The Changes of Gut Microbiota in Drug-Naïve First-Episode MDD Patients Under Treatment by SSRIs

CURRENT STATUS: Under Review

BMC Psychiatry BMC Series

Yang Shen, Xiao Yang, Gaofei Li, Xiaoxi Xing, Zhiyong Li, Yichen Huang, Jiayu Gao, Liang Ying

Yang Shen
Peking University Sixth Hospital

Xiao Yang
Henan University of Science and Technology

Gaofei Li
Beijing Hospital of Chinese Traditional and Western Medicine

Xiaoxi Xing
Peking University Sixth Hospital

Zhiyong Li
Peking University Sixth Hospital

Yichen Huang
Peking University Sixth Hospital

Jiayu Gao
Henan University of Science and Technology

Liang Ying
National Clinical Research Center for Mental Disorders,Peking University Sixth Hospital
liangying1980@bjmu.edu.cn***Corresponding Author***

Prescreen

10.21203/rs.3.rs-25341/v1

Subject Areas

Psychiatry

Keywords

Gut microbiota, Brain-gut axis, Major depressive disorder, 16S rRNA sequence, SSRIs

Abstract

Background Recently, gut microbiota has been found to play an important role during the development of MDD in mice models. Meanwhile, the antidepressant treatment with SSRIs could correct the abnormal performance in mice, and played a dual role of antibiotics. Therefore, this study aims to further explore whether the gut microbiota of MDD patients can be reshaped after receiving SSRI drugs.

Methods Thirty patients with drug-naive first-episode MDD and thirty healthy controls were recruited and their samples were collected to complete 16S rRNA sequencing. All patients received standardized treatment with ecitalopram. When the reduction rate of HAMD scale score ≥ 50%, their fecal samples were collected again to sequence as follows group. Bioinformatics analysis was used to understand the changes of gut microbiota before and after treatment.

Results A significant difference in gut microbiota abundance was found after treatment with escitalopram. The Firmicutes/Bacteroides ratio decreased significantly in the follows group. After treatment, the differences in the classification and metabolic pathways of gut microbiota still existed between follows group and controls. The mainly difference were found as follows: Transport and catabolism, Nervous system, Glycan biosynthesis and metabolism, etc.

Conclusions The study found that the gut microbiota of patients with first-episode MDD was similar, but was significantly different from controls. Under escitalopram treatment, the gut microbiota diversity of MDD patients tended to transform to normal. However, there were still some differences of structures and metabolic pathways in microbes between patients and controls, which might be related to the relapse of MDD.

Background

As a common mental disorder accompanied by high disability and suicide, major depressive disorder(MDD)has become a worldwide issue, thus making it become an international research hotspot.[1] Because of the deficient pathogenesis reported in literature, psychiatrists empirically treat patients through regulating morbific reduction of several neurotransmitters in clinic.[2] Serotonin, also known as 5-HT, is an acknowledged psychoactive agent to mediate the nervous excitation. Selective serotonin reuptake inhibitors（SSRIs）, the chemical analogues of 5-HT, have been reported to present great curative effects in the clinical treatment of MDD.[3] However, the slow effect and relapse, leading to long-term and reduplicated uses, create a severe disadvantage for patients and even build the barriers for SSRIs applications.[4, 5] Therefore, it suggests to further reveal the underlying mechanisms in details other than simply descriptions of decreased 5-HT in the occurrence of MDD.

In recent years, more and more evidences were reported to indicate that gut microbes might take part in the course of MDD.[6–8] In several paralleled researches, MDD patients showed specific features of gut microbes differ from normal controls.[9–12] Several studies have reported that transplantation of feces from MDD patients into rodents could cause depression-like behaviors, which had be used to establish animal models of MDD.[9, 13, 14] The similar phenomenon were observed in fecal bacteria transplantation between animal models. Moreover, it also observed that fecal bacteria transplantation could lead to the increase of microglial cell density and expression of IL-1 in the ventral hippocampus.[15] Taken together, the change of gut microbiota could confirmed to correlate with the occurrence of MDD.

Besides, several SSRI’s drugs, including sertraline, fluoxetine, paroxetine and escitalopram, could present antibacterial effects directly.[16, 17] For example, staphylococcus and enterococcus are especially vulnerable to sertraline, fluoxetine and paroxetine.[17–19] The effects could target on potential enterotoxigenic bacteria as well. Therefore, SSRIs have demonstrated both antidepressant and antimicrobial properties.[20] The mechanism underlying this interactive dual effects remains as an ongoing challenge.

Overall, SSRIs could directly or indirectly influence the changes of gut microbiota which might play the key role

in the development of MDD the purpose of this study was to further explore the changes of gut microbiota in MDD patients under SSRIs treatment. The current work conducted 16S rRNA gene sequencing analysis on the feces of patients with first episode MDD by SSRIs treatment to investigate into the changes and effects of gut microbiota.

2. Method

2.1 Participants

The subjects recruited in this study were from residents in Beijing, China. Age was between 18–65 years old, and body mass index(BMI) was between 18–28 Kg / m^2. The inclusion criteria for patients with depression described as follows: 1. the diagnosis of depression to be defined by two psychiatrists according to the Mini-International Neuropsychiatric Interview.[21] 2. No history of treatment with antipsychotic medication.3. Duration of symptoms was more than 1 month and less than 24 months. 4. No history of treatment with antidepressant and antipsychotic medication. 5. Currently in the acute episode with the HAMD score ≥ 24. Other mental disorders as axis I, personality disorder and mental retardation were excluded. Psychotropic drugs were never used. The control group requested the subjects and the first-degree relatives didn't have any history of mental disorder and psychotropic substance use. Their HAMD-17 score was < 7. According to the questionnaire, their life events that may affect the mood, such as examinations, unemployment, and bereavement in the last six months and the study period, were surveyed and recorded. In addition, a series of exclusion criteria developed in this study were based on the previous work to exclude the factors affecting gut microbiota.[22] Those include: 1. No somatic diseases known to affect the intestinal flora such as inflammatory bowel disease, immune system diseases, diabetes, etc.; 2. Without antibiotics, large doses of probiotics or biological agents used in recent 3 months. 3. Without the history of medical examination or surgery through the gastrointestinal tract in recent 6 months; 4. Without obvious changes in dietary habits or the presence of obvious diarrhea, constipation and other symptoms in recent 1 month.

All of subjects in this study were required to sign an informed consent. According to the Helsinki Declaration, the protocol for sample collection and analysis was approved by the Ethics Committee of Peking University Sixth Hospital and Beijing Hospital of Chinese Traditional and Western Medicine.

2.2 Sample Collection

Fecal samples were required after two groups of subjects were enrolled. Subjects were instructed by staff to discharge feces into a clean container. After defecation, the staff collected 2 g of fecal sample and quickly placed it into a container containing liquid nitrogen. The samples were then transferred to -80 ℃ refrigerator within 1 hour and stored in frozen.

2.3 Treatment

All patients with depression received standardized treatment with escitalopram. The starting dose of escitalopram was 5 mg / d from day 1- day 7 and increased to 10 mg / d from day 8. According to the individual response, the dose of escitalopram could then be adjusted, and the maximum dose was 20 mg / d. The psychiatrist followed the patients for 4–6 weeks until the HAMD reduction rate was $\geq 50\%$. Fecal sample was then collected for the second time.

2.4 16S rRNA Amplification of V3-V4 region and Illumina Sequencing

The experiments followed the manufacturer's instructions to use a PowerSoil DNA kit (MoBio, USA) to extract 200 mg fecal samples for DNA extraction. KAPA HiFi HotStart ReadyMix (KAPA, USA) was used to amplify the 16S rRNA (V3-V4) gene marker. Each DNA sample of the bacterial 16S rRNA gene was amplified with primers 341F (GGACTACHVGGGTWTCTAAT) and 805R (ACTCCTACGGGAGGCAGCAG). The primers include a unique 8-nucleotide barcode and an Illumina adapter. Polymerase chain reaction (PCR) conditions as follows: initial denaturation at 95℃ for 5 minutes, 98℃ denaturation for 20 cycles for 20 seconds, 58℃ annealing for 30 seconds, 72℃

extension for 30 seconds, and 72℃ final extension for 5 minutes. The amplicons obtained by PCR were analyzed on 1.5% agarose gel electrophoresis, and a band of a desired size was purified using a QIAquick gel extraction kit (QIAGEN, Germany). The product was submitted to the second-generation sequencing laboratory of Beijing institute of bioinformatics for sequencing on Illumina HiSeq 2500 platform.

2.5 Bioinformatics Analysis

The QIIME software was used to filter and sequence the original sequence to obtain optimized sequences (Tags).[23] Remove fragments that contain ambiguous characters in the sequence or that contain more than two nucleotide mismatched primers. Usearch software was used to cluster Tags at a similarity level of 97% to obtain OTUs.[24] OTUs were annotated based on the Silva (bacterial) and UNITE (fungi) taxonomy databases. QIIME software was used to generate species richness tables at different taxonomic levels, and R language tools were used to draw community structure maps at each taxonomic level of the sample. Obtain the community structure map of each sample at the level of taxonomy, class, order, family, genus, species.

In order to study the difference in microbial community richness between the two groups of samples, the Metastats software was used to perform a T test on the species richness data between two groups to obtain the p-value.[25] The q-value was obtained by correcting the p-value. Species were selected based on p-values or q-values that caused differences in the composition of the two groups of samples. The analysis was performed at the level of phylum, class, order, family, genus, species taxonomy to analyze the significance between groups.

Mothur (version v.1.30) software was used to evaluate the Alpha Diversity Index of the samples.[26] The species diversity within a single sample was studied by Alpha Diversity Analysis, and the Ace, Chao1, Shannon, and Simpson indices of each sample at the 97% similarity level were counted; Beta diversity analysis was performed using QIIME software. Beta diversity analysis mainly uses the binary jaccard algorithm to calculate the distance between samples to obtain the β value between samples. Based on the distance matrix obtained from the Beta diversity analysis, PCoA analysis was performed using R language tools to further demonstrate the differences in species diversity between samples.[27] Hierarchical clustering was performed on samples using unweighted paired average method (UPGMA) to determine the similarity of species composition among samples.

PICRUSt software was used to compare the species composition information obtained from 16S sequencing data to infer the functional gene composition in the samples, thereby analyzing the functional differences between different groups.[28] Based on the use of the KEGG orthology database (KOs) in the Kyoto Encyclopedia of Genes and Genomics (KEGG) database, the changes in metabolic pathways of functional genes of microbial communities between different groups of samples were observed through differential analysis of KEGG metabolic pathways.[29]

2.5 Statistics Analysis

Statistical analysis was performed using SPSS19.0 software. Participants' gender, tobacco and alcohol consumption were expressed in terms of proportional or percentages. Independent t-tests, Welch t-tests, and White non-parametric t-tests were used for continuous variables. Pearson chi-square test or Fisher's exact test were used for classification variables. All significance tests were two-sided tests, and $p < 0.05$ or adjusted $p < 0.05$ was considered statistically significant.

3. Results

3.1 Clinical data

In this study, thirty patients with drug-naive first-episode MDD and thirty healthy controls were recruited as patients’ group and controls group, respectively. There were no statistically significant differences between patients’ group and controls group in terms of age, height, weight, and tobacco and alcohol consumption (Table 1). Patients group received a standardized treatment. The average dose of escitalopram was 16.33 ± 3.46 mg / d. Patients improved significantly when the mean time of taking drugs was 34.53 ± 5.18 days and the

HAMD score decreased by more than 50%.

Table 1

Demographic characteristics of patients and controls

	Patients (n = 30)		Controls (n = 30)		*p*-value
		M ± SD		M ± SD	
Age		44.83 ± 11.00		43.97 ± 10.57	0.757
Height(m)		1.68 ± 0.07		1.70 ± 0.05	0.171
Weight(Kg)		67.83 ± 6.86		69.21 ± 7.14	0.447
BMI (kg/m2)		23.99 ± 2.05		23.83 ± 2.08	0.761
tobacco(%)*		46.67%		30.00%	0.288
alcohol(%)*		53.33%		33.33%	0.192

*Chi-square test; compared with HCs, $P < 0.05$; BMI: body mass index

3.2 Sequencing Data And Bacterial Taxonomic Composition

Total 4,790,651 original sequences were obtained from 60 samples. After double-end Reads splicing and filtering, a total of 4,444,748 Clean tags were generated. Each sample generated at least 12,039 Clean tags, and an average value were 49,386 Clean tags. Taxonomic annotation of OTUs based on Silva (bacterial) and UNITE (fungi) taxonomic databases. At phylum level, the dominated gut microbiota were Bacteroidetes, Firmicutes, Protebacteria, and Actinobacteria in three groups. Bacteroidetes and Firmicutes accounted for nearly 90% of the total gut microbiota. The Firmicutes / Bacteroides ratio of Patients group, Follows group, and Controls group were 0.64, 0.46, and 0.70, respectively. The ratio in Follows group was significantly lower than other two groups. (Fig. 1)

At the genus level, the relative abundance of Parasutterella, Prevotella_9, Fusobacterium, Prevotella_2, Christensenellaceae_R-7_group, Odoribacter, and [Eubacterium] _ruminantium_group in Patients group was significantly lower than that in Controls group,and ParabacteroidesLactobacillusAnaerostipes Ruminococcaceae_UCG-014Dialister was significantely increased in Patients group.

After treatment with escitalopram, gut microbiota with low abundance in Patients group were significantly increased in Follows group, such as: Christensenellaceae_R-7_group, [Eubacterium] _ruminantium_group and Fusobacterium. The higher abundance of gut microbiota in Patients group was also found to decrease in Follows group, such as Lactobacillus. The mainly change of gut microbiota abundance in Follows group was Bacteroides. (Fig. 2)

In addition, there are also several differences in the gut microbiota between Follows group and Controls group. In Follows group, the relative abundance of Parabacteroides, Prevotellaceae, Ruminiclostridium_6, Flavonifractor were increased significantly, and Prevotella_2, Lachnospira, Collinsella, and Clostridium_sensu_stricto_1 were decreased significantly. Among them, the abundance of Faecalibacterium and Lachnoclostridium in Follows group and Patients group were significantly lower than those in Controls group. (Supplementary Table S1)

3.3 Diversity Analysis

Alpha diversity mainly reflects the richness and diversity of the species in samples. As shown in Fig. 3, the Chaos1, Ace, and Shannon indices of Patients group were significantly higher than those of Follows group and Controls group, and the Simpson index was significantly lower in Patients group than others. This showed that the number and the diversity of gut microbiota in Patients group were significantly higher than those of Follows group and Contros group, and there were statistically differences. Four indices value of Follows group were between the values of other two groups, which were significantly different from that of Patients group, but not statistically different from that of Controls group. This meant that the Alpha diversity of gut microbiota in patients returned to the normal level. The statistics of Alpha diversity index values of each group were showed in Table 2.

Table 2

Richness and diversity index values of patients, follows and controls

	Patients	Follows	Controls	
ACE	254.88 ± 2.30	187.39 ± 11.09	173.43 ± 3.80	
Chao1	257.38 ± 2.63	188.93 ± 11.07	172.69 ± 4.46	
Shannon	3.57 ± 0.07	3.17 ± 0.11	2.99 ± 0.09	
Simpson	0.09 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	

Mothur (version v.1.30) software was used to calculate the Alpha diversity index for samples. The larger of the index values of Ace and Chao1shows the greater number of species in the samples. The larger of the Shannon index value and the smaller of the Simpson index value shows more species category of the sample.

Beta diversity was used to compare the similarity of species diversity between different groups. The binary jaccard algorithm was used to calculate β diversity, and there were statistical differences among three groups ($R = 0.273$, $p = 0.001$) (Fig. 4). The gut microbiota of Patients group was significantly different from Controls group, and the gut microbiota within Patients group was more similar. Observing the distribution of Follows group, it could be found that the gut microbiota of a part of patients who accepted escitalopram treatment was like that of Controls group, while the others remained like Patients group. The unweighted paired average method (UPGMA) was used in the R language tool to perform hierarchical clustering of each group. It also found that the gut microbiota of Patients group was significantly different from that of Controls group, and Follows group was more like Controls group. This means that the Beta diversity of the patients' gut microbiota tended to return to normal. (Supplementary Figure S2)

3.4 Functional Properties Predicted By Picrust

The study considered that the structure of gut microbiota in Follows group could not completely return to normal. The PICRUSt software was used to compare the species composition information obtained from 16S sequencing data to infer the functional gene composition between two groups. Through the annotation of the KEGG metabolic pathway, it finally found that there were differences in the metabolic pathways of Transport and catabolism, Nervous system, Glycan biosynthesis and metabolism, Cell motility, and Membrane transport between Follows group and Controls group (Fig. 5). In Patients group, the pathways above also had several differences, but it wasn't significantly different from those of Follows group. It thus suggested that the gut microbiota of Follows group might still contribute to the occurrence of depression (Supplementary Table S3).

Discussion

This study demonstrated that the gut microbiota from patients of drug-naive first-episode MDD was significantly different from healthy controls, and the composition and structure of the gut microbiota within patients was more similar. This suggested that the occurrence and development of MDD may be caused by a special and similar group of gut microbiota. In 2011, Manimozhiyan Arumugam *et al*. proposed the concept of enterotypes.[30] In the sequencing results, they reported that the human gut microbiome could be divided into three robust clusters, including Prevotella-enterotype, Bacteroides-enterotype, and Ruminococcus-enterotype. Different enterotypes have characteristic advantages of their respective functional states.[31] For example, enterotype P has stronger fermentation ability and can generate more short-chain fatty acids, but enterotype B has more specific enzymes sufficiently to degrade sugars and proteins.[32] Therefore, further research on the enterotypes and corresponding functional changes of gut microbiota would be helpful to understand the impact of gut microbiota on the development of MDD.

Secondly, the gut microbiota tended to return to the normal gut microbiota structure under treatment by SSRIs, thus indicating a positive effect of SSRI antidepressants on the change of gut microbiota. However, the gut microbiota was still different from healthy controls in Follows group. The results suggested that the recovery of the gut microbiota might take longer than the improvement of depressive symptoms. The recovered gut microbiota might still partially maintain the pathological state, which would be a trigger for depression recurrence. Currently, there’re no effective biomarkers to indicate recurrence in patients. The dynamic changes of gut microbiota under antidepressant treatment provided the possibility to become the potential biomarkers.[33] Therefore, it is expected to determine the duration and degree of recovery through detecting gut microbiota of patients in the future.

In addition, some studies have found that several antibiotics have antidepressant effects, such as ceftriaxone, minocycline, and doxycycline.[34–36] This might further support that the changes of the gut microbiota had a positive effect on the treatment of MDD. Current studies have claimed that minocycline could be used as a synergistic agent for flvoxamine in the treatment of moderate and severe obsessive-compulsive disorder.[37] This provided a basis for the effectiveness and safety of SSRI drugs combined with antibiotics in the treatment of mental illness. Moreover, the transplantation of normal gut microbiota has been reported to be a treatment for MDD.[38, 39] The related treatments were established on the hypothesis of the re-normal of gut microbiota in depression patients. In the future, promotion of healthy gut microbiota thus could be a new way to explore in the prevention of depression occurrence.

The outcomes of current study were limited to the relatively small sample size and short follow-up time. The effect of dietary factors on the gut microbiota was not evaluated. Those should be improved and the information of relapse could be collected as well in future studies. Moreover, metabolomics and proteomics omics technologies are strongly suggested to be employed to further detail the relationship between gut microbiota and depression.

Conclusion

In conclusion, this study found that the gut microbiota of patients with first-episode depression was similar and less different, and it was significantly different from healthy controls. After depressive patients received escitalopram treatment, their gut microbiota diversity tended to transform to normal. However, there were still several structures and metabolic pathways difference in the gut microbiota between followed patients and healthy controls, which might be relate to the relapse of depression.

Abbreviations

MDD

major depressive disorder

SSRIs

Selective serotonin reuptake inhibitors

16S rRNA

16S ribosomal Ribonucleic Acid

HAMD

Hamilton Depression Scale

BMI

Body mass index

OUTs

Operational taxonomic units

KEGG

Kyoto Encyclopedia of Genes and Genomes

UPGMA

unweighted paired average method

PCoA

Principal coordinate analysis

Declarations

Ethics approval and consent to participate

The study was explained to all participants both verbally and in writing, and written informed consent was obtained from each participant. According to the Helsinki Declaration, the protocol for sample collection and analysis was approved by the Ethics Committee of Peking University Sixth Hospital and Beijing Hospital of Chinese Traditional and Western Medicine.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Seed fund of Jinqiao project of Beijing Association for science and technology, China (NO. JQ18005).

Author contributions

YS, XY, JYG and YL designed the study and wrote the protocol. XXX and XY, JYG managed the literature searches and analyses. YS, ZYL and YCH undertook the statistical analysis, and YS, XY and GFL wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Acknowledgements

We thank all the subjects who took part in this study.

References

1. Roberts T, Miguel Esponda G, Krupchanka D, Shidhaye R, Patel V, Rathod S. Factors associated with health service utilisation for common mental disorders: a systematic review. BMC Psychiatry. 2018;18(1):262.
2. Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y, Leucht S, Ruhe HG, Turner EH, Higgins JPT, et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. Lancet. 2018;391(10128):1357–66.
3. Dale E, Bang-Andersen B, Sanchez C. Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. Biochem Pharmacol. 2015;95(2):81–97.
4. DiazGranados N, Ibrahim LA, Brutsche NE, Ameli R, Henter ID, Luckenbaugh DA, Machado-Vieira R, Zarate CA Jr. Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. J Clin Psychiatry. 2010;71(12):1605–11.
5. Clevenger SS, Malhotra D, Dang J, Vanle B, IsHak WW. The role of selective serotonin reuptake inhibitors in preventing relapse of major depressive disorder. Ther Adv Psychopharmacol. 2018;8(1):49–58.
6. Inserra A, Mastronardi CA, Rogers G, Licinio J, Wong ML. Neuroimmunomodulation in Major Depressive Disorder: Focus on Caspase 1, Inducible Nitric Oxide Synthase, and Interferon-Gamma. Mol Neurobiol. 2019;56(6):4288–305.
7. Inserra A, Rogers GB, Licinio J, Wong ML. The Microbiota-Inflammasome Hypothesis of Major Depression. Bioessays. 2018;40(9):e1800027.
8. Molina-Torres G, Rodriguez-Arrastia M, Roman P, Sanchez-Labraca N, Cardona D. Stress and the gut microbiota-brain axis. Behav Pharmacol. 2019;30(2):3-Spec Issue):187–200. and.
9. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, Zeng L, Chen J, Fan S, Du X, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol Psychiatry. 2016;21(6):786–96.
10. Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linlokken A, Wilson R, Rudi K. Correlation between the human fecal microbiota and depression. Neurogastroenterol Motil. 2014;26(8):1155–62.
11. Lin P, Ding B, Feng C, Yin S, Zhang T, Qi X, Lv H, Guo X, Dong K, Zhu Y, et al. Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. J Affect Disord. 2017;207:300–4.
12. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, Wang W, Tang W, Tan Z, Shi J, et al. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav Immun. 2015;48:186–94.
13. Li B, Guo K, Zeng L, Zeng B, Huo R, Luo Y, Wang H, Dong M, Zheng P, Zhou C, et al. Metabolite identification in fecal microbiota transplantation mouse livers and combined proteomics with chronic unpredictive mild stress mouse livers. Transl Psychiatry. 2018;8(1):34.
14. Luo Y, Zeng B, Zeng L, Du X, Li B, Huo R, Liu L, Wang H, Dong M, Pan J, et al. Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus. Transl Psychiatry. 2018;8(1):187.
15. Pearson-Leary J, Zhao C, Bittinger K, Eacret D, Luz S, Vigderman AS, Dayanim G, Bhatnagar S. **The gut microbiome regulates the increases in depressive-type behaviors and in inflammatory processes in the ventral hippocampus of stress vulnerable rats**. *Mol Psychiatry* 2019.
16. Lieb J. The immunostimulating and antimicrobial properties of lithium and antidepressants. J Infect. 2004;49(2):88–93.

17. Munoz-Bellido JL, Munoz-Criado S, Garcia-Rodriguez JA. Antimicrobial activity of psychotropic drugs: selective serotonin reuptake inhibitors. Int J Antimicrob Agents. 2000;14(3):177–80.
18. Ayaz M, Subhan F, Ahmed J, Khan AU, Ullah F, Ullah I, Ali G, Syed NI, Hussain S. Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. J Biol Res (Thessalon). 2015;22(1):4.
19. Coban AY, Tanriverdi Cayci Y, Keles Uludag S, Durupinar B. [Investigation of antibacterial activity of sertralin]. Mikrobiyol Bul. 2009;43(4):651–6.
20. Kruszewska H, Zareba T, Tyski S. Examination of antimicrobial activity of selected non-antibiotic medicinal preparations. Acta Pol Pharm. 2012;69(6):1368–71.
21. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59(Suppl **20**):22–33;quiz 34–57.
22. Shen Y, Xu J, Li Z, Huang Y, Yuan Y, Wang J, Zhang M, Hu S, Liang Y. Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: A cross-sectional study. Schizophr Res. 2018;197:470–7.
23. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335–6.
24. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996–8.
25. White JR, Nagarajan N, Pop M. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput Biol. 2009;5(4):e1000352.
26. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Program NCS, Bouffard GG, Blakesley RW, Murray PR, et al. Topographical and temporal diversity of the human skin microbiome. Science. 2009;324(5931):1190–2.
27. Sakaki T, Takeshima T, Tominaga M, Hashimoto H, Kawaguchi S. Recurrence of ICA-PCoA aneurysms after neck clipping. J Neurosurg. 1994;80(1):58–63.
28. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 2014;30(21):3123–4.
29. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013;31(9):814–21.
30. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174–80.
31. Chen T, Long W, Zhang C, Liu S, Zhao L, Hamaker BR. Fiber-utilizing capacity varies in Prevotella- versus Bacteroides-dominated gut microbiota. Sci Rep. 2017;7(1):2594.
32. Vieira-Silva S, Falony G, Darzi Y, Lima-Mendez G, Garcia Yunta R, Okuda S, Vandeputte D, Valles-Colomer M, Hildebrand F, Chaffron S, et al. Species-function relationships shape ecological properties of the human gut microbiome. Nat Microbiol. 2016;1(8):16088.
33. Buckman JEJ, Underwood A, Clarke K, Saunders R, Hollon SD, Fearon P, Pilling S. Risk factors for relapse and recurrence of depression in adults and how they operate: A four-phase systematic review and meta-synthesis. Clin Psychol Rev. 2018;64:13–38.
34. Mineur YS, Picciotto MR, Sanacora G. Antidepressant-like effects of ceftriaxone in male C57BL/6J mice. Biol Psychiatry. 2007;61(2):250–2.
35. Miyaoka T, Wake R, Furuya M, Liaury K, Ieda M, Kawakami K, Tsuchie K, Taki M, Ishihara K, Araki T, et al. Minocycline as adjunctive therapy for patients with unipolar psychotic depression: an open-label study. Prog Neuropsychopharmacol Biol Psychiatry. 2012;37(2):222–6.
36. Mello BS, Monte AS, McIntyre RS, Soczynska JK, Custodio CS, Cordeiro RC, Chaves JH, Vasconcelos SM, Nobre HV Jr, de Sousa F. FC et al: Effects of doxycycline on depressive-like behavior in mice after lipopolysaccharide (LPS) administration. J Psychiatr Res. 2013;47(10):1521–9.
37. Esalatmanesh S, Abrishami Z, Zeinoddini A, Rahiminejad F, Sadeghi M, Najarzadegan MR, Shalbafan MR, Akhondzadeh S. Minocycline combination therapy with fluvoxamine in moderate-to-severe obsessive-compulsive disorder: A placebo-controlled, double-blind, randomized trial. Psychiatry Clin Neurosci.

2016;70(11):517–26.
38. Xu Z, Liu Z, Dong X, Hu T, Wang L, Li J, Liu X, Sun J. Fecal Microbiota Transplantation from Healthy Donors Reduced Alcohol-induced Anxiety and Depression in an Animal Model of Chronic Alcohol Exposure. Chin J Physiol. 2018;61(6):360–71.
39. Kurokawa S, Kishimoto T, Mizuno S, Masaoka T, Naganuma M, Liang KC, Kitazawa M, Nakashima M, Shindo C, Suda W, et al. The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: An open-label observational study. J Affect Disord. 2018;235:506–12.

Figures

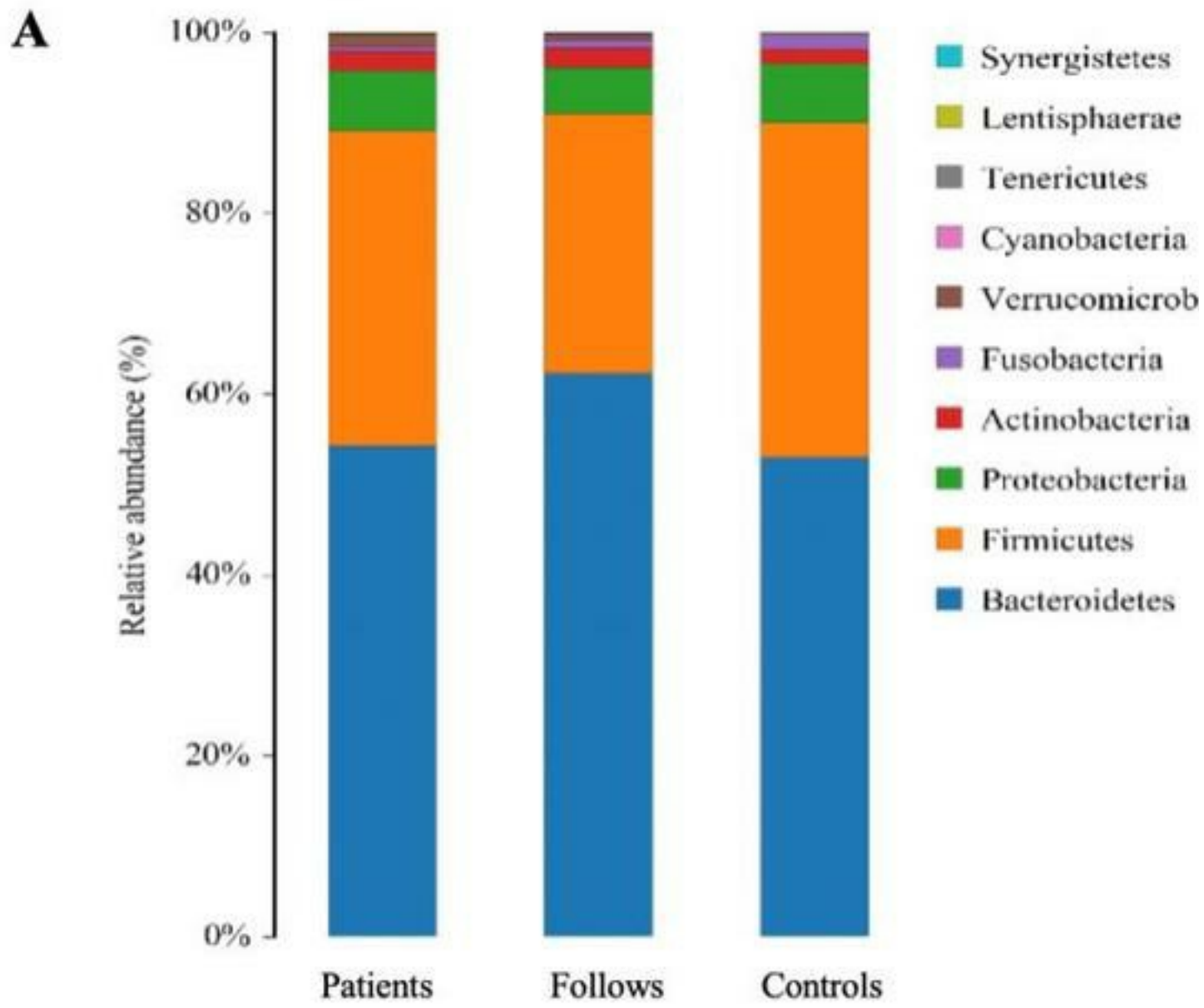


Figure 1

Histogram of species distribution. QIIME software was used to generate species abundance tables at different taxonomic levels, and R language tool was used to draw community structure charts at different taxonomic levels. (A) relative proportions of species distribution at the gate level; (B) relative proportions of species distribution at the genus level.

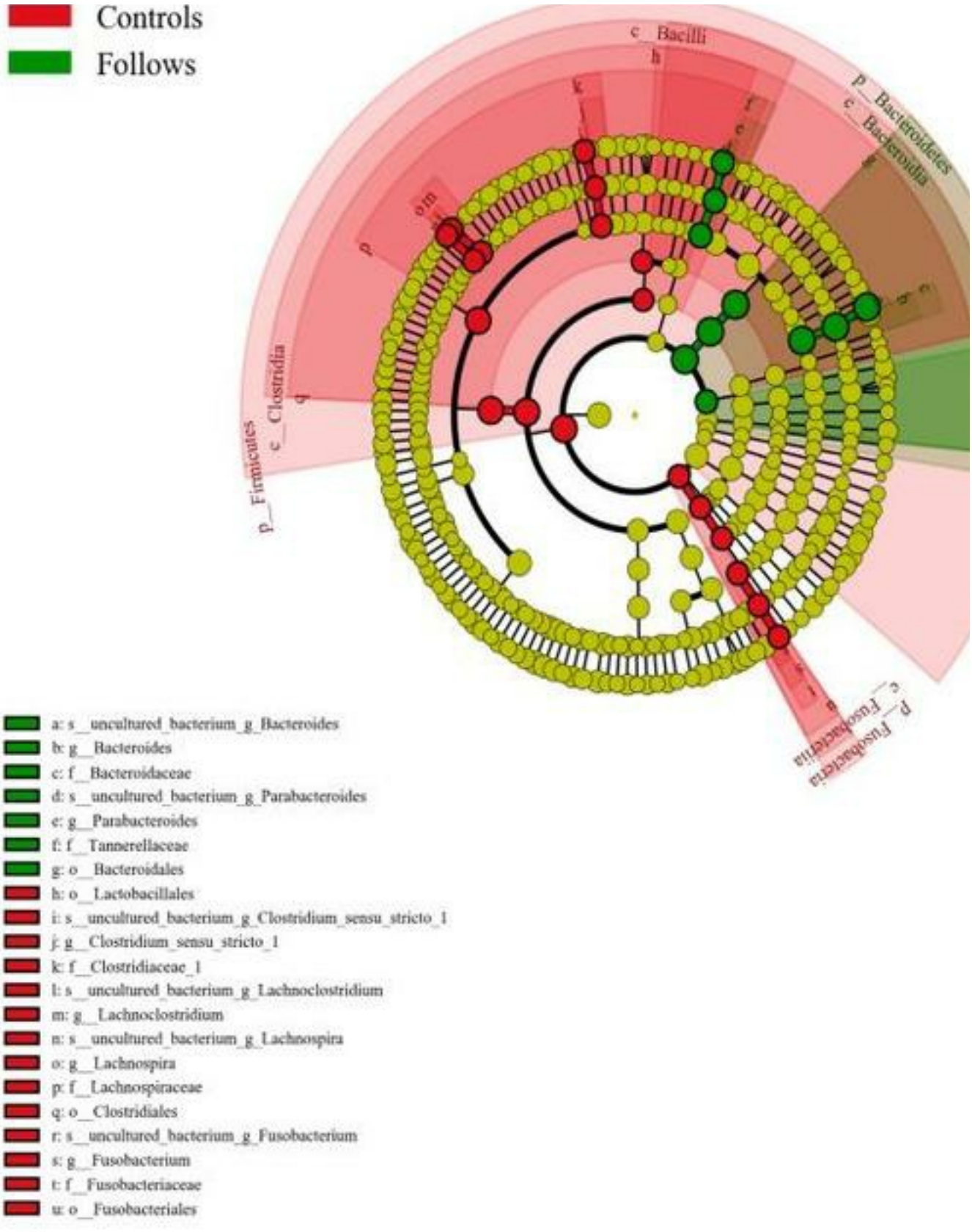
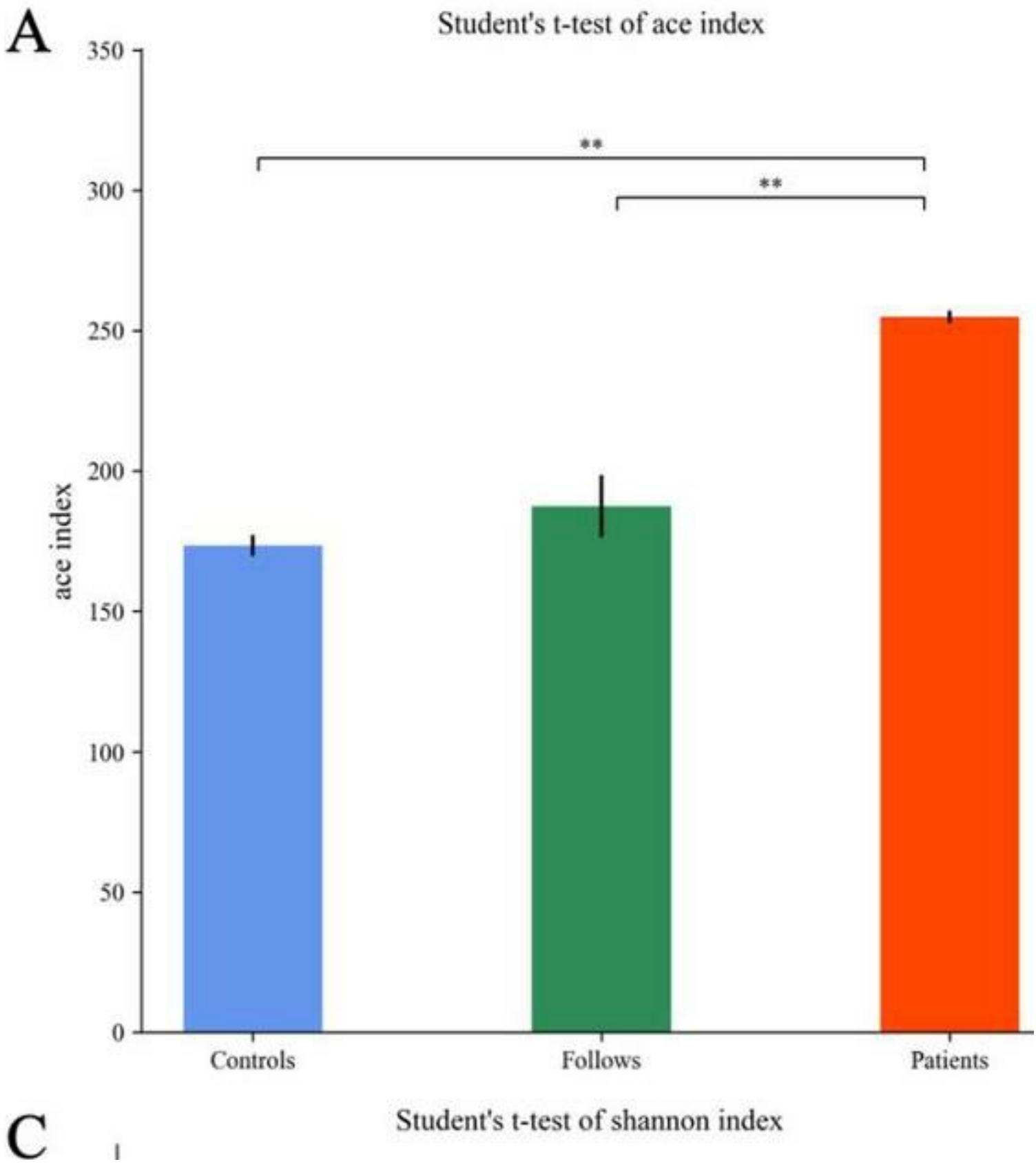


Figure 2

LEfSe was used to analyze the difference of gut microbiota between two groups. (A) the circles radiated from inside to outside in the LEfSe Cladogram represent the classification level from phylums to species; Each small

circle at a different classification level represents a classification at that level, and the diameter of the small circle is proportional to the relative abundance. Red is the enriched taxa of the control group; Green are the clusters enriched in the patient group. (B) represent the visualization of taxon satisfying LDA threshold > 3.5.



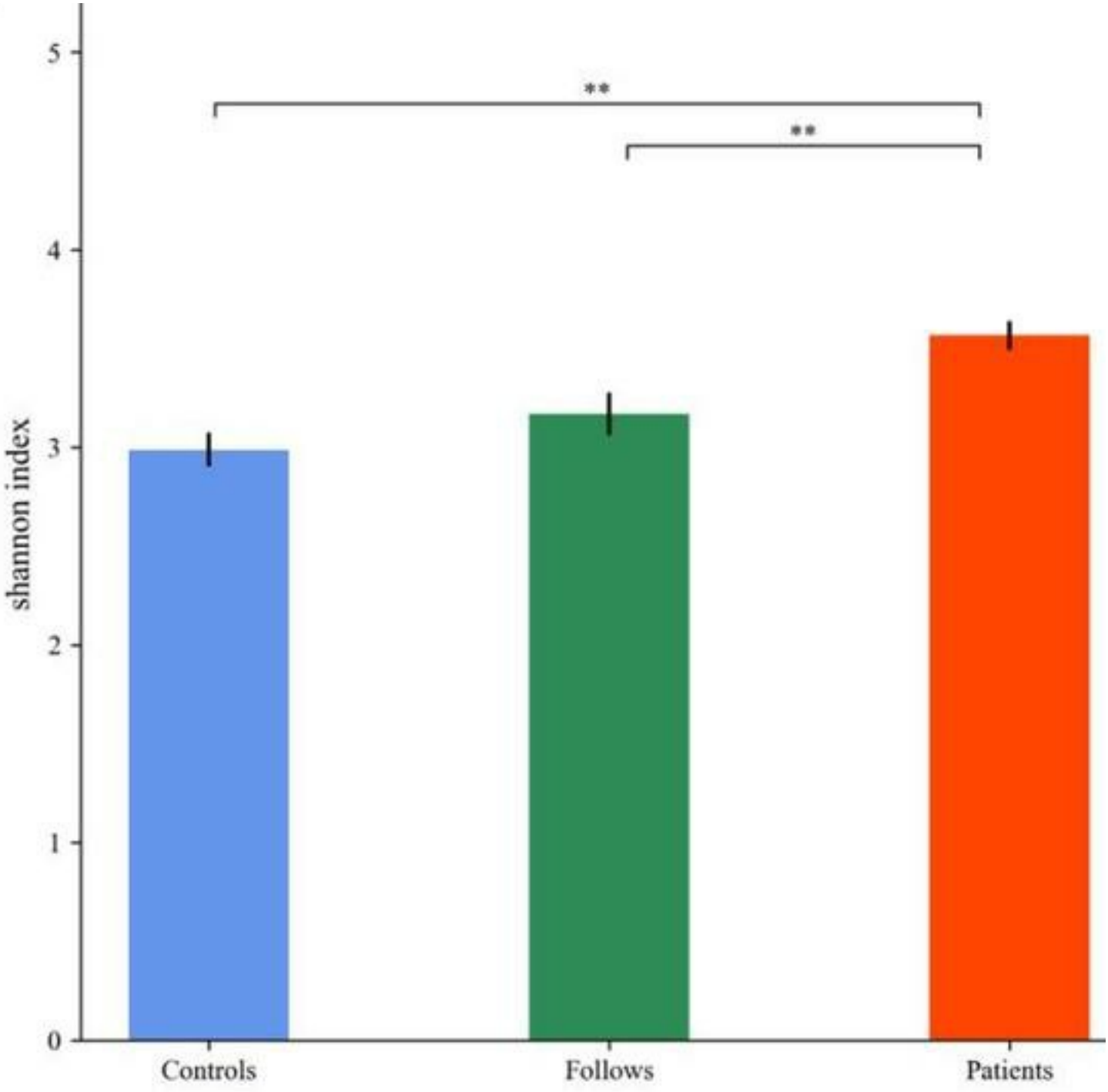
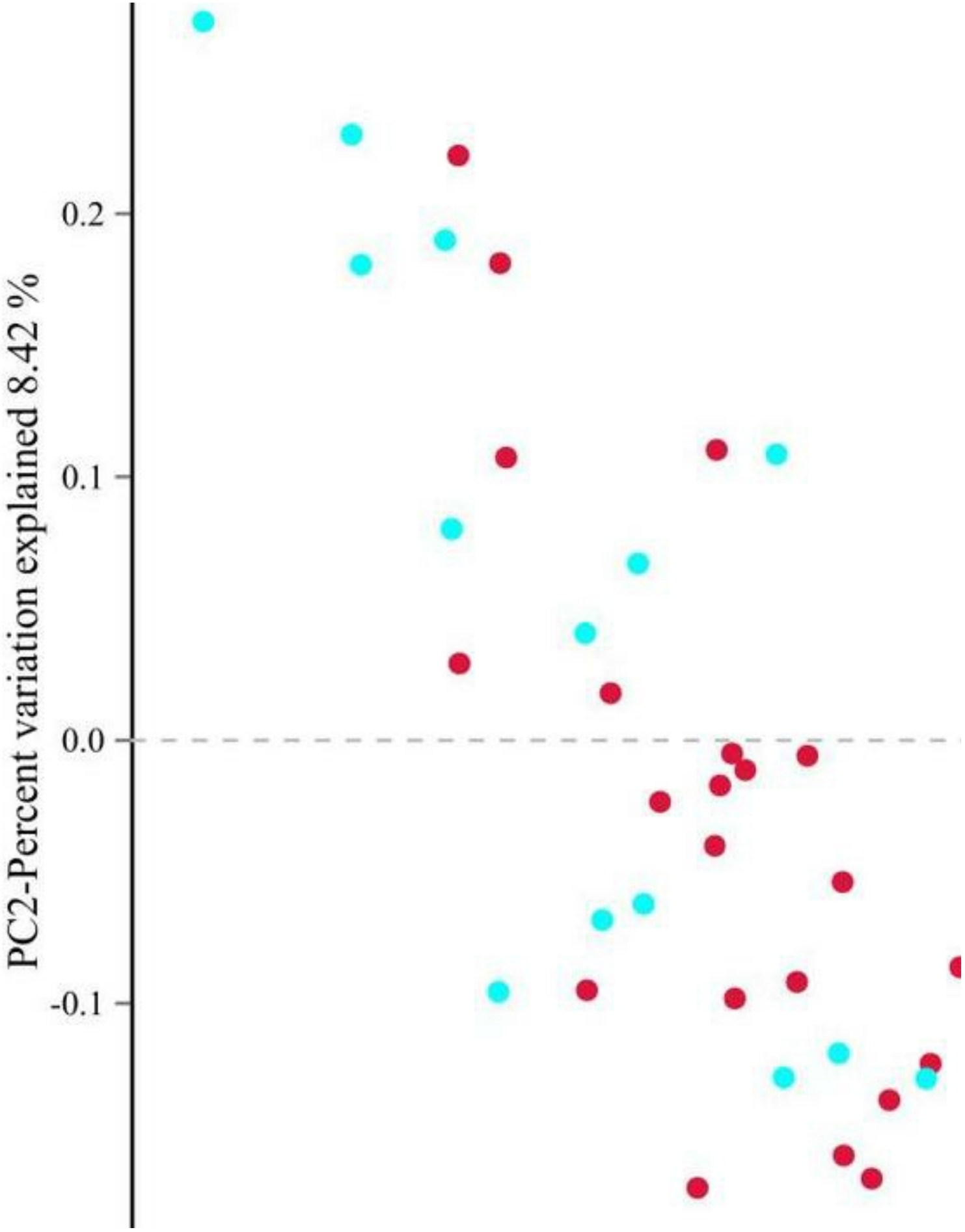


Figure 3

Visualization of Alpha diversity index. (A) Ace index; (B) Chao1 index; (C) Shannon index; (4) Simpson index. ** means the statistical difference between the two groups, $p < 0.05$


PC2-Percent variation explained 8.42 %
0.2
0.1
0.0
-0.1

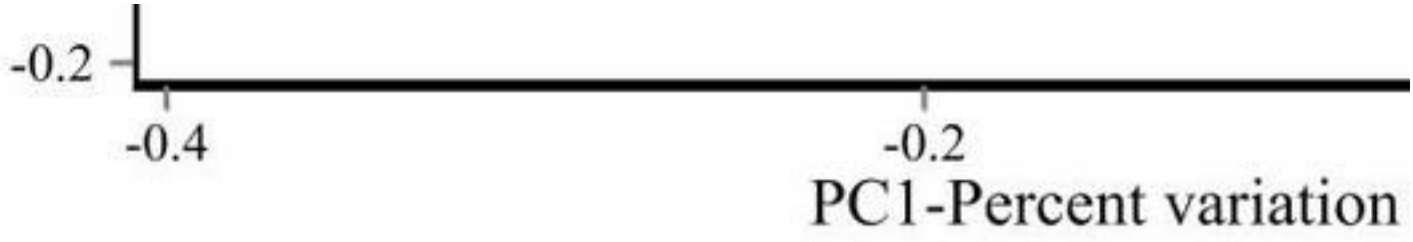


Figure 4

PCoA analysis shows the distribution coordinate diagram of samples: In the graph, the distance between the dots represents the similarity of the samples. Samples with high similarity tend to cluster together. The yellow dots represent the patient group, the blue dots represent the follow-up group, and the red dots represent the control group. The results showed a statistically significant difference among the three groups (R=0.273, p=0.001).

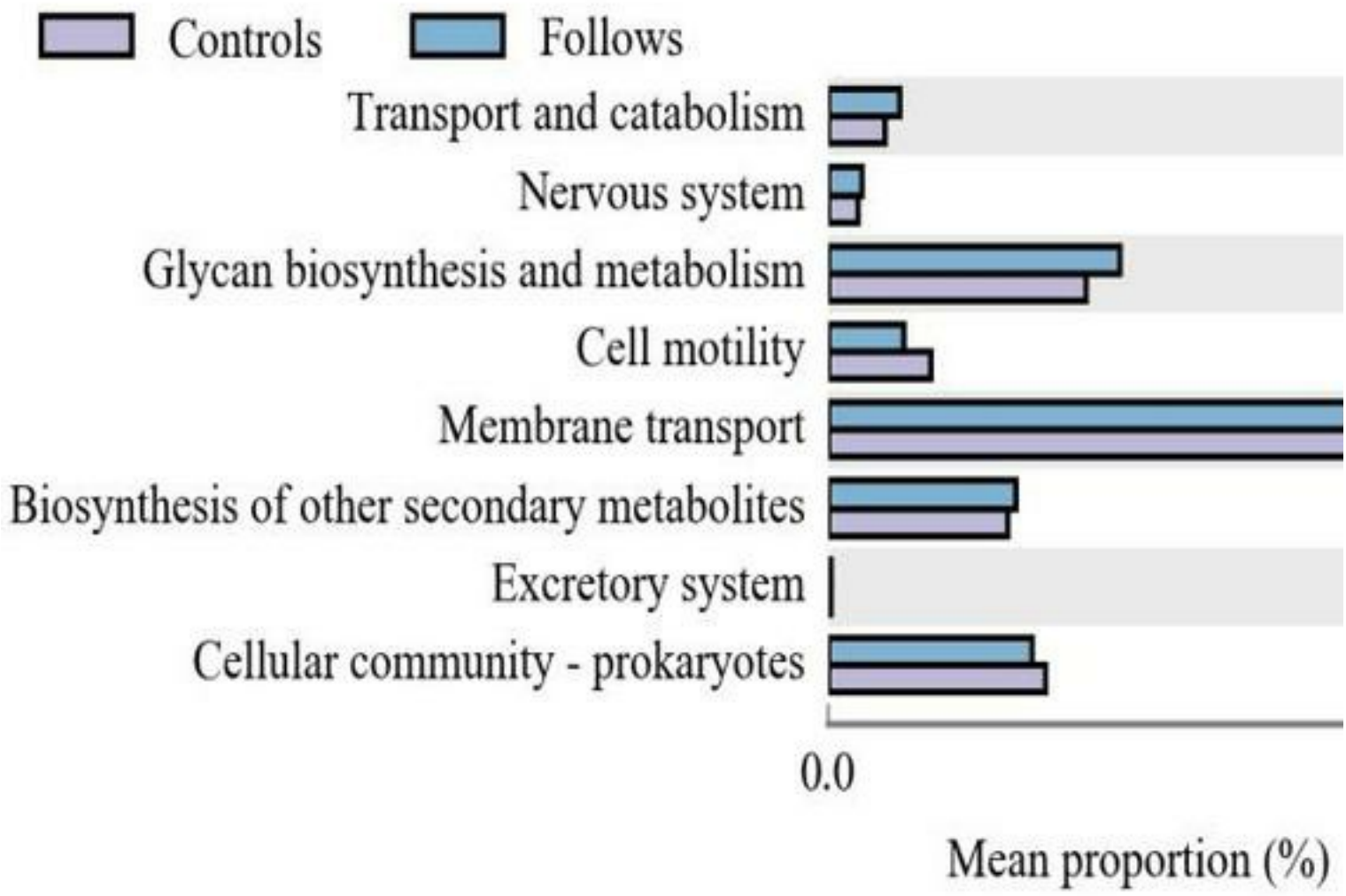


Figure 5

KEGG metabolic pathway analysis. The left side of the figure shows the abundance ratio between the two groups. The middle section shows the proportional variation in functional abundance within the 95% confidence interval. The p-value is on the right.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformations.docx