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Peritoneal dialysis promotes microbial-driven biosynthesis pathways of sesquiterpenes and triterpenes compounds in end-stage renal disease patients

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Abstract

The concept of the gut-kidney axis is gaining significant attention due to the close relationship between gut microbiota and kidney disease. Peritoneal dialysis is recognized as a crucial renal replacement therapy for end-stage renal disease (ESRD). The alterations in gut microbiota and related mechanisms after receiving this dialysis method are not fully understood. This study conducted shotgun metagenomic sequencing on fecal samples from 11 end-stage renal disease patients who did not receive dialysis (ESRD_N) and 7 patients who received peritoneal dialysis (ESRD_P). After quality control and correlation analysis of the data, our study is aimed at exploring the impact of peritoneal dialysis on the gut microbiota and health of ESRD patients. Our research findings indicate that the complexity and aggregation characteristics of gut microbiota interactions increase in ESRD_P. In addition, the gut microbiota drives the biosynthesis pathways of sesquiterpenes and triterpenes in ESRD_P patients, which may contribute to blood purification and improve circulation. Therefore, our research will lay the foundation for the prevention and treatment of ESRD.

Keywords End-stage renal disease, Peritoneal dialysis, Gut microbiota, Metagenome Shotgun sequencing, Sesquiterpenoid and triterpenoid biosynthesis

Introduction

Recent statistics indicate that chronic kidney disease (CKD) affects approximately 15–20% of adults worldwide [1]. Alarmingly, it is projected to become the fifth leading cause of death globally by 2040, experiencing the largest anticipated increase among all major causes of death [2]. End-stage renal disease (ESRD) is the final stage of CKD, characterized by a glomerular filtration rate (GFR) below 15 mL/min/1.73 m^2 [3]. As one of the main therapy methods for ESRD patients, peritoneal dialysis has been widely used in clinical treatment [4, 5]. An important part of peritoneal dialysis is using the peritoneum as a semi-permeable membrane to get dialysate into the abdominal cavity. The primary objective is

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to facilitate the elimination of metabolic byproducts and toxic substances and the correction of water and electrolyte imbalances in the body via diffusion and permeation [6]. Notably, peritoneal dialysis offers advantages over traditional hemodialysis, including better preservation of residual kidney function, enhanced patient quality of life, and an improved post-kidney transplantation prognosis [7].

Increasing evidence suggested a close relationship between gut microbiota and kidney diseases, leading to growing attention on the concept of the gut-kidney axis [8]. Notably, research has demonstrated significant differences in the gut microbiota composition between patients with ESRD and those with CKD but without ESRD [9]. This suggests an association between the gut microbiota and the occurrence and progression of kidney disease. Furthermore, studies comparing rats that received fecal microbiota transplants from healthy people to those that received them from people with ESRD showed that the latter group had higher levels of serum uremic toxins, as well as worsened renal fibrosis and oxidative stress [10]. Additionally, investigations have identified distinct variations in the gut microbiota of ESRD patients undergoing dialysis compared to those not receiving dialysis (ESRD_N). Interestingly, ESRD patients undergoing peritoneal dialysis (ESRD_P) exhibited gut microbiota compositions that were more like those of healthy individuals than ESRD patients undergoing hemodialysis [11, 12].This observation may be attributed to the potential benefits associated with peritoneal dialysis. However, the precise alterations in the gut microbiota and the underlying mechanisms in ESRD patients following peritoneal dialysis are not fully understood.

Therefore, to find out how peritoneal dialysis affects people with ESRD, we performed shotgun metagenomic sequencing on ESRD_P patients and ESRD_N patients. We aimed to investigate alterations in the gut microbiota and elucidate the associated underlying mechanisms. Our findings have significant implications, as they will provide solid groundwork for the development of ESRD-related drugs and further treatment of ESRD.

Methods and materials

Study subjects and sample collection

18 participants, including 11 ESRD_N patients and 7 ESRD_P patients (continuous regular peritoneal dialysis>1 year), were recruited from the Department of Nephrology at the Third Xiangya Hospital of Central South University in Changsha, Hunan, China. Exclusion criteria included previous history of intestinal disease, cancer, diabetes, kidney transplant, hemodialysis, special diets (such as vegan, pure meat, etc.), as well as taking antibiotics, hormones, immune agents, probiotics, yogurt, laxatives, antipurgatives in the last six months. Additionally, patients with a BMI greater than 28 were also excluded. This study was approved by the Ethics Committee of the Third Xiangya Hospital according to the ethical guidelines of the Declaration of Helsinki (NO. 23836). All participants were interviewed, and they signed informed consent forms. Recorded the clinical and demographic information of each patient, including age, gender, condition, clinical examination, and other information. All fecal samples collected were first stored at -80 °C, labeled, and then simultaneously subjected to shotgun metagenomic sequencing [13]. The fecal samples were collected from May 2023 to June 2023.

DNA extraction and metagenome shotgun sequencing

All fecal samples were subjected to DNA extraction and metagenome shotgun sequencing. According to the manufacturer's instructions, DNA from microbial genomes in samples was extracted using the Omega Soil DNA Kit (D5625-01). The extracted DNA is stored at -20 °C for further evaluation. After that, a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the concentration of the extracted DNA. Agarose gel electrophoresis was then used to measure the quality of the DNA. The qualified microbial DNA was processed to construct metagenome shotgun sequencing libraries with insert sizes of 400 bp by using the Illumina TruSeq Nano DNA LT Library Preparation Kit. Each library was sequenced by the Illumina HiSeq X-ten platform (Illumina, USA) with the PE150 strategy at Personal Biotechnology Co., Ltd. (Shanghai, China).

Metagenomic analysis

Raw sequencing reads were processed to obtain quality-filtered reads for further analysis. Cutadapt was used to remove sequencing adapters from the sequencing reads [14]. Then, Fastp was chosen to remove low quality reads by using a sliding window algorithm and achieve data quality control [15]. Bowtie2 was employed to filter the sequences from humans (hg37dec_v0.1) to make sure that the reads were not influenced by host [16]. Kraken2 was used for taxonomic classification of high-quality reads based on a custom Kraken2 microbial database [17]. Bracken was used to estimate the relative abundance of different microbial taxa at different levels [18]. Subsequently, high-quality reads were used for gene function prediction. Megahit was used to assemble contigs from the selected reads [19]. Prodigal was used to predict coding sequences (CDSs) in the generated contigs [20]. The redundant predicted genes were removed by using CD-HIT [21]. Samlon was used to estimate gene quantification across different samples [22]. Functional gene annotation was performed using eggNOG-mapper [23]. The Kyoto Encyclopedia of Genes and Genomes (KEGG)

was generated from the annotation results of eggNOG-mapper. Finally, the gene abundance table was obtained by adding the same orthologs (OGs) annotated by egg-NOG- mapper. The obtained microbial abundance table and gene abundance table were used for further statistical analysis. All analyses refer to previous methods [24, 25].

Exploration of abundance and correlation of species

All statistical analyses were conducted using R (V6.2.561) [26]. The microbial co-occurrence networks of two groups were constructed using the "Hmisc" package to establish the Spearman correlation matrix of the microbiota [27]. The "igraph" package was used to correct the *P* value matrix [28]. The Benjamini and Hochberg false discovery rate (FDR) was employed to adjust the *P* value. The Spearman correlation coefficient and the adjusted *P* value were 0.7 and 0.05, respectively. The visualization of the network graph was performed using the Gephi software (https://gephi.org/) [29].The Wilcox test was employed for differential analysis of the microbial species, considering a significance threshold of $P<0.05$ to identify differential species. The "Psych" package was used to find the Spearman correlation between clinical indicators and microbiota that was significantly different. The Benjamini and Hochberg false discovery rate (FDR) was then used to fix the *P* values [30]. The Spearman correlation coefficient and the adjusted *P* value were 0.7 and 0.05, respectively. Additionally, the relative abundance changes of significant microbiota were visualized using the "Pheatmap" package [31]. Unless otherwise specified, all results were visualized using the "ggplot2" package [32].

Analysis of differential genes

The Wilcox test was used for screening genes with significant changes based on abundance. The *P* values were adjusted using the Benjamini and Hochberg false discovery rate (FDR). The "log2 FoldChange" was defined as log2 FoldChange=log2[(A+1)/(B+1)], where A and B represent the abundance of OGs in different groups. Genes meeting the criteria of $P<0.05$ and $|log2FC| > 1$ were considered to have significant differences [33]. The "Psych" package was used to calculate the Spearman correlation between top 10 genes with significant differences and differential species, with the *P* values being adjusted using the Benjamini and Hochberg method for false discovery rate (FDR) [30]. The Spearman correlation coefficient and the adjusted *P* value were 0.7 and 0.05, respectively. Additionally, the "Pheatmap" package was used to visualize the correlation [31].

Detection of KEGG pathway and other statistical analysis

Enrichment of KEGG pathways was performed on the top 10 genes with significant differences, and 12 KEGG pathways were found. Besides, to identify significantly changed KEGG pathways, we calculated the *P* value using the Wilcox test. $P<0.05$ was considered to have a significant difference. The "Venn Diagram" package was used to create Venn diagrams, illustrating the intersection between enriched pathways and differential pathways [34]. Meanwhile, clinical indicators showing significant changes were identified using the Wilcox test ($P<0.05$). Unless otherwise specified, all results were visualized using the "ggplot2" package [32].

Results

Peritoneal dialysis significantly changes clinical indicators in ESRD patients

To investigate the impact of peritoneal dialysis on the body, the demographic characteristics of the ESRD_N and ESRD_P groups are presented in Table 1. In the baseline data, there were no significant differences in gender or age between these two groups of patients ($P>0.05$). Furthermore, we found significant changes in some clinical indicators of ESRD patients after receiving peritoneal dialysis (Fig. 1A). Among them, HDL-C, eGFR, Cl, K (Fig. 1B), and ALB showed significant decreases, while Cr and CO_2 significantly increased (Fig. 1C).

Effects of peritoneal dialysis on gut microbiota in ESRD patients

We analyzed the changes in gut microbiota between the ESRD_N and ESRD_P groups. Firstly, the differences in microbial interactions between ESRD_N and ESRD_P groups were evaluated through network analysis. In the ESRD_N group, the network graph showed 199 nodes and 1379 edges (Fig. 1D), while the ESRD_P group had 199 nodes and 1808 edges (Fig. 1E). Furthermore, we found that the weighted degree and clustering were

Table 1 Characteristics of study subjects

Variables	ESRD_N (*n* = 11)	ESRD_P (*n* = 7)	*P* value
Age [year, median(range)]	56 (40–75)	59 (22–66)	0.659
Gender, n (%)			0.285
female	5 (45.5%)	2 (25%)	
male	6 (54.5%)	6 (75%)	

ESRD_N, No dialysis treatment; ESRD_P, Peritoneal dialysis treatment

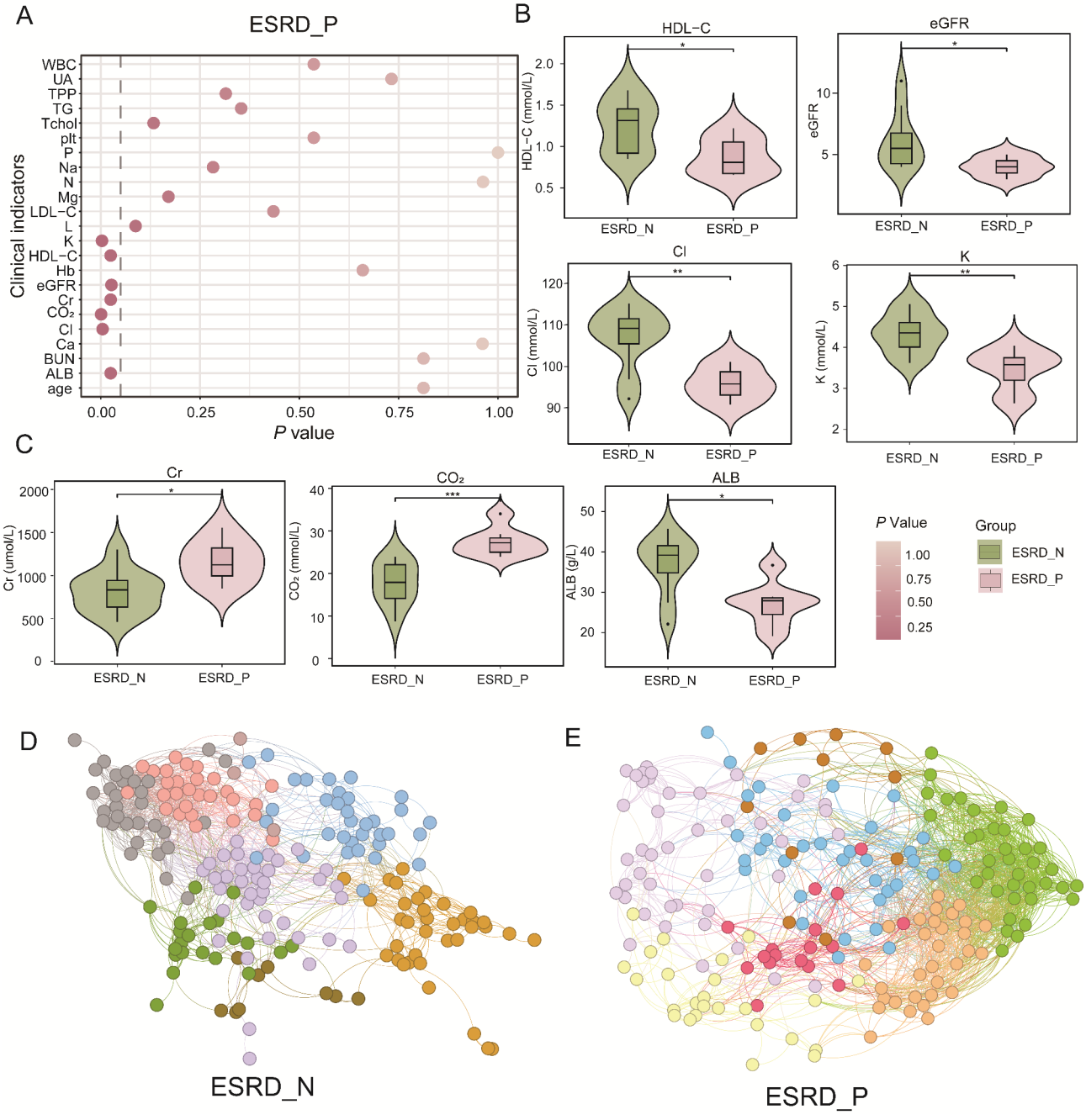

Fig. 1 Changes in clinical indicators and gut microbiota stability in peritoneal dialysis patients. **(A)** The changes in clinical indicators after peritoneal dialysis in ESRD patients. The difference in clinical indicators HDL-C, eGFR, Cl, K **(B)**, Cr, CO2, and ALB **(C)** between the ESRD_P group and the ESRD_N group (Wilcox test, $*P < 0.05$, $**P < 0.01$). The co-occurrence network of microbiota in the ESRD_N group **(D)** and the ESRD_P group **(E)**. ESRD_N, no dialysis treatment; ESRD_P, peritoneal dialysis treatment

higher in the ESRD_P group compared to the ESRD_N group (Table S1). This suggested that after peritoneal dialysis, there was increased complexity and clustering characteristics in the interaction of gut microbiota in patients. To investigate how peritoneal dialysis affects the gut microbiota, we selected the top 12 species in relative abundance from the ESRD_N and ESRD-P groups for the display of relative microbial abundance. After peritoneal dialysis, the ESRD_P group had changes in the composition of their gut microbiota (Fig. 2A). To learn how peritoneal dialysis affects the microbiota in the gut, we used the Wilcox test on the 3341 species that had been annotated. This showed that 83 species had significantly different relative abundances (Fig. 2B). We conducted the Spearman correlation analysis between these 83 species and 7 different clinical indicators in the ESRD_P group

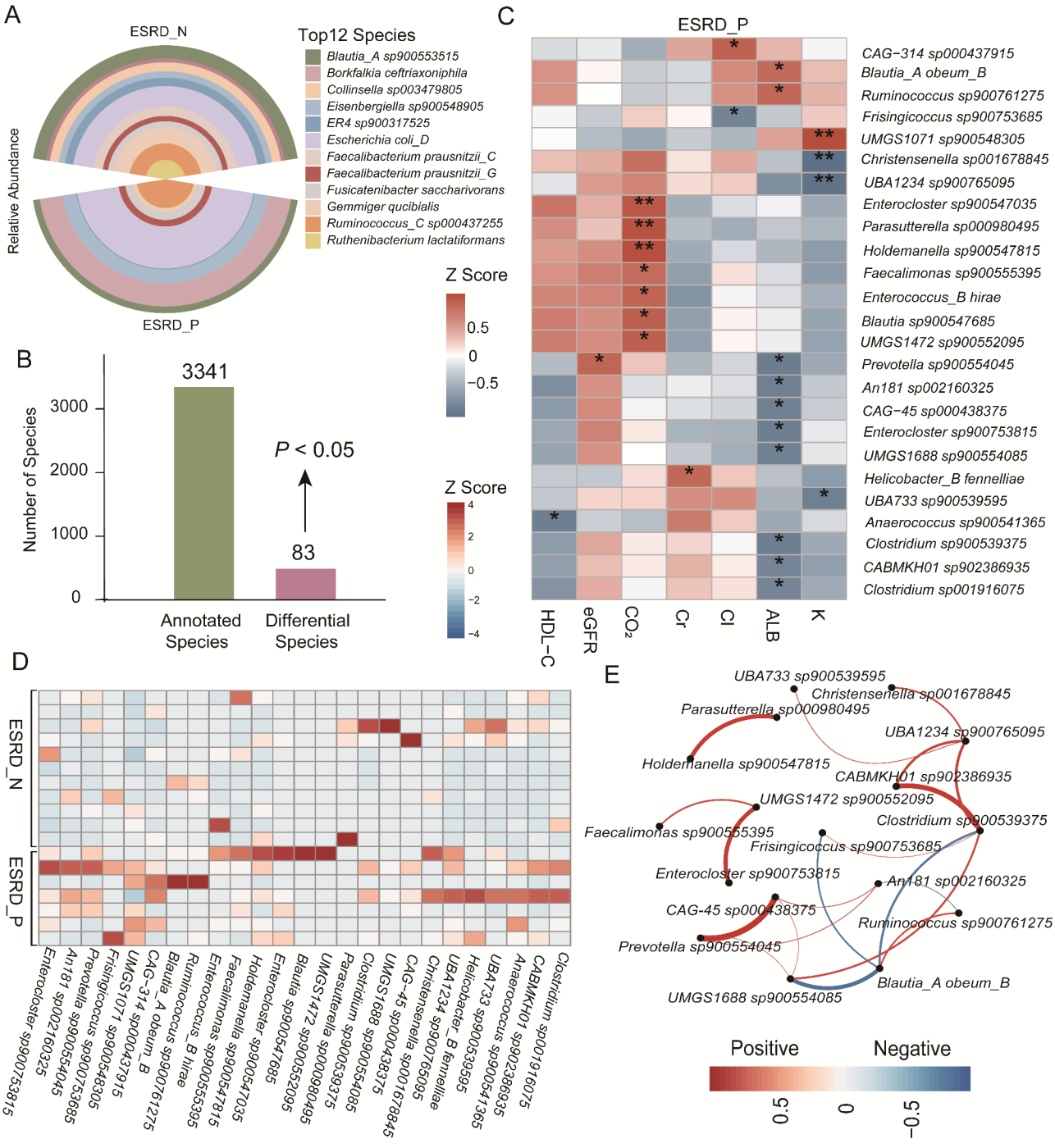

Fig. 2 Gut microbial composition and correlation analysis in the ESRD_N and ESRD_P patients. **(A)** The specific changes in the top 10 abundance microbiota in the ESRD_N and ESRD_P groups. **(B)** Difference abundance analysis at species level based on the Wilcox test. The *P* values less than 0.05 are considered differential bacteria. **(C)** The Pearson correlation between differential species and differential clinical indicators. The color bar represented the Pearson correlation index after Z score standardization. **(D)** The heatmap showed the relative abundance differences of 25 different species in the ESRD_N and ESRD_P groups. The color bar represented the relative abundance of different species after Z score standardization. **(E)** The correlation and network analysis among 25 species with significant differences in the ESRD_P group. The color bar represented the strength of the correlation. Edges in different colors between two genera represented the positive or negative correlation. ESRD_N, no dialysis treatment; ESRD_P, peritoneal dialysis treatment

and found 25 species that showed significant correlations (Table S2, Fig. 2C). The results indicated that these 25 species were primarily associated with changes in CO_2, ALB, and K. We further visualized the distribution of these 25 species in the ESRD_N and ESRD_P groups through a heatmap (Fig. 2D). We also used spearman correlation to look at the relationships between these 25 species and found important connections between *UBA733 SP900539595* and *UBA1234 SP900765095.* Both interactions were negatively related to changes in K.

The influence of peritoneal dialysis on gut microbiota genes and metabolism in ESRD patients

To further explore the changes in gut microbiota and their functional implications after peritoneal dialysis, we analyzed the ESRD_P group on the gene level. The Wilcox test and log2Fold Change were used to determine the significant changes in OGs in the ESRD_P group. The $P<0.05$ and $|Log2FoldChange| > 1$ were considered significantly different. Compared to the ESRD_N group, ESRD_P showed that 187 OGs were significantly lower ($P<0.05$ and $Log2FC < -1$), while 7 OGs were significantly higher ($P<0.05$ and $Log2FC>1$) (Fig. 3A). We selected the top 10 OGs with the most significant changes and performed Spearman correlation analysis on the 25 differential species (Fig. 3B). The results showed a significant correlation between K00511 and *UBA733 SP900539595*. Furthermore, we demonstrated the enriched pathways for these 10 OGs (Fig S1). A total of 12 KEGG pathways were enriched by these 10 OGs. We plotted a bar plot showing the read counts of these 12 pathways in the ESRD_N and ESRD_P groups (Fig. 3C). We found that the enzymes with the EC number pathway had the highest read counts in the two groups. The Wilcox test was used to see how the ESRD_N and ESRD_P groups were different to learn more about how the KEGG pathways changed after peritoneal dialysis. Meanwhile, we found 16 KEGG pathways that were significantly different (Fig. 3D). Among them, 6 pathways showed changes that were different in OGs.

Sesquiterpene and triterpene biosynthesis critical to the impact of peritoneal dialysis on ESRD patients

We performed an intersection analysis between the 12 KEGG enrichment pathways and the 16 KEGG differential pathways that were enriched by the top 10 differential OGs. The Venn diagram showed that sesquiterpenoid and triterpenoid biosynthesis might be the main pathways affected in ESRD patients after peritoneal dialysis (Fig. 3E). In the sesquiterpenoid and triterpenoid biosynthesis pathways, we found that the differentially expressed gene K00511 (*SQLE*) participates in the conversion of squalene to (S)-Squalene–2,3-epoxide (Fig. 4A). In this pathway, K00801 (*FDFT1*), which is also differentially expressed, was involved as well. Both *SQLE* and *FDFT1* exhibited a significant increase in the ESRD_P group, suggesting that they have a synergistic regulatory effect in promoting the formation of triterpenoid compounds. There is a significant correlation between *SQLE* and *UBA733 SP900539595*, with *UBA733 SP900539595* being related to K metabolism. Therefore, based on these results, we found that gut microbiota drives the biosynthesis pathways of triterpenes and triterpenoid compounds in ESRD patients after peritoneal dialysis, which may contribute to blood purification and improve circulation (Fig. 4B).

Discussion

In this study, we investigated the differences in gut microbiota between ESRD_N and ESRD_P patients through shotgun metagenomic sequencing. We found that peritoneal dialysis affects the composition and function of the gut microbiota. The changes in gut microbiota were correlated with some biochemical indicators of the human body, suggesting that peritoneal dialysis may affect the complications and prognosis of ESRD by changing gut microbiota. This is consistent with the conclusion of the study by Dan Luo et al. [12]. Disruption of the normal gut microbiota may lead to intestinal barrier dysfunction and bacterial translocation. Meanwhile, the dynamic change of the gut microbiota can produce excessive uremic toxins, such as indolyl sulfate and trimethylamine-N-oxide, which cause oxidative stress damage to the kidneys, cardiovascular system, and endocrine systems [35]. When the gut microbiota of ESRD patients was compared with healthy controls, approximately 190 microbial operational taxonomic units were significantly different in the relative abundance. The abnormal gut microbiota of ESRD patients shapes a deleterious metabolome that aggravates clinical symptoms [10, 36]. Some uremic toxins can be removed by peritoneal dialysis, along with water, electrolyte, and acid-base imbalances. This keeps the body's internal environment stable and improves the outlook for people with ESRD [6].

To further explore the potential mechanism of peritoneal dialysis, we comprehensively analyzed the gut microbiota, gut microbiota genes, and relative KEGG pathways. It was found that there were significant differences between K and *UBA733 SP900539595* in the ESRD_P group. This change in the microbial composition also affects the upregulation of the *SQLE* gene, which makes it easier for squalene to break down and makes it easier for triterpenes to form. These effects may contribute to blood purification and improved circulation.

The kidney is the main organ that maintains potassium ion balance, so ESRD patients are at a higher risk of developing hyperkalemia [37]. ESRD patients with hyperkalemia are at increased risk for subsequent adverse

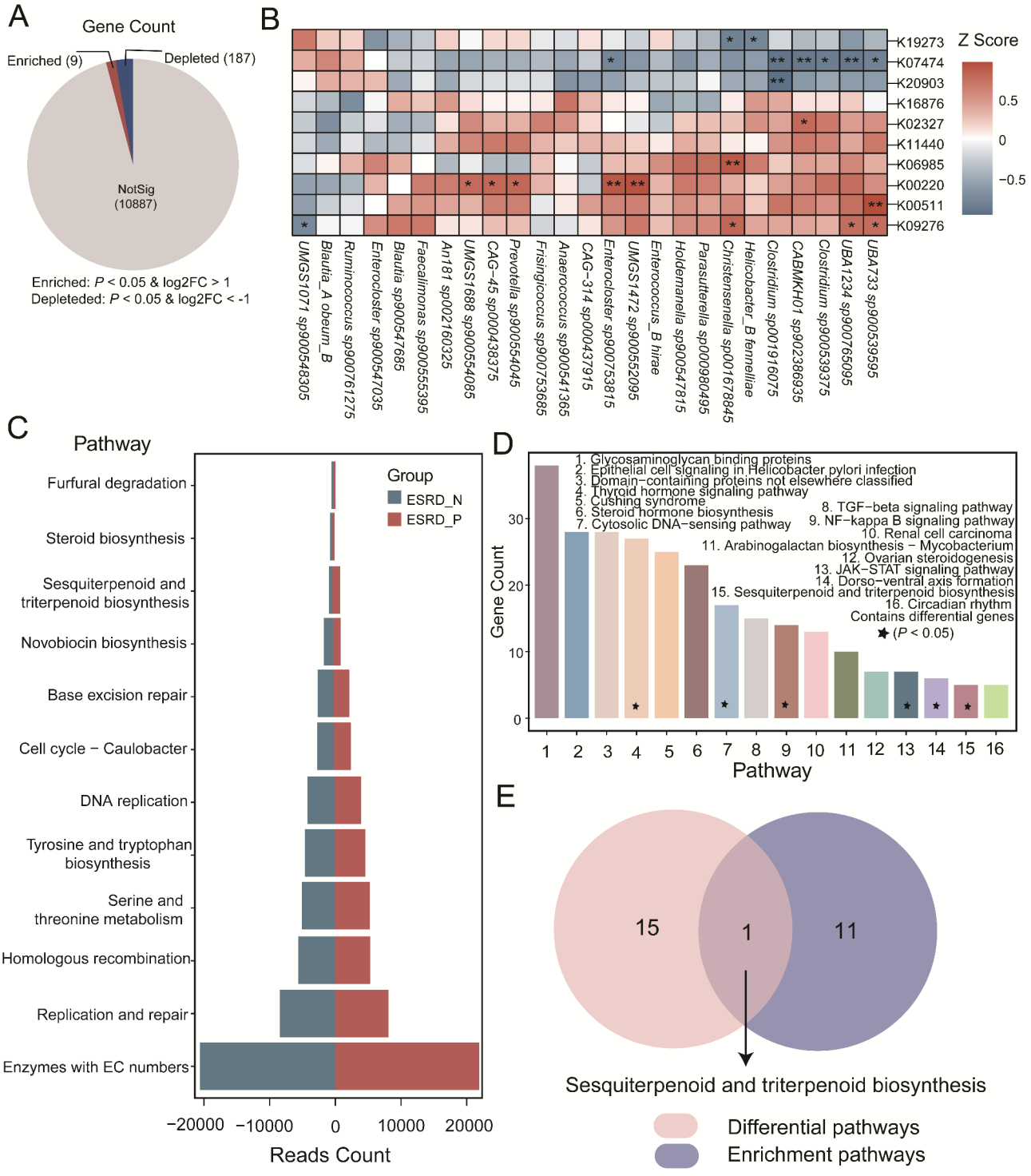

Fig. 3 Identification of differential genes and their enriched metabolic pathways of the gut microbiota in the ESRD_N and ESRD_P Patients. **(A)** The differential gene analysis of ESRD_N and ESRD_P based on the Wilcox test. (Enriched, $P < 0.05$ & Log2FC > 1; Depleted, $P < 0.05$ & Log2FC < -1.) **(B)** The Spearman correlation between the top 10 genes with the most significant differences and 25 different species. The color bar represented the Spearman correlation index after Z score standardization. **(C)** The total number of gene reads counts in the 12 metabolic pathways enriched by the top 10 differential genes. **(D)** Differential analysis showed 16 differential pathways in the ESRD_P group compared to the ESRD_N group. **(E)** The commonalities between 16 differential pathways and 12 enrichment pathways. ESRD_N, no dialysis treatment; ESRD_P, peritoneal dialysis treatment

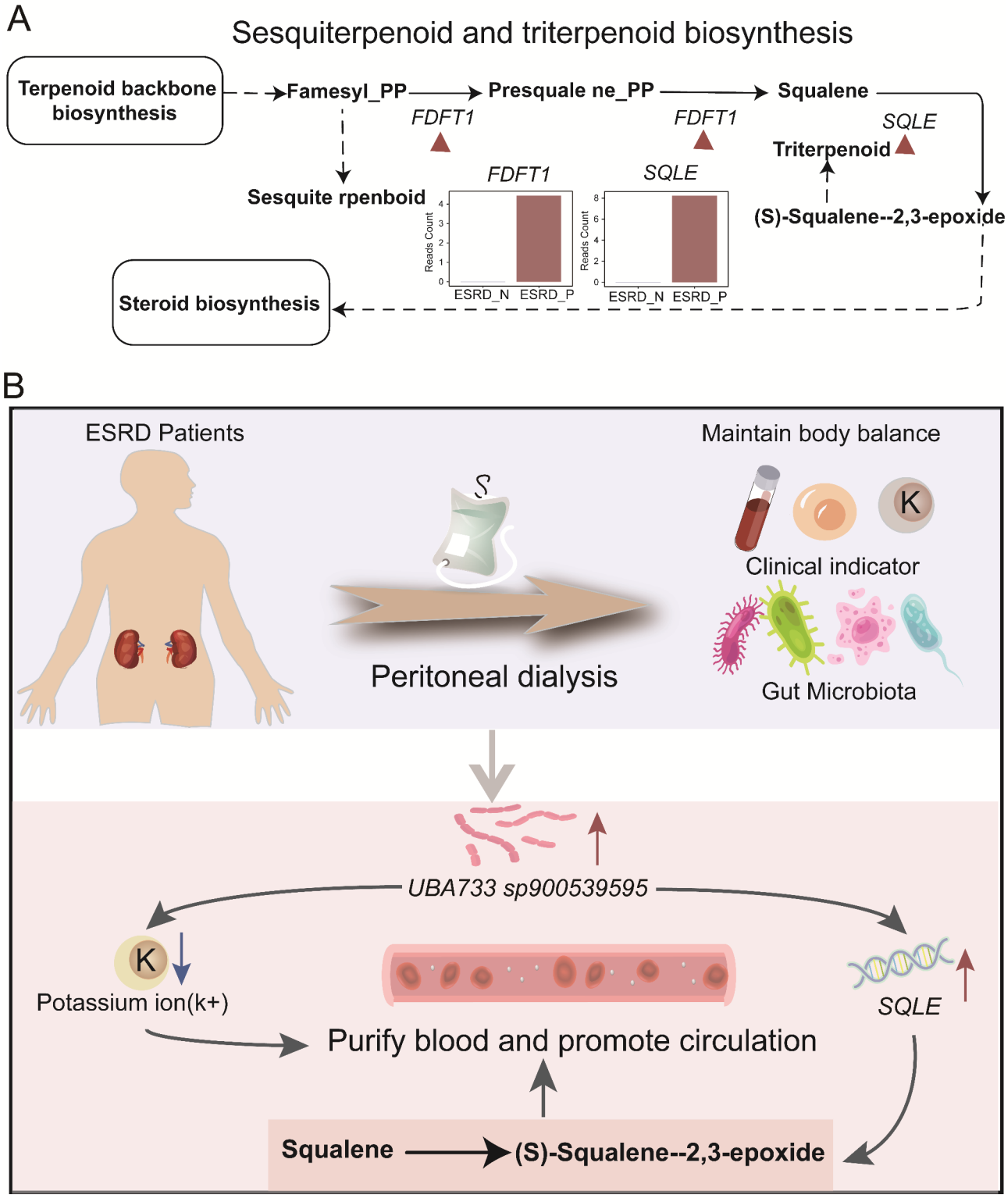

Fig. 4 Sesquiterpene and triterpene biosynthesis plays a key role in patients on peritoneal dialysis. **(A)** The specific steps for differentially expressed genes to participate in this pathway and the changes in the reads count of differential genes (Wilcox test, $*P < 0.05$, $**P < 0.01$). The red triangle indicates differential genes. The straight arrow represents a direct reaction, while the dashed arrow represents an indirect reaction. **(B)** Peritoneal dialysis affects the blood potassium and intestinal microbiota composition of patients, promotes the synthesis of triterpenoids, and ultimately achieves blood purification and circulation promotion. ESRD_N, No dialysis treatment; ESRD_P, Peritoneal dialysis treatment

events, including cardiovascular morbidity and mortality [38]. After peritoneal dialysis, patients' blood potassium will decrease. Compared with ESRD_N, ESRD_P patients are less likely to develop hyperkalemia [39]. There is currently a paucity of literature on serum potassium and gut microbiota. Our study found that *UBA733 SP900539595* are related to hypokalemia after peritoneal dialysis. Studies have found that in ESRD patients, Bacteroidetes are positively correlated with serum K, while Dorea is negatively correlated with serum K [12]. Research about oral microbiota demonstrates that periodontal pocket potassium levels increase the virulence of the entire oral community and change the immune response of the gingival epithelium. It suggested that potassium levels in periodontal pockets may be an important factor in oral microbiome dysbiosis [40]. These seem to indicate that potassium ions are closely related to the microbiota.

Our study found that the differentially expressed gene *K00801* (*FDFT1*) is involved in the formation of squalene. Squalene is a naturally occurring triterpene hydrocarbon. It is a polyunsaturated hydrocarbon produced during metabolic processes and found in many natural products, such as olive oil and shark liver oil. Squalene currently has a wide range of medical uses, including antibacterial, anticancer, and anti-inflammatory. In vitro animal experiments have found that adding squalene to the diet can increase the abundance of probiotics and promote the antioxidant capacity of the blood and jejunal mucosa, affecting blood biochemistry index [41]. Besides, squalene also increased HDL cholesterol and paraoxonase 1 and reduced oxidative stress [42]. Studies have also found that multi-drug nanoparticles of squalene can improve uncontrolled inflammation in rodents [43]. Squalene is non-polar in nature and has a high affinity for this unbound compound, helping to remove xenobiotic compounds from the body and acting as a detoxifier [44]. This may be related to the potential benefit of the differentially expressed gene K00801 (*FDFT1*) in ESRD_P patients.

The study also discovered that the differentially expressed gene *K00511* (*SQLE*) helps turn squalene into (S)-squalene-2,3-ethylene oxide, which then promotes the generation of triterpenoids. Triterpenoids have anti-cancer, anti-inflammatory, and other effects. Many studies have shown that taking triterpenoid drugs can correct gut microbiota disorders and improve prognosis in patients with chronic kidney disease [45, 46]. Besides, changes in specific gut microbiota can stimulate the production of triterpenoids [47, 48]. Research indicates that asiatic acid, a triterpenoid compound, induces significant shifts in the abundance of specific intestinal flora, displaying anti-renal fibrosis effects [45, 49]. Another triterpenoid substance, asiaticoside, inhibits TGF-β1-induced mesothelial-mesenchymal transition and oxidative stress through Nrf2 activation, safeguarding the peritoneum and preventing peritoneal fibrosis [50]. Studies with lab-grown animals have shown that two triterpenoids, called Poria A and Poria, can lower the number of glycine-conjugated compounds and polyamine metabolites in the blood. This reduction has shown efficacy in mitigating both hypertension and renal fibrosis [46]. Triptolide, yet another triterpenoid substance, mitigates oxidative stress and vascular calcification in chronic kidney disease by upregulating heme oxygenase-1 [51]. Hence, based on these results, we found that alterations in gut microbiota following peritoneal dialysis stimulate the synthesis of triterpenoids. The triterpenoids can demonstrate the ability to prevent and diminish renal and peritoneal fibrosis, counteract oxidative stress, resist vascular calcification, and regulate blood pressure.

Although our research sample size needs to be further increased, our study innovatively found that peritoneal dialysis promotes microbial-driven biosynthesis pathways of sesquiterpenes and triterpenes compounds in end-stage renal disease patients. This will lay the foundation for the development of ESRD-related drugs and further treatment of ESRD.

Conclusion

In conclusion, we performed shotgun metagenomic sequencing on ESRD_N patients and ESRD_P patients and comprehensively analyzed it at the level of gut microbiota, gut microbiota genes, and related KEGG pathways. We found that there is a noteworthy impact on *K* and *UBA733 SP900539595* after peritoneal dialysis. The changes of microbiota further influence an increase in *SQLE*, facilitating the decomposition of squalene and ultimately promoting the formation of triterpenoids, which may contribute to blood purification and improve circulation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03539-3.

Supplementary Material 1

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Author contributions

JW and JH conceived the study. XW, XY and YL collected the fecal sample form ESRD patients. XW, SY and ZY analyzed the data, wrote, and edited its final manuscript. All authors read and approved the final manuscript.

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Data availability
The Metagenome Shotgun Sequencing data reported in this paper have been deposited in the Genome Sequence Archive (GSA) (https://ngdc.cncb.ac.cn/gsa/), under GSA Project ID CRA014207 (https://ngdc.cncb.ac.cn/gsa/browse/CRA014207).

Declarations

Ethics approval
This study was approved by the Ethics Committee of the Third Xiangya Hospital according to the ethical guidelines of the Declaration of Helsinki (NO. 23836). All participants were interviewed, and they signed informed consent forms.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Commercial relationships disclosures
None.

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