grams of stool specimens within three days of enrollment. These samples were stored in sampling boxes and subsequently subjected to 16S rRNA analysis.

#### 2.2.3.2 Fecal sample processing

DNA was extracted from approximately 0.25 grams of each fecal sample using the QIAamp Fast DNA Stool Mini Kit (Qiagen, CA, USA), as per the manufacturer's instructions. The concentration and purity of the extracted DNA were verified using spectrophotometry with a Multiskan™ GO instrument (Thermo Fisher Scientific, USA). Additionally, the quality of the DNA extracts was assessed through agarose gel electrophoresis using a 1.5% agarose gel in 1× Tris-Acetate-EDTA buffer. The samples were stored at -20°C until further use.

For the amplification of 16S rRNA genes, specific primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) were used. Each PCR reaction was set up in a 20µL volume, containing 10µL of KAPA HiFi HotStart ReadyMix (KAPA Biosystems, USA), 0.2µM of both forward and reverse primers, and roughly 10 ng of template DNA. The thermal cycling conditions consisted of an initial denaturation at 95°C for 3 minutes, followed by 30 cycles of 20 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C, and a final extension at 72°C for 10 minutes.

Post-PCR, the products were mixed with an equal volume of 1X loading buffer containing SYB green and subjected to electrophoresis on a 2% agarose gel. Samples that displayed a bright primary band between 400-450bp were selected for further experiments. These PCR products were mixed in equidensity ratios and then purified using the GeneJET Gel Extraction Kit (Thermo Scientific).

Sequencing libraries were prepared using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina), following the manufacturer's recommendations. Index codes were added to the libraries, whose quality was subsequently assessed using the Qubit® 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system. Finally, the libraries were sequenced on an Illumina MiniSeq platform, generating 150 bp paired-end reads.

### 2.2.4 Untargeted metabolomics
2.2.4.1 Blood Sample Collection

Blood samples were drawn from the elbow vein of participants in the early morning following enrollment. After collection, the tubes were gently inverted 4 to 5 times to mix the blood. The samples were then centrifuged at 4°C and 3000 rpm for 10 minutes. The resulting supernatant serum was transferred to lyophilized tubes and stored in a -80°C freezer for later analysis.

2.2.4.2 Serum sample processing

Upon thawing at 4°C, 100 µL aliquots of each serum sample were mixed with 400 µL of cold methanol/acetonitrile (1:1, v/v) to precipitate proteins. The mixtures were centrifuged at 14,000g and 4°C for 15 minutes. The supernatant was then dried using a vacuum centrifuge. For LC-MS analysis, samples were reconstituted in 100 µL of acetonitrile/water (1:1, v/v). Quality control (QC) samples, prepared by pooling 10 µL from each sample, were inserted and analyzed every 5 samples to monitor instrument stability and repeatability.

2.2.4.3 LC-MS/MS Analysis

Analyses were conducted using an ultra-high-performance liquid chromatography (UHPLC) system (1290 Infinity LC, Agilent Technologies) coupled with a quadrupole time-of-flight mass spectrometer (AB Sciex TripleTOF 5600). For Hydrophilic Interaction Liquid Chromatography (HILIC) separation, a 2.1 mm × 100 mm ACQUITY UPLC BEH 1.7 µm column (Waters, Ireland) was used. The mobile phase consisted of A = 25 mM ammonium acetate and 25 mM ammonium hydroxide in water, and B = acetonitrile. The gradient started at 85% B, was reduced linearly to 65% over 11 minutes, further reduced to 40% in 0.1 minute, held for 4 minutes, and then returned to 85% B in 0.1 minute, followed by a 5-minute re-equilibration period.

Electrospray ionization (ESI) source conditions were set as follows: Ion Source Gas1 (Gas1) at 60, Ion Source Gas2 (Gas2) at 60, curtain gas (CUR) at 30, and source temperature at 600°C. IonSpray Voltage Floating (ISVF) was set at ± 5500 V. For MS-only acquisition, the m/z range was 60-1000 Da with an accumulation time of 0.20 s/spectra. For auto MS/MS acquisition, the m/z range was 25-1000 Da with an accumulation time of 0.05 s/spectra. Information-dependent acquisition (IDA) was used with high sensitivity mode selected. The collision energy (CE) was set at 35 V with a range of ± 15 eV, and the declustering potential (DP) was set at 60 V for positive ion mode and − 60 V for negative ion mode. Isotopes within 4 Da were excluded, and up to 10 candidate ions were monitored per cycle.

2.2.4.4 Data processing

Raw mass spectrometry (MS) data in the form of wiff.scan files were first converted to MzXML format using the ProteoWizard MSConvert tool. These files were then imported into the open-source XCMS software for peak picking and analysis. Peak picking parameters were set as follows: centWave m/z = 25 ppm, peakwidth = c(10, 60), and prefilter = c(10, 100). For peak grouping, the parameters bw = 5, mzwid = 0.025, and minfrac = 0.5 were used.
Annotation of isotopes and adducts was carried out using CAMERA (Collection of Algorithms for MEtabolite pRofile Annotation). Only ion features with more than 50% non-zero measurement values in at least one group were retained for further analysis. Identification of individual metabolites was based on the comparison of accurate m/z values (with a tolerance of < 25 ppm) and MS/MS spectra against an in-house database established with authentic standards.

2.3 Statistical Analysis

Data were analyzed using SPSS version 25.0. For continuous variables, the Shapiro-Wilk test was applied to assess normality. Variables that did not conform to a normal distribution were expressed as the median, accompanied by the minimum and maximum values [M(P25, P75)], and comparisons between two groups were made using the two-sample rank-sum test. Variables that did conform to a normal distribution were presented as mean ± standard deviation (\(\bar{x} \pm s\)), and the independent samples t-test was employed for between-group comparisons. For categorical variables, expressed as either counts or percentages, the \(\chi^2\) test was used. Multivariate statistical analyses included Principal Component Analysis (PCA) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA). To identify differentially abundant gut microbiota between groups, Linear Discriminant Analysis Effect Size (LEfSe) was employed, with a set Linear Discriminant Analysis (LDA) threshold of 3.8.

Pathway enrichment analyses for differential metabolites were carried out by searching the KEGG and HMDB databases through MetaboAnalyst 3.0 software. Receiver Operating Characteristic (ROC) curve analysis was utilized for the identification of potential biomarkers. Correlation assessments were conducted using the Spearman rank correlation analysis, considering a \(P\)-value of < 0.05 as statistically significant.

3. Results

3.1 Comparison of general demographic information

No statistically significant differences were observed between the two groups in terms of age, gender, BMI, years of education, smoking, drinking, family history, marital status, or dietary habits \((P > 0.05)\) as shown in Table 1. However, significant differences were found between the two groups concerning age at onset and duration of disease \((P < 0.05)\), also detailed in Table 1.

3.1 Comparison of scale scores

The demographic information of both groups was detailed in Supplementary Table 1. For the HAMD-24, HAMA, somatic anxiety, TMT-A, and DSST scores, no statistically significant differences were observed between the two groups \((P > 0.05)\) as indicated in Table 1. In contrast, significant differences were found in mental anxiety, DST, DST-forward, and DST-reverse scores between the two groups \((P < 0.05)\), also presented in Table 1.
3.2 Microbiome analysis

3.2.1 Analysis of sequencing results

A total of 82 stool samples were sequenced using 16S rRNA. After initial sequencing, Flash software was employed for read splicing and quality control, yielding 6,247,810 high-quality clean reads. These were subsequently subjected to chimera filtering. OTUs were then clustered using QIIME software, resulting in 1,899 OTUs at 97% sequence similarity. At the OTU level, 836 OTUs were common to both BD and MDD groups, while 609 OTUs were unique to the BD group and 454 OTUs were unique to the MDD group, as depicted in Figure 1.

The Shannon-Wiener curve plateaued, indicating that the sequencing depth was sufficient to capture the majority of gut microbiota in the samples (Figure 2).

Fig.1 Venn diagram of species

Fig.2 Shannon-Wiener curve.

Note: The horizontal axis represents different sequencing depths in the sample, the vertical axis represents the Shannon Wiener index at the corresponding depth, and the curves of different colors represent the Shannon Wiener curves of different samples.

3.2.2 Analysis of intestinal flora diversity

Alpha diversity metrics, including Chao1, ACE, and Observed species indices, represented species richness, while Shannon and Simpson indices were used for species diversity, and the J index indicated community evenness. The Chao1, ACE, and Observed species indices were significantly lower in the BD group compared to the MDD group ($P < 0.05$) as shown in Figure 3. However, no significant differences were found in Shannon, Simpson, and J indices between the two groups ($P > 0.05$), also shown in Figure 3, suggesting similar species diversity and evenness but differing richness between the two groups.

In this study, PCA based on OTU abundance data was used to compare the microbial community structure between samples. Significant differences were observed in the $\beta$-diversity index between the BD and MDD groups ($P = 0.001$) as shown in Figure 4, indicating distinct intestinal microbial structures between the two groups.

Fig.3 Alpha diversity index box diagram.

Note: The abscissa represents the grouping, and the ordinate is Alpha Index.

Fig.4 PCA analysis results.

3.2.3 Analysis of differences in microbial composition
To identify distinct microbial species between the BD and MDD groups and isolate the dominant flora in each, LEfSe analysis was conducted with a set LDA threshold of greater than 3.8. This analysis revealed 17 microbial groups that were significantly different between the two groups ($P < 0.05$, LDA > 3.8), as shown in Figures 5a and 5b.

Fig. 5a Histogram of LDA effect value of species with significant difference (LDA > 3.8)

Fig. 5b Branch diagram of LDA effect value of species with significant difference (LDA > 3.8)

3.2.4 ROC curve analysis

ROC curve analysis was performed on the differential flora at the genus level to evaluate their discriminative power between the BD and MDD groups. The results are presented in Figure 6 and Table 2. The AUC for the combination of these four differential genera was calculated to be 0.925, suggesting that this combination holds strong diagnostic potential for differentiating BD from MDD.

Fig. 6 ROC analysis of genus level differential bacteria and combined model.

3.2.5 Analysis of correlation between differential intestinal genera and clinical symptoms

A correlation study was performed to explore the relationships between the four differential genera and various clinical symptom scores, the results of which are depicted in Figure 7. Specifically, the genus g__un_f_Muribaculaceae was found to be negatively correlated with HAMD-24, HAMA, and somatic anxiety scores in the BD group ($r = -0.423$, $P < 0.01$; $r = -0.345$, $P < 0.05$; $r = -0.313$, $P < 0.05$). In contrast, this genus was positively correlated with DST-reverse scores ($r = 0.329$, $P < 0.05$), DSST scores ($r = 0.415$, $P < 0.01$), and TMT-A time use ($r = -0.423$, $P < 0.01$) in the MDD group.

Fig. 7 Correlation analysis between different gut microbiota and clinical symptoms

Note: A positive correlation is red, and the deeper the red, the higher the positive correlation. A negative correlation is blue, and the deeper the blue, the higher the negative correlation. ** 0.01 * 0.05.

3.3 Untargeted metabolome

3.3.1 Identification Results and Statistical Analysis

A total of 40 blood samples were analyzed—22 from the BD group and 18 from the MDD group. Utilizing an untargeted metabolomics approach based on UHPLC-MS, 1168 metabolites were identified across both groups. Of these, 514 were identified through negative ionization and 654 through positive ionization. These metabolites were subsequently categorized based on their chemical classifications, as illustrated in Figure 8.

Fig. 8 Proportion of identified metabolites in each chemical classification.

3.3.2 Validation of the model
OPLS-DA was employed to observe the overall sample distribution, depicted in Figures 9a and 9b. To confirm the model's stability, a 7-fold cross-validation was carried out. The analysis demonstrated that the metabolic data effectively distinguished between the BD and MDD groups (Positive ions: $R^2=0.164$, $Q^2=0.433$; Negative ions: $R^2=0.146$, $Q^2=0.382$). A permutation test was conducted to validate the model and prevent overfitting, as displayed in Figures 10a and 10b. The model was deemed robust when the $R^2$ and $Q^2$ values of the permutation model gradually decreased.

Fig. 9a OPLS-DA score of positive ion.

Fig. 9b OPLS-DA score of negative ion.

Note: t [1] represents principal component 1, to [1] represents principal component 2, and the ellipse represents a 95% confidence interval. Dots of the same color represent various biological duplications within a group, and the distribution of dots reflects the degree of difference between and within groups.

Fig.10a Positive ion OPLS-DA replacement test.

Fig.10b Negative ion OPLS-DA replacement test.

Note: The abscissa in the figure represents the degree of displacement retention, that is, the proportion consistent with the order of the Y variables in the original model, and the ordinate represents the values of $R^2$ and $Q^2$. The green dot represents $R^2$, the blue dot represents $Q^2$, and the two dashed lines represent the regression lines of $R^2$ and $Q^2$, respectively. The $R^2$ and $Q^2$ in the upper right corner indicate that the displacement retention is equal to 1, which is the $R^2$ and $Q^2$ values of the original model.

3.3.3 Differential metabolite screening

In this study, a total of 50 differential metabolites were identified using VIP scores greater than 1 and a P-value less than 0.05 as the criteria for significance (Figures 11a and 11b). In the positive ion mode, 13 differential metabolites were found, nine of which were elevated in the BD group. Meanwhile, in the negative ion mode, 37 differential metabolites were identified, 24 of which were elevated in the BD group.

Fig.11a Multiple analysis of significant difference in metabolite expression in positive ion

Fig.11b Multiple analysis of significant difference in metabolite expression in negative ion

Note: The abscissa in the figure represents the log2 FC value of the differential metabolite, that is, the logarithmic value of the differential multiple of the differential metabolite based on 2, and the ordinate represents the significant differential metabolite. Red indicates that the BD group upregulates differential metabolites, while green indicates that the BD group downregulates differential metabolites.

3.3.4 Metabolic pathway analysis of differential metabolites
KEGG pathway analysis was performed on the differential serum metabolites to identify the most relevant metabolic pathways between the BD and MDD groups. The analysis revealed six metabolic pathways that were most affected: Taurine and Hypotaurine Metabolism, Neuroactive Ligand-Receptor Interactions, Pyrimidine Metabolism, Vitamin B6 Metabolism, Purine Metabolism, and ABC Transporter (Figures 12a and 12b).

Fig. 12a KEGG enrichment pathway diagram (bubble diagram)

Fig. 12b KEGG enrichment pathway diagram (histogram)

3.3.5 ROC curve analysis

Given the significant differences in serum metabolic profiles between the BD and MDD groups, ROC curve analysis was conducted on the differential metabolites to identify specific biomarkers. The top 10 metabolites with VIP scores greater than 1 were selected for this analysis (Table 3 and Figure 13). Eight metabolites demonstrated an AUC greater than 0.7: adenosine, glycerophosphocholine, Phe-phe, 1-hydroxy-2-naphthoic acid, hypoxanthine, DL-lactate, taurine, and isocitric acid (AUC values as follows: 0.818, 0.737, 0.889, 0.770, 0.720, 0.760, 0.730, 0.715). These metabolites appear to have strong diagnostic potential.

Fig.13 ROC analysis of the first 10 metabolites with high VIP value difference

3.3.6 Correlation analysis of differential metabolites and clinical symptoms

A correlation analysis was conducted between the serum levels of the eight metabolites with strong diagnostic value and the clinical symptom scores (Figure 14). In the MDD group, 1-hydroxy-2-naphthoic acid showed a positive correlation with DSST score (r=0.616, P<0.01) and a negative correlation with TMT-A (r=-0.633, P<0.01). DL-lactate was positively correlated with both DSST score and DST-reverse (r=-0.614, P<0.01; r=0.486, P<0.05) and negatively correlated with TMT-A (r=-0.554, P<0.05). In the BD group, no differential metabolites were found to correlate with the severity of clinical symptoms.

Fig.14 Correlation analysis between differential metabolites and clinical symptoms

Note: A positive correlation is red, and the deeper the red, the higher the positive correlation. A negative correlation is blue, and the deeper the blue, the higher the negative correlation. ** 0.01 * 0.05.

3.4 Integrated analysis of microbiome and metabolome

To investigate the potential relationship between differential intestinal genera and differential serum metabolites, a Spearman correlation analysis was conducted (Figure 15). The analysis indicated a substantial interaction between the differential metabolites and the differential gut microbiota.

Fig. 15 Correlation analysis of differential gut microbiota and serum differential metabolites
4. Discussion

The study included 82 participants, with 38 individuals in the MDD group and 44 in the BD group. Demographic variables and dietary habits were well-matched between the two groups. The BD group had an earlier age of onset and a longer duration of illness, aligning with general characteristics of bipolar disorder. While both groups had similar levels of depression, the BD group showed more severe anxiety and poorer cognitive function, corroborating previous studies [34].

We utilized 16S rRNA sequencing and serum untargeted metabolomics to examine the variations in gut microbiota and serum metabolites between the two groups. Though the alpha diversity showed no significant difference, indicating similar gut flora diversity in both groups, the BD group had a richer composition. Beta diversity analysis revealed distinct microbial structures between the groups. Additionally, our LEfSe analysis found 17 significantly different gut flora, with 13 enriched in the BD group and 4 in the MDD group. These distinct microbial profiles suggest potential biomarkers for differentiating between MDD and BD. To further differentiate between unipolar and bipolar depressive episodes, LEfSe analysis identified 17 significantly different gut flora, with 13 enriched in the BD group and 4 in the MDD group. This suggests that the microbial profiles of unipolar and bipolar depressive episodes are notably distinct. Numerous studies have consistently found elevated levels of the actinomycete phylum in both MDD and BD patients compared to healthy controls [35–38], highlighting a potential link between actinomycete levels and depression. This study also found a higher abundance of specific genera in the BD group compared to the MDD group, such as g__Bifidobacterium, g__Prevotella_7, and g__un_f_Muribaculaceae. Our ROC curve analysis of these four differential genera yielded an AUC of 0.925, suggesting their potential utility as biomarkers to distinguish BD from MDD.

While Bifidobacterium is usually considered beneficial [39], our study found no link between its abundance and depression severity, a finding consistent with some previous research but contradictory to others [35, 40–42]. Similarly, the genus g__un_f_Muribaculaceae, known for its anti-inflammatory properties, differed in abundance between the BD and MDD groups. [43] [44–46]. It was negatively correlated with depression and anxiety severity in the BD group, suggesting its role as a potential severity marker for both types of depression.

Using UHPLC-MS, we analyzed serum samples and identified 1168 metabolites, revealing significant metabolic differences between the BD and MDD groups. We found 50 differential metabolites involved in pathways like Taurine metabolism and Purine metabolism. These metabolites, including adenosine and lactate, could serve as potential biomarkers for distinguishing between unipolar and bipolar depression. In the MDD group, certain metabolites like 1-hydroxy-2-naphthoic acid and lactate were positively correlated with cognitive function.
Adenosine, a key neuromodulator [47–52] was found in this study to have differing levels between unipolar and bipolar depression, opening new research avenues. Isocitric acid, involved in multiple metabolic pathways [53–55], showed differing metabolism between the two depression types, adding complexity to our understanding. Finally, lactate, produced mainly by gut bacteria like Lactobacillus and Bifidobacterium [56–66], had reduced levels in the MDD group compared to BD and was positively correlated with cognitive function in MDD. This supports lactate's role not just as an energy substrate but also as a signaling molecule in brain function.

Our findings highlight the intricate relationship between the gut microbiome and serum metabolites. Bifidobacterium, a prevalent genus in our study, showed positive associations with multiple serum differential metabolites such as glycerophosphatidylcholine, phenylalanine, 1-hydroxy-2-naphthoic acid, lactic acid, and taurine. Additionally, g__un_f_Muribaculaceae correlated positively with phenylalanine and hypoxanthine but negatively with adenosine. These correlations indicate a complex interplay between differential gut flora and differential metabolites, reinforcing the idea that gut microbes and serum metabolites are closely linked. This aligns with previous studies suggesting abnormalities in lactate metabolism in both BD and MDD, which could be attributed to dysfunctional mitochondrial activity [67, 68].

This study stands out for its dual-disease focus, employing an integrated microbiome and metabolome analysis to distinguish between unipolar and bipolar depression. It identifies differential biomarkers between the two conditions and correlates these biomarkers with the severity of mood and cognitive symptoms, thereby providing a theoretical foundation for precision medicine. However, the study is not without limitations. The sample size is relatively small, limiting the generalizability of the findings. Future work will aim to expand the sample size for more robust conclusions. Additionally, the study did not control for or assess the potential impact of diet and psychotropic medications on gut microbiota. Lastly, it's important to note that the study does not establish a causal relationship between differential gut microbes, serum metabolites, and the diseases under investigation.

5. Conclusion

This study revealed that gut microbes and serum metabolites can effectively differentiate between unipolar and bipolar depressive episodes. A total of seventeen gut flora and fifty endogenous serum differential metabolites were identified as significantly different between the two groups. Four genera and eight serum metabolites show promise as valuable biomarkers for differential diagnosis. Some of these differential genera and metabolites also appear to correlate with clinical symptoms, suggesting a potential interaction between gut microbes and serum metabolites.

Abbreviations

MDD
Major Depressive Disorder
BD
Bipolar Disorder
AUC
Area Under the Curve
OTUs
Operational Taxonomic Units
DSM-5
Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
HAMD
Hamilton Depression Scale
YMRS
Young Mania Rating Scale
BMI
Body Mass Index
HAMA
Hamilton Anxiety Scale
TMT-A
Trail Making Test A
DSST
Digit Symbol Substitution Test
DST
Digital Span Test
UHPLC
ultra-high-performance liquid chromatography
HILIC
Hydrophilic Interaction Liquid Chromatography
ESI
Electrospray ionization
CUR
Curtain Gas
ISVF
IonSpray Voltage Floating
IDA
Information-dependent acquisition
CE
Collision Energy
DP
Declustering Potential
MS
Mass Spectrometry
PCA
Principal Component Analysis
OPLS-DA
Orthogonal Projections to Latent Structures Discriminant Analysis
LEfSe
Linear Discriminant Analysis Effect Size
LDA
Linear Discriminant Analysis
ROC
Receiver Operating Characteristic

Declarations

Acknowledgements

Y. Jing and L. Wang contributed equally to this work.

Authors’ contributions


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Availability of data and materials

All data generated in the present study may be requested from the corresponding author.

Ethics approval and consent to participate

All studies confirm to the principles outlined in the Declaration of Helsinki and have been approved by the Ethics Committee of the First Hospital of Hebei Medical University (ethical approval number: 20210719).

Consent for publication
Not applicable.

Competing interests

The funder had no role in study design, data collection, analysis, or writing of the manuscript. The authors declare that they have no conflict of interest to report.

References


Tables

Table 1 Comparison of scales scores between MDD group and BD group

<table>
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<tr>
<th>Scale/factor</th>
<th>MDD(n=38)</th>
<th>BD(n=44)</th>
<th>t/Z value</th>
<th>P value</th>
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<td>HAMD-24</td>
<td>28.0(25.8,34.3)</td>
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<td>HAMA</td>
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<td>19.5(14.3,25.0)</td>
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<td>Somatic anxiety</td>
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<td>47.313.7</td>
<td>-0.071</td>
<td>0.944</td>
</tr>
<tr>
<td>DST</td>
<td>15(12 16)</td>
<td>11.5(10 13)</td>
<td>-3.046</td>
<td>0.002</td>
</tr>
<tr>
<td>DST-forward</td>
<td>8(7 9)</td>
<td>7(6 8)</td>
<td>-2.021</td>
<td>0.043</td>
</tr>
<tr>
<td>DST-reverse</td>
<td>6.5(5 7)</td>
<td>5(4 5)</td>
<td>-3.499</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 2 ROC analysis of different gut microbiota at genus level

<table>
<thead>
<tr>
<th>Differential genera</th>
<th>AUC</th>
<th>95%CI</th>
<th>P-value</th>
<th>Sensitivity(%)</th>
<th>Specificity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g__Bifidobacterium</td>
<td>0.878</td>
<td>0.799-0.957</td>
<td>0.001</td>
<td>93.2</td>
<td>71.1</td>
</tr>
<tr>
<td>g__un_f_Muribaculaceae</td>
<td>0.749</td>
<td>0.635-0.862</td>
<td>0.001</td>
<td>75</td>
<td>89.5</td>
</tr>
<tr>
<td>g__Parasutterella</td>
<td>0.654</td>
<td>0.533-0.775</td>
<td>0.017</td>
<td>75</td>
<td>55.3</td>
</tr>
<tr>
<td>g__Prevotella_7</td>
<td>0.615</td>
<td>0.493-0.736</td>
<td>0.075</td>
<td>34.1</td>
<td>100</td>
</tr>
<tr>
<td>Union</td>
<td>0.925</td>
<td>0.863-0.986</td>
<td>0.001</td>
<td>95.5</td>
<td>81.6</td>
</tr>
</tbody>
</table>

Note: Union is the joint predicted probability of 4 intestinal differential bacterial genera.

Table 3 ROC analysis of the first 10 serum metabolites with high VIP value in BD and MDD
<table>
<thead>
<tr>
<th>Metabolites</th>
<th>AUC</th>
<th>95%CI</th>
<th>P value</th>
<th>Sensitivity(%)</th>
<th>Specificity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>0.818</td>
<td>0.687-0.949</td>
<td>0.001</td>
<td>100</td>
<td>55.6</td>
</tr>
<tr>
<td>Glycerophosphocholine</td>
<td>0.737</td>
<td>0.577-0.898</td>
<td>0.011</td>
<td>63.6</td>
<td>83.3</td>
</tr>
<tr>
<td>Phe-phe</td>
<td>0.889</td>
<td>0.790-0.988</td>
<td>0.001</td>
<td>63.6</td>
<td>100</td>
</tr>
<tr>
<td>1-hydroxy-2-naphthoic acid</td>
<td>0.770</td>
<td>0.612-0.929</td>
<td>0.004</td>
<td>77.3</td>
<td>72.2</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.720</td>
<td>0.556-0.883</td>
<td>0.018</td>
<td>81.8</td>
<td>61.1</td>
</tr>
<tr>
<td>DL-lactate</td>
<td>0.760</td>
<td>0.603-0.917</td>
<td>0.005</td>
<td>72.7</td>
<td>77.8</td>
</tr>
<tr>
<td>N-oleoyl-d-erythro-sphingosylphosphorylcholine</td>
<td>0.669</td>
<td>0.495-0.843</td>
<td>0.069</td>
<td>90.9</td>
<td>44.4</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.730</td>
<td>0.571-0.888</td>
<td>0.013</td>
<td>95.5</td>
<td>44.4</td>
</tr>
<tr>
<td>3-hydroxyphenylacetic acid</td>
<td>0.662</td>
<td>0.490-0.833</td>
<td>0.082</td>
<td>45.5</td>
<td>88.9</td>
</tr>
<tr>
<td>Isocitric acid</td>
<td>0.715</td>
<td>0.555-0.873</td>
<td>0.021</td>
<td>72.7</td>
<td>33.3</td>
</tr>
</tbody>
</table>

**Figures**
Figure 1

Venn diagram of species
Figure 2

Shannon-Wiener curve

Figure 3

Alpha diversity index box diagram.
Note: The abscissa represents the grouping, and the ordinate is Alpha Index.

Figure 4

PCA analysis results
Figure 5

5a Histogram of LDA effect value of species with significant difference (LDA  3.8)

5b Branch diagram of LDA effect value of species with significant difference (LDA  3.8)

ROC-Curve

Source of the Curve
- g__Bifidobacterium
- g__un_f_Murbaculaceae
- g__Parasutterella
- g__Prevotella_7
- g__Prevotella_7
- Union
- Reference
Figure 6

ROC analysis of genus level differential bacteria and combined model.

Figure 7

Correlation analysis between different gut microbiota and clinical symptoms

Note: A positive correlation is red, and the deeper the red, the higher the positive correlation. A negative correlation is blue, and the deeper the blue, the higher the negative correlation. ** 0.01 * 0.05.
Figure 8

Proportion of identified metabolites in each chemical classification

Figure 9

9a OPLS-DA score of positive ion. 9b OPLS-DA score of negative ion.
Figure 10

10a Positive ion OPLS-DA replacement test.

10b Negative ion OPLS-DA replacement test.

Figure 11

11a Multiple analysis of significant difference in metabolite expression in positive ion

11b Multiple analysis of significant difference in metabolite expression in negative ion
Figure 12

12a KEGG enrichment pathway diagram (bubble diagram)

12b KEGG enrichment pathway diagram (histogram)

Figure 13

ROC analysis of the first 10 metabolites with high VIP value difference
**Figure 14**

Correlation analysis between differential metabolites and clinical symptoms

Note: A positive correlation is red, and the deeper the red, the higher the positive correlation. A negative correlation is blue, and the deeper the blue, the higher the negative correlation. **0.01 * 0.05.
Figure 15

Correlation analysis of differential gut microbiota and serum differential metabolites

Note: A positive correlation is red, and the deeper the red, the higher the positive correlation. A negative correlation is blue, and the deeper the blue, the higher the negative correlation. ** 0.01  * 0.05

Supplementary Files

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- SupplymentaryTable.docx