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ORIGINAL ARTICLE

# Intestinal flora composition and fecal metabolic phenotype in elderly patients with sleep disorders combined with type 2 diabetes

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## Abstract

**Objectives:** This study aimed to determine whether type 2 diabetes (T2D) is an independent risk factor for sleep disorders in the elderly and explore the possible intestinal flora factors of sleep disorders combined with T2D in this population.

**Methods:** All hospitalized patients with sleep disorders aged ≥65 years between June and November 2023 were retrospectively analyzed, and they were divided into a sleep disorder group ($n = 134$) and a control group ($n = 109$). The logistic regression method was utilized to clarify the causal relationship between T2D and sleep disorders. For stool analyses, 42 patients were randomly extracted, which included the control group ($n = 14$), diabetes group ($n = 14$), and elderly patients with sleep disorders combined with the T2D group (ESdD) ($n = 14$). The composition feature of intestinal flora and metabolomics in the ESdD group was described through high-throughput 16S rDNA sequencing and nontargeted analysis based on liquid chromatography–mass spectrometry.

**Results:** Gender, body mass index (BMI), T2D, intestinal discomfort, and anxiety depression were independent risk factors for sleep disorders in the elderly. Notably, older individuals with T2D were 3.3 times more likely to experience sleep disorders than normal individuals. Compared with the control group, the ESdD group had decreased relative abundance of *Barnesiella* and *Marvinbryantia*, with 47 metabolites upregulated and 53 metabolites downregulated. The ESdD group showed a decrease in *Lachnospiraceae_UCG_010*, with 62 metabolites upregulated and 43 metabolites downregulated, compared with the diabetes group.

**Conclusions:** Diabetes is an independent risk factor for sleep disorders in the elderly patients. Variations in intestinal flora and metabolism significantly influence the onset and progression of the ESdD group.

KEYWORDS
intestinal flora, metabolomics, sleep disorder, the elderly, type 2 diabetes

Zhuohao Yin, and Huaze Xie contributed equally to this work.

## 1 | INTRODUCTION

Sleep disorders in the elderly population have become an urgent clinical challenge worldwide, which can augment the vulnerability to depression, anxiety, other mental health disorders,[1] and even cardiovascular diseases.[2] A study showed that type 2 diabetes (T2D) might be a potential risk factor for sleep disorders in the elderly.[3] Over 50% of individuals with T2D also suffer from sleep disorders. Within this group, approximately 8% experience delayed sleep onset, 23% struggle with maintaining sleep, and 26% experience both delayed sleep onset and poor sleep maintenance.[4] Moreover, sleep disorders can exacerbate sleep interruptions, inhibit the overactivity of the orexin neuropeptide and hypothalamic–pituitary–adrenal (HPA) axis, increase insulin resistance and glucose intolerance, and accelerate T2D development and its complications.[5] The treatment of sleep disorders was found to improve metabolic parameters, insulin sensitivity, and blood glucose variability.[6]

A study revealed a strong association between intestinal flora imbalance and sleep disorders.[7] In a clinical trial involving 32 medical students, probiotic *Lactobacillus* along and CP2305 consumption led to significant enhancement in sleep quality, as indicated by changes in Pittsburgh sleep quality index (PSQI) scores.[8] Daily consumption of PS128 was found to alleviate the symptoms of depression, cortical excitement, and fatigue and enhance the quality of deep sleep.[9] Additionally, a clinical study found differences in intestinal flora between patients with T2D and healthy controls.[10] At the genus level, the most significant decreases were observed in *Bacteroides* and *Prevotella*, whereas *Escherichia–Shigella*, *Lachnospiraceae_incertae_sedis*, *Subdoligranulum*, *Enterococcus*, and *Klebsiella* exhibited various degrees of abundance in the T2D group.[11] However, the relationship between intestinal flora and ESdD remains unclear.

The present study aimed to determine whether T2D is an independent risk factor for sleep disorders and focused on the characteristics of the intestinal flora of ESdD. The intestinal flora characteristics of patients with ESdD were utilized to discuss the intergroup difference of the relative abundance at the genus level and fecal metabolic phenotypes.

## 2 | METHODS

The materials and methods are shown in Figure 1.

### 2.1 | Study participants

The study included 243 participants (age ≥ 65 years) who presented to the Xijing Hospital (Xi'an City, China) between June and November 2023. The PSQI was utilized to divide the participants into a sleep disorder group (SD, PSQI >7, $n = 139$) and a normal control group (CON, PSQI ≤7, $n = 104$). Single-factor analysis and binary logistic regression were adopted to screen the risk factors for elderly sleep disorder patients.

Patients with severe sequelae resulting from stroke, neuropsychiatric disorders, organic encephalopathies, complications due to severe damage to the liver and kidneys or organic diseases affecting the digestive tract, and acute or terminal stages of malignant tumors or other serious health conditions were excluded.

### 2.2 | Research design and sample collection

In the regression analysis conducted in the first part of this study, T2D (odds ratio [OR] 3.30, $P = 0.001$) emerged as a significant independent risk factor for elderly patients with sleep disorders. To delve deeper into the effects of intestinal flora on patients with ESdD in clinical research, two fecal samples were collected from 42 participants for comprehensive intestinal flora sequencing and metabolomics analysis. All fecal samples were collected at 8 am on the same day and immediately stored in a refrigerator at −80°C.

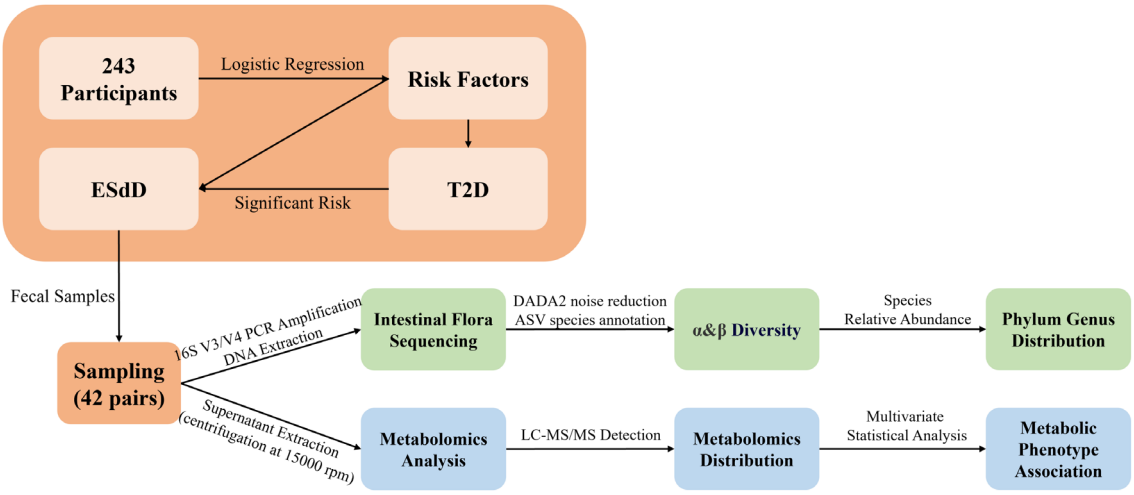


FIGURE 1 The workflow of sample collection for intestinal flora and metabolomics processing.

For the 42 pairs of fecal samples, each participant was strictly screened from the initial 243 individuals who did not use foods and drugs that inhibit probiotics, antibiotics, and corticosteroids within 6 months before enrollment.[12] The 42 participants were evenly allocated into three groups, guaranteeing the absence of significant discrepancies in age, gender, or BMI: the elderly normal control group (EC, $n=14$), the elderly T2D patients without sleep disorders group (ED, $n=14$), and ESdD group ($n=14$).

### 2.3 | Intestinal flora sequencing

The TianGen extraction kit was utilized to extract DNA from 42 fecal samples, where DNA purity and concentration were detected through 1% agarose gel electrophoresis. This study focused on the V3–V4 highly variable region of 16SrDNA from the total DNA as the target fragment for amplification, where polymerase chain reaction products were detected through 2% agarose gel electrophoresis. The target bands were recovered through the TianGen universal DNA purification and recovery reagent. Lastly, the software framework NovaSeq 6000 of PE250 was used for machine sequencing.

Software QIIME2 (2022.02) was used to process DNA sequencing data and obtain Amplicon sequence variants. The Divisive Amplicon Denoising Algorithm 2 (DADA2) package for R language was utilized to reduce noise. The software of linear discriminant analysis effect size was used to screen bacteria with significant differences. In addition, the Shannon index, Simpson index, Chao1 index, and rank-sum test were applied in the α diversity analysis. Weighted and unweighted UniFrac distances were used to observe variations in the compositional complexity of community structures.

### 2.4 | Metabolomics analysis

For metabolomics analysis, tissues (100 mg) were individually grounded with liquid nitrogen, and the homogenate was resuspended with prechilled 80% methanol by good vortex. The samples were incubated on ice for 5 min and then were centrifuged at 15,000 rpm, 4°C for 20 min. Some supernatants were diluted to a final concentration containing 53% methanol through liquid chromatography–mass spectrometry (LC–MS)-grade water. The samples were subsequently transferred to a fresh Eppendorf tube and then centrifuged at 15000 rpm, 4°C for 20 min. Finally, the supernatant was injected into the LC–MS/MS system analysis.

Raw data were imported into software CD (3.3) for comprehensive analysis, yielding both the identification and relative quantification of various metabolites. Subsequently, partial least squares-discriminant analysis (PLS-DA) was adopted to systematically screen for differential metabolites. The screening process adhered to stringent criteria: VIP >1, $p<0.05$, and FC>1.2 or FC<0.833.

### 2.5 | Statistical analysis

IBM SPSS Statistics for Windows version 27.0 (IBM Corp., Armonk, NY, USA) was utilized for the statistical and regression analyses of the clinical data of the participants. The significance level was set at $P<0.05$. The Spearman correlation analysis was conducted to explore the relationships between metabolites and intestinal flora.

## 3 | RESULTS

### 3.1 | Risk factor analyses

A comprehensive risk factor analysis of sleep disorders among 243 participants was conducted and 139 who met all assessment criteria for sleep disorders were included in the SD group. The results of the univariate analysis are shown in Table 1, and there were no significant differences among age, hypertension, and lipid status ($P>0.05$). The differences were noted in gender, BMI, T2D history, intestinal discomfort, and anxiety depression ($P<0.05$) between the SD and the CON groups. In Table 2, further binary logistic regression analysis results showed that gender, BMI, T2D history, intestinal discomfort, and anxiety depression were independent risk factors for elderly sleep disorders. Notably, the older population with T2D had a significantly increased risk for sleep disorders, with a prevalence 3.3 times higher than that of normal individuals.

### 3.2 | Intestinal flora sequencing results

The overview of intestinal flora sequencing results is illustrated in Figure 2; graph D demonstrates that the current sample size was sufficient for effective classification unit identification. Graphs A–C, combined with the Wilcoxon rank-sum test, show the same three indices of ED and ESdD ($P>0.05$). In graph C, a significant difference was found between the EC and ESdD groups under the Chao1 index ($P<0.05$). The ordination technique of the principal coordinate analysis is shown in graphs E and F, which picked up the main elements and structures from reduced multidimensional data series of eigenvalues and eigenvectors. The percentage on each axis indicated the contribution of each group to the discrepancy among samples, and the analysis indicated a notable dissimilarity in community structure between EC and ESdD relativity, with $R=0.09618$, $P=0.044$ signifying a significant difference. For the ED and ESdD groups, the community structures did not show a difference ($R=0.0134$, $P=0.307$).

In graphs I and J, no significant difference was found at the phylum level among the three groups. For the classification results of the genus level shown in shadows of graph I, the abundance levels of *Flavonifractor* and *Klebsiella* were higher in the ESdD group than in the EC group, whereas the abundance levels of 15 genera, such as *Barnesiella* and *Marvinbryantia* were higher in the EC group than in the ESdD group ($p<0.05$). For graph J, the abundance levels of *Intestinibacter* increased in the ESdD group, and the abundance

**TABLE 1** Demographic characteristics and health status of elderly patients with sleep disorders.

| Project | SD ($n=139$) | CON ($n=104$) | Statistics | *P*-value |
|---|---|---|---|---|
| Age | 72.42 ± 7. 50 | 72.72± 8.12 | 0.298 | 0.766 |
| Gender | | | | |
| Man | 64 | 68 | 8.969 | 0.003 |
| Woman | 75 | 36 | | |
| Educational level | | | | |
| Junior high school and below | 54 | 40 | 5.839 | 0.322 |
| High school and junior college | 71 | 49 | | |
| Bachelor degree and above | 14 | 15 | | |
| The resident manner | | | | |
| Live alone | 16 | 11 | 4.800 | 0.308 |
| Living with a spouse | 105 | 81 | | |
| Live with your children | 16 | 10 | | |
| Live with a nanny | 1 | 0 | | |
| Other | 0 | 2 | | |
| Family atmosphere | | | | |
| Poor | 0 | 0 | 3.582 | 0.310 |
| Normal | 0 | 1 | | |
| Preferably | 10 | 6 | | |
| Fine | 129 | 97 | | |
| Economic pressure | | | | |
| No | 111 | 92 | 4.238 | 0.237 |
| Yes | 28 | 12 | | |
| Number of trips per year | | | | |
| No | 89 | 56 | 3.268 | 0.352 |
| 1–2 | 42 | 38 | | |
| ≥3 | 8 | 10 | | |
| Smoking history | | | | |
| No | 109 | 76 | 1.773 | 0.621 |
| YSE | 13 | 13 | | |
| Quit | 16 | 14 | | |
| Weekly outdoor activity duration (*h*) | | | | |
| ≤4 | 31 | 22 | 0.255 | 0.968 |
| 4–20 | 84 | 61 | | |
| ≥20 | 24 | 16 | | |
| Weekly indoor activity duration (*h*) | | | | |
| ≤4 | 42 | 34 | 3.734 | 0.292 |
| 4–20 | 86 | 62 | | |
| ≥20 | 11 | 8 | | |
| Daily smartphone usage duration (*h*) | | | | |
| ≤1 | 34 | 33 | 4.106 | 0.392 |
| 1–4 | 74 | 50 | | |
| ≥4 | 30 | 21 | | |
| Time spent in reading per day (*h*) | | | | |
| ≤1 | 113 | 85 | 0.334 | 0.988 |
| 1–4 | 25 | 18 | | |

TABLE 1 (Continued)

| Project | SD ($n=139$) | CON ($n=104$) | Statistics | *P*-value |
|---|---|---|---|---|
| ≥4 | 1 | 1 | | |
| BMI | 23.50 ± 2.82 | 24.46 ± 3.40 | 2.371 | 0.019 |
| Hypertension | 104 (74.82) | 68 (66.02) | 2.229 | 0.135 |
| Coronary heart disease | 53 (38.41) | 37 (35.58) | 0.203 | 0.625 |
| Type 2 diabetes | 89 (64.03) | 47 (45.19) | 8.565 | 0.003 |
| Stroke | 56 (40.58) | 29 (28.16) | 5.535 | 0.063 |
| Carotid stenosis | 3 (2.56) | 1 (1.20) | 0.502 | 0.478 |
| Vertebral artery stenosis | 5 (4.27) | 1 (1.20) | 1.761 | 0.185 |
| Cerebral atrophy | 21 (22.34) | 20 (28.17) | 0.736 | 0.391 |
| TC(mmol/L) | 4.04 ± 1.18 | 3.88 ± 1.07 | −1.030 | 0.304 |
| TG(mmol/L) | 1.49 ± 0.85 | 1.46 ± 0. 99 | −0.196 | 0.845 |
| HDL(mmol/L) | 1.17 ± 0. 31 | 1.11 ± 0.27 | −1.519 | 0.130 |
| LDL(mmol/L) | 2.44 ± 1.07 | 2.36 ± 0.97 | −0.580 | 0.562 |
| GSRS | 8.63 ± 7.51 | 5.38 ± 5.16 | −3.792 | <0.001 |
| GDS-30 | 6.50 ± 4.69 | 4.66 ± 3. 05 | −3.486 | 0.001 |

Abbreviations: CON, control group; SD, elderly patients with sleep disorders.

TABLE 2 Logistic regression analysis of influencing factors for sleep disorders in elderly inpatients ($n=243$).

| Variable | *B* | S. E. | Wald | OR | *P*-value | 95% CI | |
|---|---|---|---|---|---|---|---|
| | | | | | | Floor | Upper |
| Type 2 diabetes | 1.194 | 0.313 | 14.547 | 3.300 | <0.001 | 1.787 | 6.094 |
| GSRS | 0.071 | 0.027 | 7.025 | 1.074 | 0.008 | 1.019 | 1.132 |
| GDS-30 | 0.086 | 0.042 | 4.117 | 1.089 | 0.042 | 1.003 | 1.183 |
| Gender | −0.860 | 0.318 | 7.326 | 0.423 | 0.007 | 0.227 | 0.789 |
| BMI | −0.112 | 0.051 | 4.799 | 0.894 | 0.028 | 0.809 | 0.988 |

levels of four genera, such as *Lachnospiraceae_UCG_010* were reduced in the ESdD compared with those in the ED group ($p<0.05$). Overall, the genus abundance results showed the specificity of intestinal flora such as *Flavonifractor*, *Klebsiella*, and *Intestinibacter* in the ESdD group.

### 3.3 | Metabolomics results

The results of the metabolomic analysis of the fecal samples are presented in Figure 3, where graphs A and B show the difference in metabolic phenotypes, and graphs C and D present the trend visualization of metabolites. For the results of the PLS-DA classification model, R2Y ($A=0.92$, $B=0.91$) was utilized to represent the percentage of Y matrix information, which can be explained, and Q2Y ($A=-0.19$, $B=-0.14$) was calculated through cross-validation. The fecal samples used in the PLS-DA task of the three groups in graphs A and B demonstrated predictive ability.

Graphs C and D show the results of differential metabolite screening, and this study identified 47 upregulated and 53 downregulated differential metabolites in the EC compared with that in the ESdD group. In particular, N-acetylputrescine was significantly upregulated in the ESdD group. In contrast, trigonelline and glycitin were significantly downregulated. In addition, 62 upregulated and 43 downregulated differential metabolites were found in the ED compared with that in the ESdD group, where protectin D1 and cannabidiolic acid were significantly downregulated.

Graphs E and F show the results of the Spearman correlation analysis, which was performed to investigate the correlation between intestinal flora and differential metabolites. The results demonstrated a significant correlation between *Klebsiella* and N-acetylputrescine ($P<0.01$, $R=0.57$) and a positive correlation between *Barnesiella* and trigonelline ($P=0.04$, $R=0.40$).

## 4 | DISCUSSION

This study identified several independent risk factors for elderly sleep disorders, including gender, BMI, T2D, intestinal discomfort, and anxiety depression. Notably, T2D emerged as a significant independent risk factor, with older individuals with T2D being 3.3 times more likely to experience sleep disorders compared with normal individuals. This observation aligns with prior research findings.[13] Nevertheless, very few studies have explored the mechanisms

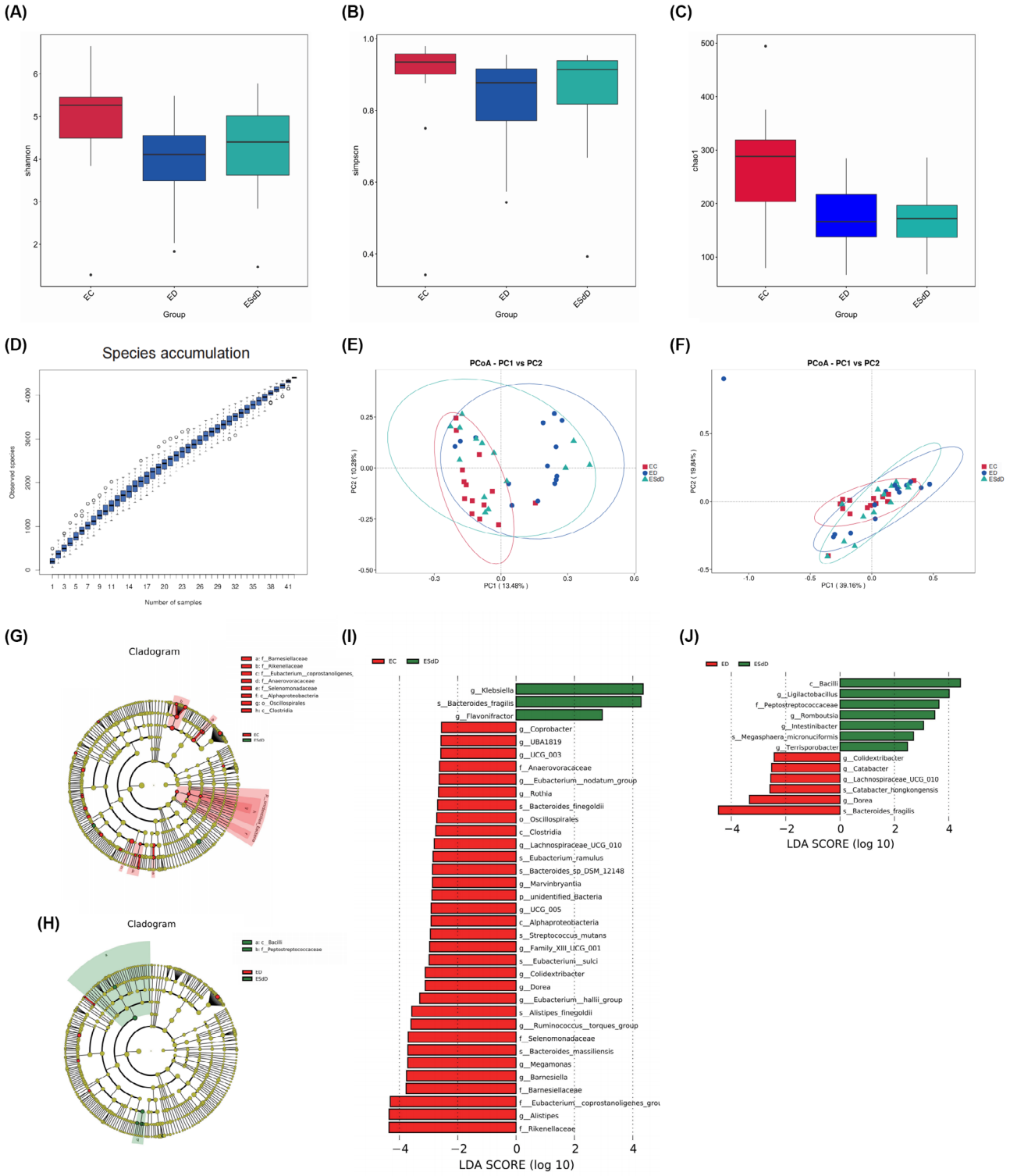


FIGURE 2 Overview of intestinal flora sequencing results. where graphs (A, B, C) are the box plots for alpha diversity from the Shannon index, Simpson index, and Chao1 index, respectively; graph (D) is a box plot of species accumulation; graphs (E, F) are PCoA classification based on unweighted E and weighted F Unifrac distance. The LEfSe statistical analysis and bar chart of the distribution of the LDA values. Graphs (G, H) show the evolutionary branch map, where each circle stands for a distinct taxon at corresponding taxonomic rank and the diameter of each circle represents proportionally the relative abundance of each taxon. Yellow stands for taxons with non-significant differences. Red nodes mean these microbiota contribute a lot in the group covered by red color, so do the green nodes. Letters above the circles and corresponding species are annotated on the right side. Graphs (I, J) show the LDA bar chart of phylum (p_), genus (g_), and others (c_, f_, s_) intestinal flora abundance distribution, where the histogram of the LDA scores represents the group differences.

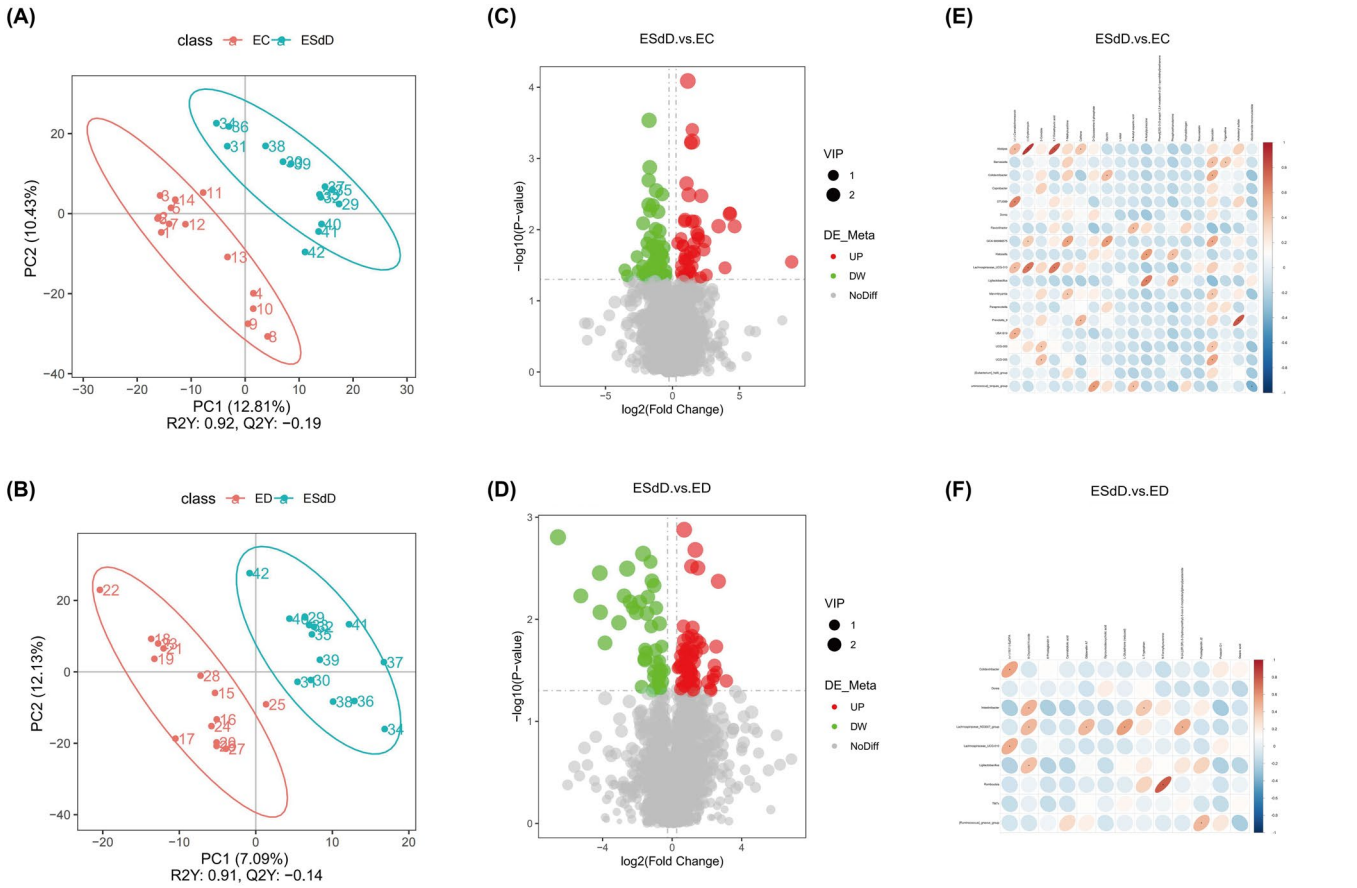


**FIGURE 3** Comparison of differential metabolites between three groups and corcorrelation of intestinal flora and differential metabolites. Graphs (A, B) of PLS-DA results are utilized to screen for differential metabolites systematically. Graphs (C, D) are volcano maps of differential metabolites, which display the distribution of different metabolites. The abscissa represents the multiple change of the difference of the metabolites in different groups (log2 (Fold Change)), and the ordinate represents the level of significance of the difference (−log10 (*p*-value)). Each point in the volcano map represents a metabolite, the significantly up-regulated metabolite is represented by a red dot, the significantly down-regulated metabolite is represented by a green dot, and the size of the dot represents the VIP value, where the heat maps show the correlation of graph (E) EC and ESdD groups, and graph (F) ED and ESdD groups. Deep red indicates that the flora is positively correlated with metabolites, and deep blue indicates that the flora is negatively correlated with metabolites. "*" indicates a significant difference between the two groups ($p < 0.05$).

underlying diabetes-induced sleep disorders. Diabetes can adversely affect sleep through multiple pathways. For instance, discomfort from nocturia, a common complication arising from peripheral neuropathy or inadequate blood sugar management, frequently disrupts normal sleep patterns, thereby precipitating sleep disorders.[14]

A comprehensive analysis of the relationship between intestinal flora and the metabolic phenotype of the ESdD group was conducted. α and β diversity analyses revealed significant changes in the diversity and composition of intestinal flora between the ESdD and EC groups, thereby demonstrating the risk of dysbiosis in the intestinal flora of the ESdD group. The analysis of intestinal flora at the genus level revealed notable findings in the ESdD group, with significant enrichment of *Flavonifractor* and *Klebsiella* and marked reductions in *Barnesiella* and *Marvinbryantia* when compared with those in the EC group.

*Flavonifractor* is associated with DNA damage caused by oxidative stress.[15] The marked increase in the abundance of *Flavonifractor* has been identified as a primary risk factor for colorectal cancer and inflammatory bowel disease in rat experimental models.[16] *Klebsiella* is a key factor that initiates the production of proinflammatory cytokines in the intestinal mucosa, resulting in DNA damage and cell cycle arrest.[17] *Barnesiella* is a competitive factor that suppresses pathogenic bacteria and regulates immune responses,[18] effectively mitigating intestinal inflammation.[19] *Marvinbryantia* positively correlates with energy metabolism and butyric acid production in intestinal epithelial cells.[20] It exhibits anti-inflammatory properties and enhances mucin production,[21] thereby modulating intestinal flora imbalance in mice with chronic colitis.[22]

The ESdD group exhibited a significant enrichment of *Intestinibacter* and a concurrent decrease in *Lachnospiraceae_UCG_010* at the genus level compared to the ED group. *Lachnospiraceae_UCG_010* is a beneficial bacterium in the colon that degrades plant fibers to produce short-chain fatty acids (SCFAs), positively affecting intestinal development and health.[23] The high

abundance of *Intestinibacter* has been linked to various diseases, including inflammatory bowel disease and sleep disorders.[24] A study showed that a decrease in the abundance of *Intestinibacter* can be beneficial for regulating the intestinal flora structure in fatigued mice.[25]

In the metabolic phenotype analysis of ESdD, the fecal samples of patients were assessed, and alterations in the fecal metabolic profile associated with ESdD were observed. Compared with the EC group, the ESdD group demonstrated significant upregulation of N-acetylputrescine and concurrent downregulation of trigonelline and glycitin. By inhibiting the NF-kB pathway, glycitin exerts its anti-inflammatory effects.[26] In Alzheimer's disease, glycitin has been shown to alleviate neurobehavioral and neurochemical abnormalities while facilitating improvements in learning and memory.[27] Trigonelline can decrease inflammation markers and reactive oxygen species, modulate MPO expression,[28] and lower tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels.[29] A clinical study revealed a high plasma concentration of N-acetylputrescine in Parkinson's disease (PD) patients, which was significantly associated with PD rating scale scores.[30] *Klebsiella* showed a positive correlation with N-acetylputrescine ($p<0.01$, $R=0.57$), whereas *Barnesiella* and *trigonelline* demonstrated a positive association ($p=0.04$, $R=0.40$).

Furthermore, the metabolic phenotype analysis of ED and ESdD groups showed significant downregulation of protectin D1 and cannabidiolic acid. The influence of protectin D1 extends to mitigating age-associated deficits in spatial learning and memory formation in Alzheimer's disease models, alongside curbing microglia activation and nerve sheath acetylase. Furthermore, within rodent models, it exerts regulatory effects on hippocampal excitability and epileptic seizures.[31] Systemic treatment of protectin D1 can reduce inflammation caused by colitis and intestinal ischemia/reperfusion in mice.[32] Cannabidiolic acid possess the ability to mitigate the inhibitory effects of calcium, thereby exerting a protective influence on primary neurons. A study suggested that cannabidiolic acid could mitigate memory loss and enhance hippocampal functionality, positioning it as a potentially effective treatment for Alzheimer's disease.[33]

Increasing evidence indicates that intestinal flora plays a pivotal role in digestive, immune, and metabolic processes and regulates sleep, circadian rhythms, and mental state. The brain–gut axis serves as a bidirectional communication system that links the gastrointestinal tract with the central nervous system. This interaction results in the transmission of information to the central nervous system and ultimately affects brain function.[34] SCFAs are the primary by-products of dietary fiber fermentation by intestinal flora. They serve multiple roles, including maintaining intestinal barrier permeability and countering the proinflammatory effects of cytokines such as IL-6 and TNF. Recent studies have also suggested that SCFAs act as intermediaries between the intestinal flora and brain mechanisms controlling sleep.[35] Changes in proinflammatory cytokines have been linked to sleep disorders, and inflammation and immune dysfunction have been proposed as potential mechanisms underlying these sleep disturbances.[36] The signals generated by the intestinal flora can trigger switches and regulate sleep. The intestinal flora and sleep/wake regulation is achieved through three main pathways: neuronal, immune, and metabolic/endocrine. In the neuronal pathway, the intestinal microbiota and its metabolites can interact with neurons in the enteric nervous system and the vagus nerve input pathway, affecting neural circuits involved in sleep/wake regulation. In the immune pathway, intestinal immune mediators can transmit to the brain via the circulatory system and vagus nerve, thereby influencing sleep. For example, lipopolysaccharides and SCFAs can affect immune cell responses and inflammatory homeostasis, activating microglial cell and influencing sleep/wake regulation. In the metabolic and endocrine pathway, the neurotransmitters and metabolites produced by the intestinal flora, intestinal endocrine cells, or intestinal chromaffin cells in the gut can affect sleep/wake regulation via the circulatory system.[37] In addition, changes in brain functions are closely linked to sleep disorders.[38]

In conclusion, this study identified T2D as an independent risk factor for elderly sleep disorders. We posit that intestinal flora and fecal metabolites play pivotal roles in the pathogenesis of ESdD. An increase in the abundance of *Flavonifractor*, *Klebsiella*, and *Intestinibacter*, coupled with a decrease in *Barnesiella* and *Marvinbryantia* and a reduction in the levels of glycitin and trigonelline, is associated with heightened intestinal inflammation. The reduction in the abundance of *Lachnospiraceae_UCG_010* and *Marvinbryantia* was correlated with a decrease in SCFA production. Conversely, high levels of N-acetylputrescine and increased expression of protective protectins D1 and cannabidiolic acid were linked to altered brain function. These changes may contribute to ESdD development.

This study has several limitations. First, most participants come from the same region and lack representativeness. This study cannot clarify the causal relationship between sleep disorders in older individuals with T2D and intestinal flora disorder. To address these limitations, studies collecting more cases from multicenters are warranted. In addition, clinical studies and animal experiments are desired to clarify the causal relationship and related mechanisms between sleep disorders in the elderly with type 2 diabetes and intestinal flora disorders. This paves the way for new prevention and treatment strategies for ESdD.

## AUTHOR CONTRIBUTIONS

**Zhuohao Yin:** Conceptualization, data curation, methodology, software, visualization, and writing original draft. **Huaze Xie:** Software, visualization, supervision, writing original draft, and writing review & editing. **Fuyuan Liu:** Data curation, software, visualization, and supervision. **Xue Kong:** Data curation, methodology, and writing review & editing. **Wei Chen:** Data curation, Methodology, Software, Visualization, and Writing review & editing. **Yangfan Gong:** Methodology, Software, Visualization, and Writing review & editing. **Wei Ge:** Conceptualization, Funding acquisition, Project administration, Supervision, and Writing review & editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

## ETHICS STATEMENTS

This study was performed according to the principles of the Declaration of Helsinki of the World Medical Association. This study was approved by the Medical Ethics Committee of Xijing Hospital (No. KY2023-2138-F-1) and all participants signed informed consents.

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