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Analysis of the characteristics of intestinal microbiota in patients with different severity of obstructive sleep apnea

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Intestinal microbiota imbalance plays an important role in the progression of obstructive sleep apnea (OSA), and is considered to be the main mediator that triggers metabolic comorbidities. Here, we analyzed the changes in intestinal microbiota in patients with different severities of OSA based on apnea hypopnea index (AHI) classification, and explored the role of intestinal microbiota in the severity of OSA. This study included 19 healthy volunteers and 45 patients with OSA [5 ≤ AHI < 15 (n = 14), 15 ≤ AHI < 30 (n = 13), AHI ≥ 30 (n = 18)]. Relevant sleep monitoring data and medical history data were collected, and microbial composition was analyzed using 16S rRNA high-throughput sequencing technology. The diversity analysis of intestinal microbiota among different groups of people was conducted, including alpha diversity, beta diversity, species diversity, and marker species as well as differential functional metabolic pathway prediction analysis. With the increase of AHI classification, the alpha diversity in patients with OSA significantly decreased. The results revealed that the severity of OSA is associated with differences in the structure and composition of the intestinal microbiota. The abundance of bacteria producing short-chain fatty acids (such as *Bacteroides*, *Ruminococcacea*, and *Faecalibacterium*) in severe OSA is significantly reduced and a higher ratio of *Firmicutes* to *Bacteroidetes*. Random forest analysis showed that *Parabacteroides* was a biomarker genus with important discriminatory significance. The differential metabolic pathway prediction function shows that the main function of maintaining intestinal microbiota homeostasis is biosynthetic function. Our results show that the differences in the composition of intestinal microbiota in patients with different severities of OSA are mainly related to short-chain fatty acid-producing bacteria. These changes may play a pathological role in OSA combined with metabolic comorbidities.

Keywords Obstructive sleep apnea, Apnea hypopnea index, Intestinal microbiota, Short-chain fatty acid

Obstructive sleep apnea (OSA) is the most common sleep apnea disorder characterized by recurrent hypoventilation or respiratory interruption caused by upper respiratory collapse during sleep, leading to chronic intermittent hypoxia (CIH) and hypercapnia[1]. Among them, CIH is the main pathogenesis and an important factor causing systemic multisystem damage. It has been confirmed that OSA is closely related to cardiovascular and cerebrovascular diseases, type 2 diabetes, metabolic syndrome and other multi system diseases[2,3]. However, its mechanism is currently unclear. Epidemiological research shows that the incidence rate of OSA is high among the elderly and obese people. In the 30–60 age group, 24% of men and 9% of women have OSA, and the incidence rate of men and women aged 65 and above has further increased, as high as 40–60%[4,5]. This is an extremely significant group and a significant issue that cannot be ignored by the medical community and society.

With the development of high-throughput sequencing technology, some scholars have explored the close relationship between the occurrence and development of OSA and changes in intestinal microbiota[6,7]. Clinical

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studies have found that there is a significant imbalance in intestinal microorganisms in patients with OSA, including *Clostridium*, *Ruminococcus*, *Escherichia coli*, and bacteria that produce short-chain fatty acid (SCFA) in large quantities[6,8]. The decrease in species diversity of intestinal microbiota in patients with OSA is closely related to apnea and hypoxia[9]. In animal models of OSA, it has been found that CIH and sleep fragmentation can lead to substantial changes in the intestinal microbial community[10–12]. Changes in intestinal microbiota affect body metabolism and physiological functions, thereby participating in the progression of OSA disease, including hypertension and impaired glucose metabolism[13,14]. CIH regulates microorganisms (such as *Clostridium*, *Lactococcus*, and *Bifidobacterium*) and important functional metabolites (such as free fatty acids and bile acids), promoting the occurrence of lipid metabolic disorders[15]. In addition, OSA induces intestinal dysbiosis, leading to neuroimmune response and intestinal inflammation[16].

At present, continuous positive airway pressure (CPAP) is still the main treatment method for patients with moderate to severe OSA, but due to its low acceptance and poor long-term compliance, about 40–70% of patients do not accept or cannot adhere to CPAP treatment for a long time. However, the emergence of new strategies to improve intestinal dysbiosis through interventions such as probiotics, prebiotics, and fecal microbiota transplantation may be an effective approach to address ecological imbalance mediated by OSA.

However, there are few studies on the intestinal microbiota of patients with different severities of OSA, which is related to multiple organ damage in OSA. In order to explore the role of changes in intestinal microbiota in the progression of OSA disease, this study performed high-throughput sequencing and feature analysis on the intestinal microbiota of patients with different severities of OSA, evaluating the composition and functional differences of intestinal microorganisms in patients with different degrees of OSA. This study provides strategies for early intervention and treatment of patients with OSA.

Materials and methods

Participants

This study included 19 healthy volunteers and 45 OSA patients who underwent overnight PSG monitoring at the Sleep Medicine Center of the Second Hospital of Shanxi Medical University. According to the AASM diagnostic criteria, all participants were divided into four groups based on their apnea hypopnea index (AHI) scores: no OSA (Control, AHI < 5 events/hour), mild OSA (L-OSA, 5 ≤ AHI < 15 events/hour), moderate OSA (M-OSA, 15 ≤ AHI < 30 events/hour), and severe OSA (S-OSA, AHI ≥ 30 events/hour)[17–19]. Exclusion criteria: Age < 18 years old; Pregnant women; Taking drugs that affect sleep, such as benzodiazepines, barbiturates, or sedatives; Accept relevant treatments, including but not limited to surgery and mechanical ventilation; Concomitant gastrointestinal diseases, such as ulcerative colitis and irritable bowel syndrome; Patients who have taken intestinal microbiota preparations, antibiotics, or immunosuppressants within 2 months prior to enrollment; People with tumors, diabetes, liver and kidney dysfunction, rheumatic immune diseases or other diseases that may affect intestinal microbiota imbalance. The Ethics Committee of the Second Hospital of Shanxi Medical University approved the trial protocol, and all methods were conducted in accordance with relevant guidelines and regulations. All subjects provide written informed consent.

Inclusion criteria for the healthy Control group: Age > 18 years old; No history of sleep apnea, insomnia, or other sleep disorders. Exclusion criteria: Individuals who have taken probiotics, antibiotics, or immunosuppressants within the two months prior to enrollment; Pregnant women; Significant changes in dietary structure or living environment within one month prior to enrollment; People with digestive system diseases, tumors, diabetes, liver and kidney dysfunction, rheumatic immune diseases or other diseases that may affect intestinal microbiota imbalance.

OSA assessment

All subjects underwent PSG monitoring at the Sleep Medicine Center of the Second Hospital of Shanxi Medical University. The PSG was conducted by a sleep technician from 10 pm to 7 am the next day. The raw PSG data was manually scored and interpreted by an experienced sleep technician, and then reviewed by a professional sleep physician.

16S rRNA gene amplification and sequencing

Clean and collect fecal samples from patients who wake up in the morning, and transfer them to a − 80 °C ultra-low temperature freezer for freezing within 30 min. Use OMEGA Soil DNA kit (D5625-01) to extract the total genomic DNA of the bacteria in the sample, and store it at − 20 °C. Use NanoDrop ND-1000 spectrophotometer (Thermo Fisher Science, Waltham, Massachusetts, USA) for quantitative analysis and 1.2% agarose gel electrophoresis for quality inspection.

PCR amplification, purification, quantification, and sample mixing: PCR amplification was performed on the highly variable V3-V4 region of the bacterial 16S rRNA gene. The PCR amplicon was purified using Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN). The PCR product was quantified using Quant-iT PicoGreen dsDNA Assay Kit on a microplate reader (BioTek, FLx800), and then mixed according to the required data volume for each sample.

Library construction and high-throughput sequencing: The sequencing library was prepared using Illumina's TruSeq Nano DNA LT Library Prep Kit. The library system was screened and purified by magnetic beads, and the final fragments were selected and purified by 2% agarose gel electrophoresis. Quality inspection of the library was performed on the Agilent Bioanalyzer, and the Quant it PicoGreen dsDNA Assay Kit was used to quantify the library on the Promega QuantiFluor fluorescence quantification system. Finally, a NovaSeq sequencer was used for dual end sequencing.

Bioinformatics analysis: For the raw sequence data obtained on the Illumina NovaSeq platform, QIIME2 bioinformatics platform is used for DADA2 processing of raw data, including primer removal, quality filtering, denoising, splicing, and de-chimerization steps. Each de-duplicated sequence generated after DADA2 quality control is called ASV (amplicon sequence variant), or feature sequence, and high-quality sequences are finally obtained. The Greengenes database is selected for species annotation of each ASV feature sequence. At the same time, the method of rarefaction is used to sample the ASV table to ensure that each sample is processed at the same sequencing depth level. Finally, QIIME2 bioinformatics platform is used for feature sequence analysis.

Statistical analysis

The bioinformatics-related statistics were analyzed using the QIIME2 software. The measurement data were expressed as mean ± standard deviation ($\overline{X}$±S). Data with homogeneity of variance is tested using one-way analysis of variance, while data without homogeneity of variance is tested using Kruskal Wallis H-test. $P < 0.05$ indicates statistical significance.

Results

Clinical characteristics

A total of 45 patients with OSA (L-OSA: N = 14, M-OSA: N = 13, H-OSA: N = 18) and 19 controls were recruited for this study after PSG monitoring (Fig. 1). There were no significant differences in gender, age, body weight, height, systolic pressure, and diastolic pressure among the four groups. The AHI in the OSA group was significantly higher than that in the Control group. Compared with the Control group, the SpO2min in the moderate and severe OSA groups was significantly increased. Additionally, the BMI and SpO2mean in the severe OSA group were significantly higher than those in the Control group (Table 1).

OTU differences

Stool samples from patients were collected for the analysis of the composition and function of the intestinal microbiota using 16S rRNA gene sequencing targeting the V3-V4 region. The OTU clustering analysis results showed a total of 1934 common OTUs among the four groups. The Control group included 10,743 OTUs, the L-OSA group included 8862 OTUs, the M-OSA group included 8042 OTUs, and the S-OSA group included 6889 OTUs (Fig. 2A). The species accumulation curve (Fig. 2B).

Analysis *of alpha* diversity

To comprehensively evaluate the alpha diversity of the microbial community, this study utilized the Chao1 and Observed Species index to characterize richness, and the Simpson and Shannon indices to represent diversity. The results indicated no significant difference in the observed Chao1 index between the Control group and OSA patients. Compared with mild OSA, severe OSA patients have a significant decrease in Chao1 index (Fig. 3A). In terms of the Simpson, Shannon and Observed species indices, there was a significant decrease in severe OSA patients compared to healthy controls. Additionally, we observed that the Simpson, Shannon and Observed species indices of severe OSA patients were significantly lower than those of mild OSA patients (Fig. 3B–D).

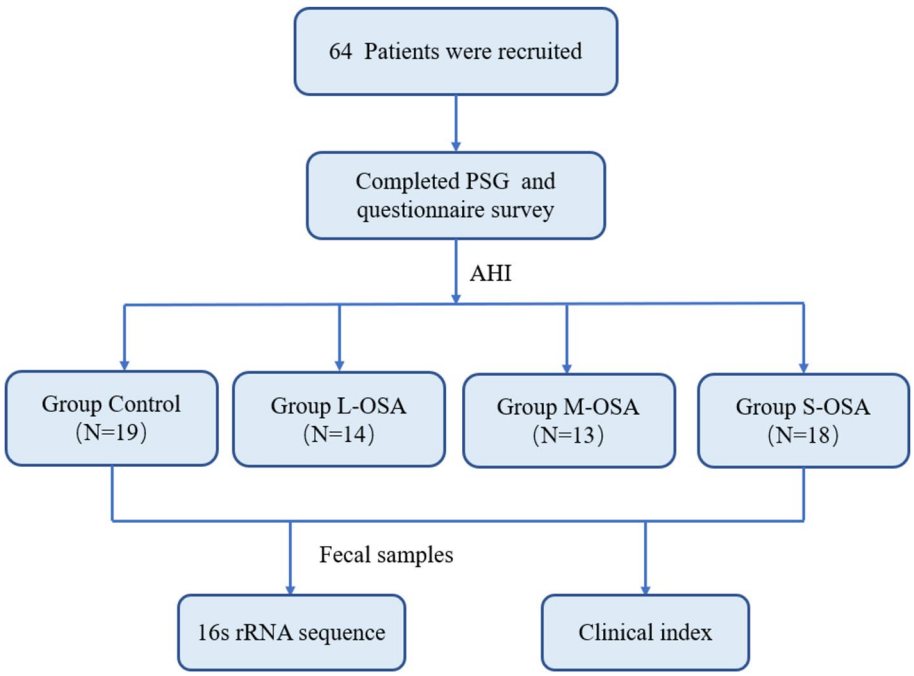

Fig. 1. Patient Ęow chart.

	Control (N = 19)	L-OSA (N = 14)	M = OSA (N = 13)	S-OSA (N = 18)
Gender (male/female)	13/6	11/3	6/7	13/5
Age (years, mean ± SD)	41.74 ± 16.71	52.71 ± 14.67	61.23 ± 21.84**	49.44 ± 13.30
Weight (kg)	72.79 ± 21.14	68.24 ± 9.24	75.29 ± 8.89	81.00 ± 10.43
Height	169.53 ± 13.73	164.93 ± 9.50	166.46 ± 6.29	171.39 ± 5.09
BMI (kg/m^2)	24.74 ± 4.91	25.11 ± 2.75	27.11 ± 2.19	27.57 ± 3.34*
Systolic pressure	122.84 ± 18.68	125.36 ± 18.66	125.92 ± 15.20	127.72 ± 14.47
Diastolic pressure	79.16 ± 11.19	80.79 ± 13.24	82.69 ± 9.05	85.50 ± 8.00
AHI (events/h)	2.41 ± 1.32	7.71 ± 1.44***	19.38 ± 2.24***	58.28 ± 14.57***
SpO_2 min (%)	88.32 ± 3.51	86.00 ± 3.44	82.23 ± 6.18*	70.83 ± 13.09***
SpO_2 mean (%)	94.98 ± 1.66	94.54 ± 2.04	93.12 ± 4.39	92.00 ± 2.90**

Table 1. Characteristics of all participants. Data are presented as mean ± standard deviation or percentage. * indicates $P < 0.05$ compared with the Control group, ** indicates $P < 0.01$, and *** indicates $P < 0.001$. AHI: apnea–hypopnea index; SpO_2 min represents the lowest oxygen saturation; SpO_2 mean represents the average oxygen saturation.

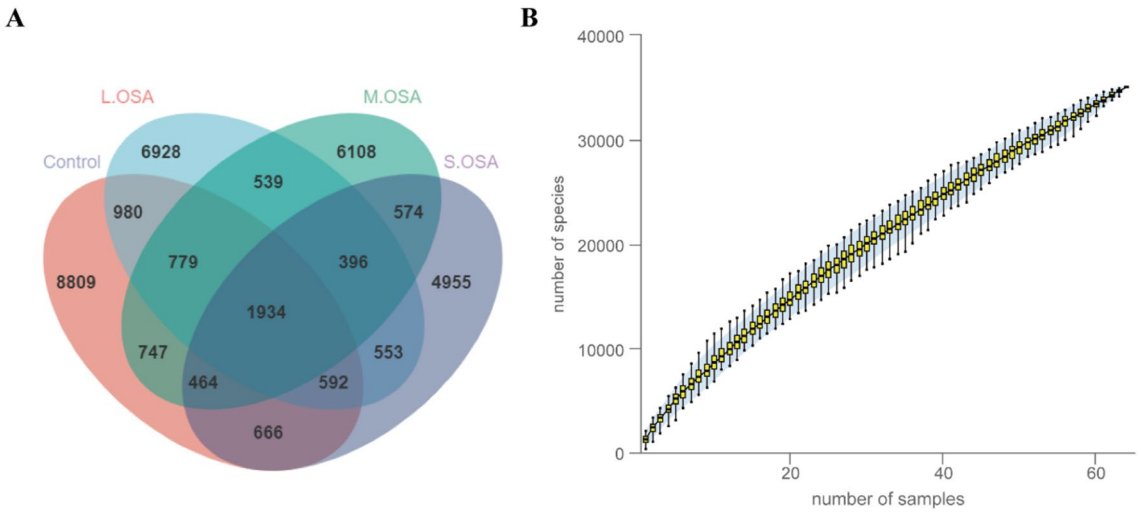

Fig. 2. (**A**) Outward Wayne diagram; (**B**) species accumulation curve, which is widely used to measure and predict the increase in species richness in a community as the sample size increases, and is widely used to determine whether the sample size is sufficient and estimate community richness.

Analysis of β diversity

β diversity analysis based on PCoA and NMDS principal coordinate analysis using bray_curtis distances revealed complete separation between the control group and OSA patient samples (Fig. 4A). Permanova analysis demonstrated that the inter-group differences between severe OSA patients and healthy controls, as well as between severe and mild OSA patients, were significantly greater than the intra-group differences (Fig. 4B).

Composition of Intestinal microbiota

Phylum level

We further evaluated the differences in microbial communities among different groups. Analysis of the top 10 phylum level differences among groups demonstrated that the dominant phyla in each group were primarily composed of *Firmicutes*, *Bacteroidetes, Proteobacteria*, and *Actinobacteria*. There were no significant differences in the relative abundances of *Firmicutes* among the groups. The abundance of *Bacteroidetes* in the moderate and severe OSA groups was significantly lower than that in the Control group. Compared with mild OSA, the abundance of *Bacteroidetes* decreases more significantly in severe OSA. In addition, the *Firmicutes/Bacteroidetes* (F/B) ratio in the severe OSA group was significantly higher than that in the Control and mild OSA groups (Fig. 5A–D).

Family level

At the family level, the dominant microbial communities among each group are mainly composed of *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidaceae*, and *Veillonellaceae*. The abundance of *Lachnospiraceae* in the moderate and severe OSA groups was significantly higher than that in the Control group. In addition, compared with the Control group, the relative abundance of *Ruminococcaceae* in OSA patients was significantly reduced.

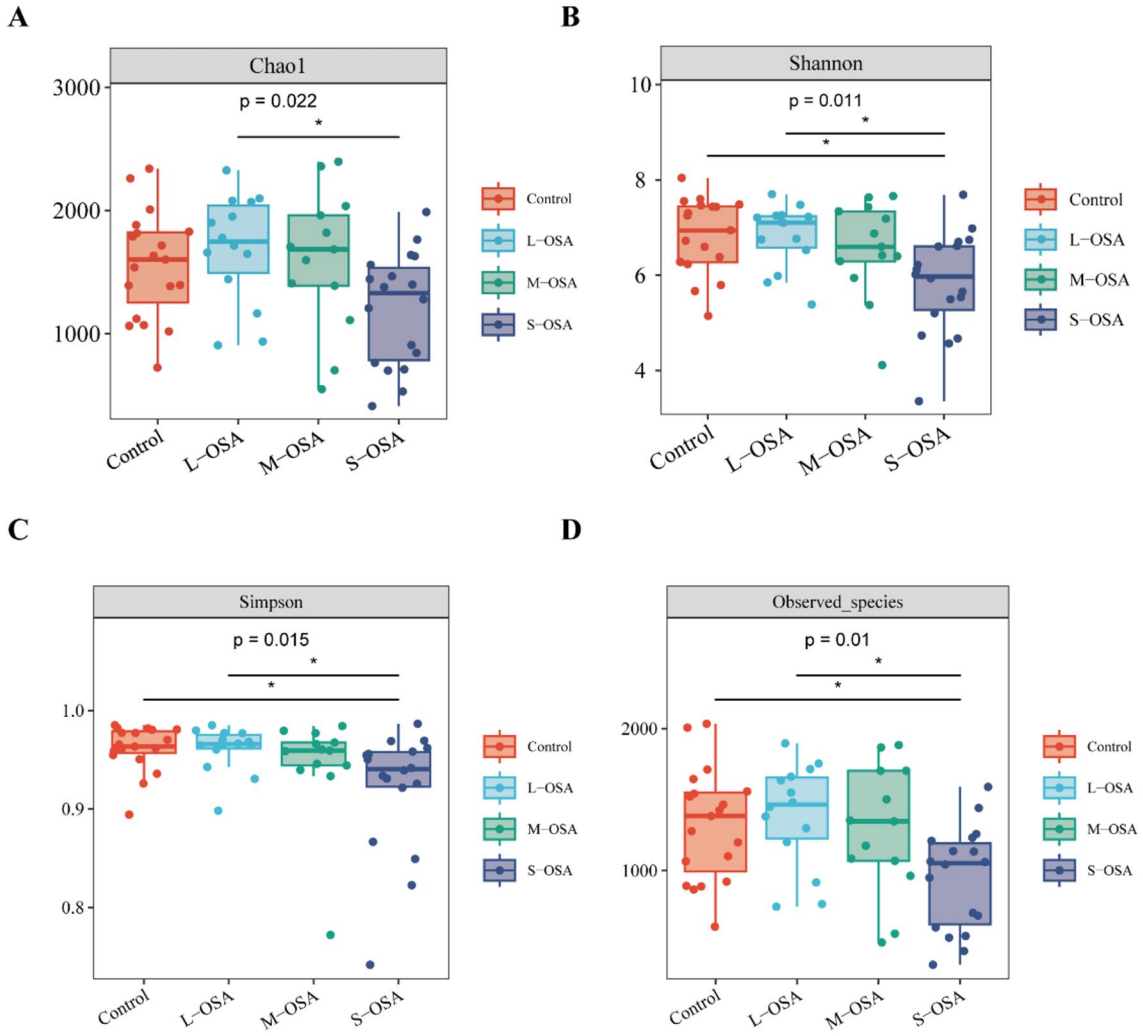

Fig. 3. Alpha diversity index represents the diversity of species within their habitat; (**A**) Chao1; (**B**) Shannon; (**C**) Simpson; (**D**) Observed_species.

The relative abundance of *Ruminococcaceae* in the moderate and severe OSA groups was significantly lower than that in the mild OSA group (Fig. 5E–G).

Genus level

At the genus level, we found that the relative abundance of *Roseburia* significantly increased in moderate and severe OSA groups compared to the Control group. The relative abundance of *Faecalibacterium* in moderate and severe OSA groups was significantly lower than that in mild OSA group. In addition, compared to the Control group, the number of *Blautia* significantly increased and the number of *Oscillospira* significantly decreased in severe OSA group (Fig. 6A–D). To further express the differences between groups, we used Lefse analysis to determine the main differences between the Control group and OSA patients. The results showed that mild OSA was enriched in genus level with *g–lachnobacterium*, moderate OSA was enriched with *g–Acidovorax*, *g–Acidaminococcus*, *f_Comamonadaceae*, severe OSA was enriched with *f_Sphingomonadaceae*, *g_Sphingomonas* (Fig. 6E). Random forest analysis showed that *Parabacteroides* had the highest importance in identifying the four groups (Fig. 6F).

Analysis of the factors affecting community distribution by RDA

The RDA analysis was used to explore the correlation between the influencing factors and the distribution of samples. The results showed that among the multiple factors of physical examination indicators, weight ($r^2 = 0.157$, $P = 0.007$), BMI ($r^2 = 0.184$, $P = 0.003$), AHI ($r^2 = 0.246$, $P = 0.002$), and SpO_2 min ($r^2 = 0.138$, $P = 0.014$), SpO_2 mean ($r^2 = 0.147$, $P = 0.005$) have significant effects on the community distribution of intestinal microbiota (Table 2, Fig. 7).

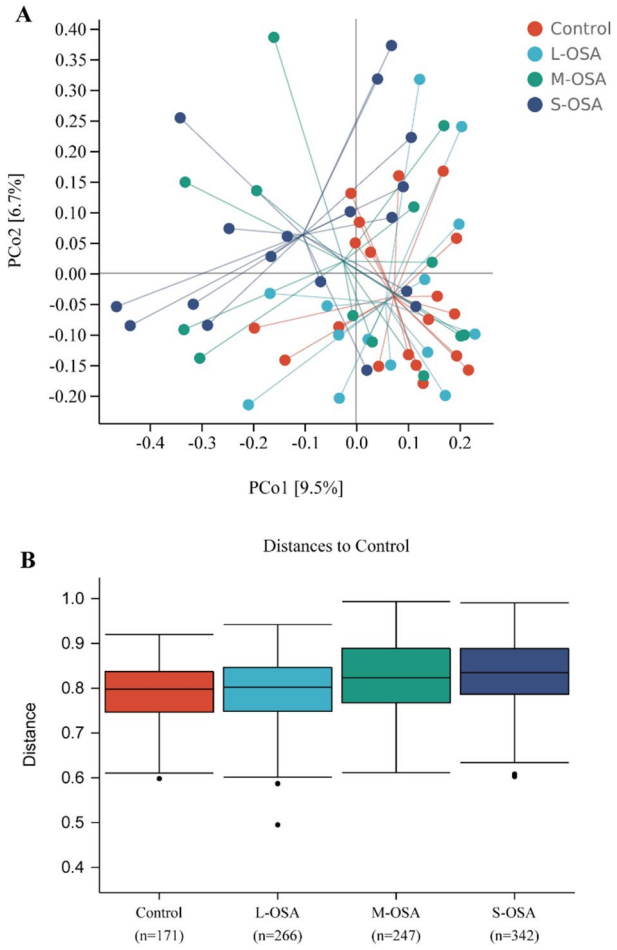

Fig. 4. (**A**) Principal component analysis of PCoA based on bray_curtis distance; (**B**) Inter group difference analysis.

Functional analysis

Based on the MetaCyc database, the results of predictive analysis by PICRUSt2 in this study reveal that the intestinal microbiota, which plays a major role in maintaining the stability of the intestinal microbiota in the host, is primarily involved in biosynthesis functions. The main functions are the synthesis of amino acids and nucleotides (Fig. 8).

Discussion

The intestinal microbiota is a microbial community in the human body that plays a crucial role in maintaining human health and disease development. Research has found that the intestinal microbiota plays a vital role in regulating the risk of various chronic diseases, maintaining intestinal immunity, and systemic homeostasis. For example, it is of significant importance in conditions such as obesity, cardiac metabolic abnormalities, inflammatory bowel disease, and mental disorders[20]. In recent years, an increasing number of studies have identified a certain association between the intestinal microbiota and OSA. OSA and its unique pathological manifestations (CIH and sleep fragmentation) contribute to intestinal dysbiosis, leading to systemic low-grade chronic inflammatory changes. Ultimately, intestinal dysregulation may trigger or exacerbate multi-organ damage caused by OSA in susceptible individuals. However, these studies are very preliminary and the specific underlying mechanisms remain unclear. In this study, we investigated the impact and difference of intestinal microbiota in different severity levels of OSA. We found alterations in the composition of intestinal microbiota in patients with different severity levels of OSA. In severe OSA patients, the relative abundance of SCFA producing bacteria such as *Bacteroidetes*, *Ruminococcaceae* and *Faecalibacterium*, significantly decreased, while the F/B ratio and the abundance of harmful bacteria such as *Roseburia* and *Lachnospiraceae* significantly increased. In summary, our results indicate that differences in the composition of intestinal microbiota in OSA, which are mainly related to SCFA producing bacteria. These changes may play a pathological role in the metabolic comorbidities associated with OSA.

This study utilized 16S rRNA high-throughput sequencing technology to perform diversity and microbiota analysis on healthy control subjects as well as patients with mild, moderate, and severe OSA. The results revealed significant differences in the abundance and evenness of intestinal microbiota among OSA patients with different

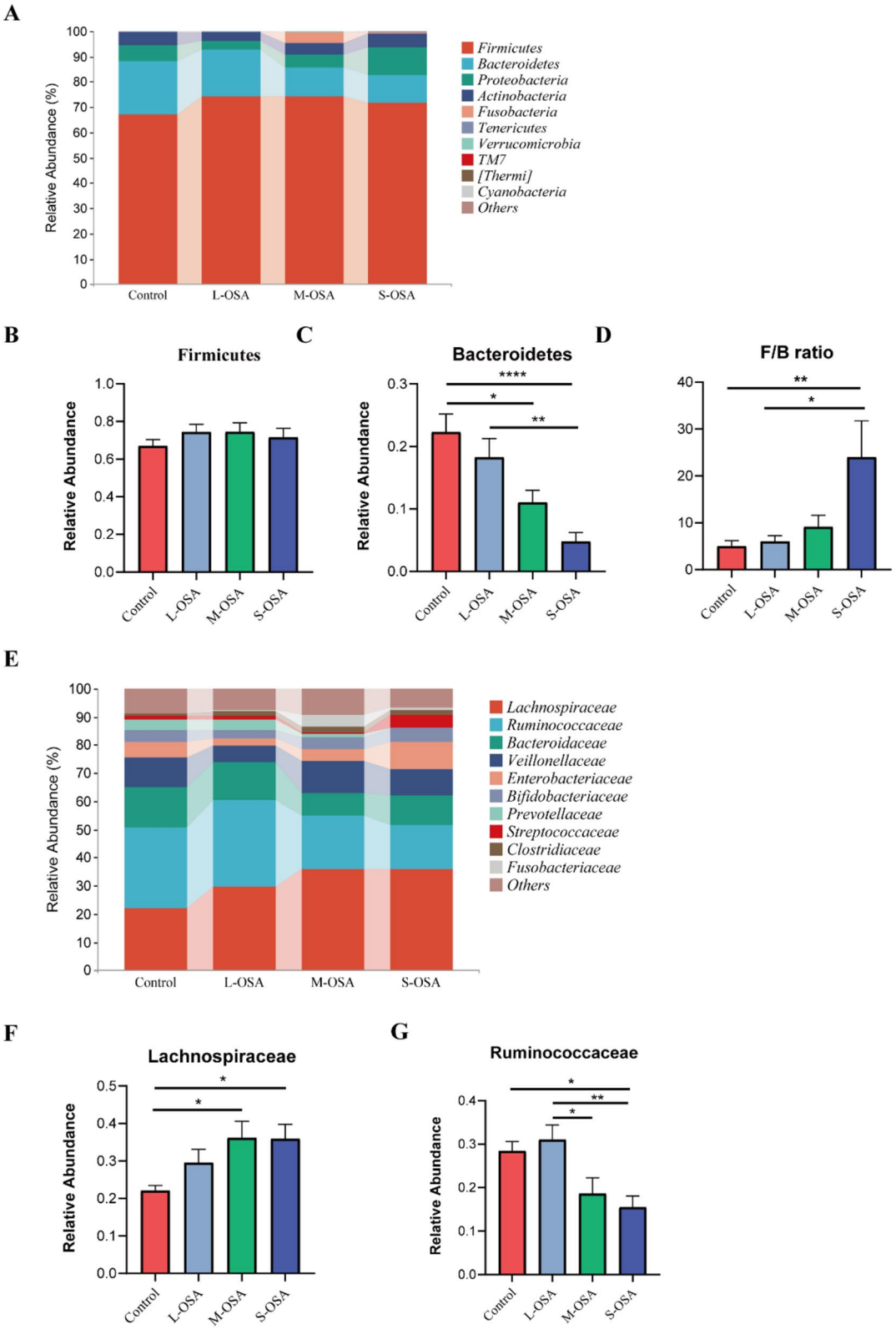

Fig. 5. (**A**–**D**) Relative abundance of microbial communities at the phylum level; (**E**–**G**) Relative abundance of bacterial communities at the family level, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. $^{****}P<0.0001$.

severity levels, and characteristic intestinal genera predictive of different severity levels of OSA were identified. Alpha diversity analysis of the microbiota showed significant differences between OSA patients of different

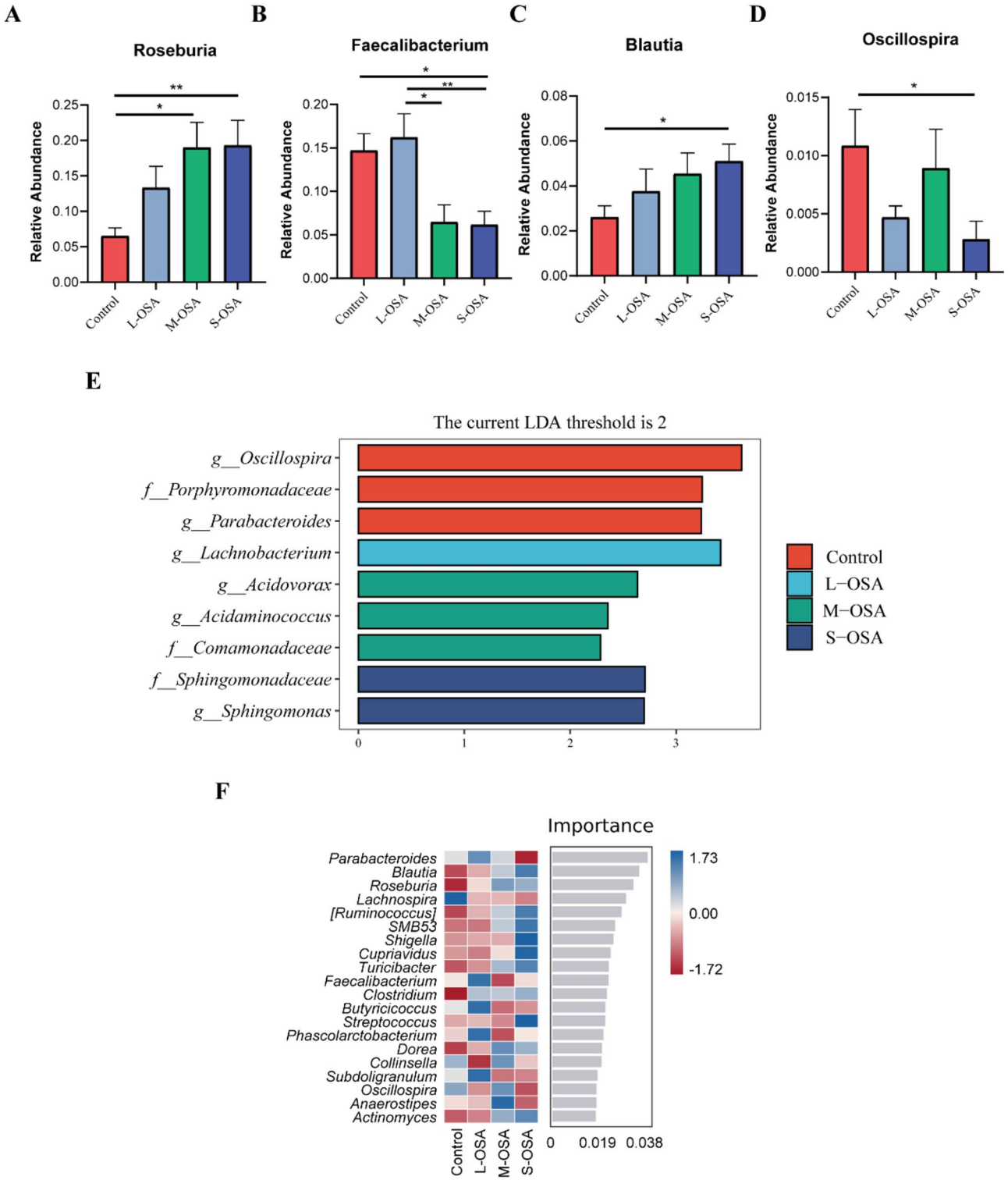

Fig. 6. Differences in microbial communities at the (**A**–**D**) genus level. (**E**) Lefse analyzed the taxonomic units with significant differences between groups. (**F**) Random forest analysis. $^{*}P<0.05$, $^{**}P<0.01$.

severity levels and the healthy control group. The Shannon, Simpson indices and Observed species of severe OSA patients were significantly lower than those of healthy subjects. Moreover, as the AHI increased, the diversity of OSA patients decreased gradually. Compared with mild OSA, the diversity of severe OSA is significantly reduced. β diversity analysis indicated significant differences in intestinal microbiota alterations between mild and severe OSA patients, with inter-group differences being more pronounced than intra-group differences. These findings suggest a close relationship between the severity of OSA and the composition of intestinal microbiota.

Factors	RDA1	RDA2	r^2	*P*
Age	−0.996	0.0876	0.0764	0.092
Weight	−0.381	0.924	0.157	0.007
Hight	−0.497	0.868	0.026	0.439
BMI	−0.250	0.968	0.184	0.003
Systolic pressure	−0.657	−0.754	0.023	0.527
Diastolic pressure	−0.582	0.813	0.014	0.65
AHI	−0.366	0.931	0.246	0.002
SpO_2 min	0.025	−1.000	0.138	0.014
SpO_2 mean	0.414	−0.910	0.147	0.005

Table 2. RDA analysis of the influence factors of community distribution. RDA1 and RDA2 represent two sorting axes, namely principal components 1 and 2.

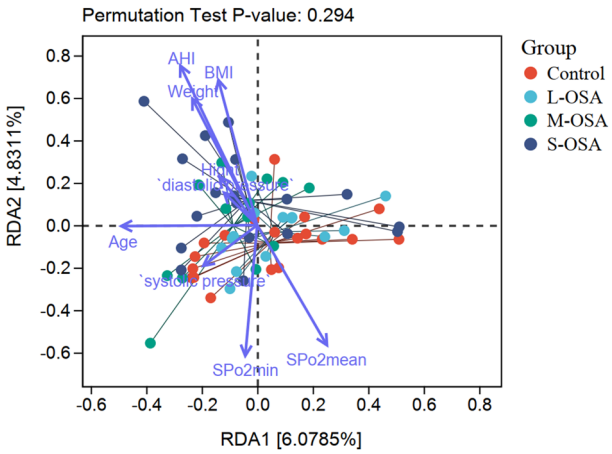

Fig. 7. RDA analysis of the correlation between related influencing factors and intestinal microbiota.

Furthermore, we found that the severity of OSA is associated with differences in the structure and composition of the intestinal microbiota. The abundance of SCFA producing bacteria, such as *Bacteroidetes*, *Ruminococcaceae*, and *Faecalibacterium*, significantly decreased in patients with severe OSA. Studies have found that SCFA is closely related to insulin resistance and the pathological process of type 2 diabetes[21]. It has been reported that the phylum *Bacteroidetes* can produce SCFAs such as acetate, which is closely associated with reduced production of inflammatory mediators[22]. Inflammation has been linked to various OSA-related conditions such as hypertension, coronary heart disease, obesity, and diabetes[23–27]. SCFAs are produced from dietary fiber in fermented foods of the bacteria, including acetate, propionate, and butyrate salts. They play an important role in the energy metabolism of bacteria and the physiological and biochemical stability of the intestinal tract[28–30]. The SCFAs-producing microbiota is believed to be closely related to human metabolism and cardiovascular disease. Reduced production of SCFAs can lead to intestinal barrier dysfunction[31,32]. SCFAs promote mucin synthesis, reduce bacterial translocation, maintain intestinal mucosal integrity, and thereby reduce intestinal inflammation[33–35]. Consumption of an unhealthy diet or intermittent hypoxia can lead to dysbiosis of the intestinal microbiota, resulting in the depletion of significant amounts of SCFAs. This depletion can cause dysfunction of colonic cells, manifested by weakened tight junctions between epithelial cells and the protective intestinal epithelial barrier. Consequently, the intestine becomes more permeable. Moreover, the process of hypoxia/reoxygenation itself exerts direct toxicity on tight junctions and increases the risk of intestinal permeability[30]. Previous studies have also observed alterations in SCFAs-producing microbiota and increased levels of inflammation in patients with OSA[36]. In this study, SCFA producing bacteria, including *Bacteroidetes*, *Ruminococcaceae*, and *Faecalibacterium*, were highly enriched in the control group, which helped to increase intestinal anti-inflammatory activity[37]. In OSA patients, the reduction of SCFA producing bacteria leads to a decrease in some anti-inflammatory activity of the body, thereby increasing inflammation. This suggests that changes in the SCFAs producing microbiota in OSA patients may be associated with the progression of OSA. Furthermore, we also observed a significant increase in the relative abundance of harmful bacteria *Roseburia* and *Lachnospiraceae* in moderate to severe OSA cases. Studies have indicated that *Roseburia* can induce infection[38]. *Lachnospiraceae* (belonging to the *Firmicutes* phylum) has been shown to be associated with an increased risk of obesity[39]. This indicates that alterations in SCFAs-producing microbiota in OSA patients may be linked to their pathophysiological processes. Therefore, qualitative and quantitative analysis of metabolomics related to SCFA will be a key focus of our research. Targeted analysis and modification of specific microbial communities and metabolites will further contribute to our

Fig. 8. Functional potential prediction based on PICRUSt.

understanding of the relationship between intestinal microbiota and the occurrence and development of OSA, potentially making the regulation of intestinal microbiota a novel alternative treatment for OSA.

Dysfunction of intestinal microbiota is related to increased permeability of the intestinal mucosal barrier, leading to increased local and systemic inflammatory responses as well as metabolic disorders[40,41]. The increased F/B ratio is considered a marker of intestinal dysbiosis associated with obesity and hypertension, and is widely used to evaluate the pathological state of the body[42–44]. Studies have suggested that manipulating the F/B ratio through interventions could potentially aid in weight loss[45]. Animal studies have revealed that intestinal microbiota dysbiosis in OSA is primarily characterized by an increase in *Firmicutes* or a decrease in *Bacteroidetes*, leading to an elevated F/B ratio, which subsequently results in an increase in *lactobacilli* and a decrease in butyrate-producing bacteria[10,46]. Clinical trials from OSA patients also observed an increase in the F/B ratio[47]. In this study, we found that severe OSA exhibited more pronounced disruption of intestinal microbiota. It is worth noting that compared to mild OSA, patients with severe OSA exhibit lower abundance of *Bacteroidetes* and higher F/B ratio. This suggests that there is a correlation between intestinal microbiota disorder and different degrees of OSA, and intervening in the intestinal F/B ratio may be one of the ways to reduce the risk of OSA disease.

To further identify target bacterial genera influencing intestinal microbiota homeostasis in OSA patients of varying severity, this study employed a multidimensional model analysis to explore and validate intestinal microbiota biomarkers in OSA patients. The Lefse analysis revealed that individuals with mild OSA exhibited enrichment of *g–lachnobacterium* at the genus level, while those with moderate OSA showed increased abundance of *g–Acidovorax*, *g–Acidaminococcus*, *f_Comamonadaceae*. Those with severe OSA demonstrated enrichment of *f_Sphingomonadaceae* and *g_Sphingomonas*. Random forest analysis highlighted the significance of *Parabacteroides* in discriminating among the four groups. Functional prediction analysis suggested that alterations in the

intestinal microbiota of OSA patients may play a crucial role in the progression of OSA by affecting biosynthesis signaling pathways.

Conclusion

In summary, this study suggested that differences in the structure and composition of intestinal microbiota in patients with different severities of OSA, indicating that alterations in intestinal microbiota may contribute to metabolic comorbidities associated with OSA. These discoveries have potential implications for profiling the microbiome of specific OSA patients and OSA-related diseases, as well as for targeted therapies such as probiotics or prebiotics.

Limitations

Our study has some limitations. Firstly, the sample size in our study was small, which may limit the robustness of our findings and necessitates a cautious interpretation of the results and conclusions. Secondly, our study revealed a significant BMI difference between severe OSA and healthy control groups. Given the potential influence of BMI on intestinal microbiota, future studies should include control and OSA groups with comparable BMIs. We consider this an area for improvement in our study. Thirdly, as dietary intake can alter intestinal microbiota and lead to adverse outcomes, the absence of dietary data in our study may impact the research findings. Future studies are needed to rectify and further validate our research. Finally, as a small-scale cross-sectional study, it cannot establish a definitive causal relationship between varying degrees of OSA severity and intestinal microbiota. Thus, future studies should focus on larger-scale longitudinal research across different levels of OSA severity to corroborate our findings. Despite these limitations, the data on the composition, diversity, and functional alterations of microbial taxa from our study form a critical foundation for understanding the intestinal microbiome in patients with various degrees of OSA.

Data availability

The raw sequence data of microbiota that corroborate our study's conclusions have been added to the NCBI SRA with accession number PRJNA1121898.

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Author contributions

P.-P.W.: Data analysis, literature review and draft writing. L.-J.W. and Y.-Q.F.:Integrate results and draft writing. Z.-J.D. and J.-X.H.: Sample selection and review literature. B.W.: Experimental design guidance.

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Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Informed consent has been obtained from all subjects for this study.

Additional information

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