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ORIGINAL RESEARCH

Comprehensive Analysis of the Microbiome and Metabolome: Unveiling Interactions Between Intestinal Flora and Metabolites in Schizophrenia

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Background: Schizophrenia (SZ) is a complex, multifactorial neurodevelopmental disorder characterized as a severe mental illness. Despite extensive research, its etiology and pathogenesis remain largely elusive. Recent studies suggest that the intestinal microbiota and its metabolites may play critical roles in the onset and regulation of SZ. This study aims to examine the characteristics of the intestinal microbiota and metabolomics in patients with SZ and to explore their correlation with clinical symptoms.

Methods: This study involved thirty-five patients diagnosed with schizophrenia (SZ group) and thirty healthy control participants (HC group). Fecal samples were collected and analyzed using microbiome (16S rRNA gene sequencing) and metabolome (UPLC-MS) techniques. Bioinformatics and statistical analyses were conducted to interpret the data.

Results: Age, sex, and BMI were comparable between the SZ and HC groups. Microbial diversity sequencing identified 837 operational taxonomic units (OTUs) across both groups. Although alpha diversity metrics showed no significant difference, beta diversity metrics revealed notable disparities. At the genus level, differences were observed in nine bacterial groups: *Turicibacter, Toprococcus, Campylobacter, Eubacterium, Blautia, Sarcina, Catenibacterium, Lactobacillus, and Porphyromonas*. At the family level, *Erysipelotrichaceae, Turicibacteraceae, Campylobacteraceae, Desulfovibrionaceae, Lactobacillaceae, and Lachnospiraceae* showed significant variations. Metabolomics analysis identified 946 metabolites, with 54 showing significant differences, primarily in caffeine metabolism and cysteine and methionine metabolism. Notably, correlations were found between differential gut microbes and metabolites, and between these metabolites and psychiatric clinical symptoms.

Conclusion: The study reveals significant abnormalities in the intestinal flora and metabolites of SZ patients, correlating with clinical severity. These findings offer new insights into the interactions between the gut microbiome and metabolites, potentially contributing to the understanding of the pathophysiology of schizophrenia.

Keywords: schizophrenia, gut microbiota, intestinal metabolites, 16S rRNA, UPLC-MS

Introduction

Schizophrenia (SZ) is a severe and complex mental disorder, characterized by clinical symptoms such as hallucinations, delusions, and cognitive impairment.[1] In 2019, schizophrenia accounted for the third-highest proportion of mental disorder disability-adjusted life years (DALYs), following depression and anxiety disorders, imposing a significant health and economic burden on both patients and society.[2] Despite advancements in antipsychotic treatments and physical therapy,[3,4] outcomes for SZ patients often fall short of expectations. Furthermore, the neuropathological processes and pathogenesis of SZ remain only partially understood. Recurrent episodes among many patients with schizophrenia lead to considerable psychological distress for the individuals and their families, exacerbating societal and economic burdens.[5]

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Approximately 40% of the metabolites in the human body are believed to be influenced by the microbiota, highlighting its vital importance to human health.[6] Research into the relationship between microbiota and the central nervous system has emphasized the critical role of gut microbiota within the gut-brain axis.[7] Investigations into schizophrenia have identified significant abnormalities in the microbiomes of affected individuals, including alterations in diversity, composition, and metabolic pathways[8–10.] These findings suggest pronounced dysbiosis in the intestinal microbiomes of patients with SZ. Moreover, the abundance of certain gut microbiota has been linked to the severity of schizophrenia symptoms.[11]

Metabolomics technologies have been instrumental in providing a more comprehensive understanding of the pathophysiological mechanisms underlying various diseases, thereby shedding light on disease onset and progression.[12] Numerous studies have demonstrated that patients with schizophrenia exhibit abnormalities in metabolites, including serum γ-glutamylcysteine, linoleic acid, glutathione, 5-hydroxytryptophan, tryptophan, and glutamic acid.[13–15] Consistently, ten metabolites—N-acetyl aspartate, lactate, tryptophan, kynurenine, glutamate, creatine, linoleic acid, D-serine, glutathione, and 3-hydroxybutyrate—have been identified as biomarkers for schizophrenia or psychosis across several independent metabolomics studies.[16] Generally, the metabolites relevant to patients with schizophrenia or psychosis encompass lipids, lipid-like molecules, carbohydrate metabolism, and organic acids, along with their derivatives.[17] These metabolites associated with schizophrenia were not only altered but their metabolic pathways were also disrupted, including those regulating glucose and amino acid metabolism.[18,19] Fecal metabolomics analysis has shown that patients with SZ exhibit increased concentrations of various proteolytic metabolites, such as amino acids, urea, and branched short-chain fatty acids.[20] Overall, while most metabolomics studies on schizophrenia have focused on serum and plasma, analyses of fecal metabolomics remain relatively limited.

The gut microbiota significantly influences host physiology by generating a diverse array of metabolites, which serve as signaling molecules and metabolic substrates within the host[21] (Krautkramer et al 2021). These metabolic interactions between the intestinal microbiota and host cells commence from birth and are pivotal for health, including the integrity of the blood-brain barrier and overall brain health.[15,22] Gut microbiota produces a spectrum of bioactive compounds, such as neurotransmitters, amino acids, sugars, and organic acids. These compounds are implicated in the development of central nervous system disorders, including autism, schizophrenia, and Huntington's disease.[23–25] Notably, *Lactobacillus brevis* and *Bifidobacterium dentium* in the human gut efficiently synthesize GABA, a major inhibitory neurotransmitter in the CNS, dysfunction of which is linked to depression, anxiety, autism, and schizophrenia.[26] Furthermore, gut microbiota-generated short-chain fatty acids (SCFAs) act as mediators that connect internal microbiota with the brain, influencing brain physiology and behavior.[27] Schizophrenia is associated with alterations in the microbiome and dysbiosis, as well as disruptions of the intestinal barrier and bacterial translocation. Environmental factors, such as stress, infections, medications, and diet, can alter gut bacterial metabolism, affecting neuropsychiatric disorders. Hence, the microbiome's metabolic function is deemed more critical than the presence of specific bacterial species.[24] Emerging research has been focusing on the differences in the gut microbiome of individuals with schizophrenia.[10]

Current research on the linkage between intestinal flora and related metabolites in schizophrenia remains sparse. In this study, we focused on schizophrenia patients as the subject group, contrasting their profiles with healthy controls. By analyzing stool samples through 16S rDNA sequencing technology and non-targeted metabolomics, our main objective was to investigate the differences in gut microbiota and metabolites between schizophrenia patients and normal controls. In addition, we sought to examine the correlations between gut microbiota, metabolites, and psychiatric symptoms in schizophrenia, providing a possible basis for research into the pathophysiology of schizophrenia.

Methods

Study Design and Participant Recruitment

This was a case-control study that included 35 SZ patients and 30 healthy controls who visited Hefei Fourth People's Hospital from August 2020 to January 2022. We collected demographic and clinical data at baseline, including body mass index (BMI), gender, age, and Positive and Negative Syndrome Scale (PANSS) scores. The inclusion criteria for SZ patients were as follows: (1) Diagnosis of SZ according to DSM-5 criteria by two independent, experienced psychiatrists;

(2) No antipsychotic drugs were taken within one month before enrollment.(3) No current allergies, autoimmune diseases, or infections; (4) No use of immunosuppressants or anti-inflammatory drugs; and (5) Aged between 16–60 years. Exclusion criteria included: (1) A history of traumatic brain injury, neurological disease, or other major physical illnesses; (2) A history of alcohol or substance abuse or dependence, or current substance abuse; (3) Mental retardation or inability to complete cognitive function tests; (4) Pregnancy or lactation; and (5) Diagnosis with another mental disorder or co-existing chronic illness, such as immune deficiency diseases, autoimmune diseases, cancer, inflammatory bowel disease, or severe diarrhea. The healthy control group was matched with the patient group by age, gender and BMI, and was recruited from Anhui region to control the effects of age, gender, BMI and dietary habits on the fecal microbiota.

Positive and Negative Syndrome Scale (PANSS)

The PANSS is extensively used to assess severe psychopathology in patients with SZ. It has good reliability and validity, with a Cronbach's alpha coefficient and intra-class coefficient were 0.928 and 0.878, respectively.[28] The versions of the three-factor model have been utilized for the assessment of positive symptoms, negative symptoms, and general psychopathology.

Specimen Collection and Analysis

Fecal samples were collected from both groups and stored at −80°C for further analysis using 16S rDNA gene sequencing and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). The 16s rRNA gene sequencing and liquid chromatography-mass spectrometry were performed by Hua da Genomics Technology Service Co.

Ethical Considerations

Written informed consent was obtained from all participants before their inclusion in the study, which was conducted in accordance with the Clinical Research Ethics Committee of Hefei Fourth People's Hospital's guidelines (Ethics No. HSY-IRB-PJ-JZB-001) and complies with the Declaration of Helsinki. The trial clinical registration number was chiCTR1800019343(06/11/2018).

16S rDNA Gene Sequencing and Data Processing

Microbial community DNA was extracted using the MagPure Stool DNA KF Kit B (Magen, China). Preparation involved five 96-well deep plates, each added with 600 μL Buffer containing magnetic beads, 20 μL Proteinase K, and 5 μL RNase A, followed by 700 μL of Wash 1, Wash 2, and Wash 3, respectively, and 100 μL Elution Buffer. Approximately 100–200 mg of the sample was transferred to a centrifuge tube with grinding beads. After adding 1 mL Buffer ATL/PVP-10, the sample was ground using a grinding machine and then incubated at 65°C for 20 minutes. Post-centrifugation at 14,000×g for 5 minutes, the supernatant was transferred to a new tube, mixed with 0.6 mL Buffer PCI, and vortexed for 15 seconds. After a subsequent centrifugation at 18,213×g for 10 minutes, the supernatant was transferred to a deep well plate with magnetic beads binding solution. The Kingfisher machine was then used as per the corresponding program to process the samples. The DNA was finally transferred to a 1.5 mL centrifuge tube. DNA quantification was performed using a Qubit Fluorometer with the Qubit dsDNA BR Assay Kit (Invitrogen, USA), and quality was assessed on a 1% agarose gel.

The V4 variable regions of the bacterial 16S rRNA gene were amplified using degenerate PCR primers, 515F (5′-GTGCCAGCMGCCGCGGGTAA-3′) and 806R (5′-GGACTACHV GGGTWTCTAAT-3′). The PCR products were purified using Agencourt AMPure XP beads and eluted in Elution Buffer. Libraries were evaluated with an Agilent Technologies 2100 bioanalyzer and sequenced on the Illumina HiSeq 2500 platform (BGI, Shenzhen, China), generating 2×250 bp paired-end reads. The USEARCH software (v7.0.1090)[29] was utilized for clustering the assembled tags into Operational Taxonomic Units (OTUs) based on 97% sequence similarity. OTU representative sequences were aligned against the Greengene database for taxonomic annotation using the RDP classifier (v2.2) software (sequence identity is set to be 0.6). The mothur software (v.1.31.2)[30] was applied for analyzing Alpha diversity, while QIIME (v1.80)[31] was used for Beta diversity analysis. Spearman correlation analysis was conducted to establish correlation data between the differential gut microbiome and metabolites. All diagrams were generated using https://www.omicstudio.cn/tool.

UPLC-MS Analysis Protocol

After gradually thawing the sample at 4°C, weigh 25 mg and transfer it into a 1.5 mL Eppendorf tube. Add 800 μL of extraction solution (methanol: acetonitrile: water = 2:2:1, v/v/v, pre-cooled to −20°C) and 10 μL of an internal standard. Introduce two small steel balls and subject the mixture to grinding in a tissue grinder at 50 Hz for 5 minutes. Follow this with ultrasonication in a 4°C water bath for 10 minutes. Allow the sample to stand at −20°C for 1 hour. Centrifuge at 25,000 g for 15 minutes at 4°C. Transfer 600 μL of the supernatant to a freeze vacuum concentrator for drying. Redissolve the residue in 600 μL of complex solution (methanol: H2O = 1:9, v/v), vortex for 1 minute, and ultrasonicate again in a 4°C water bath for 10 minutes. Centrifuge at 25,000 g at 4°C for 15 minutes, and transfer the supernatant to an autosampler vial. To evaluate the repeatability and stability of the LC-MS analysis process, mix 50 μL of the supernatant from each sample to prepare synthetic quality control (QC) samples.

This experiment utilized the Waters UPLC I-Class Plus system (Waters, USA) coupled with a Q Exactive high-resolution mass spectrometer (Thermo Fisher Scientific, USA) for metabolite separation and detection. Chromatographic separation was performed on a Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters, USA) with the column temperature maintained at 45°C. The mobile phase comprised 0.1% formic acid (A) and acetonitrile (B) in positive mode, and 10 mM ammonium formate (A) and acetonitrile (B) in negative mode. Gradient conditions were as follows: 0–1 min, 2% B; 1–9 min, 2%-98% B; 9–12 min, 98% B; 12–12.1 min, 98% to 2% B; 12.1–15 min, 2% B. The flow rate was 0.35 mL/min with an injection volume of 5 μL.

Mass spectrometry conditions included a full scan range of 70–1050 m/z with a resolution of 70,000. The automatic gain control (AGC) target for MS acquisitions was set to 3e6, with a maximum ion injection time of 100 ms. The top 3 precursors were selected for MS/MS fragmentation with a maximum ion injection time of 50 ms and a resolution of 17,500. AGC for MS/MS was set to 1e5. Stepped normalized collision energy was set to 20, 40, and 60 eV. ESI parameters included a sheath gas flow rate of 40, aux gas flow rate of 10, spray voltages of 3.80 kV in positive-ion mode and 3.20 kV in negative-ion mode, a capillary temperature of 320°C, and an aux gas heater temperature of 350°C.

Offline mass spectrometry data were analyzed using Compound Discoverer 3.3 (Thermo Fisher Scientific, USA), incorporating the BMDB (BGI Metabolome Database), MZCloud, and ChemSpider databases for metabolite identification. Data preprocessing in MetaX involved normalizing the data using Probabilistic Quotient Normalization (PQN), correcting batch effects with quality control-based robust LOESS signal correction, and removing metabolites with a coefficient of variation greater than 30% in their relative peak area in QC samples.

Pathway function annotation utilized the KEGG PATHWAY database to identify principal biochemical metabolic pathways involved. Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) was employed to link metabolite expression with sample categories for prediction. The Variable Importance for the Projection (VIP) metric assessed the impact strength and explanatory power of metabolite expression patterns on the classification and discrimination of sample groups, aiding in the identification of metabolic biomarkers. Prior to OPLS-DA model construction, data underwent log10 transformation, with pareto scaling applied. The model was validated through seven iterations of interactive verification.

Statistical Analysis

Descriptive analysis was conducted utilizing SPSS software, version 23.0. Quantitative data adhering to a normal distribution were presented as mean ± standard deviation (SD). For comparing two datasets that exhibited non-normal distributions, the Mann–Whitney *U*-test was employed. Continuous variables that conformed to normal distribution were analyzed using the independent samples *T*-test. Across all analyses, a *p*-value of less than 0.05 was considered indicative of statistical significance.

Results

Demographic and Clinical Characteristics

All participants, including patients with SZ and HC, were Han Chinese from Hefei and its surrounding regions in Anhui Province, sharing similar dietary habits. The demographic and clinical characteristics of both groups were closely matched, showing no significant differences in gender, age, or BMI, which suggests the absence of confounding factors that could influence group differentiation (all p>0.05) (Table 1).

Table 1 Demographic Data and Clinical Characteristics of SZ Patients and HCs

Variables	All Participants (n=65)	SZ (n=35)	HCs (n=30)	X^2/Z/T	*p-value*
Sex				0.711[a]	0.399
Female	34(52.3%)	20(58.8%)	14(41.2%)		
Male	31(47.7%)	15(48.4%)	16(51.6%)		
Age	33.1±10.3	33.1±10.3	31.8±9.3	−0.596[b]	0.551
BMI	21.7±3.3	21.2±3.7	22.3±2.6	−1.401[c]	0.166
PANSS		83.8±18.1			
Positive Symptom		22.4±6.4			
Negative Symptom		20.1±6.2			
General Psychopathology		41.3±9.4			

Notes: [a]Chi-square test, [b]mann–Whitney *U*-test, [c]T-test.
Abbreviations: SZ, Schizophrenia; HC, Healthy control.

Bacterial Diversity of Fecal Microbiota in SZ and HC

In our recent microbiome investigation, we analyzed a total of 3,466,097 high-quality 16S rRNA reads. Classification and identification efforts yielded 837 OTUs, with 628 OTUs shared between SZ and HC groups. The species accumulation curve (Figure S1) for all samples validates the sufficiency of our sampling efforts. Through alpha and beta diversity analyses, we examined the variations in gut flora diversity between the two groups. No statistically significant differences were observed in Shannon (p = 0.6332), Chao (p = 0.681), Sobs (p = 0.626), Ace (p = 0.552), Simpson (p = 0.421), and Coverage (p = 0.612) indices (Figure S2). However, both unweighted (p = 2.438e^{-12}) and weighted (p = 4.824e^{-06}) UniFrac metrics indicated a clear separation between the groups (Figure S3).

Alterations in the Composition of Fecal Microbiota in SZ and HC

We further analyzed the intestinal flora composition of these 65 samples, drawing species composition bar graphs at the family and genus levels for SZ and HC groups (Figure 1). Species with an abundance of less than 0.05% in all samples and those without annotations were categorized as "Others".

Taxon-dependent analysis identified twelve families within both the SZ and HC groups, with *Bacteroidaceae, Prevotellaceae, Veillonellaceae, Lachnospiraceae*, and *Ruminococcaceae* emerging as the dominant families. Notably, Bacteroidaceae was the most prevalent, constituting 40.3% in the SZ group and 33.2% in the HC group, respectively (Figure 1A). Subsequent analysis at the family level revealed significant differences between the two groups in the

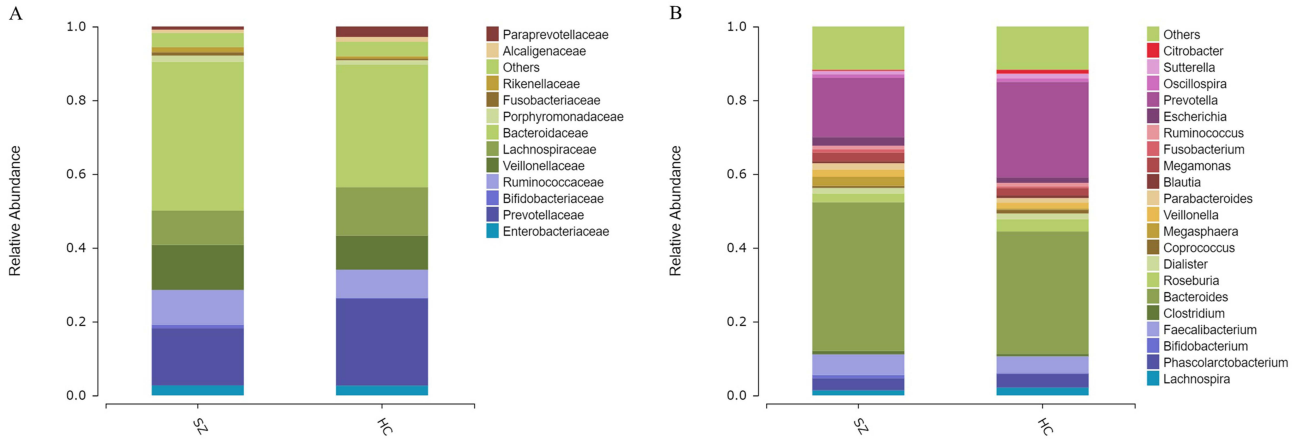

Figure 1 Composition of the Gut Microbiome at the Family (**A**) and Genus (**B**) Levels. The horizontal axis represents the sample names, while the vertical axis shows the relative abundance of the species annotated.
Abbreviations: SZ, Schizophrenia; HC, Healthy control.

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abundance of *Erysipelotrichaceae, Turicibacteraceae, Campylobacteraceae, Desulfovibrionaceae, Lactobacillaceae*, and *Lachnospiraceae* being more prevalent in HCs than in SZ individuals with *p*-values of 0.001, 0.004, 0.021, 0.036, 0.040, and 0.041 respectively.(Table 2). At the genus level, Bacteroides, *Prevotella, Faecalibacterium, Phascolarctobacterium*, and *Megasphaera* were identified as the top five genera in both groups (Figure 1B). Among these, *Bacteroides, Prevotella*, and *Faecalibacterium* exhibited the highest proportions in the SZ group (40.3%, 16.1%, and 5.6%, respectively). Differential abundance testing at the genus level highlighted distinct intestinal flora between the two groups. *Turicibacter, Coprococcus, Campylobacter, Eubacterium, Blautia, Sarcina, Catenibacterium, Lactobacillus*, and *Porphyromonas* were among the intestinal microbes showing significant differences between the SZ and HC groups with *p*-values of 0.004, 0.021, 0.021, 0.024, 0.031,0.034, 0.035, 0.040, and 0.047 respectively (Table 3).

Linear discriminant analysis effect size (LEfSe) was employed to identify species with notable abundance differences between groups, pinpointing those with significant variations as potential biomarkers (Figure 2). The LEfSe analysis yielded 17 distinct species, characterized by an Linear Discriminant Analysis(LDA) score greater than 2 and a P-value below 0.05. In the SZ group, there was a pronounced prevalence of species such as *Lactobacillus, Campylobacteraceae, Desulfovibrionales, Epsilonproteobacteria, Lactobacillaceae, Desulfovibrionaceae, Sarcina, Campylobacterales, Campylobacter, Deltaproteobacteria*, and *Clostridiaceae*. Conversely, the HC group exhibited a higher enrichment of *Turicibacter, Catenibacterium, Turicibacterales, Blautia, Turicibacteraceae*, and *Coprococcus*. These findings suggest that the differential abundance of these species could serve as insightful biomarkers, offering a deeper understanding of the microbial composition associated with schizophrenia compared to healthy states.

The findings from our study indicate substantial alterations in the gut microbiota of individuals with SZ. Given the pivotal role of the gut microbiome in modulating intestinal metabolites, these observations led us to hypothesize that the microbial composition in SZ patients significantly impacts intestinal metabolic pathways. To explore this hypothesis further, we conducted UPLC-MS analysis on stool samples collected from 35 SZ patients and 30 healthy controls. This

Table 2 Family-Level Abundance Differences SZ and HC

Family	SZ (mean ± SD)	HC (mean ± SD)	*p* value	FDR
Erysipelotrichaceae	0.079 ±0.099	0.195±0.182	0.001	0.052
Turicibacteraceae	0.032±0.064	0.125±0.644	0.004	0.148
Campylobacteraceae	0.002±0.006	0.001±0.005	0.021	0.455
Desulfovibrionaceae	0.457±0.557	0.267±0.626	0.036	0.455
Lactobacillaceae	0.046±0.106	0.006±0.032	0.040	0.455
Lachnospiraceae	9.350±7.061	13.160±8.333	0.041	0.455

Abbreviations: SZ, schizophrenia; HC, healthy control; SD, standard deviation; FDR, false discovery rate.

Table 3 Differences in the Abundance of SZ and HC at Genus Level

Genus	SZ (mean ± SD)	HC (mean)	*p* value	FDR
Turicibacter	0.032±0.064	0.125±0.644	0.004	0.450
Coprococcus	0.554±0.912	1.002±1.336	0.021	0.450
Campylobacter	0.002±0.006	0.001±0.005	0.021	0.450
Eubacterium	0.021±0.026	0.0315±0.069	0.024	0.450
Blautia	0.583±0.900	0.705±0.542	0.031	0.450
Sarcina	0.036±0.162	0.000±0.000	0.034	0.450
Catenibacterium	0.004±0.013	0.063±0.131	0.035	0.450
Lactobacillus	0.046±0.106	0.006±0.032	0.040	0.450
Porphyromonas	0.004±0.012	0.000±0.001	0.047	0.450

Abbreviations: SZ, schizophrenia; HC, healthy control; SD, standard deviation; FDR, false discovery rate.

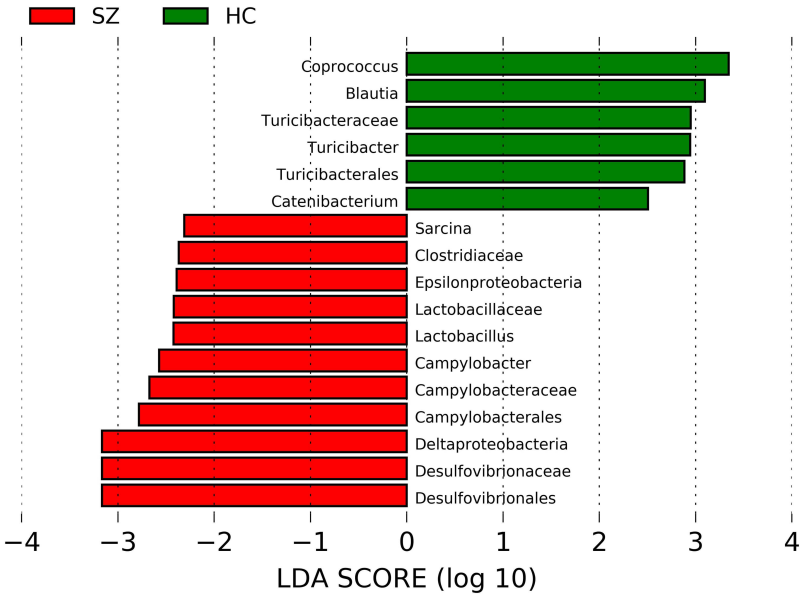

Figure 2 Presents the results of a LEfSe comparison between individuals with SZ and HC. The colors of the bar chart represent the respective groups, and the length represents the LDA score.
Abbreviations: SZ, Schizophrenia; HC, Healthy control; LDA, Linear Discriminant Analysis.

analytical approach aimed to delineate the specific metabolic differences attributable to the altered gut microbiota in schizophrenia, thereby enhancing our understanding of the microbiome-metabolome interaction in this condition.

Overall Fecal Metabolome Analysis

The UPLC-MS-based untargeted metabolomics approach successfully identified and quantified 946 metabolites across the two groups. These metabolites were classified and annotated using the KEGG and HMDB databases, resulting in the classification of 690 metabolites. Notably, fatty acids, comprising 86 metabolites, represented the largest category (12.5%). The KEGG database facilitated the labeling of identified metabolites, allowing for an in-depth understanding of their functional characteristics and the elucidation of the primary biochemical metabolic and signal transduction pathways involved. Among these, 436 metabolites were associated with metabolic pathways, regulated by 12 distinct pathways including amino acid metabolism (126 metabolites), lipid metabolism (85 metabolites), and carbohydrate metabolism (38 metabolites).

Differential Fecal Metabolites Between Groups

Principal Component Analysis (PCA) was employed to analyze the metabolite abundance in both groups (Figure 3). This analysis simplifies and reduces the dimensionality of complex, high-dimensional data, reflecting the overall variability and highlighting the differences between and within the SZ and HC groups based on the first two principal components (PC1=16.5%, PC2=6.5%), without significant within-group differences or outliers. In contrast to PCA, Partial OPLS-DA is a supervised method that maximizes the separation between groups. OPLS-DA revealed distinct intestinal metabolite profiles between the SZ and HC groups, indicating specific alterations in the intestinal metabolites of SZ patients (Figure 4A). The reliability and predictive accuracy of the OPLS-DA model were confirmed through 200 response permutation tests (RPT), demonstrating stability and a high prediction effect (R2Y(cum)=0.930; Q2(cum)=0.628). Furthermore, Variable Importance in Projection (VIP) scores derived from the OPLS-DA model (Figure 4B) identified metabolites with the most significant impact on group classification and discrimination. A VIP score greater than 1 is considered indicative of a metabolite's significant influence on the categorization of sample groups. Collectively, these findings strongly suggest the presence of specific fecal metabolites in individuals with schizophrenia, highlighting the potential for these metabolites as biomarkers.

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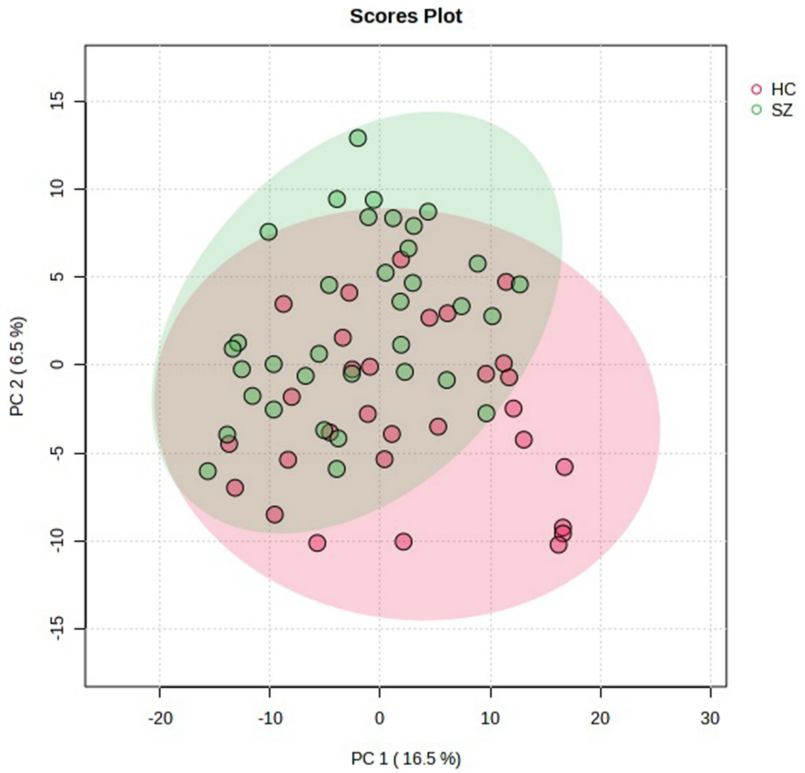

Figure 3 PCA analysis on the metabolite abundance of the two groups. The X-axis represents Principal Component 1 (PC1), which explains 16.5% of the variance. The Y-axis represents Principal Component 2 (PC2), which explains 6.5% of the variance.
Abbreviations: SZ, Schizophrenia; HC, Healthy control.

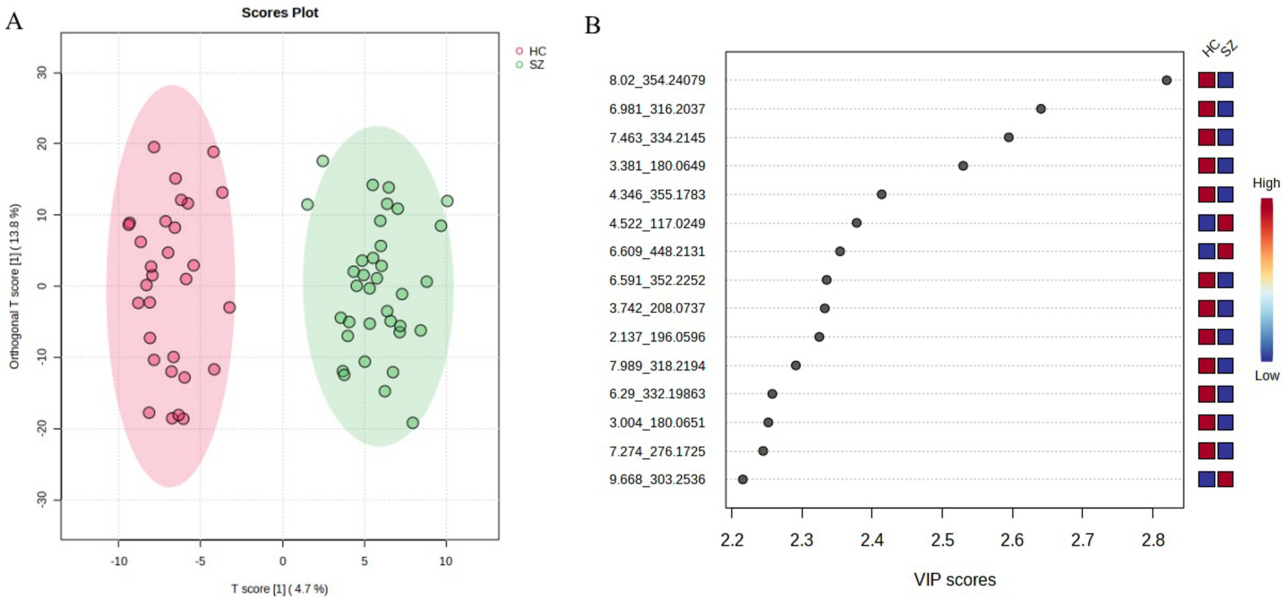

Figure 4 OPLS-DA scores displaying the discrimination between SZ and HC(**A**). Variable importance in projection (VIP) obtained from OPLS-DA between SZ and HC(B). In (**B**), the ordinate is the metabolite ID, and the corresponding specific substances can be seen in Table S1.
Abbreviations: SZ, Schizophrenia; HC, Healthy control; OPLS-DA, Orthogonal Partial Least Squares-Discriminant Analysis.

Fold change (FC) analysis was employed to evaluate the ratio of metabolite levels between the SZ and healthy control (HC) groups, with statistical significance assessed using the Mann–Whitney *U*-test followed by false discovery rate (FDR) correction. Metabolites meeting the criteria of statistical significance were considered differential. Screening

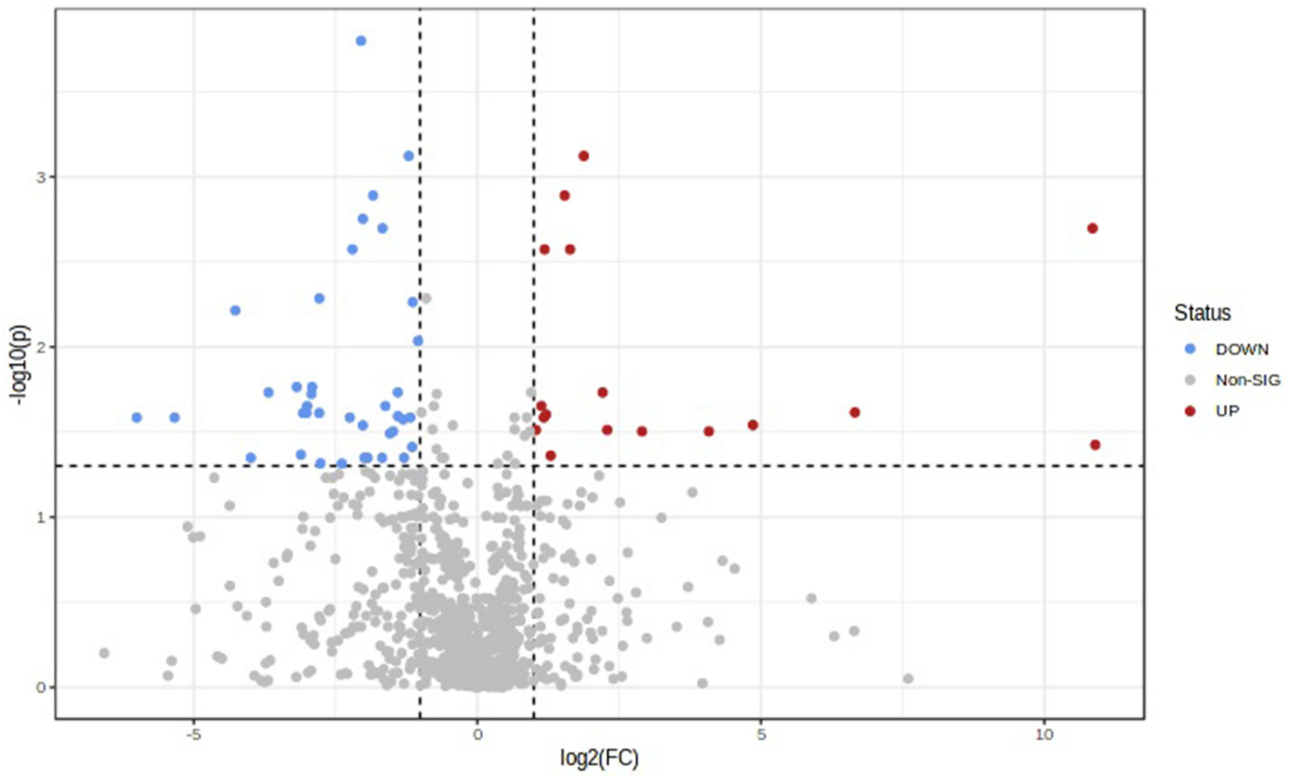

Figure 5 Volcano plot showing significantly altered metabolites between SZ and HC. Violet is the down-regulated differential metabolite, red is the up-regulated differential metabolite.
Abbreviation: SZ, Schizophrenia; HC, Healthy control.

criteria for differential metabolites included: 1) a Variable Importance in Projection (VIP) score from the OPLS-DA model ≥ 1, 2) a Fold Change ≥ 2 or ≤ 0.5, and 3) a *p*-value < 0.05. To identify significant metabolite changes potentially relevant to the development of SZ, a volcano plot was constructed to visualize the differences between groups (Figure 5). This analysis revealed 55 metabolites significantly altered in SZ compared to HC. After excluding one metabolite with a VIP score below 1, 54 metabolites remained, including 17 up-regulated (eg, kumitol, rotenone, xanthohumol, aripiprazole, noscapine, protopine) and 37 down-regulated metabolites (eg, catechin gallate, dihydrocapsaicin, 3,4-dimethoxycinnamic acid, taurodeoxycholic acid, paraxanthine, taurocholic acid 3-sulfate). Detailed information on these 54 metabolites was provided in Table S1. Enrichment analysis of these metabolites highlighted significantly affected pathways, notably in caffeine metabolism and cysteine and methionine metabolism, suggesting altered metabolic pathways in SZ (Figure S4).

Microbe-Associated Metabolite Identification in SZ

Leveraging fecal microbiome and metabolomics data, Spearman correlation analysis identified microbe-associated metabolites in SZ (Figure 6). Correlations between differential flora and metabolites were predominantly negative. *Lachnospiraceae* showed a notable correlation with several metabolites, including a strong positive relationship with 13,14-dihydro-15-keto prostaglandin A2 (r = 0.53). Other significant correlations included negative associations with 2-methoxyresorcinol, protopine, aflatoxin G2, noscapine, and a positive correlation with rotenone and aflatoxin G2. The strongest negative correlation was observed between *Eubacterium* and *dihydrocapsaicin* (r = −0.52). Taurochenodeoxycholic acid exhibited the most negative correlation with intestinal microbes, particularly *Turicibacter, Turicibacteraceae, Campylobacter*, and *Campylobacteraceae* (r ≈ −0.35 to −0.36), indicating potential interactions between differential metabolites and gut microbiota.

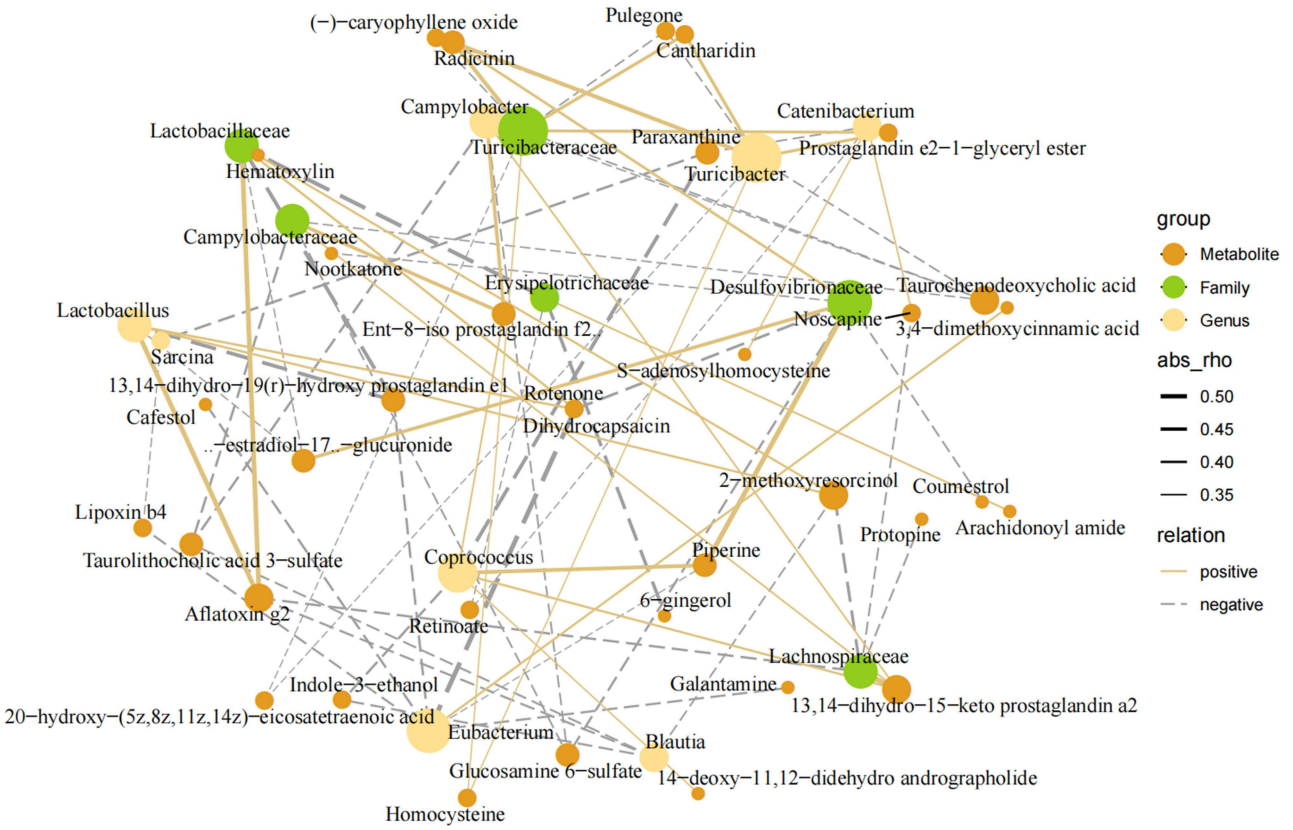

Figure 6 Correlation network diagram illustrating potential associations between different microbes and intestinal metabolites in SZ. The Orange dots represent metabolite. The green dots represent gut flora at the family level. The yellow dots represent gut flora at the genus level. The yellow solid line indicates a positive relation. The gray dotted line represents a negative relation. The thickness of the line represents the absolute value of the correlation coefficient.
Abbreviations: SZ, Schizophrenia; HC, Healthy control.

Correlation Between Differential Flora, Metabolites, and Clinical Symptoms

Our analysis revealed significant correlations between specific metabolites and clinical symptoms of schizophrenia, as measured by the PANSS. Radicinin, celastrol, and biotin showed positive correlations with overall PANSS scores, indicating an association with the severity of psychiatric symptoms. Conversely, noscapine exhibited a negative correlation with PANSS scores, suggesting a potential mitigating effect on symptoms. *Tetrahydrocortisone* was specifically linked to positive symptoms. A broader range of metabolites, including noscapine, 14-deoxy-11,12-didehydroandrographolide, benzoylecgonine, ent-8-iso prostaglandin F2α, 5-trans-prostaglandin E2, and lipoxin B4, were associated with negative symptoms, highlighting their potential roles in the modulation of specific SZ symptomatology (Figure 7).

Potential Interactions Between Abnormal Gut Microbes, Metabolites, and Clinical Symptoms

To synthesize the complex relationships between abnormal gut microbes, their metabolites, and psychiatric symptoms in SZ, we employed a Sankey diagram. This visualization tool elucidated the connections between 10 abnormal intestinal microbial metabolites, 5 aberrant gut microbes, and psychiatric clinical symptoms. Notably, the diagram illustrated associations such as *Eubacterium* and *Sarcina* with interleukin B4, which in turn, was linked to negative symptoms (Figure 8).

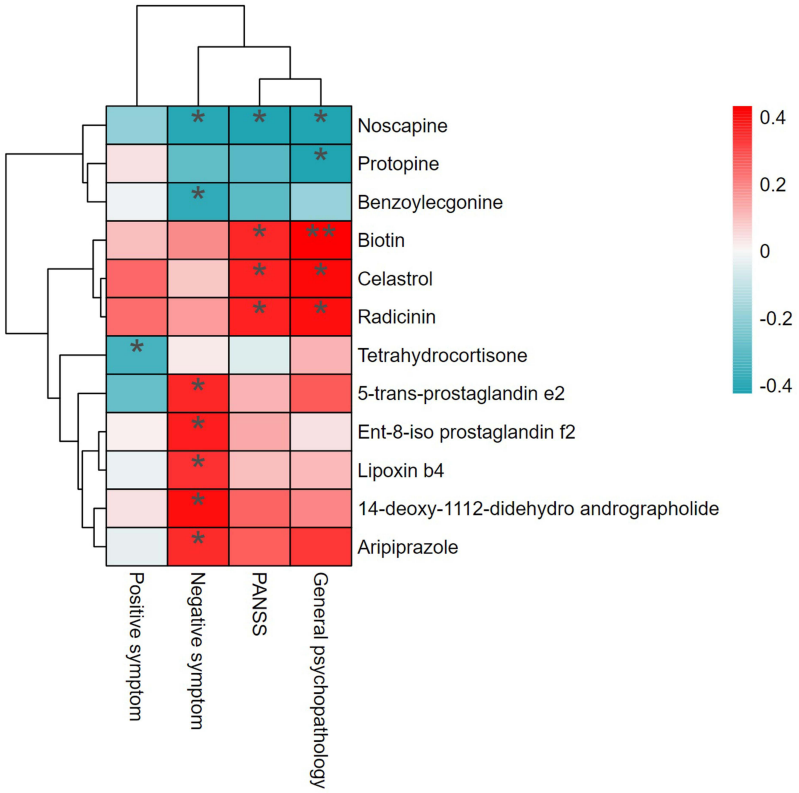

Figure 7 Illustrates the correlation between differential gut flora, metabolites, and clinical symptoms in schizophrenia, highlighting how specific metabolites correlate with PANSS scores and symptom dimensions. *p<0.05; **p<0.01. Color: Red - Positive correlation; Green - Negative correlation.

Discussion

Recent research increasingly highlights the crucial role of gut microbiota and their metabolites in the development of schizophrenia. This study integrates microbiome and metabolomics analyses to delineate distinct structural and metabolic profiles of the gut microbiota in schizophrenia, uncovering specific disease-related interactions. Our results reveal significant differences in the composition of intestinal microbiota and fecal metabolic phenotypes between patients with schizophrenia and healthy controls. Importantly, we observed that disturbances in the gut microbiome are significantly associated with unique metabolite profiles. These altered intestinal metabolites exhibit close correlations with the clinical manifestations of schizophrenia. Using a Sankey diagram, we demonstrated the interconnectedness of gut microbiota, metabolites, and the severity of schizophrenia symptoms, providing new insights into the pathophysiological mechanisms underpinning schizophrenia.

The complex ecosystem of the intestinal flora plays a significant role in neurodevelopmental disorders' etiology, including schizophrenia. Growing evidence links alterations in gut microbiota composition to schizophrenia's pathogenesis. Although most studies, including ours, do not report significant differences in α-diversity between schizophrenia patients and healthy controls, they consistently observe substantial disparities in β-diversity,[32,33] suggesting a microbial imbalance in schizophrenia. Our findings reveal an increased relative abundance of *Lactobacillaceae* and *Lactobacillus* in the schizophrenia group compared to healthy controls. Probiotic strains of *Lactobacillus* are known to influence neurotransmitter levels such as tryptophan, kynurenine, and serotonin[34] and promote the secretion of pro-inflammatory cytokines including interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), IL-12p70, and IL-6.[35] The elevated presence of *Lactobacillus* in psychiatric disorders, including schizophrenia, correlates with clinical symptoms.[36] Additionally, we observed an increased relative abundance of *Epsilonproteobacteria* and a decrease in *Eubacterium, Turicibacteraceae*, and *Blautia* in schizophrenia patients, aligning with previous research.[8,37–39] These gut floras are implicated in short-chain fatty acid regulation and contribute to the disease's pathogenesis. Metabolites produced by gut microbiota, such as

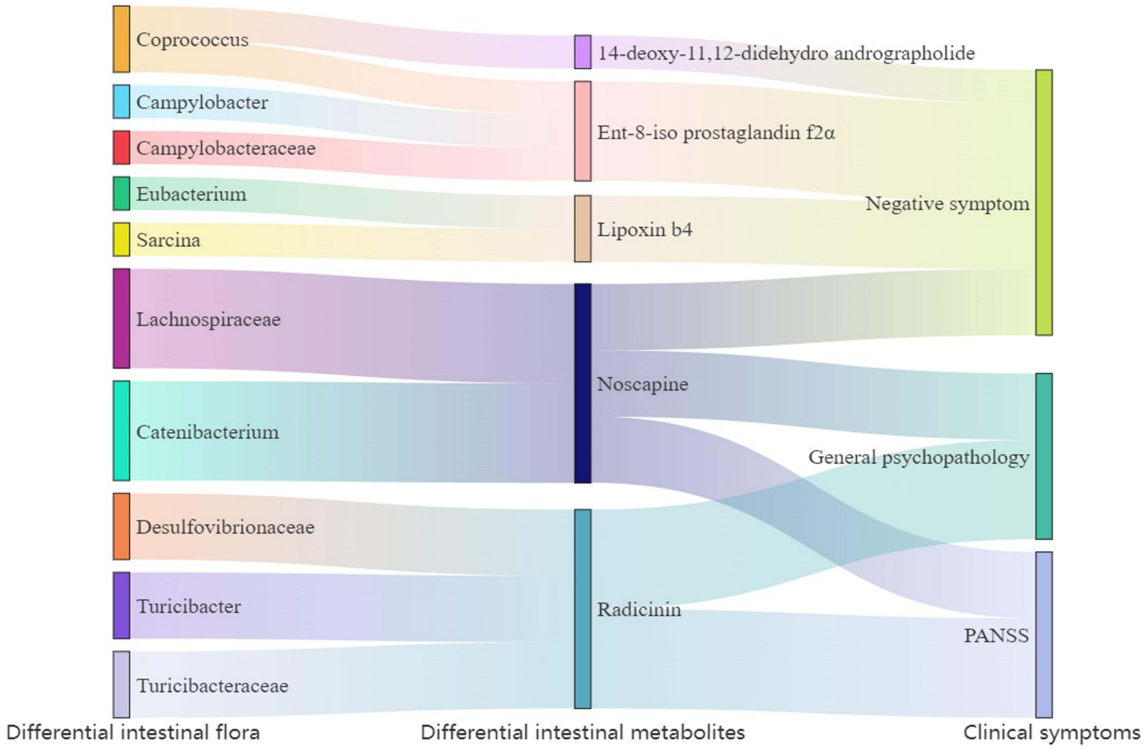

Figure 8 A Sankey diagram summarizing potential interactions between abnormal gut microbes, microbial metabolites, and psychiatric symptoms. The far left represents differential fecal microbiota, the middle represents fecal Metabolites, and the far right represents Clinical Symptoms. The connecting lines imply that there is a correlation.

neurotransmitters and amino acids, also play a crucial role in psychiatric disorders.[8] An imbalance of Proteobacteria in the intestines of schizophrenia patients may be particularly pronounced. The study further notes changes in the abundance of *Clostridiaceae*, associated with carbohydrate fermentation and short-chain fatty acid production, crucial for intestinal barrier integrity.[40–42] Reduced abundance of these bacteria in schizophrenia patients may facilitate pathogen colonization and disease development. Additionally, abnormal phenylalanine metabolites in *Clostridium* difficile infections have been linked to increased urinary excretion in schizophrenia patients, affecting brain catecholamine levels and causing autism-like symptoms in animal models.[43] The decreased abundance of *Turicibacterales* in schizophrenia, previously observed in SZ and Alzheimer's disease (AD) patients,[44,45] and their role in serotonin production and regulation,[46,47] highlights the complex interplay between gut microbiota, metabolic pathways, and psychiatric disorders, underscoring the necessity for further research.

In our investigation, significant deviations in metabolites were detected among schizophrenia patients compared to healthy controls, with 54 metabolites identified as notably altered. This aligns with previous observations of altered peripheral proline levels in schizophrenia, which contrast with our findings of decreased fecal proline concentrations in patients,[48] suggesting a complex interplay between systemic and gastrointestinal metabolite environments that warrants further exploration. Notably, the reduction of levodopa in schizophrenia patients emphasizes its potential role in symptomatology, given levodopa's capacity to cross the blood-brain barrier and modulate dopamine levels, thereby influencing psychiatric symptoms.[49] Our study also revealed significant shifts in lipid-related metabolites, including bile acids and fatty acid derivatives, which are integral to neurotransmission and inflammatory processes.[50] Specifically, reductions in taurolithocholic acid 3-sulfate and taurochenodeoxycholic acid in schizophrenia patients highlight their potential as biomarkers and their roles in cell signaling and immune modulation.[51,52] The observed decrease in eicosanoic acid, a key regulator of inflammation and neurotransmitter signaling, further underscores the metabolic alterations in schizophrenia that may impact immune response.[53,54] Additionally, our findings of altered sterol lipids and their potential link to estrogen signaling pathways suggest a gender-specific aspect of schizophrenia pathophysiology that merits deeper investigation.[55,56] The study also sheds light on the critical roles of homocysteine and S-adenosylhomocysteine in the

cysteine and methionine metabolism pathways, implicating them in the neurochemical imbalances observed in schizophrenia,[57–59] and points to glutathione's antioxidant defenses as a key area of interest in understanding the disease's neurodegenerative aspects.[60–62]

Our investigation into the metabolic alterations associated with schizophrenia has highlighted significant pathway disruptions, particularly in caffeine metabolism, arginine and proline metabolism, and tryptophan metabolism. These findings suggest a broader spectrum of metabolic dysfunction during the development of schizophrenia beyond individual metabolite changes. Notably, aberrations in caffeine metabolism, including altered levels of paraxanthine, theobromine, and 1,7-dimethyluric acid, point towards distinctive consumption patterns of caffeine and nicotine among schizophrenia patients compared to healthy controls.[63] This aspect of caffeine metabolism may reflect on its potential role in the pharmacokinetics of antipsychotic medications, as caffeine clearance and CYP1A2 activity—crucial for the metabolism of drugs like clozapine—are significantly interrelated.[64] The observed metabolic irregularities in our study, particularly the unique profiles of bile acids and eicosanoids, underscore the complexity of schizophrenia's pathophysiology. These metabolic pathways not only participate in cellular signaling and immune modulation but may also influence neurotransmitter receptor functionality, further implicating their roles in the disease's underlying mechanisms. Our findings pave the way for future investigations to decipher the intricate relationships between these metabolic pathways and schizophrenia, aiming to enhance our understanding of the disease and potentially uncover novel therapeutic targets.

Exploring the metabolic intricacies within the human body reveals a sophisticated interplay between the gut microbiota and host cells, a relationship that begins at birth and is mediated through the production of metabolites.[22] In this study, we delved into the correlations between differential metabolites and gut flora to uncover their interactive roles. By integrating data on differential flora and metabolites, we identified a robust link between Trichoderma and specific gut metabolites. The Trichosporonaceae family, known for its butyrate-producing capabilities, suggests anti-inflammatory properties,[65] highlighting the potential therapeutic benefits of targeting these interactions. A strong correlation was observed between *Trichoderma* and 13,14-dihydro-15-keto prostaglandin A2, indicating a close relationship between *Trichoderma* activity and fatty acid production. Furthermore, the neurotoxic insecticide rotenone, which is used in models to induce schizophrenia-like symptoms due to its inhibition of mitochondrial complex I,[66] showed a significant elevation in schizophrenia patients and a positive correlation with *Trichosidae*. This suggests that certain gut flora may influence the onset and progression of schizophrenia through their impact on intestinal metabolites. Additionally, dietary influences, such as a low-protein, high-carbohydrate diet, have been shown to mitigate dyskinesia in Parkinson's disease through microbial metabolites like tauroursodeoxycholic acid (TUDCA) and taurine, playing key roles along the gut-microbe-brain axis.[67] Our findings suggest taurochenodeoxycholic acid (TCDCA) might have a similar role in schizophrenia, emphasizing the potential of intestinal bile acids in modulating disease through enterohepatic circulation.[68] The correlation between differential metabolites and clinical symptoms underscores the significant role of fecal metabolites in the pathogenesis and symptomatology of schizophrenia, suggesting that targeting gut microbes and their metabolomics could offer new insights into the disease's management. This study underscores the complexity of interactions between gut microbiota, metabolites, and schizophrenia, highlighting the need for further research to unravel these relationships fully.

Despite the insights provided, our study is subject to several limitations. Primarily, while 16s rRNA gene sequencing serves as a widely adopted method for microbiome profiling, its resolution is insufficient for exhaustive gene identification, potentially overlooking critical microbial constituents. Moreover, this study did not take into account the effects of diet, smoking habits, alcohol consumption, and overall lifestyle on the concentration of fecal metabolites. This makes it difficult to attribute these changes to schizophrenia solely based on observing metabolic variations. Additionally, the study's sample size and its single-center nature may limit the generalizability of our findings, necessitating caution in extrapolating these results to broader populations. Finally, as a cross-sectional survey, it is hard to infer any causality from the findings.

Conclusions

Our investigation into SZ has unveiled profound alterations within the gut microbiota at various taxonomic levels, including class, order, family, genus, and species, when compared to healthy controls. These microbial changes are accompanied by significant shifts in intestinal metabolites and metabolic pathways, notably in caffeine metabolism and cysteine and methionine metabolism, underscoring a disrupted metabolic equilibrium in SZ patients. Through network correlation analysis, we identified a direct and significant correlation between distinct bacterial taxa and specific intestinal metabolites, highlighting the intricate interplay within the gut ecosystem of SZ patients. Furthermore, we discovered that certain intestinal metabolites exhibit a close association with the clinical symptoms of SZ, suggesting their potential roles in the manifestation and severity of the disorder. Collectively, our study sheds light on the complex relationship between altered gut microbiota, fecal metabolite profiles, and schizophrenia, marking a significant step forward in our comprehension of how gastrointestinal microbial dysbiosis may contribute to the pathophysiology of psychiatric conditions. This novel insight into the gut-brain axis provides a potential avenue for therapeutic interventions targeting schizophrenia, which may alter gut metabolism by regulating the gut microbiota, thereby informing the treatment of mental illness. In future longitudinal studies, we will focus on exploring the effects of drug treatment on the fecal microbiota and intestinal metabolites.

Availability of Clinical Trial Data

The data supporting the results of this study were used under the license of this study and therefore cannot be publicly available. However, with the permission of the Hefei Fourth People's Hospital, the author can reasonably request the corresponding author to consult these materials. Sequencing of the raw data has been submitted and save at the National Center for Biological Information (NCBI) (https://submit.ncbi.nlm.nih.gov/) under the accession number SUB9453991.

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Disclosure

All authors declare no conflict of interest.

References

1. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2018;392(10159):1789–1858. doi:10.1016/S0140-6736(18)32279-7
2. Collaborators GBDMD. Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Psychiatry*. 2022;9(2):137–150. doi:10.1016/S2215-0366(21)00395-3
3. Zheng W, Cai D-B, Yang X-H, et al. Short-term efficacy and tolerability of lurasidone in the treatment of acute Schizophrenia: a meta-analysis of randomized controlled trials. *J Psychiatric Res*. 2018;103:244–251. doi:10.1016/j.jpsychires.2018.06.005
4. Wen K-S, Yang X-H, Zhang N, et al. Adjunctive intermittent theta-burst stimulation for Schizophrenia: a systematic review and meta-analysis of randomized double-blind controlled studies. *Alpha Psychiatry*. 2024;25(6):676–684. doi:10.5152/alphapsychiatry.2024.241799
5. Pennington M, McCrone P. The cost of relapse in Schizophrenia. *Pharmacoeconomics*. 2017;35(9):921–936. doi:10.1007/s40273-017-0515-3
6. Vernocchi P, Del Chierico F, Putignani L. Gut microbiota profiling: metabolomics based approach to unravel compounds affecting human health. *Front Microbiol*. 2016;7:1144. doi:10.3389/fmicb.2016.01144
7. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol*. 2015;28(2):203–209.

8. Eicher TP, Mohajeri MH. Overlapping mechanisms of action of brain-active bacteria and bacterial metabolites in the pathogenesis of common brain diseases. *Nutrients*. 2022;14(13):2661. doi:10.3390/nu14132661
9. Golofast B, Vales K. The connection between microbiome and schizophrenia. *Neurosci Biobehav Rev*. 2020;108:712–731. doi:10.1016/j.neubiorev.2019.12.011
10. Schwarz E, Maukonen J, Hyytiainen T, et al. Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. *Schizophr Res*. 2018;192:398–403. doi:10.1016/j.schres.2017.04.017
11. Zheng P, Zeng B, Liu M, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Sci Adv*. 2019;5(2):eaau8317. doi:10.1126/sciadv.aau8317
12. Zhang A, Sun H, Wang X. Serum metabolomics as a novel diagnostic approach for disease: a systematic review. *Anal Bioanal Chem*. 2012;404(4):1239–1245. doi:10.1007/s00216-012-6117-1
13. Amdanee N, Shao M, Hu X, et al. Serum metabolic profile in Schizophrenia patients with antipsychotic-induced constipation and its relationship with gut microbiome. *Schizophr Bull*. 2023;49(3):646–658. doi:10.1093/schbul/sbac202
14. Fukushima T, Iizuka H, Yokota A, et al. Quantitative analyses of schizophrenia-associated metabolites in serum: serum D-lactate levels are negatively correlated with gamma-glutamylcysteine in medicated schizophrenia patients. *PLoS One*. 2014;9(7):e101652. doi:10.1371/journal.pone.0101652
15. Parker A, Fonseca S, Carding SR. Gut microbes and metabolites as modulators of blood-brain barrier integrity and brain health. *Gut Microbes*. 2020;11(2):135–157. doi:10.1080/19490976.2019.1638722
16. Li C, Wang A, Wang C. Metabolomics in patients with psychosis: a systematic review. *Am J Med Genet B Neuropsychiatr Genet*. 2018;177(6):580–588. doi:10.1002/ajmg.b.32662
17. Davison J, O'Gorman A, Brennan L, Cotter DR. A systematic review of metabolite biomarkers of schizophrenia. *Schizophr Res*. 2018;195:32–50. doi:10.1016/j.schres.2017.09.021
18. Quintero M, Stanisic D, Cruz G, Pontes JGM, Costa T, Tasic L. Metabolomic biomarkers in mental disorders: bipolar disorder and Schizophrenia. *Adv Exp Med Biol*. 2019;1118:271–293. doi:10.1007/978-3-030-05542-4_14
19. Tasic L, Pontes JGM, Carvalho MS, et al. Metabolomics and lipidomics analyses by (1)H nuclear magnetic resonance of schizophrenia patient serum reveal potential peripheral biomarkers for diagnosis. *Schizophr Res*. 2017;185:182–189. doi:10.1016/j.schres.2016.12.024
20. Liang Y, Shi X, Shen Y, et al. Enhanced intestinal protein fermentation in Schizophrenia. *BMC Med*. 2022;20(1):67. doi:10.1186/s12916-022-02261-z
21. Krautkramer KA, Fan J, Backhed F. Gut microbial metabolites as multi-kingdom intermediates. *Nat Rev Microbiol*. 2021;19(2):77–94. doi:10.1038/s41579-020-0438-4
22. Sprockett D, Fukami T, Relman DA. Role of priority effects in the early-life assembly of the gut microbiota. *Nat Rev Gastroenterol Hepatol*. 2018;15(4):197–205. doi:10.1038/nrgastro.2017.173
23. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe*. 2018;23(6):716–724. doi:10.1016/j.chom.2018.05.003
24. Chen MX, Wang SY, Kuo CH, Tsai IL. Metabolome analysis for investigating host-gut microbiota interactions. *J Formos Med Assoc*. 2019;118(Suppl 1):S10–S22. doi:10.1016/j.jfma.2018.09.007
25. Cheng S, Zhu Z, Li H, et al. Rifaximin ameliorates depression-like behaviour in chronic unpredictable mild stress rats by regulating intestinal microbiota and hippocampal tryptophan metabolism. *J Affect Disord*. 2023;329:30–41. doi:10.1016/j.jad.2023.02.086
26. Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, Stanton C. gamma-Aminobutyric acid production by culturable bacteria from the human intestine. *J Appl Microbiol*. 2012;113(2):411–417. doi:10.1111/j.1365-2672.2012.05344.x
27. Kimura I, Inoue D, Maeda T, et al. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A*. 2011;108(19):8030–8035. doi:10.1073/pnas.1016088108
28. Wu B-J, Lan T-H, Hu T-M, Lee S-M, Liou J-Y. Validation of a five-factor model of a Chinese Mandarin version of the Positive and Negative Syndrome Scale (CMV-PANSS) in a sample of 813 schizophrenia patients. *Schizophr Res*. 2015;169(1–3):489–490. doi:10.1016/j.schres.2015.09.011
29. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*. 2013;10(10):996–998. doi:10.1038/nmeth.2604
30. Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*. 2009;75(23):7537–7541. doi:10.1128/AEM.01541-09
31. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. UniFrac: an effective distance metric for microbial community comparison. *ISME J*. 2011;5(2):169–172. doi:10.1038/ismej.2010.133
32. Murray N, Al Khalaf S, Kaulmann D, et al. Compositional and functional alterations in the oral and gut microbiota in patients with psychosis or schizophrenia: a systematic review. *HRB Open Res*. 2021;4:108. doi:10.12688/hrbopenres.13416.1
33. Nguyen TT, Hathaway H, Kosciolek T, Knight R, Jeste DV. Gut microbiome in serious mental illnesses: a systematic review and critical evaluation. *Schizophr Res*. 2021;234:24–40. doi:10.1016/j.schres.2019.08.026
34. Sherwin E, Sandhu KV, Dinan TG, Cryan JF. May the force be with you: the light and dark sides of the microbiota-gut-brain axis in neuropsychiatry. *CNS Drugs*. 2016;30(11):1019–1041. doi:10.1007/s40263-016-0370-3
35. Rocha-Ramirez LM, Perez-Solano RA, Castanon-Alonso SL, et al. Probiotic lactobacillus strains stimulate the inflammatory response and activate human macrophages. *J Immunol Res*. 2017;2017:4607491. doi:10.1155/2017/4607491
36. Borkent J, Ioannou M, Laman JD, Haarman BCM, Sommer IEC. Role of the gut microbiome in three major psychiatric disorders. *Psychol Med*. 2022;52(7):1222–1242. doi:10.1017/S0033291722000897
37. Ma X, Asif H, Dai L, et al. Alteration of the gut microbiome in first-episode drug-naive and chronic medicated schizophrenia correlate with regional brain volumes. *J Psychiatr Res*. 2020;123:136–144. doi:10.1016/j.jpsychires.2020.02.005
38. Shen Y, Xu J, Li Z, et al. Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: a cross-sectional study. *Schizophr Res*. 2018;197:470–477. doi:10.1016/j.schres.2018.01.002
39. Xing M, Gao H, Yao L, et al. Profiles and diagnostic value of intestinal microbiota in schizophrenia patients with metabolic syndrome. *Front Endocrinol*. 2023;14:1190954. doi:10.3389/fendo.2023.1190954

40. Hung -C-C, Chang -C-C, Huang C-W, Nouchi R, Cheng C-H. Gut microbiota in patients with Alzheimer's disease spectrum: a systematic review and meta-analysis. *Aging*. 2022;14(1):477–496. doi:10.18632/aging.203826
41. McIntyre RS, Subramaniapillai M, Shekotikhina M, et al. Characterizing the gut microbiota in adults with bipolar disorder: a pilot study. *Nutritional Neuroscience*. 2019;24(3):173–180. doi:10.1080/1028415x.2019.1612555
42. Sasaki D, Sasaki K, Ikuta N, et al. Low amounts of dietary fibre increase in vitro production of short-chain fatty acids without changing human colonic microbiota structure. *Sci Rep*. 2018;8(1):435. doi:10.1038/s41598-017-18877-8
43. Shaw W. Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine metabolite of Clostridia spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia. *Nutr Neurosci*. 2010;13(3):135–143. doi:10.1179/147683010X12611460763968
44. Dunham SJB, McNair KA, Adams ED, et al. Longitudinal analysis of the microbiome and metabolome in the 5xfAD mouse model of Alzheimer's disease. *mBio*. 2022;13(6):e0179422. doi:10.1128/mbio.01794-22
45. Nguyen TT, Kosciolek T, Daly RE, et al. Gut microbiome in Schizophrenia: altered functional pathways related to immune modulation and atherosclerotic risk. *Brain Behav Immun*. 2021;91:245–256. doi:10.1016/j.bbi.2020.10.003
46. Sun N, Zhang J, Wang J, et al. Abnormal gut microbiota and bile acids in patients with first-episode major depressive disorder and correlation analysis. *Psychiatry Clin Neurosci*. 2022;76(7):321–328. doi:10.1111/pcn.13368
47. Fung TC, Vuong HE, Luna CDG, et al. Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. *Nat Microbiol*. 2019;4(12):2064–2073. doi:10.1038/s41564-019-0540-4
48. Clelland CL, Drouet V, Rilett KC, et al. Evidence that COMT genotype and proline interact on negative-symptom outcomes in schizophrenia and bipolar disorder. *Transl Psychiatry*. 2016;6(9):e891. doi:10.1038/tp.2016.157
49. Garfinkel PE, Stancer HC. L-Dopa and Schizophrenia. *Can Psychiatr Assoc J*. 1976;21(1):27–29. doi:10.1177/070674377602100105
50. Davidson M, Keefe RS, Mohs RC, et al. L-dopa challenge and relapse in Schizophrenia. *Am J Psychiatry*. 1987;144(7):934–938.
51. Huang F, Wang T, Lan Y, et al. Deletion of mouse FXR gene disturbs multiple neurotransmitter systems and alters neurobehavior. *Front Behav Neurosci*. 2015;9:70. doi:10.3389/fnbeh.2015.00070
52. Tao Y, Zheng F, Cui D, Huang F, Wu X. A combination of three plasma bile acids as a putative biomarker for Schizophrenia. *Acta Neuropsychiatr*. 2021;33(1):51–54. doi:10.1017/neu.2020.42
53. Wang D, Sun X, Yan J, et al. Alterations of eicosanoids and related mediators in patients with Schizophrenia. *J Psychiatr Res*. 2018;102:168–178. doi:10.1016/j.jpsychires.2018.04.002
54. Schmidt L, Ceglarek U, Kortz L, et al. Mechanisms of involvement of eicosanoids and their precursors in the pathophysiology and treatment of Schizophrenia. *Med Chem*. 2013;9(6):763–773. doi:10.2174/1573406411309060002
55. Yi W, Ji Y, Gao H, et al. Effects of urban particulate matter on gut microbiome and partial Schizophrenia-like symptoms in mice: evidence from shotgun metagenomic and metabolomic profiling. *Sci Total Environ*. 2023;857(Pt 1):159305. doi:10.1016/j.scitotenv.2022.159305
56. Xiang Y-T, Wang C-Y, Si T-M, et al. Sex differences in use of psychotropic drugs and drug-induced side effects in Schizophrenia patients: findings of the Research on Asia Psychotropic Prescription (REAP) studies. *Aust N Z J Psychiatry*. 2011;45(3):193–198. doi:10.3109/00048674.2010.538839
57. Misiak B, Frydecka D, Slezak R, Piotrowski P, Kiejna A. Elevated homocysteine level in first-episode schizophrenia patients--the relevance of family history of schizophrenia and lifetime diagnosis of cannabis abuse. *Metab Brain Dis*. 2014;29(3):661–670. doi:10.1007/s11011-014-9534-3
58. Zhilyaeva TV, Piatoikina AS, Bavrina AP, et al. Homocysteine in Schizophrenia: independent pathogenetic factor with prooxidant activity or integral marker of other biochemical disturbances? *Schizophr Res Treatment*. 2021;2021:7721760. doi:10.1155/2021/7721760
59. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. *Nutr J*. 2015;14:6. doi:10.1186/1475-2891-14-6
60. McBean GJ. The transsulfuration pathway: a source of cysteine for glutathione in astrocytes. *Amino Acids*. 2012;42(1):199–205. doi:10.1007/s00726-011-0864-8
61. Nucifora LG, Tanaka T, Hayes LN, et al. Reduction of plasma glutathione in psychosis associated with schizophrenia and bipolar disorder in translational psychiatry. *Transl Psychiatry*. 2017;7(8):e1215. doi:10.1038/tp.2017.178
62. Aoyama K. Glutathione in the Brain. *Int J Mol Sci*. 2021;22(9). doi:10.3390/ijms22095010
63. Tse MT, Piantadosi PT, Floresco SB. Prefrontal cortical gamma-aminobutyric acid transmission and cognitive function: drawing links to schizophrenia from preclinical research. *Biol Psychiatry*. 2015;77(11):929–939. doi:10.1016/j.biopsych.2014.09.007
64. van Troostwijk LJ D, Koopmans RP, Vermeulen HD, Guchelaar HJ. CYP1A2 activity is an important determinant of clozapine dosage in schizophrenic patients. *Eur J Pharm Sci*. 2003;20(4–5):451–457. doi:10.1016/j.ejps.2003.09.010
65. Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett*. 2002;217(2):133–139. doi:10.1111/j.1574-6968.2002.tb11467.x
66. Siena A, Yuzawa JMC, Ramos AC, et al. Neonatal rotenone administration induces psychiatric disorder-like behavior and changes in mitochondrial biogenesis and synaptic proteins in adulthood. *Mol Neurobiol*. 2021;58(7):3015–3030. doi:10.1007/s12035-021-02317-w
67. Chu C, Li T, Yu L, et al. A Low-protein, high-carbohydrate diet exerts a neuroprotective effect on mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinson's disease by regulating the microbiota-metabolite-brain axis and fibroblast growth factor 21. *J Agric Food Chem*. 2023;71(23):8877–8893. doi:10.1021/acs.jafc.2c07606
68. Uchida A, Yamada T, Hayakawa T, Hoshino M. Taurochenodeoxycholic acid ameliorates and ursodeoxycholic acid exacerbates small intestinal inflammation. *Am J Physiol*. 1997;272(5 Pt 1):G1249–G1257. doi:10.1152/ajpgi.1997.272.5.G1249