Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

# Gut Microbiota in T2DM Patients with Microvascular Complications: A 16S rRNA Sequencing Study

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**Objective:** This study aims to investigate the characteristics of gut microbiota in patients with microvascular complications of Type 2 Diabetes Mellitus (T2DM) using 16S rRNA high-throughput sequencing technology.

**Methods:** Patients diagnosed with T2DM were enrolled as study subjects. Based on the presence of microvascular complications, subjects were divided into a study group, a control group. Clinical fecal samples from the two groups were subjected to diversity analysis using the Illumina MiSeq high-throughput sequencing technology, comparing the richness and diversity of the gut microbiota between the two groups. The Tax4Fun software was utilized for the functional prediction of differential microbiota.

**Results:** A total of 3727 operational taxonomic units (OTUs) were identified, with 1311 OTUs common to both groups, and 1363 and 1053 OTUs unique to the study group and the control group, respectively. The study group exhibited a significant increase in the relative abundance of Clostridia and Negativicutes, and a marked decrease in Gammaproteobacteria, Bacilli, and Verrucomicrobia compared to the control group. LefSe analysis revealed significant differences in the relative abundance at two phyla, two classes, two orders, three families, and two genera levels between the groups. KEGG pathway analysis of differential microbiota identified 10 pathways with statistically significant differences ($P<0.05$).

**Conclusion:** This study reveals significant disparities in gut microbiota abundance between T2DM patients and those with microvascular complications of T2DM, suggesting potential microbial markers for diagnosing and treating microvascular complications of T2DM.

**Keywords:** 16S rRNA sequencing, type 2 diabetes, microvascular complications, gut microbiota

## Introduction

Globally, the prevalence of type 2 diabetes mellitus (T2DM) is estimated to be around 417 million, projected to rise to 700 million by 2045,[1,2] making it one of the chronic diseases that significantly impact public health. Microvascular complications are a frequent occurrence in T2DM, with the disease itself being a major risk factor.[3] The enduring exposure to "glycotoxin", coupled with microvascular proliferation and alterations in homeostasis, culminates in a state of hypoxia, resulting in disorders in various microvascular circulatory systems and basement membrane thickening in T2DM patients.[4] As the global incidence of diabetes rises, the risk of microvascular complications is notably on the rise.[5] The onset of microvascular lesions caused by diabetes is insidious and progresses rapidly, severely compromising the physical and mental health of the affected individuals.

The gut microbiota, functioning as a microbial organ, plays multiple physiological and pathological roles, affecting systemic metabolic activities and maintaining immune system stability by inhibiting pathogenic bacteria growth and fermenting primary energy substrates.[6,7] Studies have shown that the occurrence of T2DM is intricately linked not only to dietary habits but also to alterations in the gut microbiota, making it a focal point of diabetes prevention and treatment research worldwide.[8,9] Restoration of gut microbiota is beneficial for the improvement of T2DM. Patients with diabetic

Received: 29 August 2024
Accepted: 24 October 2024
Published: 13 February 2025

complications exhibit severe gut dysbiosis, such as in diabetic retinopathy and diabetic neuropathy.[10,11] However, there is limited research on the gut microbiota associated with T2DM microvascular complications, with only one study reporting gut microbiota dysbiosis in female patients with T2DM microvascular complications.[12] Microvascular complications of T2DM generate various microbial metabolites that compromise the epithelial barrier, facilitate pathogen invasion, and enhance oxidative stress.[13] A cross-sectional study on female T2DM patients highlighted the differences in gut microbial communities between those treated with and without metformin,[14] underscoring the necessity of studying gut microbiota alterations before and after metformin treatment. Other studies also showed that the restoration of gut microbiota is key to the improvement of T2DM and its complications.[15] Therefore, it is essential to gain a deeper understanding of the changes in gut microbiota in patients with T2DM microvascular complications.

Currently, there is limited reporting on the gut microbiota of patients with T2DM microvascular complications. This study, based on 16S rRNA sequencing, compares the structural changes in gut microbiota among patients with microvascular complications of T2DM, T2DM without complications. The objective is to investigate the relationship between gut microbiota and the pathogenesis of microvascular complications of T2DM, thereby contributing to the development of novel strategies for the prevention and management of these complications. A comprehensive understanding of the gut microbiota in T2DM complications provides a theoretical basis for personalized treatment based on gut microbiota in clinical practice and for improving public health.

# Materials and Methods

## Study Subjects

Patients diagnosed with T2DM treated at the Endocrinology Department of Yancheng Third People's Hospital from November 2022 to April 2023 were selected as study subjects. Based on the presence of microvascular complications, subjects were divided into a study group (T2DM with microvascular complications, n=22), a control group (T2DM alone, n=27). The inclusion criteria were as follows: (1) meeting the diagnostic criteria of the "Guideline for the Prevention and Treatment of Type 2 Diabetes Mellitus in China (2020 Edition)";[12] (2) aged 18–70 years; (3) diagnosis of any of the following microvascular complications such as diabetic peripheral neuropathy, diabetic retinopathy, or diabetic nephropathy;[13] and (4) normal communication abilities. The exclusion criteria were as follows: (1) those with type 1 diabetes mellitus or other forms of diabetes; (2) those with concurrent gastrointestinal diseases like ulcerative colitis or Crohn's disease; (3) those with other severe diseases such as cancer, liver or kidney dysfunction, cardiovascular diseases, or blood disorders; (4) those who had not taken antibiotics, steroid medications, traditional Chinese medicine, prebiotics, or intestinal microecological agents within the past two months that could affect gut microbiota.

Individuals with T2DM alone who had not taken antibiotics or intestinal microecological agents within the past two months were selected as the control group. All participants were fully informed about the study's purpose and procedures, voluntarily agreed to participate, and signed informed consent forms. The study was conducted in compliance with medical ethical standards and approved by the hospital's ethics committee.

## Diagnostic Criteria for T2DM

The international standard diagnostic criteria for T2DM[16] were applied: diagnosis is confirmed for patients presenting with polydipsia, polyuria, polyphagia, and unexplained weight loss, along with a fasting blood glucose level ≥7 mmol/L, or a 2-hour OGTT blood glucose level or random blood glucose level ≥11.1 mmol/L, or a glycated hemoglobin (HbA1c) level ≥6.5%.

## Study Methods

### Clinical Data

Cases were meticulously selected based on inclusion and exclusion criteria, documenting subjects' gender, age, BMI, duration of diabetes, and history of hypertension. Venous blood was drawn from all subjects at 6 A.M. on the day

following admission to measure various blood indices. Glycated hemoglobin (HbA1c) levels were determined using the BIO-RAD D110 instrument, and fasting blood glucose (FBG), 1-hour postprandial glucose (1hPG), 2-hour postprandial glucose (2hPG), triglycerides (TG), total cholesterol (TC), uric acid (UA), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), lipoprotein-a [Lp(a)], apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), apolipoprotein E (ApoE), fasting insulin (INS), fasting C-peptide (FC-P), and serum neuregulin 4 (NRG4 ErbB4) were quantified using the Beckman Coulter AU5800 automatic biochemical analyzer.

### Collection and DNA Extraction from Fecal Samples

Fresh fecal samples (5 g) from each group were collected using sterile spoons, placed in sterile storage tubes, and stored at −80°C until analysis. DNA was extracted from the fecal samples using the Magnetic Soil and Stool DNA Kit (TIANGEN, China, batch No. DP180427) following the instructions for use.

### PCR Amplification and High-Throughput Sequencing of Fecal Sample DNA

The high-throughput sequencing of fecal sample DNA was conducted based on published references.[17–19] PCR amplification was carried out on the bacterial 16S rRNA V3-V4 regions in fecal samples using diluted genomic DNA as the template and primers containing barcodes targeting the highly variable V3-V4 regions. The amplification conditions included an initial denaturation at 98°C for 1 min, denaturation at 98°C for 10s, annealing at 50°C for 30s, extension at 72°C for 30s, and a final extension at 72°C for 5 min. The TruSeq® PCR-Free DNA Library Preparation Kit was used for library construction, followed by quantification and validation using Q-PCR and Qubit. Sequencing was performed on the NovaSeq 6000 platform upon meeting quality standards.

## Bioinformatics Analysis and Statistics

Following a series of data processing steps, valid data were obtained for all samples. Operational taxonomic units (OTUs) were clustered and species classification analysis was performed at 97% similarity. Differences in Alpha diversity indices between groups were analyzed, and sequencing depth was assessed by coverage rates. Multiple sequence alignment of OTUs was conducted, and potential marker species were identified using LEfSe analysis. The functional prediction of the gut microbiota was evaluated using the Tax4Fun method. Data were analyzed using SPSS 26.0 statistical software. Normally distributed measurement data were expressed as $\bar{x}\pm$ sand compared between groups using independent sample *t*-tests. Non-normally distributed measurement data were expressed as median ($P_{25}$, $P_{75}$) and compared using the Mann–Whitney *U*-test. Count data were expressed as cases (percentage) [n(%)] and compared between groups using $X^2$ test. A *P*-value <0.05 was considered a statistically significant difference.

# Results

## Comparison of Clinical Data

A total of 49 subjects were included in this study, with 22 in the study group and 27 in the control group. The comparison revealed that the study group had a longer duration of disease [7.5 (2.75, 12.5) vs 5 (1, 11)] and higher levels of Lp(a) [127.75 (60.68, 258.28) vs 68.80 (42.10, 130.10)], while NRG4 levels (0.33±0.29 vs 1.93±4.30) were lower than those in the control group, with statistically significant differences ($P<0.05$) (Table 1).

## Sequencing Results and OTU Clustering of Gut Microbiota

Sequencing of the gut microbiota from 49 fecal samples yielded 4,427,6523 raw reads. After merging, quality control, and chimera filtering, 1,854,273 valid reads were obtained. At 97% similarity, a total of 3727 OTUs were identified from the valid sequences, with 1311 OTUs shared between the two groups, and 1363 and 1053 OTUs unique to the study and control groups, respectively.

## Composition and Differences in Gut Microbiota Between Groups

In the study group, the dominant bacterial genera in descending order of relative abundance were: Clostridia (42.59%), Bacteroidia (26.03%), Gammaproteobacteria (14.25%), Bacilli (7.35%), Negativicutes (4.67%), Actinobacteria (2.54%),

**Table 1** Comparison of Clinical Data Between the Two Groups

| Indicators | Study group (n=22) | Control group (n=27) | $x^2$/t/Z | P |
|---|---|---|---|---|
| Gender (Male) | 12 (54.55%) | 14 (51.85%) | 0.572 | 0.132 |
| Age | 56.23±7.53 | 52.85±12.09 | 2.831 | 0.632 |
| BMI | 25.36±5.75 | 24.88±4.30 | 1.482 | 0.146 |
| Duration of disease | 7.5 (2.75, 12.5) | 5 (1, 11) | −4.903 | 0.043* |
| History of hypertension | 13 (59.09%) | 10 (37.04%) | 1.295 | 0.062 |
| FBG | 7.77±2.64 | 8.24±2.85 | −0.325 | 0.092 |
| 1hPG | 14.61±4.27 | 15.68±3.85 | 1.104 | 0.163 |
| 2hPG | 13.99±4.25 | 15.89±5.11 | 1.648 | 0.084 |
| HbAlc | 8.49±1.87 | 8.73±2.20 | 1.594 | 0.153 |
| TG | 1.9±1.28 | 2.55±1.96 | 1.058 | 0.073 |
| TC | 4.27±0.84 | 4.58±1.64 | 1.784 | 0.281 |
| UA | 283.56±63.81 | 297.90±95.20 | 2.904 | 0.061 |
| HDL-C | 1.16±0.23 | 1.26±0.50 | 1.854 | 0.136 |
| LDL-C | 2.36±0.72 | 2.71±1.36 | 1.846 | 0.327 |
| Lp(a) | 127.75 (60.68, 258.28) | 68.80 (42.10, 130.10) | −2.794 | 0.010* |
| ApoA1 | 1.29±0.22 | 1.35±0.39 | 1.904 | 0.326 |
| ApoB | 0.88±0.21 | 0.99±0.34 | 1.673 | 0.431 |
| ApoE | 47.91±18.36 | 51.76±24.12 | 1.384 | 0.083 |
| INS | 47.34 (32.63 67.85) | 50.68 (38.13 84.54) | −2.903 | 0.104 |
| FC-P | 0.70±0.33 | 0.74±0.53 | 1.583 | 0.362 |
| NRG4 ErbB4 | 0.33±0.29 | 1.93±4.30 | 1.205 | 0.021* |

**Note**: Body mass index (BMI), Hemoglobin A1c (HbA1c), Fasting Blood Glucose (FBG), 1-hour Postprandial Glucose (1hPG), 2-hour Postprandial Glucose (2hPG), Triglycerides (TG), Total Cholesterol (TC), Uric Acid (UA), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), Lipoprotein-a [Lp(a)], Apolipoprotein A1 (ApoA1), Apolipoprotein B (ApoB), Apolipoprotein E (ApoE), Fasting Insulin (INS), Fasting C-Peptide (FC-P), and Serum Neuregulin 4 (NRG4), Erb-B2 Receptor Tyrosine Kinase 4 (ErbB4). *p<0.05.

and Verrucomicrobia (1.31%). Compared to the control group, the relative abundance of Clostridia and Negativicutes significantly increased in the study group, while the relative abundance of Gammaproteobacteria, Bacilli, and Verrucomicrobia significantly decreased, as shown in Figure 1.

## Comparative Analysis of Gut Microbiota Between Groups

The comparison of species' relative abundance differences between the two groups, as depicted in Figure 2, reveals significant disparities in the relative abundance across 2 phyla, 2 classes, 2 orders, 3 families, and 2 genera, as determined by LefSe analysis. At the phylum level, Proteobacteria served as the biomarker for the control group, whereas Firmicutes was identified as the biomarker for the study group. At the genus level, the biomarker for the control group was Escherichia Shigella, and for the study group, it was Faecalibacterium, as illustrated in Figure 2.

## Functional Prediction Analysis of Both Groups Using Tax4Fun

The top 10 functional annotations based on the highest abundance within each sample or group are presented in Figure 3. The seven most abundant KEGG pathways include metabolism, genetic information processing, environmental information processing, cellular processes, human diseases, and organ systems. A total of 10 KEGG pathways exhibited statistically significant differences ($P$<0.05), encompassing DNA repair and recombination proteins, tRNA biosynthesis, purine metabolism, pyrimidine metabolism, mismatch repair, peptidoglycan biosynthesis and degradation, homologous recombination, peptidoglycan biosynthesis, DNA replication, and D-alanine metabolism pathway, as shown in Figure 4.

# Discussion

The gut microbiota, functioning as a metabolic organ, co-evolves and coexists in harmony with the host, contributing to energy metabolism, nutrient synthesis, inflammation suppression, and immune response, thereby maintaining the

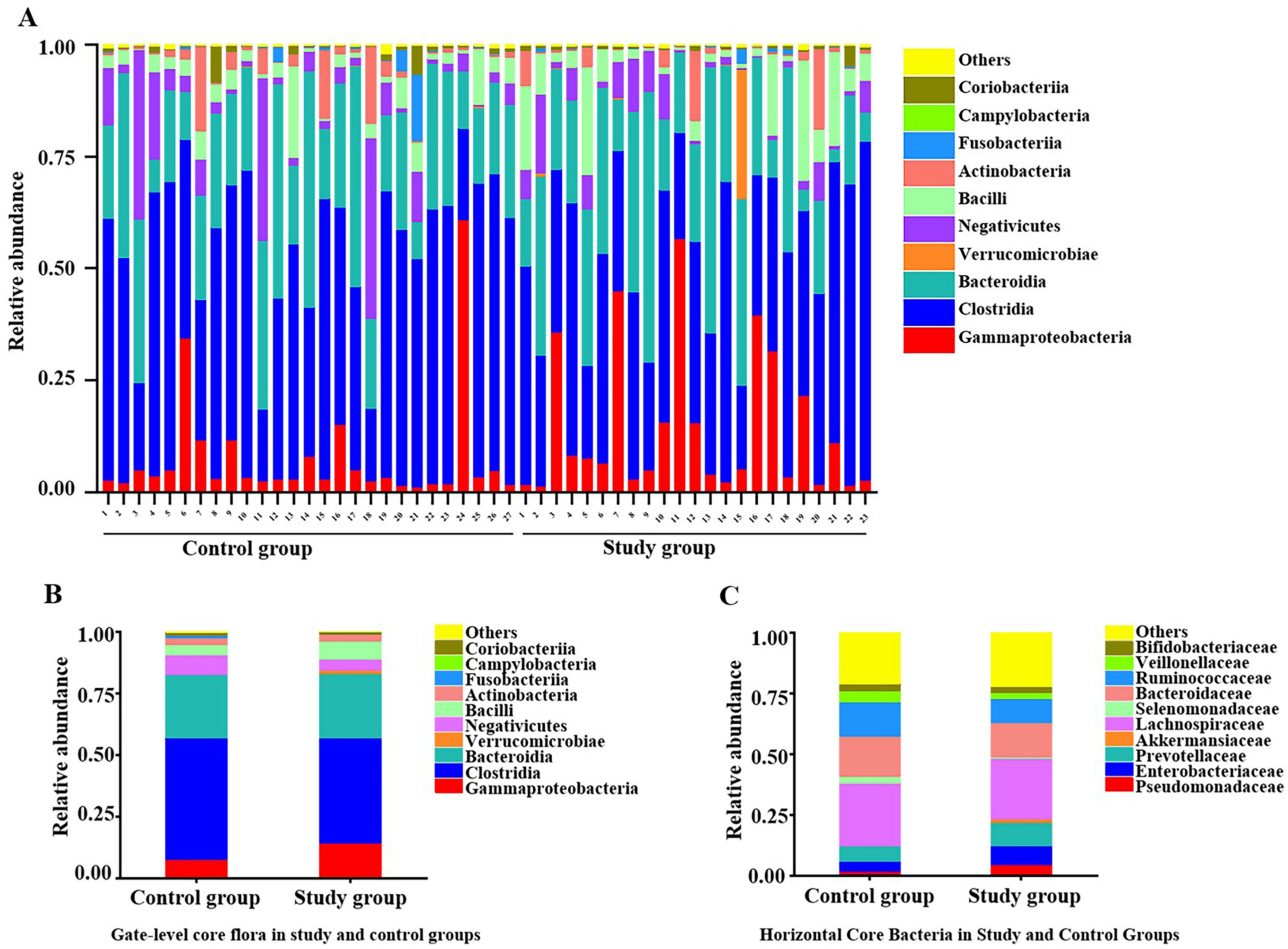


**Figure 1** (**A**) Comparative of microbial abundance between the two groups; (**B**) Core microbiota at the phylum level in both groups; (**C**) Core microbiota at the genus level in both groups.

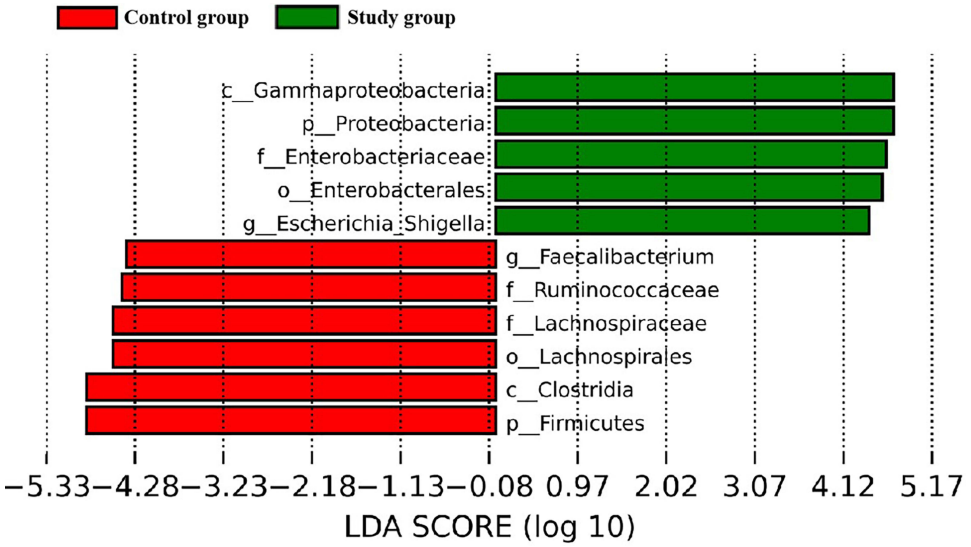


**Figure 2** Histograms of LDA distribution for the two groups.


https://doi.org/10.2147/DMSO.S493720

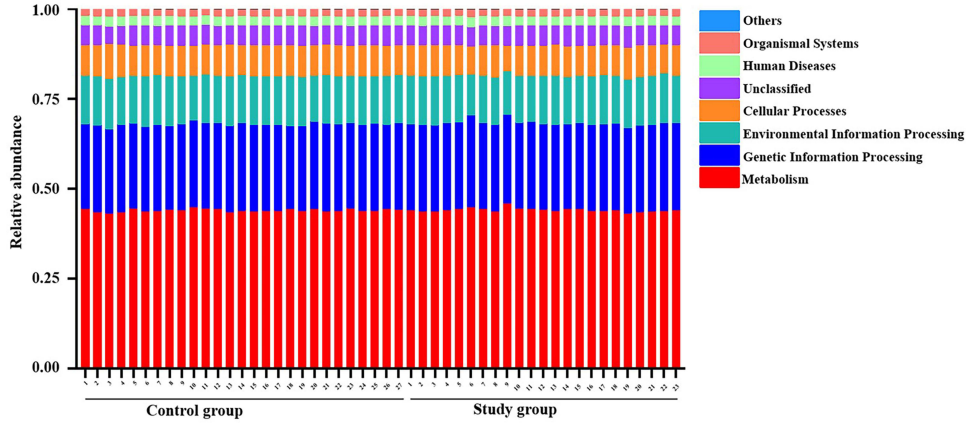


**Figure 3** Relative abundance of KEGG pathways in the two groups.

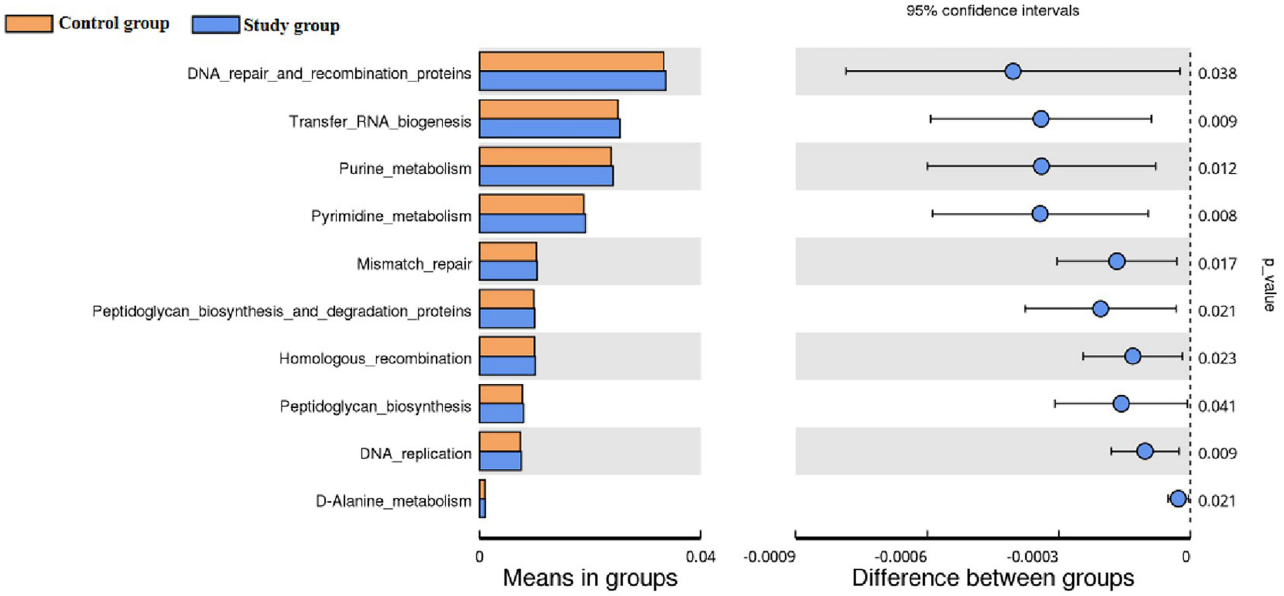


**Figure 4** Differential analysis of KEGG pathways between the two groups.

dynamic equilibrium of metabolism within the body.[20] Dysbiosis in the gut microbiota can lead to lipopolysaccharide (LPS)-induced peripheral tissue inflammation, metabolic endotoxemia, intestinal barrier dysfunction, and a decrease in short-chain fatty acid-producing bacteria. These factors may directly or indirectly participate in the development and progression of microvascular complications of T2DM.

Bacteria from the phyla Firmicutes and Bacteroidetes serve as indicators for assessing changes in the gut microbiota of T2DM patients. This study found that, compared to patients with diabetes alone, those with combined microvascular complications exhibited a significant increase in the relative abundance of Clostridia and Negativicutes, while the relative abundance of Gammaproteobacteria, Bacilli, and Verrucomicrobia significantly decreased. Gram-negative bacteria, which are relatively abundant in the gut microbiota of T2DM patients, contain LPS in their outer membranes that can induce metabolic endotoxemia. Once LPS enters the bloodstream through a leaky gut, it triggers inflammation in organs such as the kidneys, liver, and pancreas through oxidative stress, leading to disturbances in glucose metabolism.[21] Li et al[22] observed that diabetic mice with severe proteinuria had significantly higher blood LPS levels than those with mild proteinuria. The enrichment of Gram-negative bacteria, by elevating LPS levels, stimulates microvascular inflammation and exacerbates microvascular damage, playing a crucial role in the onset of microvascular complications. Tao et al[23]

noted changes in the structure and quantity of gut microbiota in patients with microvascular complications, including a significant reduction in the abundance of Prevotella, a key SCFA-producing genus, aligning with the findings of this study.

Earlier investigations have demonstrated that obesity and insulin resistance can profoundly impact the diversity and abundance of the predominant gut microbiota in the early stages of type 2 diabetes mellitus (T2DM).[24] Larsen et al,[25] using 16S rRNA sequencing, discovered that the abundance of Firmicutes and Bacteroidetes in the gut of T2DM patients was significantly lower than in healthy individuals, with the Firmicutes/Bacteroidetes ratio positively correlating with blood glucose levels. This study's findings on species relative abundance revealed that Proteobacteria was the biomarker for the control group at the phylum level, and Firmicutes for the study group. At the genus level, Escherichia Shigella was identified as the biomarker for the control group, whereas Faecalibacterium was identified as the biomarker for the study group. An increase in Escherichia Shigella may result in LPS production, causing endotoxemia, overexpression of inflammatory factors, mesangial cell proliferation, an increase in mesangial matrix, reduced glomerular blood flow, and renal insufficiency.[26] Research has shown that intermittent fasting can alter the gut microbiota in T2DM mice with microvascular complications, increasing the ratio of Firmicutes to Bacteroidetes and decreasing the ratio of Verrucomicrobia, with an elevation in the metabolic product tauroursodeoxycholic acid (TUDCA).[27] TUDCA has anti-inflammatory effects and can reduce levels of inflammatory and chemotactic factors by regulating TUDCA levels, improving retinal gliosis and capillary loss, and alleviating symptoms.[28]

T2DM patients, being in a state of low-level inflammation, are susceptible to a spectrum of pathogens. Urinary tract infections (UTIs) represent a significant health concern for diabetic patients. Enterococcus and Veillonella, frequently implicated in UTIs, can contaminate the urethral perineum, especially in females, due to the unique anatomical structure of the human body.[29] This study found no significant differences in the abundance of Enterococcus and Veillonella in the gut microbiota of subjects from both groups, considering the influence of regional, environmental, medicinal, and dietary factors on gut microbiota, and the small sample size of this study. However, efforts were made to minimize differences in gender, age, and living environment among the two groups of subjects included.

This study is constrained by its small sample size and the individual variability in daily diet and medication among the study subjects, which may affect changes in the gut microbiota. Future studies should expand the sample size and refine statistical information to further validate the experimental results.

## Conclusion

In conclusion, this study leveraged 16S rRNA gene sequencing to delineate the structural alterations in the gut microbiota among patients with microvascular complications of T2DM. It analyzed the differences in gut microbiota between these patients and those with T2DM alone, aiming to identify potential distinguishing microbial communities. The study identified Clostridia and Negativicutes as significantly increased in patients with microvascular complications, while Gammaproteobacteria, Bacilli, and Verrucomicrobia were decreased. These microbial markers could be developed into non-invasive diagnostic tests for early detection of microvascular complications, and targeted probiotic or dietary interventions could be explored as therapeutic options. The insights gained from this study offer novel perspectives for diagnosing and treating microvascular complications of T2DM.

## Ethics Approval and Consent to Participate

This study was conducted in accordance with the declaration of Helsinki. The study was approved the Ethics Committee of Yancheng Third People's Hospital. All participants were fully informed about the study's purpose and procedures, voluntarily agreed to participate, and signed informed consent forms.

## Author Contributions

Wang YY: Study design, execution, acquisition of data, analysis and interpretation; Jiang DM: acquisition of data, analysis and interpretation; Pan X: acquisition of data, analysis and interpretation; Sun K: drafting the manuscript and revising; Li TT: revising the manuscript; Cao X and Zhu XH: conception and giving the final approval of the version to be published.

## Funding

Immunosuppressive mechanism of SYTL5 regulated by transcription factor ZNF384 in thyroid cancer immune microenvironment (Z2022098) Study on Intestinal flora and Serum Metabolites in diabetic peripheral neuropathy (Z20229136).

## Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

1. Saeedi P, Petersohn I, Salpea P, et al. IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas. *Diabet Res Clin Pract*. 2019;157:107843. doi:10.1016/j.diabres.2019.107843
2. American Diabetes Association. Microvascular Complications and Foot Care: standards of Medical Care in Diabetes-2020. *Diabetes Care*. 2020;43(1):S135–S151. doi:10.2337/dc20-S011
3. Barnes JA, Eid MA, Creager MA, et al. Epidemiology and Risk of Amputation in Patients With Diabetes Mellitus and Peripheral Artery Disease. *Arterioscler Thromb Vasc Biol*. 2020;40(8):1808–1817. doi:10.1161/ATVBAHA.120.314595
4. Sun T, Yin L, Kuang H. miR-181a/b-5p regulates human umbilical vein endothelial cell angiogenesis by targeting PDGFRA. *Cell Biochem Funct*. 2020;38(2):222–230. doi:10.1002/cbf.3472
5. Singh MV, Dokun AO. Diabetes mellitus in peripheral artery disease: beyond a risk factor. *Front Cardiovasc Med*. 2023;10:1148040. doi:10.3389/fcvm.2023.1148040
6. Gurung M, Li Z, You H, et al. Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine*. 2020;51:102590. doi:10.1016/j.ebiom.2019.11.051
7. Ahmad A, Yang W, Chen G, et al. Analysis of gut microbiota of obese individuals with type 2 diabetes and healthy individuals. *PLoS One*. 2019;14(12):e0226372. doi:10.1371/journal.pone.0226372
8. Zhou Z, Sun B, Yu D, et al. Gut Microbiota: an Important Player in Type 2 Diabetes Mellitus. *Front Cell Infect Microbiol*. 2022;12:834485. doi:10.3389/fcimb.2022.834485
9. Ma Q, Li Y, Li P, et al. Research progress in the relationship between type 2 diabetes mellitus and intestinal flora. *Biomed Pharmacother*. 2019;117:109138. doi:10.1016/j.biopha.2019.109138
10. Das T, Jayasudha S, Chakravarth G, et al. Alterations in the gut bacterial microbiome in people with type 2 diabetes mellitus and diabetic retinopathy. *Sci Rep*. 2021;11(1):2738. doi:10.1038/s41598-021-82538-0
11. Huang Y, Wang Z, Ma H, et al. Dysbiosis and Implication of the Gut Microbiota in Diabetic Retinopathy. *Front Cell Infect Microbiol*. 2021;11:646348. doi:10.3389/fcimb.2021.646348
12. Gao S, Zhao LH, Tian X, et al. Characteristics of Gut Microbiota in Female Patients with Diabetic Microvascular Complications. *J Diabetes Res*. 2022;2022:2980228. doi:10.1155/2022/2980228
13. Zhang X, Wang M, Wang X, et al. Relationship between periodontitis and microangiopathy in type 2 diabetes mellitus: a meta-analysis. *J Periodontal Res*. 2021;56(6):1019–1027. doi:10.1111/jre.12916
14. Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*. 2013;498(7452):99–103. doi:10.1038/nature12198
15. Xu Z, Huang J, Wen M, et al. Gentiopicroside ameliorates glucose and lipid metabolism in T2DM via targeting FGFR1. *Phytomedicine*. 2024;132:155780. doi:10.1016/j.phymed.2024.155780
16. Iatcu CO, Steen A, Covasa M. Gut Microbiota and Complications of Type-2 Diabetes. *Nutrients*. 2021;14(1):166. doi:10.3390/nu14010166
17. Zhang S, Zhang S, Bai X, et al. Thonningianin A ameliorated renal interstitial fibrosis in diabetic nephropathy mice by modulating gut microbiota dysbiosis and repressing inflammation. *Front Pharmacol*. 2024;15:1389654. doi:10.3389/fphar.2024.1389654
18. Zheng W, Ning K, Shi C, et al. Xiaobugan decoction prevents $CCl_4$-induced acute liver injury by modulating gut microbiota and hepatic metabolism. *Phytomedicine*. 2024;135:156113. doi:10.1016/j.phymed.2024.156113
19. Isokaanta H, Tomnikov S, Vanhatolo E, et al. High-throughput DNA extraction strategy for fecal microbiome studies. *Microbiol Spectr*. 2024;12(6):e0293223. doi:10.1128/spectrum.02932-23
20. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008;57(6):1470–1481. doi:10.2337/db07-1403
21. Xu J, Liang R, Zhang W, et al. Faecalibacterium prausnitzii-derived microbial anti-inflammatory molecule regulates intestinal integrity in diabetes mellitus mice via modulating tight junction protein expression. *J Diabetes*. 2020;12(3):224–236. doi:10.1111/1753-0407.12986
22. Li Y, Su X, Gao Y, et al. The potential role of the gut microbiota in modulating renal function in experimental diabetic nephropathy murine models established in same environment. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866(6):165764. doi:10.1016/j.bbadis.2020.165764
23. Tao S, Li L, Li L, et al. Understanding the gut-kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition. *Acta Diabetol*. 2019;56(5):581–592. doi:10.1007/s00592-019-01316-7
24. Hartstra AV, Bouter KE, Bäckhed F, et al. Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care*. 2015;38(1):159–165. doi:10.2337/dc14-0769
25. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One*. 2010;5(2):e9085. doi:10.1371/journal.pone.0009085

26. López-de-Andrés A, Albaladejo-Vicente R, Palacios-Ceña D, et al. Time Trends in Spain from 2001 to 2018 in the Incidence and Outcomes of Hospitalization for Urinary Tract Infections in Patients with Type 2 Diabetes Mellitus. *Int J Environ Res Public Health*. 2020;17(24):9427. doi:10.3390/ijerph17249427
27. Beli E, Yan Y, Moldovan L, et al. Restructuring of the Gut Microbiome by Intermittent Fasting Prevents Retinopathy and Prolongs Survival in db/db Mice. *Diabetes*. 2018;67(9):1867–1879. doi:10.2337/db18-0158
28. Ouyang H, Mei X, Zhang T, et al. Ursodeoxycholic acid ameliorates diabetic retinopathy via reducing retinal inflammation and reversing the breakdown of blood-retinal barrier. *Eur J Pharmacol*. 2018;840:20–27. doi:10.1016/j.ejphar.2018.09.027
29. Woldemariam HK, Geleta DA, Tulu KD, et al. Common uropathogens and their antibiotic susceptibility pattern among diabetic patients. *BMC Infect Dis*. 2019;19(1):43. doi:10.1186/s12879-018-3669-5