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Analysis of Intestinal Microbiota in Schizophrenic Patients with Type 2 Diabetes Mellitus

ABSTRACT

Objective: Our goal is to examine the correlation between gut microbiota and the cooccurrence of schizophrenia and type 2 diabetes.

Methods: We conducted a study on the intestinal microbiota of 4 distinct groups: simple schizophrenia group (SC), schizophrenia with type 2 diabetes group (TS), type 2 diabetes group (T2DM), and normal population control group (HC), comprising a total of 35 subjects.

Results: The bacteria phyla *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria,* and *Verrucobacteria* were consistently present across all 4 groups. Significantly higher intestinal microbiota richness was observed in the T2DM compared to the other group, and the intestinal microbiota richness in TS significantly lower than that of the SC.

Conclusion: Our study suggests that the presence of type 2 diabetes in individuals with schizophrenia may affect the composition of their gut microbiota. We hypothesize that the concurrent existence of both diseases could potentially lead to alterations in the structure of gut microbiota, potentially influencing treatment effectiveness and outcomes.

Keywords: Schizophrenia, type 2 diabetes, intestinal microbiota

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Introduction

Schizophrenia is a severe mental disorder characterized by thinking, emotion, cognition, and behavioral dysfunctions. Its global incidence is high, affecting 1% of the population, and it poses a significant public health concern due to its high recurrence and disability rates.[1] The exact cause of schizophrenia is unknown but is believed to involve genetic, environmental, developmental, and immune factors.[2,3] Unfortunately, treatment options for schizophrenia are limited.

Type 2 diabetes (T2D), a prevalent metabolic disease characterized by insulin resistance, often coexists with schizophrenia.[4] Individuals diagnosed with schizophrenia have a prevalence of T2D that is 2-4 times greater than that observed in the general population. Both schizophrenia and type 2 diabetes are polygenic disorders, and there are connections between the 2 diseases.[5] Despite having few common clinical symptoms, the deletion of the Phosphate and Tension homology deleted on chromosome ten (PTEN) gene has been observed in patients with both schizophrenia and T2D,[6,7] suggesting the presence of shared pathogenic genes between T2D and schizophrenia.

The human digestive system hosts a varied population of microorganisms, collectively referred to as the intestinal microbiota, forming a intricate micro-environment.[8] With advancements in sequencing technology, researchers now have a deeper understanding of these microorganisms. It is now known that the central nervous system influences intestinal function, and in turn, the gut microbiota can impact the central nervous system.[9] Studies indicate that gut microbes regulate neurotrophic factors in the brain through pathways such as the hypothalamic-pituitary-adrenal pathway.[10] Furthermore, extensive research has been conducted on the connection between gut microbiota and T2D, exploring hypotheses

like the short-chain fatty acid theory, bile acid theory, and immune inflammation theory.[11,12]

In research related to diabetes and the gut microbiota, scientists have identified specific bacteria such as *Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia,* and *Roseburia* were negatively associated with T2D. Conversely, the genera of *Ruminococcus, Fusobacterium*, and *Blautia* were positively associated with T2D.[13] Most studies suggest that *Bacteroides* and *Bifidobacterium* may have beneficial effects for individuals with diabetes.[14] There is ample evidence indicating the substantial impact of the gut microbiota on diabetes. Yet, the precise mechanisms through which the gut microbiota affects glucose metabolism remain uncertain. Many studies propose that the gut microbiota might impact the expression of relevant genes in the intestine through microbial metabolites, subsequently influencing nutrient absorption and inflammation, which in turn affects glucose metabolism.[15,16]

In studies related to schizophrenia and the gut microbiota, it is suggested that the gut microbiota might influence schizophrenia by impacting substances related to neural function, including Gamma-aminobutyric acid (GABA) and neurotrophic factors. Specific bacteria, like *Lactobacillus* and *Bifidobacterium*, can produce GABA through the vagus nerve, leading to increased activity of GABAergic neurons in the brain.[17] Changes in the activity of GABAergic neurons play a significant role in schizophrenia.[18] The gut microbiota can also regulate the expression of various neurotrophic factors, such as Brain-Derived Neurotrophic Factor (BDNF), synapsin, and Post-Synaptic Density Protein 95 (PSD-95), which influence neural development and brain plasticity.[19,20] The neuro-immuno-endocrine-metabolic pathways connect the gastrointestinal microbiota, the gastrointestinal system, and the central nervous system, mutually regulating to maintain the body's balance. This connection is known as the "microbiota–gut–brain axis."[21]

From the above research, it is evident that Bifidobacterium demonstrates positive effects in both diabetes and schizophrenia.[22,23] The immune system plays a role in the processes of schizophrenia and diabetes influenced by the gut microbiota.[15,24,25] The potential connection between schizophrenia and diabetes might be related to the gut microbiota. Studying the gut microbiota could lead to the development of more suitable treatment strategies for individuals with both schizophrenia and diabetes. This study aims to explore the relationship between schizophrenia and T2D by analyzing the gut microbiota of patients with schizophrenia, patients with T2D, patients with both schizophrenia and T2D, and normal individuals. The PacBio platform, a high-throughput sequencing technology, was utilized for this investigation. The results of this study could provide a scientific basis for further understanding the gut microbiota in patients with both schizophrenia and T2D and provide a research foundation for developing more suitable treatment strategies for comorbid patients with schizophrenia and diabetes.

MAIN POINTS

- *The gut microbiota composition in patients with both T2DM and schizophrenia differs significantly from other groups.*
- *Reduced microbial diversity is observed in patients with both T2DM and schizophrenia compared to healthy controls.*
- *Specific bacterial taxa exhibit significant alterations in patients with both T2DM and schizophrenia, suggesting a potential link between gut microbiota composition and the comorbidity of these conditions.*
- *Changes in the gut microbiota may contribute to the pathophysiology of schizophrenia and its comorbidity with T2DM, necessitating further investigation into underlying mechanisms and potential therapeutic interventions targeting the gut microbiota.*
- *Understanding the role of the gut microbiota in schizophrenia and T2DM could pave the way for novel diagnostic and therapeutic approaches, potentially involving microbiota modulation to manage or prevent these diseases.*

Material and Methods

The Inclusion and Exclusion Criteria of Studies (sample information is presented in Supplementary Table 1):

The intestinal microbiota of different groups such as simple schizophrenia group (SC) 12 samples, TS 7 samples, type 2 diabetes group (T2DM) 9 samples, and normal population control group (HC) 7 samples, consisting of a total of 35 subjects were included in the study. This study adheres to the Declaration of Helsinki and has been approved by the Dali University Medical Ethics Committee (Approval Number MECDU-202105-17, Date 2021-05-14). Informed consent forms were signed by all volunteers or their family members.

Simple Schizophrenia Group, inclusion criteria: ages 18-65; no gender restrictions; schizophrenia diagnosis is determined according to the criteria outlined in the International Classification of Diseases, 10th edition (ICD-10). The diagnosis of schizophrenia is established using the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Patients or their family members are informed about the study and have provided signed informed consent forms. Exclusion criteria include individuals without a diagnosis of schizophrenia, those aged over 65, individuals diagnosed with T2D based on the criteria set by the American Diabetes Association (2003 version), recent use of antibiotics or probiotics within the past month, and patients with other neurological or psychiatric disorders or a significant medical history. TS, schizophrenia with type 2 diabetes group (TS), inclusion criteria: ages 18-65; no gender restrictions; diagnosis of schizophrenia based on the International Classification of Diseases, 10th edition (ICD-10) criteria; diagnosis of schizophrenia based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria; diagnosis of T2D based on the American Diabetes Association (ADA) criteria (2003 version); patients themselves or their family members are aware of this study and have signed informed consent forms. Exclusion criteria: age over 65; recent use of antibiotics or probiotics within the past month; patients with other neurological or psychiatric disorders or significant medical history.

Simple Schizophrenia Group, inclusion criteria: ages 18-65; no gender restrictions; diagnosis of T2D based on the ADA criteria (2003 version); patients themselves or their family members are aware of this study and have signed informed consent forms. Exclusion criteria: Age over 65; diagnosis of schizophrenia based on the International Classification of Diseases, 10th edition (ICD-10) criteria; diagnosis of schizophrenia based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria; recent use of

antibiotics or probiotics within the past month; patients with other neurological or psychiatric disorders or significant medical history.

Normal Population Control Group, inclusion criteria: ages 18-65; no gender restrictions; participants themselves are aware of this study and have signed informed consent forms. Exclusion criteria: age over 65; diagnosis of schizophrenia based on the International Classification of Diseases, 10th edition (ICD-10) criteria; diagnosis of schizophrenia based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria; diagnosis of T2D based on the ADA criteria (2003 version); recent use of antibiotics or probiotics within the past month; Presence of other neurological or psychiatric disorders or significant medical history.

Samples Isolation

Distributing fecal sampling boxes to all subjects, ask medical staff to assist in sampling, and instructing patients or families to collect as much fresh fecal as possible in the morning for subsequent processing. After obtaining the fecal samples, send them to laboratory for processing as soon as possible. If the samples can't be processed immediately, they should be placed in incubator with ice bag for temporary storage, the storage time should not exceed 2 h. Samples should be sub-packaged on sterile test bench, and the central part of fecal should be picked with sterile cotton swab. First, observe the color and texture of the samples, then pick about 2 g of material. Place it in frozen storage tube, sealing the nozzle with sealing film. Each sample is divided into 2 cryopreservation tubes, 1 for testing sequence and another for preservation. Before collecting all the samples, those collected previously should be stored in refrigerator at minus 80 degrees. All samples were obtained through patient donations.

Fecal Samples DNA Extraction and DNA Purification

Total genome DNA from samples was extracted using CTAB (Cetyltrimethylammonium Bromide)/SDS (Sodium Dodecyl Sulfate) Method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water.

Primer: 27F (5′-AGRGTTYGATYMTGGCTCAG-3′), 1492R (5′-RGYTAC CTTGTTACGACTT-3′). The 16S rRNA genes were amplified using specific primers with barcodes attached. PCR reactions were conducted in 30 μL volumes, comprising 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and approximately 10 ng of template DNA. Thermal cycling involved an initial denaturation step at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 60 s, with a final extension at 72°C for 5 min.

Amplicons were then extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following the manufacturer's protocol. SMRTbell libraries were subsequently prepared from the amplified DNA via blunt-ligation, adhering to the manufacturer's instructions (Pacific Biosciences). The purified SMRTbell libraries from both the Zymo and HMP mock communities were sequenced on dedicated PacBio Sequel II 8M cells utilizing Sequencing Kit 2.0 chemistry. Additionally, the purified SMRTbell libraries from pooled and barcoded samples were sequenced on a single PacBio Sequel II cell. All amplicon sequencing procedures were conducted by Shanghai Origin-gene Biotechnology Co. Ltd (Shanghai, China).

Sequencing Data Analysis

OTU Clustering and Species Annotation: The PacBio raw reads underwent processing with SMRT Link Analysis software version 9.0 (PacBio; Pacific Biosciences of California, Inc. USA) to yield demultiplexed circular consensus sequence (CCS) reads, applying the following parameters: a minimum number of passes of 3 and a minimum predicted accuracy of 0.99. Subsequently, the raw reads were subjected to filtering via SMRT Portal, where sequences were screened based on length (excluding those below 800 bp or above 2500 bp) and quality. Further refinement involved the removal of barcode and primer sequences, chimeras, as well as sequences containing 10 consecutive identical bases. Sequences were analyzed using the UPARSE software package with UPARSE-OTU and UPARSE-OTUref algorithms. Alpha and beta diversity were analyzed using in-house Perl scripts. Sequences with ≥97% similarity were grouped into the same OTUs. Representative sequences for each OTU were annotated using the RDP classifier for taxonomic information. Alpha Diversity metrics, including Chao1, Observed Species, and Shannon index, were calculated after rarifying the OTU table. Rarefaction curves were generated based on these metrics.

Phylogenetic Distance and Community Distribution

The Krona chart visually represented the relative abundance of bacterial diversity from phylum to species. Principal Component Analysis (PCA) was conducted using the prcomp function from the stats package in the R programming language, PCA was employed to reduce the dimensionality of original variables prior to cluster analysis. The QIIME software package was employed to calculate both weighted and unweighted unifrac distances, which serve as phylogenetic metrics of beta diversity. Specifically, unweighted unifrac distance was utilized for Principal Coordinate Analysis (PCoA) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Clustering.

Principal Coordinate Analysis transformed the distance matrix into orthogonal axes, highlighting maximum variation factors along principal coordinates. UPGMA Clustering, utilizing average linkage, was employed for hierarchical clustering based on the distance matrix.

Statistical Analysis

Metastats software confirmed differences in individual taxonomy abundances between the 2 groups. LEfSe quantitatively analyzed biomarkers within groups, addressing data where species outnumber samples. This method provided biological class explanations, establishing statistical significance, biological consistency, and effect-size estimation of predicted biomarkers. Differences in microbial communities between groups were assessed using ANOSIM and MRPP based on Bray-Curtis dissimilarity distance matrices. Descriptive statistics including Mean, Median, Standard Deviation and parametric statistics including One-way ANOVA was used on Data statistics. statistical graphs are created using GraphPad v8.0 (GraphPad Software, Inc. California, USA). The significance level was set at $P < .05$.

Results

Type 2 Diabetes has an Impact on the Gut Microbiota Distribution in Individuals with Schizophrenia

Sequencing data quality assessment and Species composition analysis on are presented in Supplementary Figure 1 and Supplementary Table 1. High-throughput sequencing detected

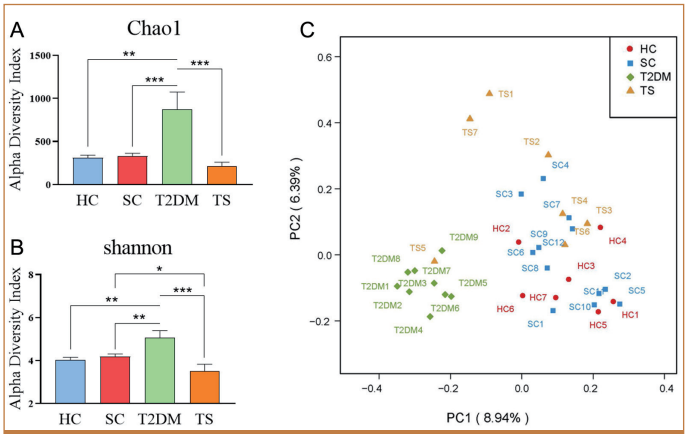

Figure 1. Alpha and beta diversity analysis. (a, b) Alpha diversity analysis; (c) PCoA analysis.

689999 sequences from 35 samples across four groups. Alpha diversity, which measures species richness and diversity, was assessed in the study. The Chao1 richness estimator of Alpha diversity index showed differences between groups ($P = .0005$) (Figure 1a, Table 1), The T2DM was significantly higher compared to the SC ($P < .001$), TS ($P = .0005$), and HC ($P = .001$). The Shannon-wiener diversity index of the Alpha diversity index also showed different between groups ($P = .001$) (Figure 1b, Table 1). The T2DM group showed significantly higher diversity than the HC group ($P = .006$), TS group ($P = .0001$) and the SC group ($P = .008$). Moreover, the diversity of the SC group significantly higher than that of the TS group ($P = .047$). These results indicate that the T2D changed the alpha diversity index of the gut microbiota, and the concurrent presence of diabetes may affect the alpha diversity index of the gut microbiota in schizophrenia patients.

One-way ANOVA Test was Used for Statistical Analysis

Principal Co-ordinates Analysis based on other distances other than Euclidean distance, through dimensionality reduction to find out potential principal components that affect difference in sample community composition. The results showed that PC1 accounted for 8.94% of the analysis, while PC2 accounted for 6.39%, PC3 accounted for 5.73%. The distribution on the PC1 axis indicated that the T2DM exhibited outliers and was the most scattered, whereas the groups with schizophrenia and type 2 diabetes (TS) showed less scatter but more than the SC and the HC (Figure 1c). This suggests that the structure and diversity of the intestinal microbiota differed among the four groups, indicating that T2D has an impact on the intestinal microbiota of patients with schizophrenia. Similar results were observed in Principal Component Analysis and Non-Metric Multidimensional Scaling (Supplementary Figure 2).

Type 2 Diabetes has an Impact on the Dominant Species in the Gut Microbiota of Individuals with Schizophrenia

The taxonomy annotation of fecal samples from the four groups revealed that *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria,* and *Verrucomicrobia* were the dominant phyla across all groups. *Tenericutes, Chloroflexi*, and others phylum made up a small portion of the sequences (Figure 2a). At the genus level, *Bacteroides, Blautia, Escherichia-Shigella*, and *Bifidobacterium* were the most abundant, representing the dominant species in the intestinal microbiota of all groups. Other notable genera included *[Eubacterium]_hallii_group, Faecalibacterium, Prebotella_9, Subdoligranulum* and *Lachnoclostridium*. The relative abundance of each genus for each sample is shown in Figures 2b.

Comparing SC (schizophrenia) with TS (schizophrenia with T2D), the content of *Bacteroides* increased (24.43% vs. 12.13%), while the content of *Blautia* (3.26% vs. 7.44%), *Shigella coli* (4.69% vs. 9.75%), and *Bifidobacterium* (3.91% vs. 8.56%) decreased. Comparing SC with T2DM (T2D), the content of *Bacteroides* increased (24.44% vs. 7.41%), as did *Shigella coli* (4.69% vs. 3.47%) while *Blautia* (3.26% vs. 8.51%) decreased. Comparing SC with HC (normal control), the content of *Bacteroides* increased (24.43% vs. 20.01%), as did *Shigella coli* (4.69% vs. 2.01%) and *Bifidobacterium* (3.91% vs. 2.38%), *Blautia* (3.26% vs. 2.60%). Comparing TS with T2DM, the content of *Bacteroides* increased (12.13% vs. 7.41%), as did *Shigella coli* (9.75% vs. 3.47%) and *Bifidobacterium* (8.56% vs. 4.46%), while *Blautia* (7.44% vs. 8.51%) decreased. Comparing TS with HC, the content of *Bacteroides* decreased (12.13% vs. 20.01%), while *Shigella coli* (9.75% vs. 20.09%), *Bifidobacterium* (8.56% vs. 2.38%), and *Blautia* (7.44% vs. 2.60%) increased. Comparing T2DM with HC, the content of *Bacteroides* decreased (7.41% vs. 20.01%), as did *Shigella coli* (3.47% vs. 2.01%) and *Bifidobacterium* (4.46% vs. 2.38%), while *Blautia* (8.51% vs. 2.60%) increased. These comparisons are shown in Figures 2b. The class classification level Results were shown in (Supplementary Figure 2).

Type 2 Diabetes has an Impact on the Species Diversity in the Gut Microbiota of Individuals with Schizophrenia

LEfSe (LDA Effect Size) results indicate differences at the genus level among the four groups. Specifically, SC shows significant differences in *Bacteroidales, Bacteroidia, Bacteroidetes, Bacteroidaceae, Bacteroides, Porphyromonadaceae, Rikenellaceae, Alistipes, Parabacteroides, Peptostreptococcaceae_g_unclassified, Lachnospiraceae_NK4A136_group, Desulfovibrionales, Desulfovibrionaceae*. TS exhibits significant differences in *Morganella*. While, T2DM shows significant

Table 1. Alpha Diversity Indices and Intergroup Comparison

Alpha Diversity Index	Mean ± SD (HC)	Mean ± SD (SC)	Mean ± SD (T2DM)	Mean ± SD (TS)	*P* Between Columns	*P* HC/SC	*P* HC/T2DM	*P* HC/TS	*P* SC/T2DM	*P* SC/TS	*P* T2DM/TS
Chao1	310.1 ± 82.53	333.2 ± 104.7	875.8 ± 596.1	215.9 ± 115.9	.0005	.879	.001	.58	.0005	.44	.0002
Ace	309 ± 75.88	324.4 ± 94.32	886.9 ± 589.4	213.1 ± 105	.0003	.917	.0008	.567	.0003	.456	.0002
Simpson	0.038 ± 0.015	0.035 ± 0.019	0.039 ± 0.022	0.072 ± 0.070	.157	.837	.959	.083	.779	.034	.075
Shannon	4.021 ± 0.346	4.188 ± 0.420	5.058 ± 1.002	3.499 ± 0.869	.001	.62	.006	.173	.008	.047	.0001
Coverage	0.997 ± 0.001	0.997 ± 0.002	0.992 ± 0.006	0.972 ± 0.070	.347	.982	.747	.134	.731	.098	.201

HC, normal population control croup; SC, simple schizophrenia group; TS, schizophrenia with type 2 diabetes group; T2DM, type 2 diabetes group.

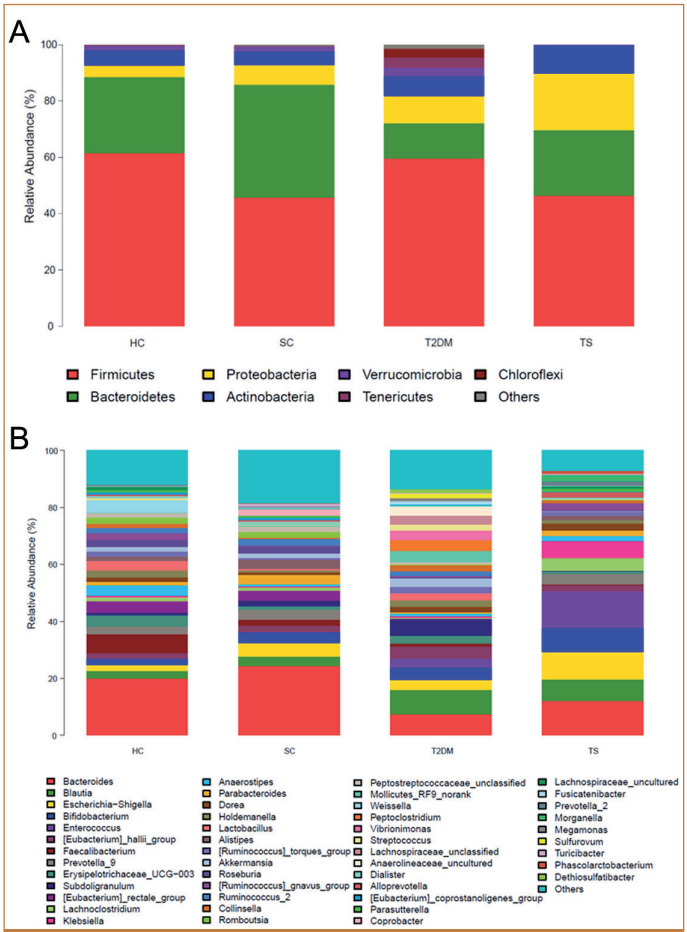

Figure 2. Species abundance analysis.

differences, including *Subdoligranulum, Peptostreptococcaceae, Sphingobacteriales, Sphingobacteriia, Chitinophagaceae, Vibrionimonas, Lachnospiraceae_g_unclassified, Comamonadaceae, Chitinophagaceae_g_unclassified, Phyllobacterium, Phyllobacteriaceae, Pseudorhodoferax, Alphaproteobacteria, Rhizobiales, Clostridiales_g_unclassified, Clostridiales_f_unclassified, Bradyrhizobiaceae, Bradyrhizobium*. Additionally, HC displays significant differences in *Firmicutes, Ruminococcaceae, Weissella, Leuconostocaceae, _Eubacterium__rectale_group,Romboutsia,Ruminococcaceae_UCG_013,Coriobacteriaceae_g_uncultured, _Eubacterium__ventriosum_group*. These findings are illustrated in Figure 3.

Discussion

This study analyzed and compared the gut microbiota structure of four subject groups. The core bacterial phyla, including *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria*, and *Verrucomicrobia*, were common among all groups. The dominant bacterial genera *Bacteroides, Blautia, Escherichia-Shigella*, and *Bifidobacterium*.

The richness and diversity of gut microbiota varied among the groups, with T2DM having the highest classification levels. Schizophrenia with type 2 diabetes had the lowest richness, possibly due to the combination of the 2 diseases. Alpha diversity analysis showed no significant differences in species richness between SC and the HC, and between TS and the control group. However, SC and T2D differed significantly in terms of species diversity. Beta diversity analysis

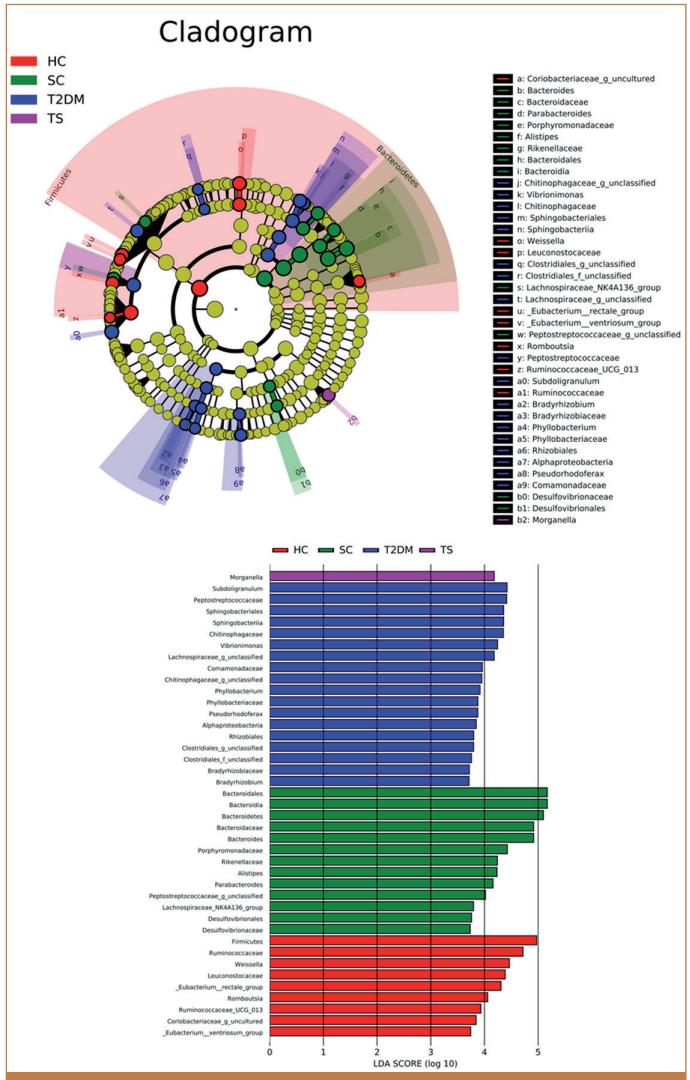

Figure 3. Species difference analysis. LDA value distribution histogram and evolutionary branch diagram of HC, SC, T2DM, and TS.

indicated distinct differences in gut microbiota distribution between the groups, with T2D being isolated from the other 3 groups. This suggests that T2D affects the distribution and diversity of gut microbiota in patients with schizophrenia. Previous studies have shown that schizophrenia patients with impaired glucose tolerance have disrupted and reduced gut microbiota diversity due to the coexistence of the 2 diseases.[26]

According to results of LEfSe analysis, the iconic species of intestinal microbiota in the SC may be the genus *Bacteroidales*. This being been found to be associated with neurodegenerative diseases, including Huntington's disease.[27] For TS, the iconic species of the intestine may be *Morganella*, *Morganella morganii* is a Gram-negative, opportunistic pathogen that can cause a variety of infections, including bloodstream infections,[28] Moreover, *Morganella* was related on neurological disease like depression,[29] which is a probiotic crucial for human health. Iconic species of intestinal microbiota in group of T2D may be *Subdoligranulum*, which is correlated positively with microbial richness and HDL cholesterol levels and negatively correlated with fat

mass, adipocyte diameter, insulin resistance, levels of leptin, insulin, CRP, and IL6 in humans,[30] and it also related on depression.[31] For the HC, the iconic species of intestinal microbiota may be *Firmicutes*, which is correlated with obesity[32] and age[33] In adulthood, the *Firmicutes* ratio is higher compared to that in early childhood and old age.[33]

This study compared the gut microbiota of patients with schizophrenia, T2D, both conditions, and a control group. The findings revealed differences in the distribution and diversity of gut microbiota among the groups. Type 2 diabetes influenced the gut microbiota of patients with schizophrenia, reducing beneficial bacteria compared to the control group. Comparing patients with T2D to those with schizophrenia, there were significant differences in microbiota structure and diversity, with the latter group exhibiting more diverse species. However, patients with schizophrenia and T2D had relatively few beneficial gut bacteria. In comparison, the T2DM had significantly different species with a higher abundance of beneficial gut bacteria. Iconic species analysis identified *Bacteroidales, Morganella, Subdoligranulum,* and *firmicutes* as representative species for each group, highlighting their potential roles in metabolism infections, insulin resistance, blood glucose regulation, and neurological disease. The study suggests that the coexistence of schizophrenia and T2D can alter gut microbiota structure, potentially impacting treatment outcomes. Our study indicates that the comorbidity of schizophrenia and diabetes further reduces the diversity of gut microbiota. This finding suggests that supplementing probiotics may be even more crucial for schizophrenia patients with comorbid diabetes

In this study, we found that comorbidity of schizophrenia and T2D affects the structure of the gut microbiota. The coexistence of diabetes and schizophrenia also impacts the gut microbiota structure, leading to differences in the diversity of gut microbiota between patients with comorbid conditions and those with either diabetes or schizophrenia alone. However, based on principal component analysis and PCoA analysis, the gut microbiota structure of patients with both conditions is closer to that of schizophrenia patients. Additionally, there were significant differences in the gut microbiota between schizophrenia and T2D patients. Therefore, this study does not provide an explanation for why individuals with schizophrenia are more prone to developing diabetes, *Subdoligranulum* (the iconic species of T2DM) correlated with both insulin resistance[30] and depression,[31] give us same suggestions on individuals with T2D may more prone to developing mental disease. Based on our results, it appears that changes in the gut microbiota may not be the sole reason why schizophrenia patients are more prone to developing T2D. These differences could be due to the small sample size.

Data Availability Statement: *Other relevant data and patient information are presented in the supplementary materials, The OUT data was deposited in Mendeley Data, 10.17632/8kn7kjvgrm.1, we deposit the Raw data in SRA database http://www.ncbi.nlm.nih.gov/bioproject/1073246.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Dali University (Approval No: MECDU-202105-17, Date: 2021-05-14).*

Informed Consent: *Informed consent was obtained from the patients who agreed to take part in the study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept – W.N., L.L., G.Z.; Design – W.N., L.L., G.Z.; Supervision – L.L.; Resources – L.L., G.Z.; Materials – W.N., Y.C., F.R., J.R., Z.L., W.W., Y.H., Y.Y.; Data Collection and/or Processing – W.N., Y.C., F.R., J.R., Z.L., W.W., Y.H., Y.Y.; Analysis and/or Interpretation – W.N., Y.C., L.L., G.Z.; Literature Search – W.N., Y.C., L.L., G.Z.; Writing Manuscript – W.N., Y.C., F.R., J.R., Z.L., W.W., Y.H., Y.Y., L.L., G.Z.*

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Declaration of Interests: *The authors have no conflict of interest to declare.*

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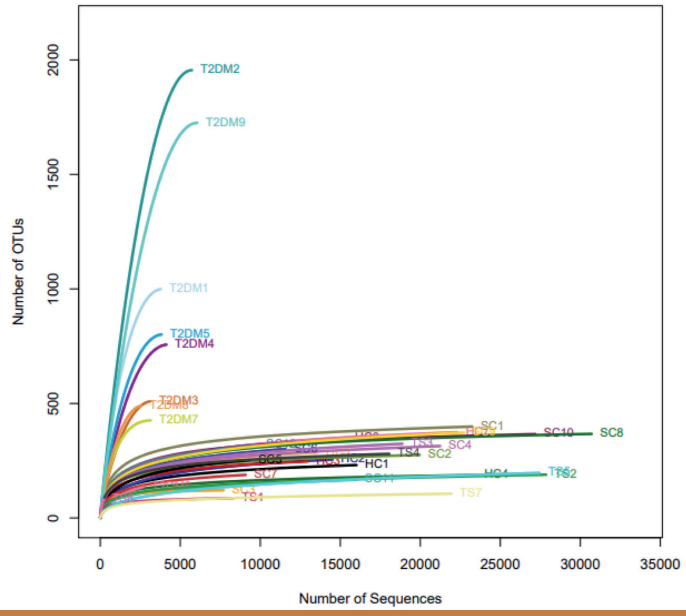
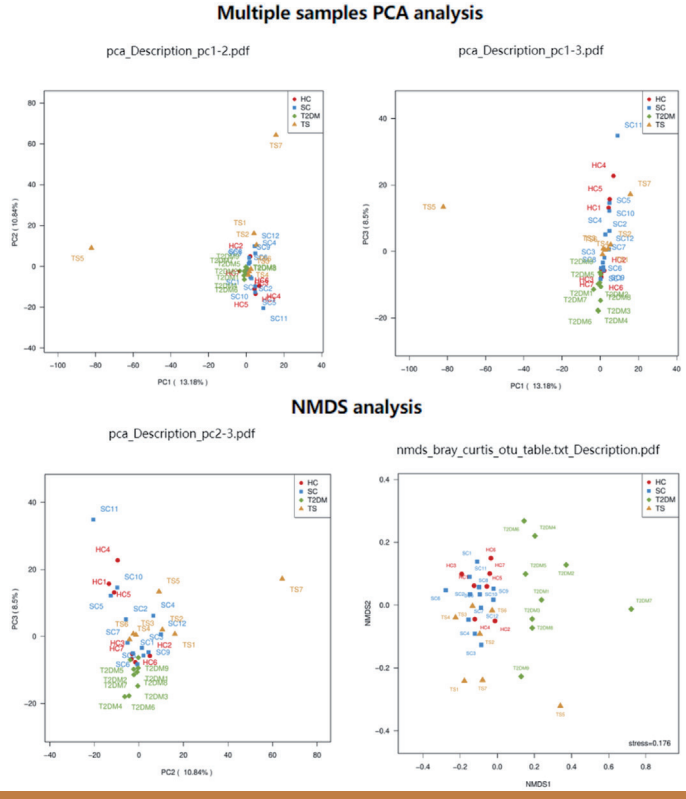
Supplementary Table 1. Basic Information of Each Group, and Sequence Counts for Each Sample

			Sequencing Result in Different Samples						
				0.97					
Sample ID	**Gender**	**Age**	**Reads**	**OTU**	**Ace**	**Chao**	**Coverage**	**Shannon**	**Simpson**
HC									
HC1	male	52	16018	231	259	258	0.99769	3.88	0.037
HC2	male	57	14510	255	291	290	0.996761	3.6	0.0559
HC3	female	52	12969	247	271	270	0.997224	4.32	0.0233
HC4	female	58	23472	187	203	195	0.999105	3.54	0.0615
HC5	female	57	13581	286	328	325	0.996539	4.28	0.0338
HC6	male	65	15396	350	400	413	0.995843	4.18	0.0319
HC7	female	50	22301	374	411	420	0.997444	4.35	0.0248
SC									
SC1	male	51	23233	399	433	454	0.997805	4.87	0.0138
SC2	female	64	19957	275	299	301	0.998246	4.42	0.0211
SC3	male	57	7696	121	128	129	0.998701	4.16	0.0205
SC4	female	50	21229	314	356	385	0.997409	4.21	0.0322
SC5	female	35	9351	253	296	304	0.994118	3.95	0.0361
SC6	female	19	11576	301	356	397	0.994471	4.02	0.0555
SC7	female	30	9092	188	213	209	0.99637	3.81	0.0444
SC8	female	52	30704	367	390	393	0.998665	4.37	0.0285
SC9	female	47	22734	373	434	450	0.996789	4.41	0.0251
SC10	female	64	27190	366	394	405	0.998382	4.46	0.0249
SC11	female	63	15997	167	230	204	0.997625	3.19	0.0848
SC12	female	25	9823	317	364	367	0.993994	4.39	0.0311
T2DM									
T2DM1	female	60	3786	999	1013	1000	0.990755	5.96	0.0138
T2DM2	male	32	5722	1955	1955	1955	1	6.41	0.0209
T2DM3	male	30	3143	510	526	512	0.988228	4.57	0.0418
T2DM4	female	57	4134	757	773	758	0.990808	4.65	0.0629
T2DM5	female	44	3833	801	812	802	0.993478	5.16	0.0405
T2DM6	male	42	2694	135	245	213	0.980698	3.2	0.0806
T2DM7	female	45	3145	426	428	426	0.997774	4.54	0.0487
T2DM8	male	39	2555	490	504	491	0.987867	4.87	0.0287
T2DM9	male	52	6058	1725	1726	1725	0.999505	6.16	0.0152
TS									
TS1	male	60	8325	87	96	95	0.998679	3.54	0.0393
TS2	female	63	27858	189	221	220	0.998744	3.61	0.0431
TS3	female	50	18880	324	376	387	0.996822	4.21	0.0337
TS4	female	65	18057	280	306	340	0.997785	4.44	0.0209
TS5	female	68	27452	196	246	240	0.998179	2.28	0.1787
TS6	male	41	192	81	118	105	0.8125	4.06	0.0211
TS7	female	57	21946	106	129	124	0.999043	2.35	0.1673



Supplementary Figure 1. Rarefaction curves, X-axis: randomly sampled sequencing data volume; Y-axis: observed OTU quantity.



Supplementary Figure 2. Results of PCA analysis and NMDS analysis.