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RESEARCH

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Functional associations of the gut microbiome with dopamine, serotonin, and BDNF in schizophrenia: a pilot study

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Abstract

Background Schizophrenia is a complex neuropsychiatric disorder with various etiologic factors. Aberrant levels of neurotransmitters or growth factors such as dopamine, serotonin, and BDNF have been shown to cause cognitive impairment in schizophrenia. Recently, the gut microbiome has also been suggested as a factor in the development of the disorder. To explore this potential link, we conducted a pilot study to examine the relationship between the gut microbiome and plasma levels of neurotransmitters and growth factors in schizophrenia. Shotgun metagenome sequencing of total RNA from fecal samples were used to profile the gut microbiome of schizophrenia patients (SCZ) and healthy controls (HC). The MetaPhlAn2 and HUMaN2 pipelines were used for bioinformatic analyses. ELISA was used to measure the plasma levels of dopamine, serotonin, and BDNF. Spearman's rank correlation coefficient was used for correlation analysis.

Results We found that butyrate-producing bacteria were enriched in HC, whereas succinate-producing bacteria, namely *Phascolarctobacterium succinatutens* and *Paraprevotella clara*, were enriched in SCZ. The gut microbiota of SCZ was enriched in lipid biosynthesis pathways related to bile-resistant bacteria, whereas phospholipid pathways linked with butyrate-producing bacteria were enriched in HC. *Alistipes indistinctus*, *Dorea longicatena*, and *Roseburia inulinivorans* were negatively correlated with dopamine levels. *Roseburia intestinalis* and *Parabacteroides goldsteini* were negatively correlated with serotonin and BDNF levels, respectively. We found a significant correlation between dopamine and serotonin levels, and the super-pathway of purine deoxyribonucleoside degradation.

Conclusions This study provides further support that gut microbiota could modulate neurotransmitter levels. The results suggest that gut microbiome-targeted therapies may help to rebalance neurotransmitter levels, offering new hope for the treatment of schizophrenia.

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Background

Schizophrenia is a devastating neuropsychiatric disorder that manifests in adolescence or early adulthood and is characterized by striking features such as hallucinations, delusions, and cognitive impairment. According to a WHO report (January 10, 2022), it affects approximately 24 million people worldwide. Despite numerous research efforts, the precise cause of schizophrenia remains unknown.

The etiology of schizophrenia involves a complex interplay of genetic and environmental factors [1]. Recent advances in next-generation sequencing technology have led researchers to investigate the role of the human microbiome, particularly the gut microbiome, in mental function and development. This emerging approach has the potential to uncover new candidate genes or molecular mechanisms related to mental disorders, which could improve management and treatment strategies [2, 3].

The human digestive tract harbors approximately 10^{13} to 10^{14} microorganisms, collectively known as the gut microbiota. The diversity and balance of these microorganisms are crucial for host health [4]. Dysbiosis of the gut microbiota has been implicated in several neurological and neuropsychiatric disorders, including multiple sclerosis, Alzheimer's disease, Parkinson's disease, autism, major depressive disorder, bipolar disorder, and schizophrenia [5].

Zheng and colleagues discovered that fecal transfer of gut microbiota from schizophrenia patients induces schizophrenia-related behaviors in germ-free mice, accompanied by imbalances in glutamate, glutamine, and gamma-aminobutyric acid (GABA) in the hippocampus [6]. Studies using 16S rRNA and shotgun sequencing have shown alterations in the gut microbiome of schizophrenia patients [7]. Dysbiosis may contribute to schizophrenia through several gut-brain axis pathways, including the hypothalamic–pituitary–adrenal (HPA) axis, toll-like receptor (TLR) pathways, the tryptophan-kynurenine pathway, the vagus nerve, and neurotransmitter pathways involving brain-derived neurotrophic factor (BDNF), dopamine, serotonin, GABA, and short-chain fatty acids [8].

Schizophrenia is associated with abnormal levels of neurotransmitters and growth factors, such as dopamine, serotonin, and BDNF. These abnormalities are linked to cognitive impairment and the regulation of reality perception, memory, and attention [9–12]. This paper aims to investigate the relationship between the gut microbiome and plasma levels of neurotransmitters and growth factors in schizophrenia.

Methods

This cohort included 10 schizophrenia patients (6 males, 4 females) and 10 healthy controls (3 males, 7 females). All participants read a written description of the study objectives and provided written informed consent.

Qualified psychiatrists at both hospitals diagnosed schizophrenia using the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (American Psychiatric Association, 2013). The severity of psychotic symptoms was assessed using the 30-item Positive and Negative Symptom Scale (PANSS). Clinical data, including age, gender, race, PANSS score, and BMI, were collected from both patients and healthy controls by evaluating psychiatrists. Schizophrenia patients were receiving a single antipsychotic medication at the time of the study: eight were on risperidone and two on olanzapine. Excluded from the study were patients with recent antibiotic use, substance addiction, alcohol consumption, HIV infection, severe neurologic problems, or mental impairment. Healthy control individuals were screened using the Structured Clinical Interview for Non-Patient Edition (SCID-NP) and were excluded if they had a history of medical or mental illnesses, drug or alcohol misuse, or recent antibiotic use.

Each participant received a fecal collection kit with explicit instructions for self-collection of feces following defecation. The kit included disposable gloves, a sterile 50 ml Falcon tube with a wooden tongue depressor for stool collection, a 15 ml Falcon tube containing 5 ml of RNA Protect Bacteria reagent (Qiagen, USA) for sample preservation, and a stand to hold the tube upright during storage, transport, and laboratory analysis. Fecal samples were collected on the same day or within two days, and participants notified researchers once the sample was collected. The samples were either analyzed immediately upon arrival at the laboratory or frozen at −80 °C for later analysis. Blood samples (2 ml) were collected via venipuncture, stored in EDTA anticoagulant tubes at −80 °C, and analyzed later.

ELISA was used to measure plasma levels of dopamine, serotonin, and BDNF. Competitive ELISA kits (Elabscience, USA) measured dopamine and serotonin, while the Human BDNF ELISA Kit PicoKine™ Pre-Coated ELISA kit (Boster Bio, USA) assessed BDNF levels. All tests followed the manufacturer's instructions. The Mann–Whitney *U* test was used to compare neurotransmitter levels between groups, and the Spearman rank correlation test assessed correlations between dopamine and serotonin levels.

RNA extraction was performed using the RNeasy Power Microbiome Kit (Qiagen, USA) with modifications in the lysis step. A total of 0.25 g of feces was transferred to a tube prefilled with 0.1 mm glass beads. The

tube was filled with 650 µl of lysis solution PM1 (chemical lysis solution) and 6.5 µl of beta-mercaptoethanol, then vortexed using the Omni Bead Ruptor 4 Bead Mill Homogenizer (Omni International, USA) at 3 m per second (m/s) for 6 min and 5 m per second (m/s) for 8 min. The remaining steps of the protocol were carried out according to the manufacturer's instructions. rRNA was removed using the Ribo-Zero rRNA Removal kit (Illumina, USA), and library quality was controlled with Qubit 2.0 (Thermo Scientific, USA). Libraries were sequenced on the Illumina HiSeq 2000 platform (Illumina, USA).

Bioinformatics analysis was performed using the ASaiM pipeline [13]. Initial sequence quality control was executed with FastQC v0.11.8 (2018, Babraham Bioinformatics, United Kingdom) to assess overall read quality. Data cleaning was carried out using Cutadapt v2.8 (2019, Marcel Martin, Germany), which removed adaptors and low-quality reads, ensuring a minimum read length of 30 base pairs and a Phred quality score threshold of 30. To differentiate between ribosomal and non-ribosomal RNA, SortmeRNA v2.1 (2016, Evguenia Kopylova, Laurent Noé, and Hélène Touzet, France) was employed, focusing on non-ribosomal RNA to represent the functional microbial community.

Microbiota community identification was conducted using MetaPhlAn2 v2.6.0 (2015, Nicola Segata, Harvard T.H. Chan School of Public Health, USA), which utilizes clade-specific marker genes from a comprehensive database of 17,000 reference genomes (including 13,500 bacterial and archaeal, 3500 viral, and 110 eukaryotic genomes). Functional analysis of microbial communities was performed using HUMaN2 v0.11.1 (2012, Curtis Huttenhower, Harvard T.H. Chan School of Public Health, USA).

The sequencing effort generated a total of 636,206,364 unprocessed sequences across 20 samples, with individual sequence counts ranging from 26,645,328 to 37,197,780. Quality metrics were assessed with Q20 and Q30 percentages ranging from 97.41 to 99.03% and 92.84 to 95.76%, respectively. These metrics corresponded to base-calling accuracies between 97.67 and 99.22%, with minimal base-calling errors ranging from 0.02 to 0.03%. All samples passed the quality control criteria for Q20 and Q30, and the GC content of all raw data was within the acceptable range of 40–60%.

Statistical analysis of clinical variables, including age, BMI, gender, neurotransmitter levels, and bacterial species abundance, was conducted using GraphPad Prism (version 9.0.0, 2020; GraphPad Software, San Diego, CA, USA). The modalities employed from GraphPad Prism included the Shapiro–Wilk test to assess whether the data followed a normal distribution, the Anderson–Darling test to provide a measure of goodness-of-fit for the distribution, and the Mann–Whitney *U* test to compare differences in non-normally distributed data between schizophrenia patients (SCZ) and healthy controls (HC).

Bacterial profiles of HC and SCZ were visually examined at the family, genus, and species levels using a TSS-normalized OTU count table. Alpha diversity metrics, including Observed, Chao1, Shannon, and Simpson indices, were calculated to quantify the diversity within each sample. These metrics were analyzed using the R function estimate richness and visualized using GraphPad Prism version 9 (2020, GraphPad Software, USA).

Beta diversity, which measures differences in microbial community composition between HC and SCZ, was assessed using the MicrobiomeAnalyst v1.0 (2017, McGill University, Canada). Bray–Curtis and Jaccard indices were calculated and visualized using non-metric multidimensional scaling (NMDS). The PERMANOVA test was applied to determine the statistical significance of observed differences in microbial community composition between the two groups.

To identify significant differences in microbial abundance and functional metabolisms between HC and SCZ, we used LEfSe v1.1.01 (2011, Harvard T.H. Chan School of Public Health, USA (Linear Discriminant Analysis Effect Size) with a Linear Discriminant Analysis (LDA) threshold >0.2 and p-value <0.05. LEfSe is designed to detect taxonomic features and functional biomarkers that differ significantly between groups, accounting for both biological significance and effect size. This analysis identified differentially abundant microbial taxa and functional pathways associated with schizophrenia.

To evaluate the diagnostic potential of different microbial species, Receiver Operating Characteristic (ROC) curves were generated using GraphPad Prism version 9 (2020, GraphPad Software, USA). ROC curves assessed the ability of specific microbial features to distinguish between HC and SCZ, providing insights into their potential as diagnostic biomarkers.

To explore the relationships between bacterial species abundance, neurotransmitter levels, and functional pathways, we conducted Spearman rank correlation analysis. This non-parametric method assessed the strength and direction of monotonic associations among these variables, providing insights into how changes in microbial communities relate to neurotransmitter levels and functional pathways.

Results

Table 1 displays the clinical characteristics of the 20 subjects chosen. There were no statistically significant differences in age, gender, or BMI between HC and SCZ ($p > 0.05$).

Table 1 Demographics and clinical characteristics of the study participants

Demographic variables	Healthy control (HC)	Schizophrenia (SCZ)	*p* value
Number	(*n* = 10)	(*n* = 10)	
Age[a] (years)	31.5	27.5	0.4917
Gender[a]			0.3698
Male	3 (30%)	6 (60%)	
Female	7 (70%)	4 (40%)	
Race			0.721
Malay	9 (90%)	10 (100%)	
Chinese	1 (10%)	0	
BMI[b]	26.84	28.77	0.502
Antipsychotic drugs	NA	Risperidone (*n* = 8, 80%) Olanzapine (*n* = 2, 20%)	
PANNS score	NA	Mean:102.7	
Dopamine[b] (pg/ml)	951.5	1178	0.0089
Serotonin[b] (ng/ml)	91.91	139.7	0.0387
BDNF[b] (pg/ml)	432.9	416.2	0.7449

[a] The variable is abnormally distributed and Mann–Whitney *U* test was applied to compare the variables between group

[b] Variable is normally distributed, and unpaired *T*-test was applied to compare the variables between groups

The *p* value <0.05 is significant

BMI body mass index, *PANNS* Positive and Negative Symptom Scale, *BDNF* brain-derived neurotrophic factor, *NA* not applicable

We compared the levels of dopamine, serotonin, and BDNF in HC and SCZ (Fig. 1). SCZ patients showed a higher level of dopamine and serotonin compared to HC ($p < 0.05$). BDNF level was higher in HC, but the difference was not statistically different ($p > 0.05$). This could be due to the effect of atypical antipsychotics, especially risperidone that aims to bring the BDNF in schizophrenia patients to normal levels [14]. Our finding is consistent with prior studies indicating that increase in dopamine in schizophrenia cause hallucinations and delusions [15]. Although the schizophrenia patients in this study are being treated with atypical antipsychotics such as risperidone and olanzapine, these medications primarily target serotonin rather than dopamine. In addition, atypical antipsychotics may enhance dopamine release in the prefrontal cortex [16, 17]. According to a previous study [18], people with schizophrenia have a greater serotonin level compared to healthy controls, which concur with our results. The schizophrenia patients in this study were given risperidone or olanzapine to rebalance their serotonin level by blocking serotonin 5-HT2 receptors. However, the serotonin levels in SCZ were still higher than those of HC, and the reason for this disparity is unknown. We also found a strong correlation between dopamine and serotonin levels among the 20 subjects (rho = 0.73 $p = 0.0002$) (Fig. 1D).

The compositional profile of the gut microbiome at the family level revealed that the top 15 families accounted for 94.6 and 90.4% of the gut microbiome in HC and SCZ, respectively. *Ruminococcaceae* (HC: 23.16%, SCZ: 16.88%) and *Veillonellaceae* (HC: 17.59%, SCZ: 16.58%) were the most abundant in both groups. This was followed by *Lachnospiraceae* (10.74%) in HC and *Acidaminococcaceae* (11.20%) in SCZ. Interestingly, *Prevotellaceae* was more abundant by 10.56% in SCZ compared to HC (HC: 0.19%, SCZ: 10.56%), while *Coriobacteriaceae* was lower by 5.88% in SCZ compared to HC (HC: 7.80%, SCZ: 1.91%) (Fig. 2A).

The top 15 genera accounted for 81.8 and 79.4% of the total microbiota profile in HC and SCZ, respectively. *Megasphaera* (HC: 16.47%, SCs: 14.5%) and *Faecalibacterium* (HC: 11.41%, SCZ: 12.43%) were the most prevalent

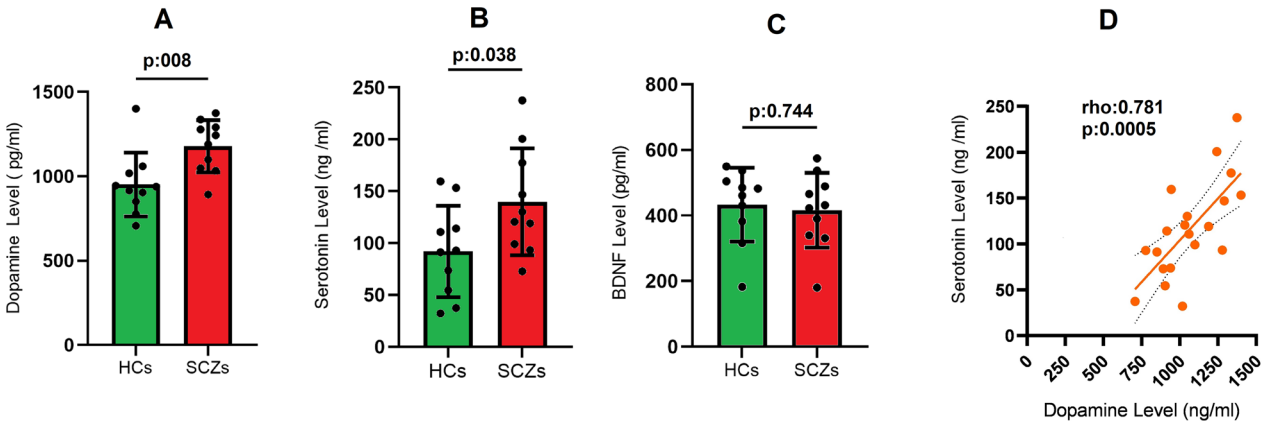

Fig. 1 Comparison of schizophrenia-related neurotransmitter levels between HCs and SCZs. Dopamine **A** serotonin **B** and BDNF **C** levels measured using ELISA. The levels of dopamine and serotonin were significantly higher in SCZ compered to HCs (Mann–Whitney test, $p < 0.05$). **D** Dopamine and serotonin levels are positively correlated in the entire cohort (Spearman correlation, $p < 0.05$)

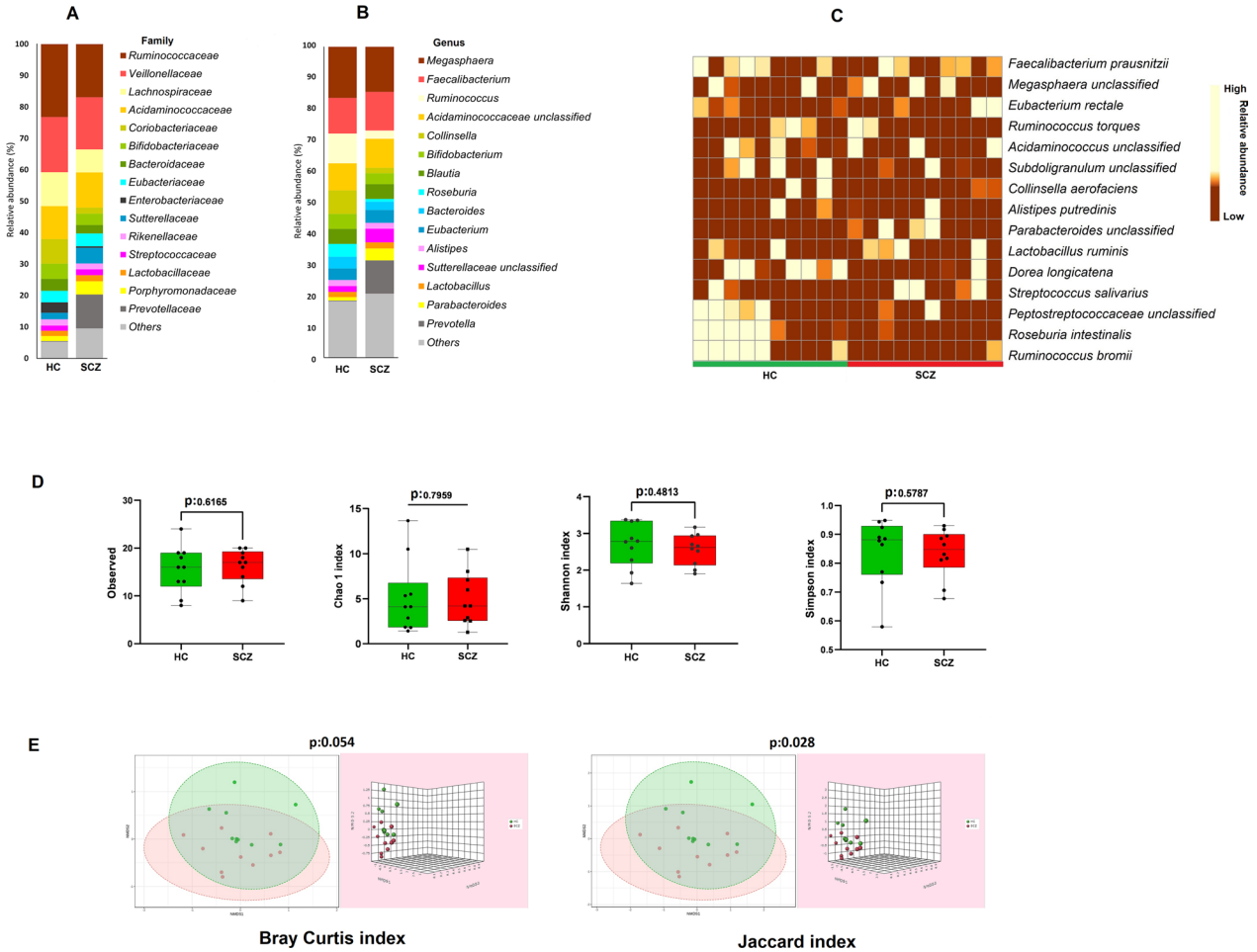

Fig. 2 The gut microbiome profiles, diversity and interpersonal variations in HCs compared to SCZs. **A** Microbiome composition profiles at the family level in HCs and SCZs. It illustrates the "top 15" families in HCs and SCZs. **B** Microbiome composition profiles at the genus level in HCs and SCZs. It illustrates the "top 15" genera in HCs and SCZs. **C** Heatmap of gut microbiome composition profiles at the species level in HCs and SCZs. It represents the top 15 species at the individual level in HCs and SCZs. **D** Boxplot of alpha diversity of Observed, Chao1, Shannon and Simpson's indices between HCs and SCZs show no significant alpha diversities between HCs and SCZs. **E** Bray Curtis and Jaccard index show there is significant difference between gut microbiome of HCs and SCZs

genera in both groups, with *Ruminococcus* (9.63%) dominating in HC and *Prevotella* (10.67%) in SCZ (Fig. 2B). *Faecalibacterium prausnitzii, Megasphaera unclassified, Eubacterium rectale,* and *Ruminococcus torques,* with median relative abundances of 0.148, 0.144, 0.020, and 0.0189, respectively, are listed among the top 4 most abundant bacteria at species level in both groups (Fig. 2C).

Next, we evaluated the alpha diversity utilizing several indexes (observed, Chao1, Shannon, and Simpson). HC exhibited a trend towards greater Shannon and Simpson metrics at the species level than SCZ, but these differences were not statistically significant ($p > 0.05$) (Fig. 2D). We found a substantial beta diversity between HC and SCZ. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity distances and Jaccard index for beta diversity revealed a statistically significant difference between the microbial communities of HC and SCZ using the PERMANOVA test ($p < 0.05$) (Fig. 2E).

To identify the specific bacterial taxa linked with schizophrenia, we utilized LEfSe to analyze the gut microbiota of SCZ and HC [19]. The LEfSe analysis showed that *Bacteroidetes* (phylum), *Bacteroidia* (class), *Bacteroidales*, *Paraprevotella* (genus) were more abundant in SCZ while *Adlercreutzia* (genus) was more abundant in HC (Fig. 3A). LEfSe analysis also revealed that 8 bacterial species were enriched in HC, these included *Roseburia intestinalis, Adlercreutzia equolifaciens, Roseburia inulinivorans, Bacteroides finegoldii, Dorea longicatena, Eubacterium eligens, Alistipes indistinctus* and *Streptococcus*

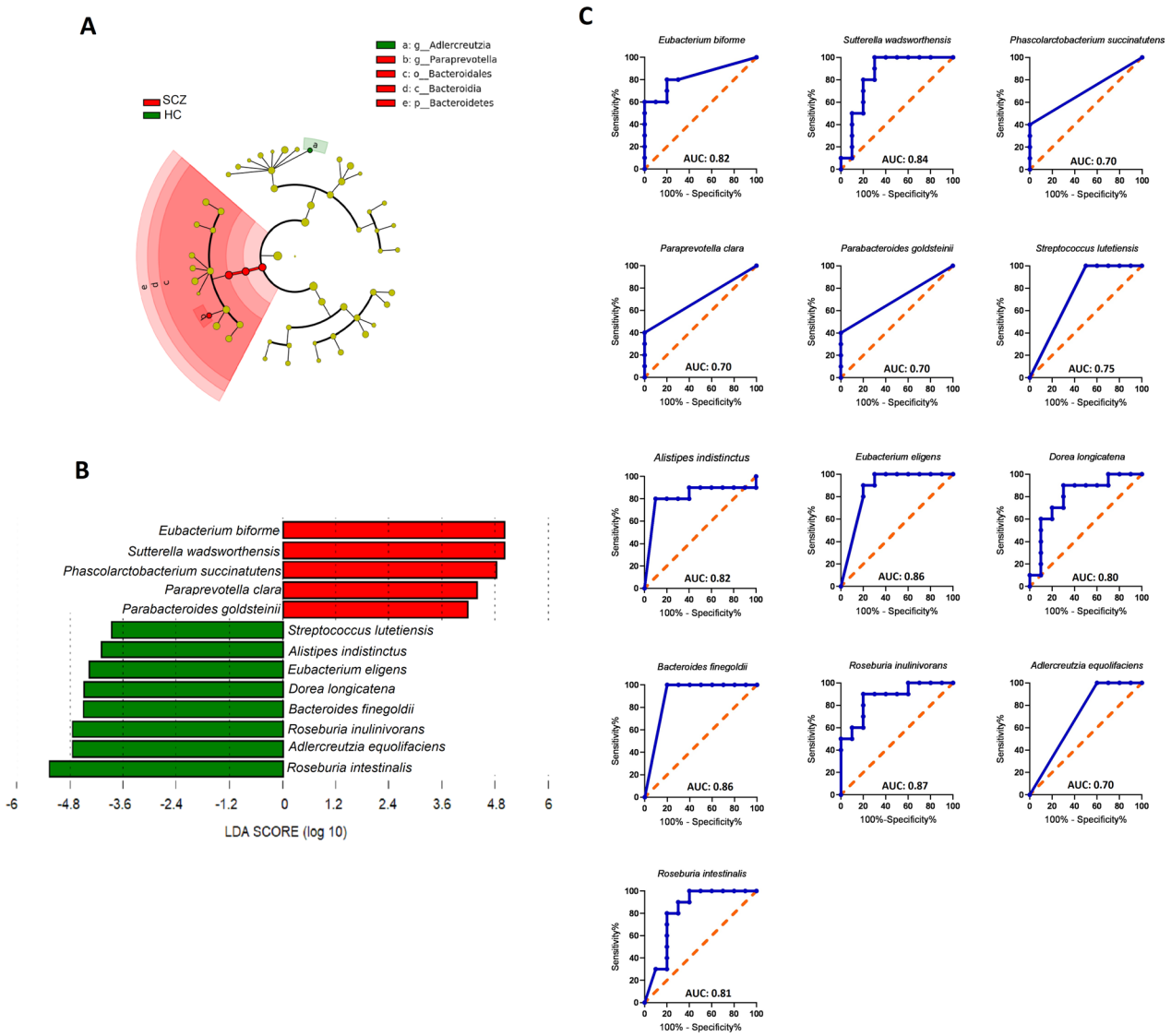

Fig. 3 Taxonomic differences of the gut microbiome between HCs and SCZs and correlation of structural signatures with the brain neurotransmitters. **A** The cladogram of LEfSe depicts taxonomic levels, with the *outer circle* representing phyla and the *inner circle* representing genera. **B** Linear discriminant analysis (LDA) effect size analysis (LEfSe) identified the most significant species between HCs and SCZs ($p < 0.05$; LDA score 2). HCs-enriched species are indicated with a negative LDA score (*green*), and species enriched in SCZs are indicated with a positive score (*red colour*). **C** The receiver operating characteristic analysis (ROC) yield significant area under curve (AUC) scores for the individual species (AUC; 95% CI, $p < 0.05$)

lutetiensis. On the other hand, the gut microbiome of SCZ was enriched with *Eubacterium biforme, Sutterella wadsworthensis, Phascolarctobacterium succinatutens, Paraprevotella clara* and *Parabacteroides goldsteinii* (Fig. 3B).

We further evaluated the discriminative power of these significant bacteria. The results of the ROC showed that the area under curve (AUC) scores of *Dorea longicatena, Roseburia intestinalis, Alistipes indistinctus, Eubacterium biforme, Sutterella wadsworthensis, Bacteroides finegoldii, Eubacterium eligens,* and *Roseburia inulinivorans* ranged from 0.80 to 0.87 (AUC; 95% CI, $p < 0.05$), indicating excellent predictive power, while *Adlercreutzia equolifaciens, Paraprevotella clara, Phascolarctobacterium succinatuten, Parabacteroides goldsteinii*, and *Streptococcus lutetiensis* had a AUC scores of 0.70 to 0.75 (AUC; 95% CI, $p < 0.05$) reflecting strong predictive capability (Fig. 3C).

Network analysis based on Spearman correlation revealed that the bacteria enriched in the HC showed a

positive correlation with each other (rho = 0.49 to 0.69, $p < 0.05$) but a negative correlation with those enriched in the SCZ (rho = −0.46 to 0.62, $p < 0.05$). Positive correlation was observed between SCZ-enriched bacteria (rho = 0.49 to 0.59, $p < 0.05$). We found that *Alistipes indistinctus, Dorea longicatena* and *Roseburia inulinivorans* (rho = −0.4682 to −0.585 $p < 0.05$) was negatively correlated with dopamine levels, *Roseburia intestinalis* negatively correlated with serotonin concentration (rho = −0.483, $p < 0.05$) and *Parabacteroides goldsteini* negatively correlated with BDNF (−0.7033, $p < 0.05$, in SCZ). There was no significant correlation between the bacteria and age and BMI (Fig. 4A).

Using LEfSe, 14 functional metabolic pathways that were different between HC and SCZ were found. Three pathways including lipid biosynthesis, tetrapyrrole biosynthesis I (from glutamate) and heme biosynthesis I (aerobic) were upregulated in SCZ, whereas the following metabolic pathways were upregulated in HC: pyruvate fermentation to acetate and lactate II, phosphopantothenate biosynthesis I, superpathway of purine deoxyribonucleoside degradation, super-pathway of L-aspartate and L-asparagine biosynthesis, L-isoleucine biosynthesis IV, phosphatidylglycerol biosynthesis I and II, inosine 5′-phosphate biosynthesis II and CDP-diacylglycerol biosynthesis I and II (Fig. 4B).

Furthermore, a significant correlation was found between levels of dopamine and serotonin with the superpathway of purine deoxyribonucleoside degradation (rho = −0.60 and rho = −0.47, respectively, $p < 0.05$). Serotonin also correlated negatively with the pathway of L-isoleucine biosynthesis IV (rho = −0.45, $p < 0.05$) and correlated positively with the pathway of inosine 5-phosphate biosynthesis II (rho = 0.48, $p < 0.05$). Dopamine

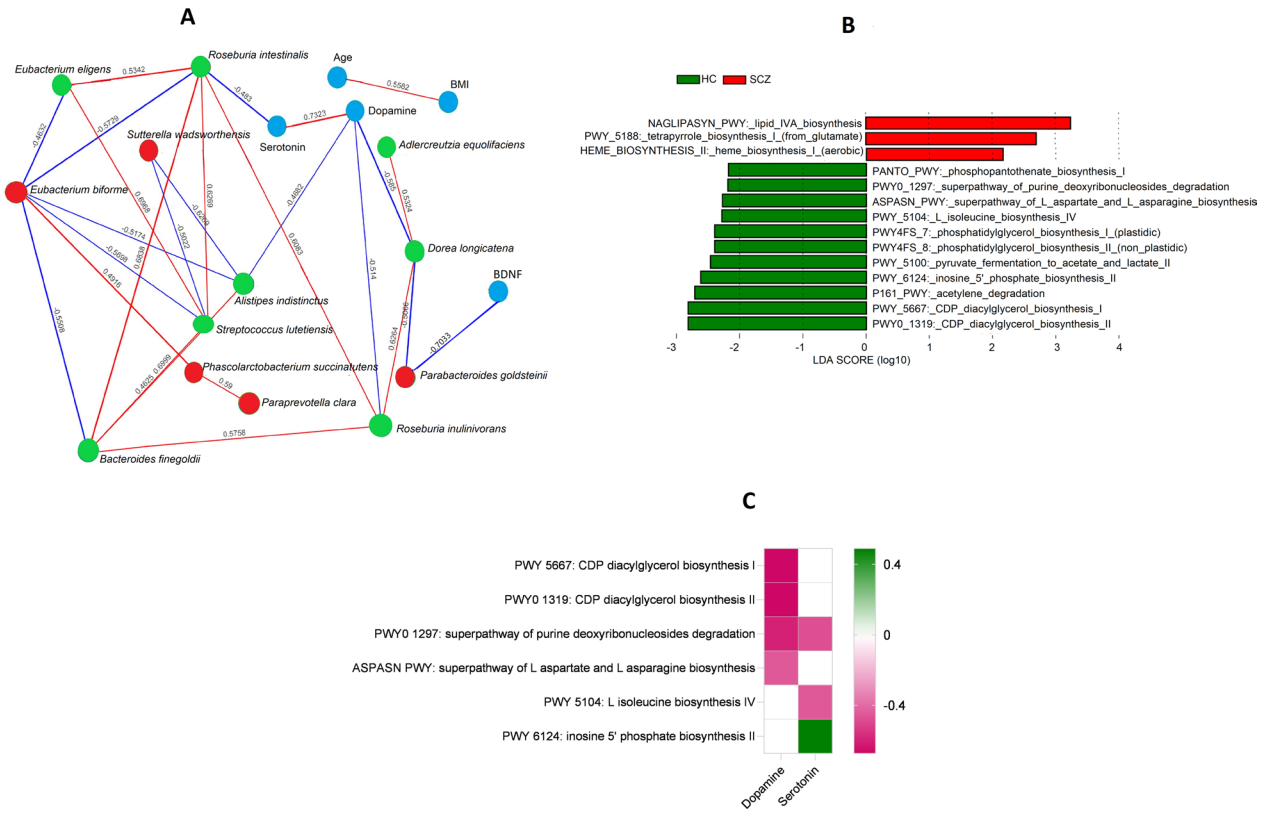

Fig. 4 Co-occurrence of microbiota and their functional characteristics in HCs and SCZs and their correlation with brain neurotransmitters. **A** Network analysis based on the Spearman correlation shows co-occurrence and anti-occurrence of gut microbiota and brain neurotransmitters as well as age and BMI. *Green nodes* represent bacteria that are enriched in HCs, red nodes represent bacteria that are enriched in SCZs, and *blue nodes* represent clinical data such as neurotransmitters, age, and BMI. *Red* and *blue lines* indicate positive and negative correlations respectively (Spearman correlation, $p < 0.05$). **B** Linear discriminant analysis (LDA) effect size analysis (LEfSe) identified the most significant functional pathways between HCs and SCZs participants ($p < 0.05$; LDA score > 2). HCs-enriched pathways are indicated with a negative LDA score (*green*), and SCZs-enriched pathways are indicated with a positive score (*red*). **C** The heatmap shows the correlation between schizophrenia-associated neurotransmitters and functional pathways triggered by the microbiota. Positive correlations are shown in *green* and negative correlations in *pink*. The intensity of the colour is proportional to the value of the correlation coefficient. The lighter green and pink, the weaker the correlation, and the *darker green* and *pink*, the stronger the correlation (Spearman correlation, $p < 0.05$)

was also found to have a negative correlation with the super pathways of L-aspartate and L-asparagine biosynthesis, as well as CDP-diacylglycerol biosynthesis I and II (rho = −0.45 and rho = −0.67, respectively, $p < 0.05$) (Fig. 4C).

Discussion

The gut microbiome plays a crucial role in mental health through the gut-brain axis. Our study examined the impact of gut microbiome composition on neurotransmitter levels related to schizophrenia and identified potential functional pathways involved in these interactions.

We observed significant differences in gut microbiome composition between schizophrenia patients and healthy controls. Specifically, *Phascolarctobacterium succinatutens* and *Paraprevotella clara*, which produce succinate, were more abundant in schizophrenia patients. Elevated succinate levels are associated with inflammation-related conditions such as obesity and type 2 diabetes, which are common comorbidities in schizophrenia [20–22].

In contrast, butyrate-producing bacteria like *Roseburia inulinivorans*, *Eubacterium eligens, Roseburia intestinalis*, and *Dorea longicatena* were more prevalent in healthy controls. Previous studies have linked decreased butyrate levels with increased inflammation and central nervous system infections in schizophrenia [23].

Although the precise mechanistic role of the gut microbiome in schizophrenia is unknown, a few potential mechanisms could explain the link between the gut microbiome, schizophrenia and its comorbidities. Irritable bowel syndrome (IBS) is one of the most common comorbidities of schizophrenia [24, 25]. In the SCZ group, we discovered some bacteria, namely *Adlercreutzia equolifaciens, Bacteroides finegoldii*, and *Alistipes,* whose deficiencies have been associated with IBS [26–28]. Furthermore, there was a high abundance of *Paraprevotella clara* species, an acetic acid-producing intestinal bacteria [29] in the SCZ group. Chronic schizophrenia associated with increased levels of acetic acid may lead to alterations in the body's acid–base homeostasis [30]. Studies have found that changes in body acid–base homeostasis are associated with panic disorder and other psychiatric disorders. There were also evidences that showed the brain pH of patients with schizophrenia and bipolar disorder was lower than in healthy individuals [31–34].

In our study, *Parabacteroides goldsteinii* was enriched in SCZ while absent in HC, and it showed a negative correlation with BDNF level (rho = −0.70 $p = 0.028$, 40% prevalence). Nobel and colleagues [35] suggested that *Parabacteroides* negatively affect hippocampus-dependent, peri-rhinal cortex-dependent memory functions and cause cognitive impairment. *Roseburia inulinivorans* was negatively correlated with dopamine levels in SCZ. A previous study by Chen and colleagues [36] showed that *Roseburia* level was deceased in SCZ. Additionally, it has been demonstrated that *Roseburia* decreases in the gut microbiota of patients suffering from anorexia nervosa, a serious psychological disorder with similar symptoms to schizophrenia [23]. Södersten and colleagues [37] found that anorexics have increased dopamine levels in the brain and this was supported by genome-wide association studies that identified metabolic factors in the etiology of anorexia nervosa [38, 39].

We further found that the super pathway of purine deoxyribonucleoside degradation was decreased in SCZ, and this was negatively correlated with both dopamine and serotonin levels. The change in this metabolic pathway was marked by the absence of butyrate-producing bacteria such as *Eubacterium eligens, Roseburia intestinalis,* and *Roseburia inulinivorans*, and the increase in the levels of *Eubacterium biformes*. Studies have shown that alteration of purine degradation affects dopamine and serotonin levels leading to abnormalities in brain function and schizophrenia [40–44]. The brain, which is 60% lipids, requires an abundance of phospholipids in the right proportions to stabilize neuronal cell membranes and promote cognitive function [45]. In the SCZ, the lipid biosynthesis pathway showed an increase while the phospholipid and asparagine biosynthesis pathways were decreased. Several studies have also found that patients with schizophrenia have higher serum lipid levels (cholesterol and triglycerides) than healthy individuals and low phospholipid levels [46–48]. Low levels of amino acid asparagine are correlated with a high level of dopamine and the *Sutterella wadsworthensis.* Association of asparagine deficiency with brain abnormality and cognitive impairment have been shown in a previous study [49].

Overall, we found that butyrate, a short-chain fatty acid known for its anti-inflammatory properties, was depleted in individuals with schizophrenia. Butyrate plays a crucial role in maintaining gut barrier integrity and reducing systemic inflammation, which can, in turn, influence the kynurenine pathway and affect kynurenic acid (KYNA) levels [50]. Since KYNA modulates dopaminergic and glutamatergic pathways—both of which are implicated in schizophrenia—alterations in gut microbiome-derived metabolites like butyrate could impact KYNA levels and contribute to the neurobiological abnormalities observed in this disorder, as discussed in a recent review [51].

This study has a few limitations. The small sample size may impact the generalizability of our findings. Additionally, the study did not specifically evaluate how

antipsychotic treatment might affect the gut microbiome, which could be a significant factor.

To address these limitations, future research should involve larger sample sizes and longitudinal studies, including both drug-naive and antipsychotic-treated schizophrenia patients. This approach will help clarify the relationships between the gut microbiome and schizophrenia more comprehensively.

Additionally, future investigations should focus on microbiome-targeted interventions as potential strategies for managing schizophrenia. Understanding how specific alterations in the gut microbiome influence neurotransmitter levels and related symptoms will be essential for developing more effective, personalized treatments. These studies could pave the way for novel therapeutic approaches that address the microbiome's role in schizophrenia.

Conclusion

This study highlights the association of the gut microbiome with schizophrenia-associated brain neurotransmitters such as dopamine, serotonin and BDNF. Furthermore, the dysbiosis of certain gut bacteria such as *Roseburia inulinivorans, Sutterella wadsworthensis, Bacteroides finegoldii* and *Eubacterium eligens* in schizophrenia could potentially serve as a biomarker for diagnostics and therapeutics. This could potentially be useful to develop gut microbiome-targeted interventions to rebalance schizophrenia-related neurotransmitters.

Abbreviations

AUC	Area under curve
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
bp	Base pairs
CI	Confidence interval
CNS	Central nervous system
ELISA	Enzyme-linked immunosorbent assay
GABA	Gamma-aminobutyric acid
HCs	Healthy controls
HPA	Hypothalamic-pituitary-adrenal
IBS	Irritable bowel syndrome
NMDS	Non-metric multidimensional scaling
PANSS	Positive and Negative Symptom Scale
PERMANOVA	Permutational multivariate analysis of variance
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
SCID-NP	Structured Clinical Interview for Non-Patient Edition
SCZ	Schizophrenia
TLRs	Toll-like receptors
WHO	World Health Organization

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Author contributions

Conceptualization: HR and LSY; data curation: MG, GBSJ, TMM, SSR, MAMR and HR; formal analysis: MG; funding acquisition, HR; investigation: MG, HR and LSY; Methodology, MG and GBSJ; writing—original draft preparation: MG and LSY; Writing – review & editing: All Authors.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the AIMST University Human Ethics Committee and the National Medical Research Register (NMRR), Ministry of Health, Malaysia (Research ID: 45540, Ref ID: KKM/NIHSEC/P19-446(13). The research was carried out in accordance with the Helsinki Declaration between December 2019 and December 2020 at Hospital Sultan Abdul Halim, Sungai Petani, Kedah, Malaysia and Hospital Sultanah Bahiyah, Alor Setar, Kedah, Malaysia. All participants read a written description of the study objectives prior to the study and provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Patel KR, Cherian J, Gohil K, Atkinson D. Schizophrenia: overview and treatment options. P T Peer-Rev J Formul Manag. 2014;39:638–45.
2. Martin CR, Osadchiy V, Kalani A, Mayer EA. The brain-gut-microbiome axis. Cell Mol Gastroenterol Hepatol. 2018;6:133–48. https://doi.org/10.1016/j.jcmgh.2018.04.003.
3. Zhu S, Jiang Y, Xu K, Cui M, Ye W, Zhao G, et al. The progress of gut microbiome research related to brain disorders. J Neuroinflammation. 2020;17:25. https://doi.org/10.1186/s12974-020-1705-z.
4. Kho ZY, Lal SK. The human gut microbiome – a potential controller of wellness and disease. Front Microbiol. 2018;9:1835. https://doi.org/10.3389/fmicb.2018.01835.
5. Cryan JF, O'Riordan KJ, Sandhu K, Peterson V, Dinan TG. The gut microbiome in neurological disorders. Lancet Neurol. 2020;19:179–94. https://doi.org/10.1016/S1474-4422(19):30356-4.
6. Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. Sci Adv 2019;5:eaau8317. https://doi.org/10.1126/sciadv.aau8317.
7. Szeligowski T, Yun AL, Lennox BR, Burnet PWJ. The gut microbiome and schizophrenia: the current state of the field and clinical applications. Front Psychiatry. 2020;11:156. https://doi.org/10.3389/fpsyt.2020.00156.
8. Ghorbani M, Rajandas H, Parimannan S, Stephen Joseph GB, Tew MM, Ramly SS, et al. Understanding the role of gut microbiota in the pathogenesis of schizophrenia. Psychiatr Genet. 2021;31:39–49. https://doi.org/10.1097/YPG.0000000000000270.

9. Di Carlo P, Punzi G, Ursini G. Brain-derived neurotrophic factor and schizophrenia. Psychiatr Genet. 2019;29:200–10. https://doi.org/10.1097/YPG.0000000000000237.
10. Fry BR, Russell N, Gifford R, Robles CF, Manning CE, Sawa A, et al. Assessing reality testing in mice through dopamine-dependent associatively evoked processing of absent gustatory stimuli. Schizophr Bull. 2020;46:54–67. https://doi.org/10.1093/schbul/sbz043.
11. Kesby J, Eyles D, McGrath J, Scott J. Dopamine, psychosis and schizophrenia: the widening gap between basic and clinical neuroscience. Transl Psychiatry. 2018;8:30. https://doi.org/10.1038/s41398-017-0071-9.
12. Sumiyoshi T, Kunugi H, Nakagome K. Serotonin and dopamine receptors in motivational and cognitive disturbances of schizophrenia. Front Neurosci 2014;8:395. https://doi.org/10.3389/fnins.2014.00395.
13. Batut B, Gravouil K, Defois C, Hiltemann S, Brugère J-F, Peyretaillade E, et al. ASaiM: a Galaxy-based framework to analyze microbiota data. GigaScience 2018;7:giy057. https://doi.org/10.1093/gigascience/giy057.
14. Yu W, Zhu M, Fang H, Zhou J, Ye L, Bian W, et al. Risperidone reverses the downregulation of BDNF in hippocampal neurons and MK801-induced cognitive impairment in rats. Front Behav Neurosci. 2019;13:163. https://doi.org/10.3389/fnbeh.2019.00163.
15. Brisch R, Saniotis A, Wolf R, Bielau H, Bernstein H-G, Steiner J, et al. The Role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: old fashioned, but still in vogue. Front Psychiatry 2014;5:47. https://doi.org/10.3389/fpsyt.2014.00047.
16. Bortolozzi A, Masana M, Díaz-Mataix L, Cortés R, Scorza MC, Gingrich JA, et al. Dopamine release induced by atypical antipsychotics in prefrontal cortex requires 5-HT1A receptors but not 5-HT2A receptors. Int J Neuropsychopharmacol. 2010;13:1299–314. https://doi.org/10.1017/S1461145710000009X.
17. Vile JM, Strange PG. Atypical antipsychotics — serotonergic mechanisms but don't forget dopamine. J Psychopharmacol (Oxf). 1997;11:24–5. https://doi.org/10.1177/026988119701100107.
18. Risch SC. Pathophysiology of schizophrenia and the role of newer antipsychotics. Pharmacotherapy. 1996;16:11–4.
19. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60. https://doi.org/10.1186/gb-2011-12-6-r60.
20. Serena C, Ceperuelo-Mallafré V, Keiran N, Queipo-Ortuño MI, Bernal R, Gomez-Huelgas R, et al. Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. ISME J. 2018;12:1642–57. https://doi.org/10.1038/s41396-018-0068-2.
21. Osuna-Prieto FJ, Martinez-Tellez B, Ortiz-Alvarez L, Di X, Jurado-Fasoli L, Xu H, et al. Elevated plasma succinate levels are linked to higher cardiovascular disease risk factors in young adults. Cardiovasc Diabetol. 2021;20:151. https://doi.org/10.1186/s12933-021-01333-3.
22. Annamalai A, Kosir U, Tek C. Prevalence of obesity and diabetes in patients with schizophrenia. World J Diabetes. 2017;8:390. https://doi.org/10.4239/wjd.v8.i8.390.
23. Mondot S, Lachkar L, Doré J, Blottière HM, Hanachi M. Roseburia, a decreased bacterial taxon in the gut microbiota of patients suffering from anorexia nervosa. Eur J Clin Nutr. 2022;76:1486–9. https://doi.org/10.1038/s41430-022-01116-3.
24. Fadgyas-Stanculete M, Buga A-M, Popa-Wagner A, Dumitrascu DL. The relationship between irritable bowel syndrome and psychiatric disorders: from molecular changes to clinical manifestations. J Mol Psychiatry. 2014;2:4. https://doi.org/10.1186/2049-9256-2-4.
25. Lee Y-T, Hu L-Y, Shen C-C, Huang M-W, Tsai S-J, Yang AC, et al. Risk of psychiatric disorders following irritable bowel syndrome: a nationwide population-based cohort study. PLoS ONE. 2015;10: e0133283. https://doi.org/10.1371/journal.pone.0133283.
26. Parker BJ, Wearsch PA, Veloo ACM, Rodriguez-Palacios A. The genus Alistipes: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol. 2020;11:906. https://doi.org/10.3389/fimmu.2020.00906.
27. Volokh O, Klimenko N, Berezhnaya Y, Tyakht A, Nesterova P, Popenko A, et al. Human gut microbiome response induced by fermented dairy product intake in healthy volunteers. Nutrients. 2019;11:547. https://doi.org/10.3390/nu11030547.
28. Yang X, Xiu W-B, Wang J-X, Li L-P, He C, Gao C-P. CO2 is beneficial to gut microbiota homeostasis during colonoscopy: randomized controlled trial. J Clin Med. 2022;11:5281. https://doi.org/10.3390/jcm11185281.
29. Morotomi M, Nagai F, Sakon H, Tanaka R. *Paraprevotella clara* gen. nov., sp. nov. and *Paraprevotella xylaniphila* sp. nov., members of the family "Prevotellaceae" isolated from human faeces. Int J Syst Evol Microbiol 2009;59:1895–900. https://doi.org/10.1099/ijs.0.008169-0.
30. Perry TL, Hansen S, Diamond S, Melançon SB, Lesk D. Acetic and benzoic acids in the urine of patients with chronic schizophrenia. Clin Chim Acta. 1971;31:181–6. https://doi.org/10.1016/0009-8981(71)90376-7.
31. Cole R. Acid-base balance in acute panic attack. Nephrol Dial Transplant. 2009;24:2007–2007. https://doi.org/10.1093/ndt/gfp139.
32. Dogan AE, Yuksel C, Du F, Chouinard V-A, Öngür D. Brain lactate and pH in schizophrenia and bipolar disorder: a systematic review of findings from magnetic resonance studies. Neuropsychopharmacology. 2018;43:1681–90. https://doi.org/10.1038/s41386-018-0041-9.
33. Hagihara H, Catts VS, Katayama Y, Shoji H, Takagi T, Huang FL, et al. Decreased brain pH as a shared endophenotype of psychiatric disorders. Neuropsychopharmacology. 2018;43:459–68. https://doi.org/10.1038/npp.2017.167.
34. Vollmer LL, Strawn JR, Sah R. Acid–base dysregulation and chemosensory mechanisms in panic disorder: a translational update. Transl Psychiatry. 2015;5:e572–e572. https://doi.org/10.1038/tp.2015.67.
35. Noble EE, Olson CA, Davis E, Tsan L, Chen Y-W, Schade R, et al. Gut microbial taxa elevated by dietary sugar disrupt memory function. Transl Psychiatry. 2021;11:194. https://doi.org/10.1038/s41398-021-01309-7.
36. Chen Y, Xu J, Chen Y. Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. Nutrients. 2021;13:2099. https://doi.org/10.3390/nu13062099.
37. Södersten P, Bergh C, Leon M, Zandian M. Dopamine and anorexia nervosa. Neurosci Biobehav Rev. 2016;60:26–30. https://doi.org/10.1016/j.neubiorev.2015.11.003.
38. Morylowska-Topolska J, Ziemiński R, Molas A, Gajewski J, Flis M, Stelmach E, et al. Schizophrenia and anorexia nervosa – reciprocal relationships. A literature review Psychiatr Pol. 2017;51:261–70. https://doi.org/10.12740/PP/OnlineFirst/63514.
39. Watson HJ, Yilmaz Z, Thornton LM, Hübel C, Coleman JRI, Gaspar HA, et al. Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa. Nat Genet. 2019;51:1207–14. https://doi.org/10.1038/s41588-019-0439-2.
40. Fox IH. Metabolic basis for disorders of purine nucleotide degradation. Metabolism. 1981;30:616–34. https://doi.org/10.1016/0026-0495(81)90142-6.
41. Jinnah HA, Sabina R l., Van Den Berghe G. Metabolic disorders of purine metabolism affecting the nervous system. Handb Clin Neurol. 2013;113:1827–36. https://doi.org/10.1016/B978-0-444-59565-2.00052-6.
42. Kristóf Z, Baranyi M, Tod P, Mut-Arbona P, Demeter K, Bitter I, et al. Elevated serum purine levels in schizophrenia: a reverse translational study to identify novel inflammatory biomarkers. Int J Neuropsychopharmacol. 2022;25:645–59. https://doi.org/10.1093/ijnp/pyac026.
43. Loeffler DA, Camp DM, Juneau PL, Harel E, LeWitt PA. Purine-induced alterations of dopamine metabolism in rat pheochromocytoma PC12 cells. Brain Res Bull. 2000;52:553–8. https://doi.org/10.1016/S0361-9230(00)00293-8.
44. Micheli V, Camici M, Tozzi MG, Ipata PL, Sestini S, Bertelli M, et al. Neurological disorders of purine and pyrimidine metabolism. Curr Top Med Chem. 2011;11:923–47. https://doi.org/10.2174/156802611795347645.
45. Schverer M, O'Mahony SM, O'Riordan KJ, Donoso F, Roy BL, Stanton C, et al. Dietary phospholipids: role in cognitive processes across the lifespan. Neurosci Biobehav Rev. 2020;111:183–93. https://doi.org/10.1016/j.neubiorev.2020.01.012.
46. Solberg DK, Bentsen H, Refsum H, Andreassen OA. Lipid profiles in schizophrenia associated with clinical traits: a five year follow-up study. BMC Psychiatry. 2016;16:299. https://doi.org/10.1186/s12888-016-1006-3.
47. Sun Z, Zhao L, Bo Q, Mao Z, He Y, Jiang T, et al. Brain-specific oxysterols and risk of schizophrenia in clinical high-risk subjects and patients with schizophrenia. Front Psychiatry. 2021;12: 711734. https://doi.org/10.3389/fpsyt.2021.711734.
48. Yang X, Sun L, Zhao A, Hu X, Qing Y, Jiang J, et al. Serum fatty acid patterns in patients with schizophrenia: a targeted metabonomics study. Transl Psychiatry. 2017;7:e1176–e1176. https://doi.org/10.1038/tp.2017.152.

49. Dalangin R, Kim A, Campbell RE. The role of amino acids in neurotransmission and fluorescent tools for their detection. Int J Mol Sci. 2020;21:6197. https://doi.org/10.3390/ijms21176197.
50. Garcez ML, Tan VX, Heng B, Guillemin GJ. Sodium butyrate and indole-3-propionic acid prevent the increase of cytokines and kynurenine levels in LPS-induced human primary astrocytes. Int J Tryptophan Res. 2020;13:1178646920978404. https://doi.org/10.1177/1178646920978404.
51. Grau-Del Valle C, Fernández J, Solá E, Montoya-Castilla I, Morillas C, Bañuls C. Association between gut microbiota and psychiatric disorders: a systematic review. Front Psychol. 2023;14:1215674. https://doi.org/10.3389/fpsyg.2023.1215674.

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