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ORIGINAL RESEARCH

Dysbiosis and Metabolic Dysregulation of Salivary Microbiota in Schizophrenia

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Background: Schizophrenia (SZ) is a chronic, severe mental disorder that presents significant challenges to diagnosis and effective treatment. Emerging evidence suggests that gut microbiota may play a role in the disease's pathogenesis. However, fewer studies have directly investigated the potential links between oral microbiota and SZ.

Purpose: This study aimed to explore the relationship between salivary microbiota dysbiosis and SZ, examining microbial and metabolic alterations that may contribute to SZ pathophysiology.

Methods: Salivary samples from 30 hospitalized patients diagnosed with SZ and 10 healthy controls were collected. The microbial and metabolic profiles were analyzed using 16S rRNA gene sequencing and metabolomic profiling. Clinical parameters, including oral health status, were also evaluated to minimize variability in sampling.

Results: Patients with SZ exhibited significantly poorer oral health compared to healthy controls, with more missing teeth and worse periodontal status. Microbiota sequencing revealed notable alterations in the overall structure and composition of the salivary microbiome in SZ patients, characterized by increased abundance of specific genera such as *Neisseria* and *Porphyromonas*. Metabolomic analysis indicated significant differences between the SZ and control groups, with upregulation of key metabolic pathways, including "β-alanine metabolism" and "vitamin digestion and absorption". Correlations between microbial dysbiosis and elevated levels of certain metabolites, such as L-methionine sulfoxide (L-MetO) and tyramine, were observed, suggesting links to oxidative stress.

Conclusion: The study highlights the presence of significant dysbiosis and metabolic dysfunction in the salivary microbiota of SZ patients, suggesting that alterations in the oral microbiome may contribute to SZ pathogenesis. These results provide new insights into potential diagnostic biomarkers and therapeutic targets for SZ. Further studies with larger sample sizes are required to validate these findings.

Keywords: schizophrenia, oral microbiome, dysbiosis, 16S rRNA, salivary metabolome

Introduction

Schizophrenia (SZ) is a chronic and severe mental disorder characterized by profound disruptions in cognition and emotion, affecting language, thought, perception, and self-awareness. With a global prevalence of approximately 1%, SZ imposes significant burdens on individuals, families, and healthcare systems.[1] Despite extensive research, the underlying causes and mechanisms of SZ remain largely unknown, which poses substantial challenges for the development of effective therapeutic interventions.

The gut microbiome, the largest microbial community in the human body, has a significant impact on human health and disease development.[2] Gut microorganisms interact with the nervous system through various pathways, and dysbiosis

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in the gut-brain axis may contribute to the pathogenesis of multiple neurological disorders.[3] Substantial evidence suggests a close relationship between gut microbiota and SZ.[4,5]

Parallel to the gut microbiome, the oral microbiome—the second most abundant microbial community—has become a central focus in microbiome research.[6] This growing interest is attributed to its easy sampling methods, diverse sites for sample collection, and its close associations with both oral and systemic health.[7] Recent studies indicate that translocation and colonization of oral microorganisms into the gut are common and widespread, implying that the oral cavity serves as an endogenous source influencing gut microbiota.[8,9]

Associations between the oral microbiota and neuropsychiatric disorders, including autism spectrum disorder and bipolar disorder, have been reported,[10,11] which has led to the emergence of the "oral-brain axis" concept in psychopathological studies.[12] Alterations in the oral microbial structure of patients with SZ have been observed,[13] and specific genera of tongue-coating microbiota have been proposed as potential biomarkers to distinguish elderly patients with SZ from healthy controls.[14] Nevertheless, further studies are necessary to detail these alterations and to identify potential metabolic pathways associated with SZ.

Therefore, the purpose of this study is to comprehensively investigate the microbial and metabolic profiles of the salivary microbiome in the context of SZ, and to find out the possible links between oral microbiota and the disease. A deeper understanding of these associations could potentially lead to novel therapeutic interventions to help alleviate the burden of this disorder.

Materials and Methods

Ethics Statement

This study was conducted in compliance with the Declaration of Helsinki, and was approved by the Ethics Committee of Shanghai Tongji Stomatological Hospital and Dental School, Tongji University (Ethical reference number: 2023-SR-77, Shanghai, China). Informed written consent was obtained from all the participants before study commencement.

Subject Recruitment

We recruited 30 hospitalized patients diagnosed with SZ and 10 healthy controls (HCs) from Zhejiang Xiaoshan Hospital. The enrolled SZ subjects were between 46 and 61 years of age, with no previous antibiotic/antifungal use within 3 months of sample collection. Gender- and age-matched healthy individuals unrelated to SZ were recruited as controls.

Sample Collection

The participants were instructed to refrain from eating and drinking, and to avoid oral hygiene practice, for at least 3 hours prior to sample collection. Approximately 1mL of non-stimulated, naturally outflowed saliva was continuously collected and transferred into 1.5 mL sterile microcentrifuge tubes (Eppendorf, Axygen, Union City, CA, USA), which were immediately placed on ice, transported to the local laboratory within 2 hours, and stored at −80°C until DNA extraction.

The oral conditions of all the participants, including the number of remaining teeth, bleeding on probing (BOP), probing depth (PD), decay-filled teeth (DFT), and decayed-missing-filled teeth (DMFT) were recorded after sampling. All clinical examinations were performed by the same experienced dentist according to routine criteria.

Microbiota Sequencing

DNA from salivary samples was extracted and 16S ribosomal RNA gene was amplified. Both 16S rRNA gene and metagenomic sequencing were performed using the Illumina platform at Personal Bio, Inc. (Shanghai, China).

Statistical Analyses

Demographics and clinical parameters of subjects between the SZ and healthy groups were compared by Chi-square test (gender), and Mann–Whitney *U*-test (age, DMFT, DFT, remaining teeth, PD, and BOP). $p<0.05$ was considered statistically

significant. Rarefaction curves were generated with Mothur to assess the current sequencing depth. The α diversity and principal coordinate analysis (PCoA) distance matrix were analyzed by Permutation Multivariate Analysis of Variance (PERMANOVA) using R software package (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria). Mann–Whitney *U*-test was used for statistical analysis of non-normally distributed variables between the patients with SZ and the healthy controls. Correlations among the microbiota, clinical parameters, and metabolites were tested and visualized using Corrplot in the R software package.

Results

Investigation of Oral Conditions

The demographic characteristics and clinical parameters of all participants are summarized in Table 1. The SZ group consisted of 30 hospitalized patients with a mean age of 50.2 years (±9.6) and an equal male-to-female ratio of 1: 1. In parallel, the HC group comprised 10 individuals averaging 51.1 years of age (±10.8), also with a balanced gender distribution.

To assess oral health status, all participants underwent comprehensive clinical examinations. The SZ group demonstrated significantly poorer oral health conditions compared to the HC group. Specifically, patients with SZ had a higher average number of decayed-missing-filled-teeth (4.2 ± 3.6 vs 3.0 ± 1.5, $p < 0.01$), fewer decay-filled teeth (0.5 ± 2.8 vs 1.3 ± 1.4, $p < 0.01$), and a reduced number of remaining teeth (21.0 ± 1.3 vs 28.3 ± 2.5, $p < 0.01$). Additionally, the mean probing depth was lower in the SZ group (3.7 ± 1.8 mm) than in the HC group (5.6 ± 7.1 mm, $p < 0.01$), the bleeding-on-probing index was significantly higher among the individuals with SZ (62.3% vs 50.0%, $p < 0.01$) (Table 1). These findings indicate an association between SZ and compromised oral health, suggesting that individuals with SZ are at an increased risk for oral health complications.

Overall Structure of the Salivary Microbiota

To investigate the role of oral microbiota in the pathogenesis of SZ, 16S rRNA sequencing of all collected salivary samples was conducted. After merging and filtering, we obtained 2,801,461 high-quality sequence reads, averaging 70,037 per sample, which were used for subsequent microbiome analyses. Across the entire cohort, 561 bacterial manipulation taxa (OTUs) with a coverage rate of 27 were identified. To assess the differences in the overall microbiome composition between SZ patients and healthy controls, alpha (α)- and beta (β)-diversity analyses of salivary microbiota was performed. Using the OTU relative abundance table, we calculated α-diversity indices—including Faith's phylogenetic diversity (Faith's pd), Good's coverage, Chao1 richness estimator, Simpson index, Shannon index, and Observed species count—to evaluate microbial diversity within samples (Figure 1A). The richness index (Chao1, Observed species, and Faith's pd) revealed significant disparities between the SZ and HC groups, whereas the Simpson and Shannon indices did not show significant differences between them. The increased species richness observed in the SZ group suggests a disruption in their microbial community. Principal Coordinate Analysis (PCoA) based on unweighted UniFrac distances demonstrated that the microbiota composition of SZ patients was significantly distinct from that of HCs (Figure 1B), and differences of β-diversity between the two groups also

Table 1 Demographic and Clinical Parameters of SZ Patients and Healthy Controls

Characteristics	Salivary samples		
	SZ (n=30)	HC (n=10)	*p* value
Age (years, Mean±SD)	50.2±9.6	51.1±10.8	NS[a]
Gender (M/F)	15/15	5/5	NS[b]
Decayed-missing-filled teeth (n, Mean±SD)	4.2±3.6	3.0±1.5	<0.01[a]
Decay-filled teeth (n, Mean±SD)	0.5±2.8	1.3±1.4	<0.01[a]
Remaining teeth (n, Mean±SD)	21.0±1.3	28.3±2.5	<0.01[a]
Probing depth (mm, Mean±SD)	3.7±1.8	5.6±7.1	<0.01[a]
Bleeding on probing (%)	62.3	50.0	<0.01[a]

Notes: [a]Mann–Whitney *U*-test. [b]Chi-square test.

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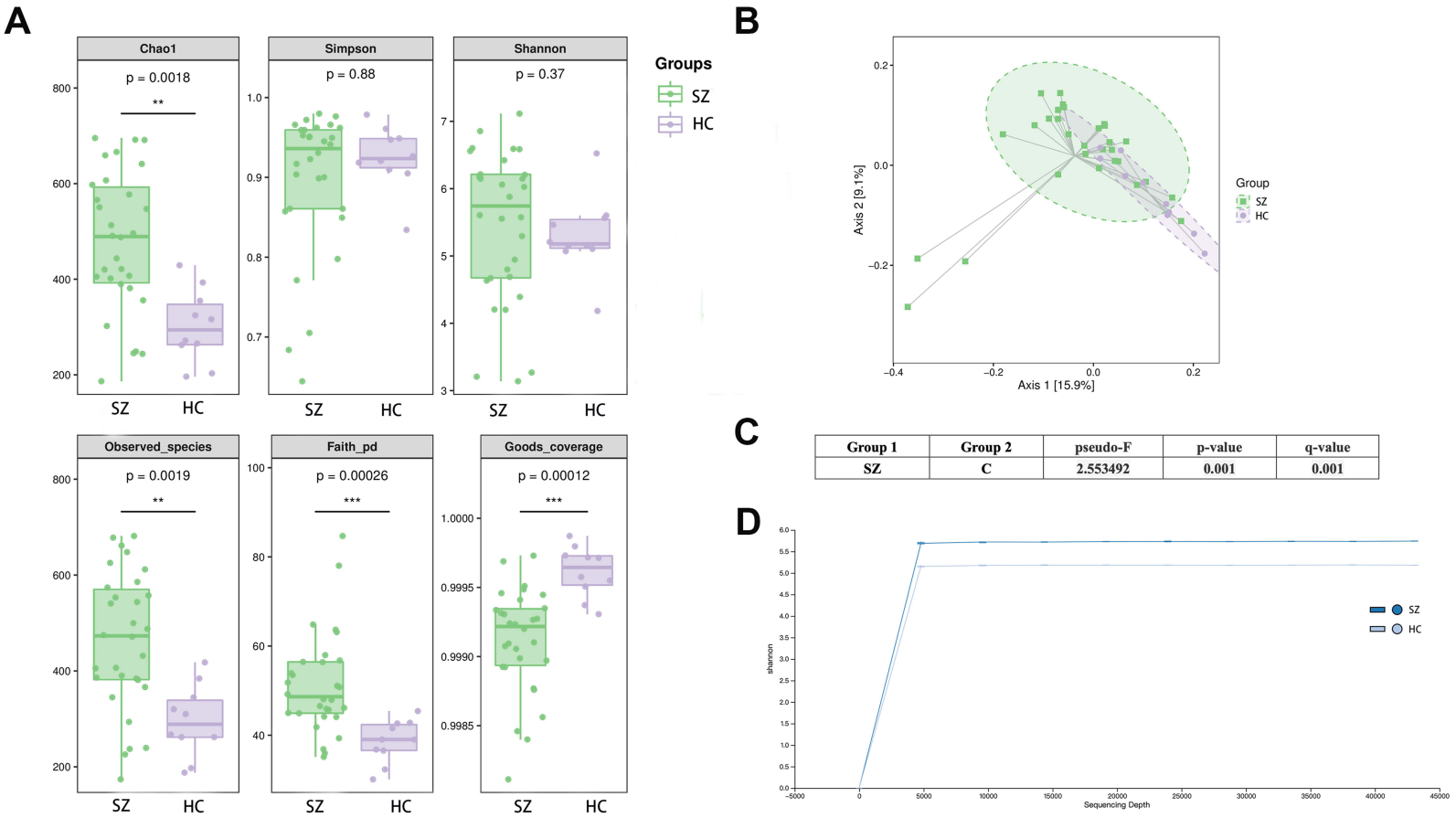

Figure 1 Changes of oral microbiota diversity in patients with SZ. (**A**) Chao1, Faith's phylogenetic diversity (Faith's pd), Shannon index, Simpson index, Observed species and Goods coverage of oral microbiota in participants with or without SZ. (**B**) The PCoA plot showed a reasonable separation of samples from SZ (green) and HC (purple). (**C**) The samples from SZ were clearly separated from HC. (**D**) Rarefaction curves of all samples. The data are presented as mean ± standard deviation. Mann–Whitney *U*-test was used to analyze the variation between the groups. **Differences significant at $p < 0.01$; ***Differences significant at $p < 0.001$.

exhibited a statistical trend ($p = 0.001$) (Figure 1C). The rarefaction curves approached asymptotes for each sample when approximately 5000 sequences were extracted and the Good's coverage estimator of each group came up to 99.7%, indicating that the sequencing depth was sufficient (Figure 1D).

Dysbiosis of Salivary Microbiota in Patients with SZ

The microbiota of SZ and HC groups were compared using a Venn diagram, and 869 shared genera were identified (Figure 2A). Stacked bar plots of relative abundance at the phylum level exhibited clear differences in the oral microbiota between patients with SZ and HCs (Figure 2B). The microbiota composition at the genus level was further analyzed. Of the top 50 genera in the salivary microbiota, 14 exhibited significant differences between the HC and SZ groups (Figure 2C). Compared with HC, SZ patients exhibited higher levels of *Bacteroidota, Synergistota, Spirochaetota, Campylobacterota*, and *Thermodesulfobacteriota* and lower levels of *Actinomycetota* and *Planctomycetota* (Figure 2D). At the genus level, there were 6 differential expressions between patients with SZ and HCs (Figure 2E). The abundance of *Neisseria, Porphyromonas*, and *Fusobacterium* were increased in those with SZ, while the abundance of *Schaalia, Rothia*, and *Actinomyces* decreased. Patients with SZ also exhibited significantly lower *Bacillus*/*Bacteroides* ratio ($p < 0.05$) (Figure 2F). These findings clearly indicate that the salivary microbiota was dysregulated in the SZ group.

Metabolites of Salivary Microbiota Were Altered in Patients with SZ

To identify the differential metabolites between SZ patients and healthy controls, we conducted metabolomic sequencing on all salivary samples. The PCA, OPLS-DA, and PLS-DA score plots all revealed a distinct separation in metabolite composition between the SZ and HC groups, indicating significant differences in their metabolomic profiles (Figure 3A). Differential metabolites were identified by metabolomic sequencing techniques (Log2FC>|2|, $p < 0.05$), resulting in a volcano plot (Figure 3B) in which 48 metabolites were upregulated while two metabolites were downregulated including deoxyinosine. Further analysis of salivary microbial metabolites was performed. The heatmap showcasing the top 150 metabolites revealed variations in their relative abundances across different samples (Figure 3C). Moreover, distinct distribution patterns between the SZ and HC groups were observed, indicating significant metabolic dysfunctions.

Potential Metabolic Pathways Linking to the Dysfunctions of Salivary Metabolites in SZ Patients

To predict the potential pathways affecting the alteration of metabolites, MetaboAnalyst was utilized to conduct Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on the altered metabolites, aiming to analyze salivary microbial function and possible metabolic pathways involved.

The results identified nine metabolic pathways, including "central carbon metabolism in cancer", "protein digestion and absorption", "β-Alanine metabolism, taste transduction", "ABC transporters", "vitamin digestion and absorption", "alanine, aspartate and glutamate metabolism", "mineral absorption", and "citrate cycle (TCA cycle)" ($p < 0.05$) (Figure 4A). The most notable differences were found in beta-alanyl-L-lysine and pantothenic acid, which exhibited increased expression in the SZ group. These two metabolites are involved in the "beta-alanine metabolism" and "vitamin digestion and absorption" pathways, respectively. On the other side, reduced expression of Deoxyinosine and Guanosine was detected in the SZ group, associated with the "ABC transporters" pathway. In addition, several remarkable metabolites, including isovaleric acid, tyramine, aspartam, L-Methionine, and L-Malic acid, exhibited significantly elevated expression levels (Figure 4B).

Possible Mechanism of Salivary Microbiota Dysbiosis and Metabolic Dysfunctions Affecting SZ

Using the Pearson correlation coefficient method, we found that the Bacillus/Bacteroidetes ratio and L-methionine-S-oxide (L-MetO) had a strong negative correlation (R=-0.66, $p < 0.01$) (Figure 5A). The expression levels of L-MetO and L-methionine (L-Met) in the SZ group were significantly higher than those in HCs ($p < 0.01$)(Figure 5C and D). The top 50 metabolites associated with L-MetO were figured out, and tyramine was most correlated with L-MetO (Figure 5B).

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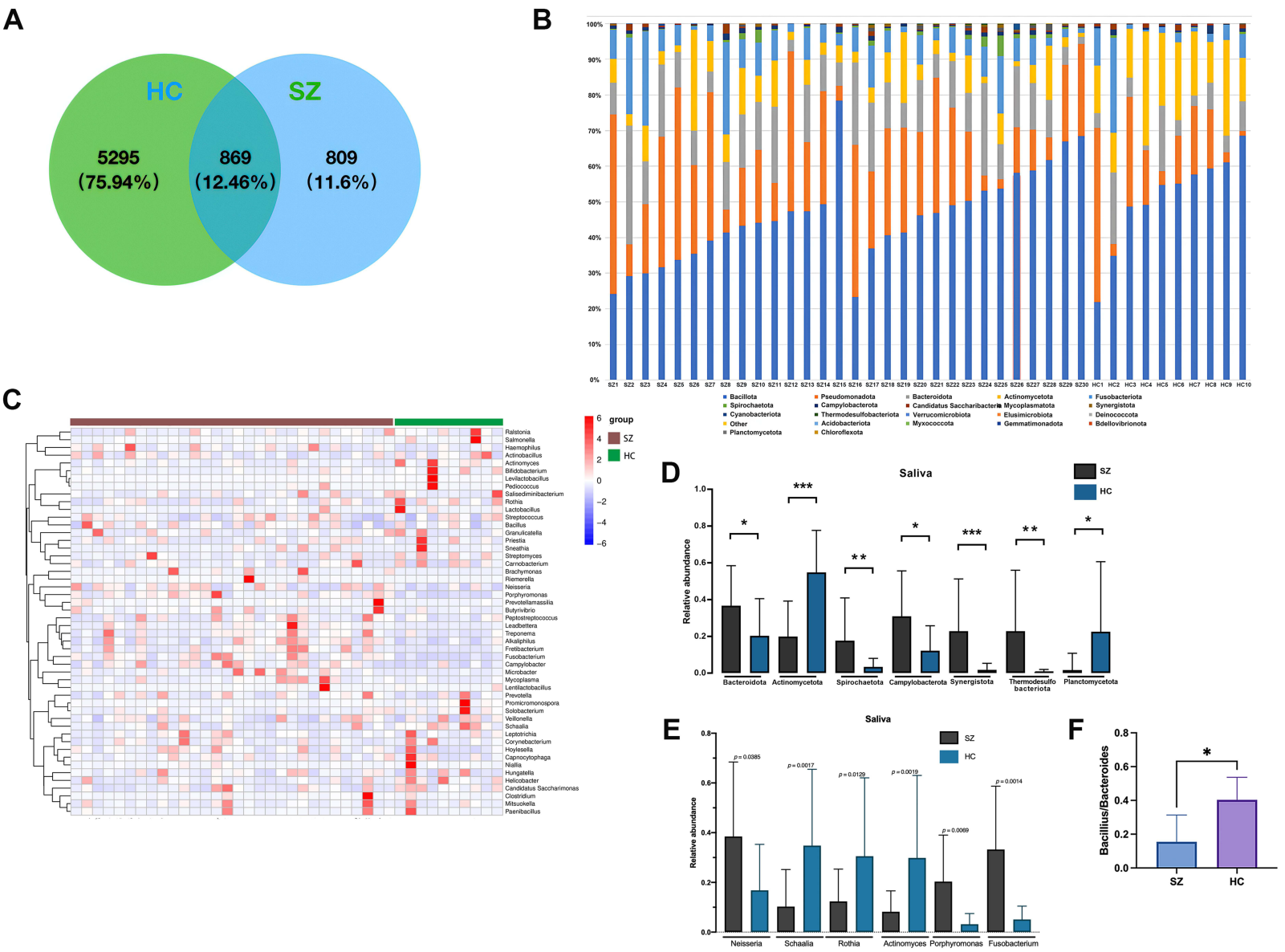

Figure 2 Changes in the composition of the oral microbiota in patients with SZ. (**A**) Venn diagrams showing the unique and shared OTUs among saliva. (**B**) Stacked bar plots showing relative abundances of oral microbiota at phylum level in participants with or without SZ. (**C**) Clustering heatmaps showing relative abundances of the top 50 genera in participants with or without SZ. (**D**) Box plots showing differential enrichment of the statistically significant phyla in participants with or without SZ. (**E**) Box plots showing differential enrichment of the statistically significant genus in participants with or without SZ. (**F**) Box plots showing differential ratio of Bacillus/Bacteroides in participants with or without SZ. All microbiota was analyzed using 16S rRNA gene sequencing. Mann–Whitney *U*-test was used for statistical analysis in (**D–F**). *Differences significant at $p < 0.05$, **Differences significant at $p < 0.01$; ***Differences significant at $p < 0.001$.

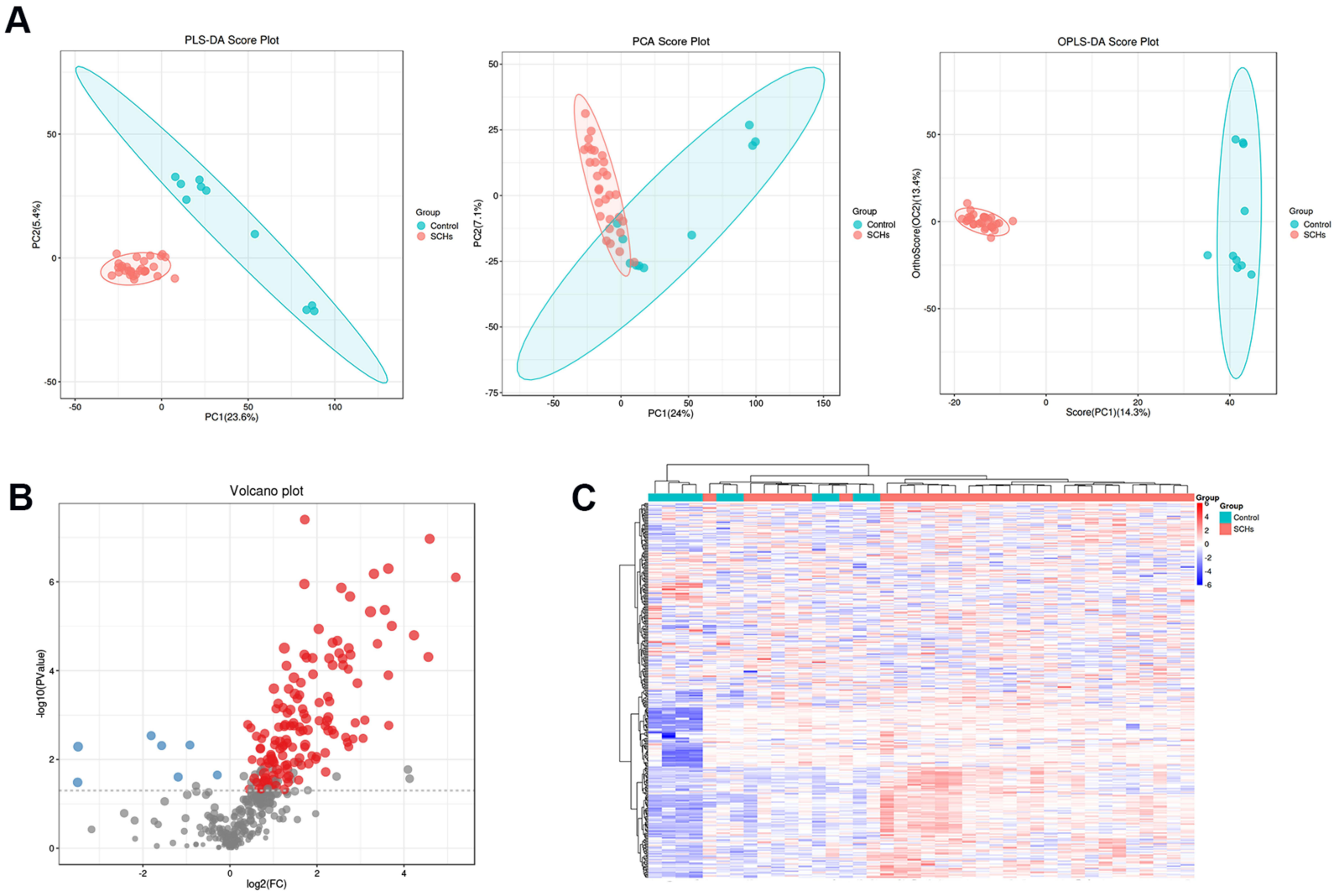

Figure 3 Changes in the diversity and composition of oral metabolites in patients with SZ. (**A**) PLS-DA, PCA, OPLS-DA of oral microbiota in participants with or without SZ. (**B**) Volcano plot showing the different metabolites in participants with or without SZ. (**C**) Clustering heatmaps showing relative abundances of the top 50 metabolites in participants with or without SZ.

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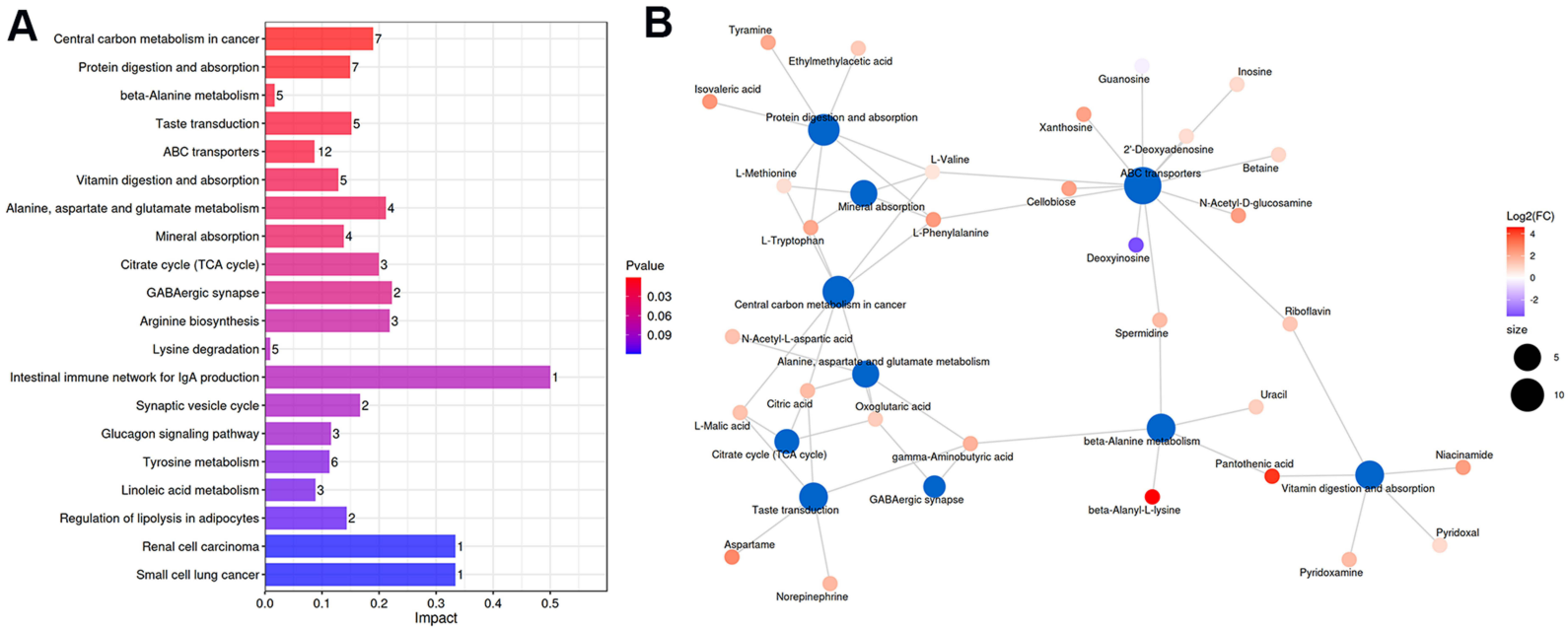

Figure 4 Functional prediction of oral metabolites in patients with SZ. (**A**) The histogram of metabolic pathway influence factor. The horizontal coordinate is the impact value enriched to different metabolic pathways, and the vertical coordinate is the enrichment pathway. The bar size indicates the corresponding number of metabolites on the pathway. The color is related to the P value, the redder the color, the smaller the P value, and the bluer the color, the larger the P value. (**B**) The network of KEGG pathways. The blue dots represent pathways, and the other dots represent metabolites. The size of the pathway point indicates the number of metabolites connected to it, the more connected the number, the larger the point, the color of the metabolite point indicates the size of the log2 (FC) value, red indicates the difference up, blue indicates the difference down, the darker the color, the greater the difference.

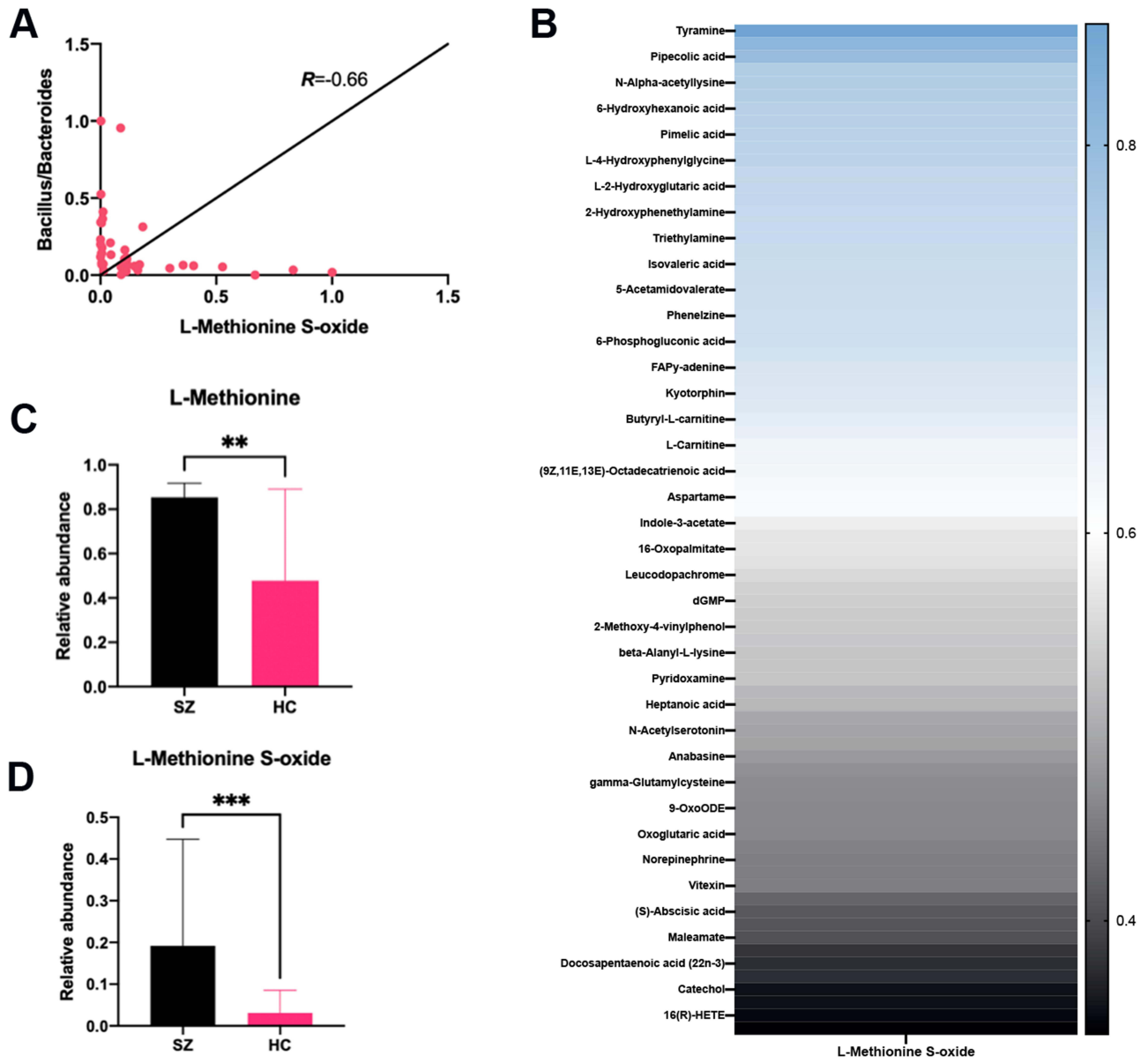

Figure 5 Association between oral microbiota and saliva metabolites in patients with schizophrenia. (**A**) The correlation of Bacillus/Bacteroides and L-methionine s-oxide. (**B**) Clustering heatmaps showing relative abundances of the L-methionine s-oxide in participants with or without SZ. (**C**) Box plots showing differential enrichment of the statistically significant L-Met in participants with or without SZ. (**D**) Box plots showing differential enrichment of the statistically significant L-MetO in participants with or without SZ. All microbiota was analyzed using 16S rRNA gene sequencing. Mann–Whitney *U*-test was used for statistical analysis in (**B** and **C**). **Differences significant at $p < 0.01$; ***Differences significant at $p < 0.001$.

Discussion

Schizophrenia (SZ) is a chronic and severe mental disorder that imposes substantial personal, social, and societal burdens. It still remains big challenges for psychiatrists and researchers, including achieving accurate diagnosis, enhancing treatment strategies, developing personalized medical interventions, and improving disease prognosis.[15] Accumulating evidence suggests that gut microbiota plays a role in the pathogenesis of SZ via the microbiota-gut-brain axis. A recent study report that the gut microbiome from patients with SZ induces SZ-relevant behaviors in germ-free recipient mice.[16] Transplanting intestinal flora from SZ patients into mice has been shown to cause disturbances in glutamate metabolism in mouse brain tissue.[17] Notably, promising results indicate that fecal microbiota transplantation (FMT) is an effective treatment for diseases in which the intestinal microbiota may play a role in the pathogenesis.[18] However, there is currently no consensus on the

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specific pattern of changes in SZ-associated oral microbiota. The results of this study provide new insights into the pathogenesis of SZ by highlighting alterations in the oral microbiome and metabolome.

The oral microbiome, the second abundant microbial community in the human body, has emerged as a focus of interest in microbiome research. Saliva, as a non-invasive and practical site for microbiome sampling, offers high sampling stability and represents the oral microbiota effectively, making it valuable for microbiome analyses. Moreover, the diverse microbial composition of saliva can reflect an individual's characteristics and disease states.[19] Patients with neuropsychiatric disorders often exhibit poor oral hygiene, largely due to a lack of awareness about oral care and difficulties in cooperating with routine dental examinations and treatments. This poor oral hygiene frequently results in an increase in pathogenic oral bacteria, leading to dysbiosis of oral microbiota[20] and exacerbate their neuropsychiatric conditions by promoting systemic inflammation or affecting neurological pathways in turn.[11] To minimize variability in oral health conditions and mitigate confounding effects from systemic factors and oral hygiene habits, the study adopted strict inclusion and exclusion criteria, specifically recruiting participants with periodontitis who exhibited similarly poor oral hygiene. This strategy was implemented to enhance the uniformity of the sample and improve the reliability of the findings. Clinical assessment revealed that SZ patients exhibited worse periodontal status and more missing teeth compared to healthy controls. Furthermore, our findings identified substantial alterations in both the structure and composition of the salivary microbiota in SZ patients, indicating a dysregulation of the oral microbiome. These results are consistent with previous research,[21] further supporting the link between oral microbiota and SZ pathogenesis.

Rothia, a Gram-positive, aerobic, rod-shaped, and non-motile bacterial genus from the phylum *Actinobacteria*, was found to be the dominant genus. *Rothia* is also associated with the occurrence of allergic diseases and alcoholic fatty liver disease.[22,23] Furthermore, the abundance of *Rothia* increased in the saliva of ASD patients,[24] and may be involved in the immune-mediated inflammatory response that contribute to allergic diseases and asthma.[25] Our analysis showed that *Rothia* levels were significantly lower in the patients with SZ compared to healthy individuals, which aligns with the findings from studies on the gut microbiota of SZ patients.[26] This reduction may be linked to the effects of antipsychotic medications commonly used by hospitalized SZ patients.

Actinomyces species are recognized as contributors to the onset and development of diseases such as dental caries and periodontitis.[27] An increased abundance of *Actinomyces* has been positively linked to higher lactic acid levels in patients with SZ,[28] with acidosis identified as a characteristic feature of this mental disorder.[29] Interestingly, *Actinomyces* enrichment is frequently detected in initial-stage semen samples but tends to diminish following the administration of psychotropic medications.[30] This observation aligns with our experimental results, indicating that antipsychotic drugs may alter the composition of the oral microbiome.

Oral treponema has been shown to induce Alzheimer's-like Tau hyperphosphorylation by activating neuroinflammation in the hippocampus.[30] In this study, we observed an increased proportion of *Treponema* in the saliva of SZ patients, which may be related to their compromised oral hygiene.[14] This findings suggests that poor oral health in SZ patients could contribute to alterations in oral microbiome that may influence neuroinflammatory pathways.[31]

We also observed an increase in the relative abundance of *Porphyromonas* in the saliva of SZ patients. According to our oral health assessments, SZ patients generally exhibited poorer periodontal condition compared to healthy individuals. *Porphyromonas gingivalis* (*P. gingivalis*) is a key pathogen associated with periodontitis, and previous studies have shown that *P. gingivalis* and/or its product gingivalis proteinase can be transferred to the brain.[32,33] Another case-control autopsy study reported that lipopolysaccharides (LPS) from periodontal pathogens could be detected in the brains of Alzheimer's disease patients, and the serum levels of antibodies of tumor necrosis factor, IgG and *P. gingivalis* were significantly elevated in these patients compared to controls.[33,34] Cunha et al[35] also found that bipolar disorder was associated with an increased risk of periodontitis and a higher bacterial load, including gingival *Actinomyces* and *Porphyromonas*. The prevalence of periodontitis and the bacterial load of *Actinomyces aggregatus* and *P. gingivalis* were notably higher during depressive periods than during manic or euthymic periods.

An elevated abundance of *Neisseria* was also observed in SZ patients. Previous research has shown that prenatal exposure to the Gram-negative bacterium *Neisseria gonorrhoeae* (NG) during the first trimester was associated with an increased risk of SZ in offspring.[36] In addition, studies have confirmed a link between vaginal bacteria and gingivitis, suggesting that the poor oral environment of SZ patients may contribute to the increased abundance of *Neisseri*a.[37,38]

The *Bacillus*/*Bacteroides* ratio is often cited as a marker of intestinal flora imbalance which is often associated with disease states.[39] The *Bacillus*/*Bacteroides* ratio was higher in the healthy controls than that in patients with breast cancer.[40] Notably, our study found that the *Bacillus*/*Bacteroides* ratio was lower in SZ patients compared to healthy controls, consistent with previous studies.[41]

Recent studies has highlighted the importance of genetic factors in SZ,[42] indicating that microbial alterations alone are unlikely to fully account for SZ-related behaviors. One potential explanation is the impact of altered salivary microbiota on metabolic processes. In our research, metabolomic sequencing revealed significant disruptions in the RNA profiles of microorganisms in SZ patients, along with notable differences in their salivary metabolic pathways compared to healthy controls. Consistent with previous studies highlighting differences in gut microbiota between SZ patients and healthy individuals,[43] our findings also identified significant changes in metabolites associated with β-alanine metabolism in the saliva of SZ patients. These results offer valuable insights into the metabolic dysregulation associated with SZ. β-alanine can be rapidly transported to the brain and accumulate in neuronal cells in various mammals, including humans. It has been shown to significantly increase carnosine levels in different regions of the rat brain, and thus improve cognitive function. Previous research reported that β-alanine level in the stool of SZ patients differed significantly from that of healthy individuals.[41] Notably, our study identified increased transcripts of "GABAergic synapse" and "Alanine, aspartate and glutamate metabolism" pathways in the salivary microbiota of SZ patients. Additionally, increased levels of citric acid, L-malic acid, and oxoglutaric acid were detected, suggesting an upregulation of the tricarboxylic acid (TCA) cycle. These findings align with previous studies,[44] providing additional evidence of metabolic disruptions in SZ.

Isovaleric acid, a short-chain fatty acid (SCFA) involved in leucine metabolism, has been reported to be elevated in the gut microbiome in patients with SZ,[40] which consists with our observations in the oral microbiome It was reported that leucine and GABA were highly expressed in SZ patients and exhibited a strong correlation. Interestingly, GABA levels in the cerebrospinal fluid have been found to vary in individuals undergoing long-term treatment. Our results revealed that GABA expression is significantly elevated in SZ patients. This correlation between GABA and isovaleric acid further supports the role of GABA in enhancing the production of SCFA.[44,45]

Methionine (Met) oxidation has been linked not only to normal aging processes, but also to mechanisms associated with Alzheimer's disease.[46,47] Notably, the oxidation of Met residues in key proteins is significantly increased in the hippocampus of aged mice.[48,49] Met interacts with the antioxidant system through methionine sulfoxide (MetO) reductase, an enzyme that reduces MetO back to Met.[50] Genetic studies have demonstrated an association between polymorphisms in oxidative stress-related genes and SZ.[51] Additionally, L-methionine has been shown to induce SZ-like behavior in adolescent mice.[52] Our findings indicated that both L-Met and L-MetO levels in the saliva of SZ patients were higher than those in HCs. The Bacillus/Bacteroides ratio, a recognized indicator of intestinal flora imbalance[41] has been associated with oxidative stress caused by dysregulated gut microbiota. Interestingly, we observed a negative correlation between the *Bacillus*/*Bacteroides* ratio and L-MetO levels, though no such relationship was found with L-Met. This suggests a potential link between oral microbiota dysregulation and oxidative stress. Furthermore, we identified a strong positive correlation between tyramine and L-MetO levels, consistent with previous studies which reported elevated tyramine levels in the urine of SZ patients.[53] These findings suggest that the oral microbiota may contribute to SZ pathogenesis by influencing oxidative stress, particularly through the regulation of L-MetO.[54]

This study has several limitations. The sample size in this study is relatively small, which may increase the risk of false-positive results from multiple testing. Thus, we employed appropriate statistical methods to account for small sample sizes, ensuring the validity and reliability of our analysis. Another limitation is that the participants in the SZ group were hospitalized SZ patients undergoing long-term pharmacological treatment, which may limit the generalizability of the results to other stages of the disorder. While our study provides evidence of significant differences in oral microbiota between schizophrenia patients and healthy controls, it is not sufficient to establish a causal relationship. Furthermore, the study did not account for potential influences from genetic variations or clinical symptoms, both of which could have impacted the observed outcomes.

Conclusions

In conclusion, this study provides novel insights into the association between salivary microbiota dysbiosis and SZ. It identifies specific microbial alterations potentially contributing to SZ pathophysiology and highlights the relevant

metabolic pathways involved. These findings suggest that salivary microbiota dysbiosis plays a crucial role in the development of SZ, which may provide new strategies for the prevention, diagnosis, and treatment of the disease. Further studies involving larger cohorts are necessary to validate these findings and to explore the underlying mechanisms.

Disclosure

The authors report no conflicts of interest in this work.

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