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Characteristics of the intestinal flora in patients with peripheral neuropathy associated with type 2 diabetes

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Abstract

Objective: To study the characteristics of the intestinal flora in patients with diabetic peripheral neuropathy (DPN) and analyze the association between the intestinal flora and clinical indicators.

Methods: We classified 80 subjects into three groups: patients with DPN (n = 45), patients type 2 diabetes without DPN (n = 21), and healthy controls (n = 14). The intestinal flora composition was compared among the three groups, and the correlation between the intestinal flora and clinical indicators was analyzed.

Results: At the phylum level, the richness of Firmicutes and Actinobacteria was elevated in the DN group, and that of Bacteroidetes was decreased. At the genus level, the richness of *Bacteroides* and *Faecalibacterium* was significantly decreased in the DPN group, whereas that of *Escherichia-Shigella*, *Lachnoclostridium*, *Blautia*, *Megasphaera*, and *Ruminococcus torques* group was increased. The homeostasis model assessment insulin resistance index was positively correlated with *Megasphaera* richness. Glycine ursodeoxycholic acid was positively correlated with *Ruminococcus gnavus* group and *Phascolarctobacterium* richness. Tauroursodeoxycholic acid was positively correlated with *Ruminococcus gnavus* group and *Parabacteroides* richness.

Conclusion: There was obvious intestinal microbiota disorder in patients with DPN, which may be related to insulin resistance. These changes may have important roles in the development of DPN.

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Keywords
Diabetic peripheral neuropathy, type 2 diabetes, gastrointestinal microbiota, microbial diversity, insulin resistance, bile acids, richness, blood chemistry

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Introduction

Diabetic peripheral neuropathy (DPN) is one of the most common complications of diabetes, but its underlying pathogenesis remains elusive. To date, most related research has focused on potential causative factors such as oxidative stress, hypoxic injury, activation of the polyol pathway, hexosamine pathway, protein kinase C, and inflammatory response.[1,2] In addition, early investigations indicated that patients with type 2 diabetes have significantly higher risks of peripheral neuropathy, DPN, diabetic retinopathy, and hypertension than those with type 1 diabetes.[3–6]

A meta-analysis of more than 2000 studies on fecal bacteria suggested that the characteristics of the intestinal microbiota can be used to differentiate diseased and healthy populations, and they can serve as biomarkers for diagnosing type 2 diabetes.[7] Insulin resistance is an essential pathogenic factor of peripheral neuropathy in type 2 diabetes. Studies illustrated that intestinal flora imbalance influences the development of type 2 diabetes by causing metabolic disorders such as insulin resistance.[8–10] On this basis, scholars found that promoting the growth of bifidobacteria and lactobacilli via probiotic supplementation, intestinal flora transplantation, or other means can reduce insulin resistance.[11,12] However, the mechanism by which intestinal flora disorder affects the initiation and progression of peripheral neuropathy related to type 2 diabetes requires further research for clarification. Bile acids can regulate metabolic processes such as glucose, fat, and energy homeostasis by activating bile acid receptors (such as farnesoid X receptor [FXR] and G-protein coupling bile acid receptor 5 [TGR5]).[12] Some hypoglycemic agents improve metabolic health by regulating the intestinal flora and changing the composition of plasma bile acid.[13]

The aims of the present study were to (i) reveal the differences of the structure, abundance, and species diversity of intestinal flora in patients with type 2 diabetes, patients with peripheral neuropathy associated with type 2 diabetes, and healthy controls, (ii) analyze the relationship between the intestinal flora composition and clinical blood biochemical indicators or insulin resistance, and (iii) explore the possible role of intestinal flora imbalance in the development of peripheral neuropathy in type 2 diabetes.

Patients and methods

Patients

The study was approved by the Ethical Committee of the Second Affiliated Hospital of Nanjing Medical University. Sixty-six patients with type 2 diabetes diagnosed at our institution between April 2018 and February 2019 and 14 healthy subjects (controls) were enrolled in this study. Signed informed consent was obtained from each participant. No subjects used antibiotics or probiotics in the prior 3 months before enrollment. Patients only

received short-term treatment with subcutaneous insulin and metformin, and the course of treatment was approximately 14 days. The 66 patients were divided into the DPN (n = 45, patients with peripheral neuropathy associated with type 2 diabetes) and DM groups (n = 21, patients with type 2 diabetes alone). Data were recorded for gender; age; duration of disease; body mass index (BMI); fasting blood glucose (FBG), 2-hour postprandial glucose, triglyceride (TG), total cholesterol (TC), C-reactive protein (CRP), and glycated hemoglobin (HbA1c) levels; and the homeostasis model assessment insulin resistance index (HOMA-IR). Plasma bile acid levels were measured using a triple quadrupole mass spectrometer (API 3200 MDTM, American Pharmaceutical Distributors, Fort Lauderdale, FL, USA).

Inclusion and exclusion criteria

The diagnoses of type 2 diabetes mellitus were established according to the diagnostic criteria of the American Diabetes Association. The criteria for DPN were as follows: spontaneous limb pain, symmetrical or unilateral limb numbness, sensation of dullness and body tension, muscle weakness, weakened or absent tendon reflexes, and significant decreases in sensory and motor nerve conduction velocities as revealed via electromyography indicating the positive involvement of two or more nerves and normal arterial pulses in the foot and back.

During the assessment, subjects were excluded upon the discovery of other types of diabetes; neuropathy caused by other causes; hypertension; gastrointestinal disease; severe vascular disease; severe cardiovascular, hepatic, and renal insufficiency; tumors; and infectious diseases such as tuberculosis, viral hepatitis, and AIDS.

Fecal samples

Approximately 2 to 5 g of fecal samples were collected in sterile fecal storage tubes within 2 hours after defecation. Samples were immediately stored in a sample transport box in a −80°C refrigerator in the laboratory, and the halfway transport process was approximately 30 minutes. Samples transported for more than 2 hours or those suspicious for contamination were discarded immediately. Subjects were not allowed to use antibiotics and probiotics in the previous 3 months or present with diarrhea or other gastrointestinal diseases within the previous month.

16S rDNA-based high-throughput sequencing

The community sequencing data of the intestinal flora were detected via 16S rDNA-based high-throughput sequencing as described previously.[13] Briefly, intestinal flora DNA was extracted from a 0.2-g thawed fecal sample using a Fast DNA® Spin Kit for soil (MP Biomedicals, LLC, Santa Ana, CA, USA) following the manufacturer's protocols. The 16s rRNA V3-V4 region was amplified using forward (5′-AACGGGAAGACAACGTACGG-3′) and reverse primers (5′-CAGATGCAGGA GGACATGTC-3′) with barcode sequences. The 16S raw sequencing reads are available in the NCBI Sequence Read Archive (SRA) database under the SRA accession number SRP168691. Matching of operational taxonomic units (OTUs) to bacteria was conducted using the SILVA reference database. Microbial composition at each taxonomic level was defined using Quantitative Insights Into Microbial Ecology).

Statistical analysis

Usearch (version 7.0 http://drive5.com/uparse/) software was used to perform

OTU cluster analysis based on 97% similarity. Classification of the representative OTU sequences with 97% similarity was analyzed using the RDP classifier Bayesian algorithm. Alpha diversity analysis was performed using the Chao1 and Shannon index, and the differences between groups were evaluated using Student's *t*-test. In the data table in the tax_summary_a folder, the R language tool was used to map the community components. The Wilcoxon rank-sum test was used to analyze differences between two groups. Correlation analysis using was analyzed using the Spearman correlation coefficient with R (pheatmap package) software.

Results

Comparison of clinical baseline data

Compared with the findings in the NC group, TC, TG, LDL-C, FBG, HbA1c, and CRP levels and HOMA-IR were significantly elevated in the DM and DPN groups (both $P < 0.05$). Detailed results are shown in Table 1.

Microbial species richness and biodiversity

In total, 4,441,044 high-quality sequences were obtained from 80 specimens among the three groups, and the average sequence length was 416.92 bp. The raw gut microbial sequence data of the 16S rRNA V3–V4 region were sorted into 1121 OTUs (>97% identity). The Venn diagram of the three groups of samples intuitively reflected the similarity and overlap of the three groups: 175 OTUs in the DPN and DM groups, 114 in the DPN and NC groups, and 485 OTUs among all three groups (Figure 1).

Alpha diversity analysis and Shannon index

According to the results of alpha diversity analysis based on Chao1 and abundance-based coverage estimator indices, the abundance was highest in the NC group and lowest in the DM group. There were statistically significant differences in the abundance between the NC and DM groups ($P < 0.05$) and between the DPN and DM groups ($P < 0.01$). Meanwhile, although the

Table 1. Comparison of clinical baseline data.

	NC (n = 14)	DM (n = 21)	DPN (n = 45)	F or χ^2	*P*
Gender (male/female)	8/6	12/9	25/20	0.020	0.990
Age	58.06 ± 6.39	59.33 ± 10.21	58.55 ± 6.61	0.127	0.881
BMI	24.49 ± 4.65	24.56 ± 5.57	23.35 ± 4.06	0.708	0.496
TG (mmol/L)	1.53 ± 0.36	2.39 ± 0.74	2.71 ± 0.76	15.07	0.000
TC (mmol/L)	3.05 ± 1.09	4.56 ± 1.03	4.73 ± 1.35	10.200	0.000
LDL-C (mmol/L)	2.16 ± 0.56	2.74 ± 0.61	2.76 ± 0.69	4.833	0.011
CRP (mg/L)	1.89 ± 0.94	3.49 ± 0.96	3.53 ± 0.93	17.340	0.000
FPG (mmol/L)	5.19 ± 0.87	6.64 ± 1.03	6.75 ± 1.01	13.710	0.000
HbA1c (%)	4.35 ± 0.66	8.24 ± 2.26	9.95 ± 2.83	28.200	0.000
FCP (ng/ml)	1.38 ± 0.06	1.39 ± 0.09	1.40 ± 0.11	0.244	0.784
FinS (pmol/ml)	11.08 ± 2.67	12.06 ± 2.73	12.09 ± 2.86	0.741	0.480
Fasting plasma glucagon	128.65 ± 13.78	129.91 ± 15.44	129.13 ± 15.03	0.033	0.967
HOMA-IR	6.59 ± 1.16	22.47 ± 6.88	26.79 ± 8.16	43.110	0.000

BMI, body mass index; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; HbA1c, glycated hemoglobin; FCP, fasting C peptide; FinS, fasting insulin; HOMA-IR, homeostasis model assessment insulin resistance index; DPN, diabetes peripheral neuropathy group; DM, type 2 diabetes mellitus group; NC, normal control group.

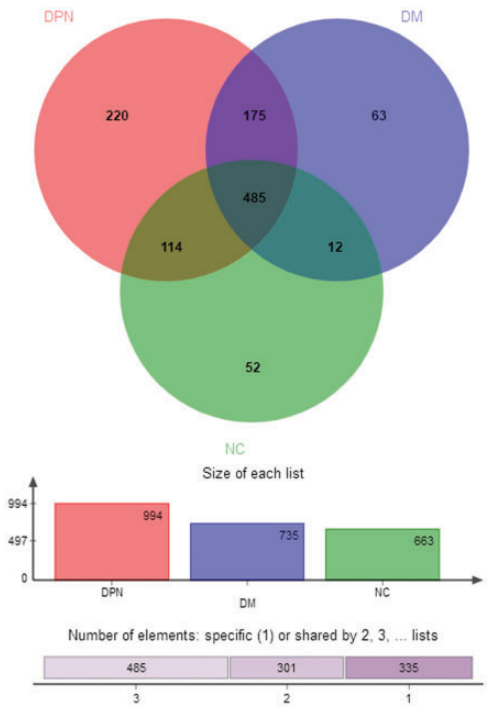

Figure 1. Venn diagram of operational taxonomic groups among the three groups. DPN, diabetes peripheral neuropathy group; DM, type 2 diabetes mellitus group; NC, normal control group.

abundance was higher in the NC group than in the DPN group, the difference was not statistically significant. In terms of the Shannon and Simpson indices, a higher Shannon index indicates greater community diversity, whereas a higher Simpson index denotes lower community diversity. This study found that according to the Shannon index, microbial diversity was greater in the DPN group than in the DM group ($P < 0.05$). Conversely, the diversity was slightly but not significantly higher in the NC group than in the DM group. Noticeably, the Simpson index was highest in the NC group and lowest in the DPN group, but no significant differences were noted among the groups (Figure 2a, 2b). Hence, both the Simpson and Shannon indices indicated that the DPN group had the most diverse bacterial community. The coverage index can genuinely reflect the actual situation of the intestinal flora in the sequencing sample. We found that the coverage index exceeded 0.99 in all three groups, indicating that the probability of undetected sequences was low for all three groups, and there were statistical differences between the DPN and DM groups and between the NC and DM groups (both $P < 0.01$, Figure 2).

Analysis of microbial species composition

Twenty phyla were detected in the three groups of samples. At the phylum level, the most dominant microbial taxa in the three groups were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria. In particular, Firmicutes accounted for 61.30%, 49.17%, and 34.16% of bacteria in the DPN, DM, and NC groups, respectively, whereas Bacteroidetes accounted for 23.27%, 27.69%, and 57.04% of bacteria, respectively, in these three groups. Proteobacteria accounted for 8.67%, 14.07%, and 3.08% of bacteria in the DPN, DM, and NC groups, respectively. Actinobacteria accounted for 5.09%, 5.98%, and 0.54% of bacteria in the DPN, DM, and NC groups, respectively, whereas Fusobacteria accounted for 1.20%, 2.09%, and 4.97% of bacteria, respectively, in these three groups (Figure 3).

In addition, 344 genera were detected in the three groups of samples. In particular, 163 Firmicutes, 37 Bacteroidetes, 67 Proteobacteria, 43 Actinobacteria, and 4 Fusobacteria genera were detected. The most commonly detected genera in the NC group were as follows: *Bacteroidetes*, 37.51%; *Prevotella* 9, 15.75%; *Faecalibacterium*, 9.31%; *Fusobacterium*, 4.97%; Megamonas, 2.92%; *Clostridium sensu stricto* 1, 2.01%; *Lachnoclostridium*, 1.80%; *Sutterella*, 1.71%; *Roseburia*, 1.66%; *Eubacterium rectale* group, 1.32%;

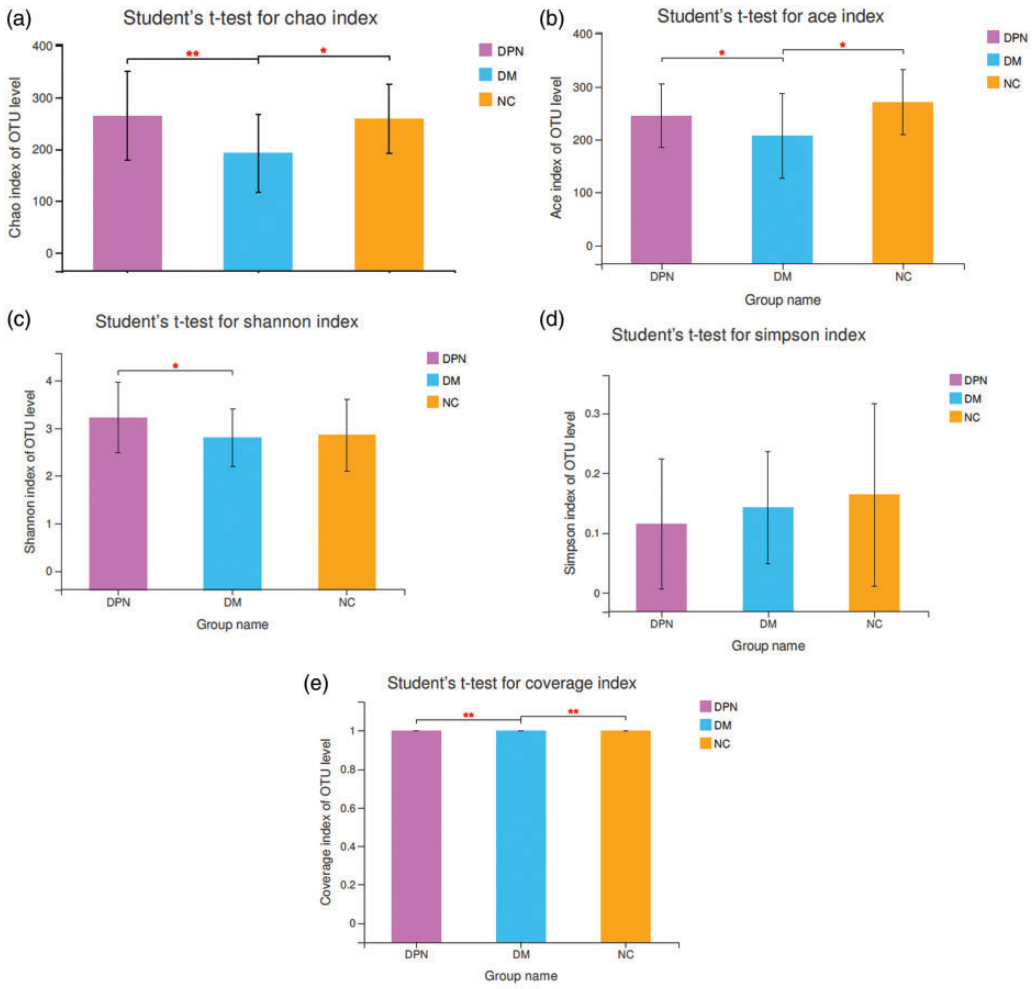

Figure 2. Alpha diversity analysis. (a) The Chao1 index of bacterial communities. (b) The abundance-based coverage estimator (ACE) index of bacterial communities. (c) The Shannon index of bacterial communities. (d) The Simpson index of bacterial communities. (e) The coverage index of bacterial communities. DPN, diabetes peripheral neuropathy group; DM, type 2 diabetes mellitus group; NC, normal control group.

and *Lachnospira*, 1.26%. The most commonly detected genera in the DPN group were as follows: *Bacteroidetes*, 14.76%; *Megamonas*, 9.79%; *Escherichia-Shigella*, 5.36%; *Prevotella* 9, 4.31%; *Faecalibacterium*, 4.29%; *Bifidobacterium*, 3.77%; *Blautia*, 3.75%; *Lachnoclostridium*, 3.68%; *Roseburia*, 2.60%; *Phascolarctobacterium*, 2.47%; *Subdoligranulum*, 2.26%; and *Megasphaera*, 2.38%. The most commonly detected genera in the DM group were as follows: *Bacteroidetes*, 21.66%; *Escherichia-Shigella*, 9.59%; *Megamonas*, 7.44%; *Blautia*, 5.17%; *Bifidobacterium*, 4.76%; *Lachnoclostridium*, 4.37%; and *Eubacterium rectale* group, 2.94%, *Subdoligranulum*, 2.37%, *Fusobacterium*, 2.09%; *Ruminococcus gnavus* group 1.86%; and *Phascolarctobacterium*, 1.74% (Figure 4).

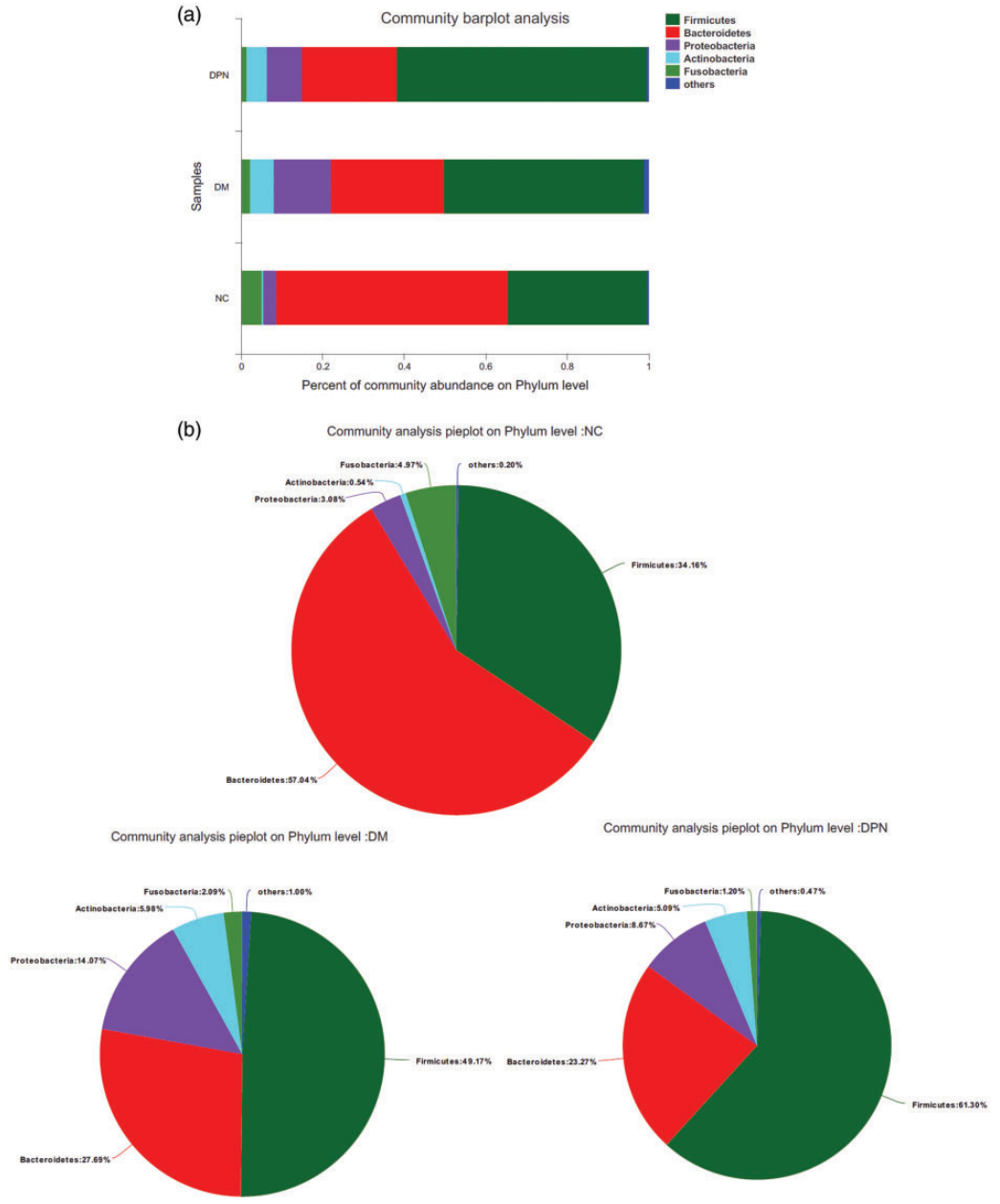

Figure 3. Microbial composition of different communities at the phylum level. DPN, diabetes peripheral neuropathy group; DM, type 2 diabetes mellitus group; NC, normal control group.

Compared with the results in the NC group, at the phylum level, the richness of Firmicutes ($P < 0.001$) and Actinobacteria ($P < 0.01$) was higher in the DPN group, whereas that of Bacteroidetes was decreased ($P < 0.001$). At the genus level, the richness of *Bacteroides* ($P < 0.001$) and *Faecalibacterium* ($P < 0.05$) was

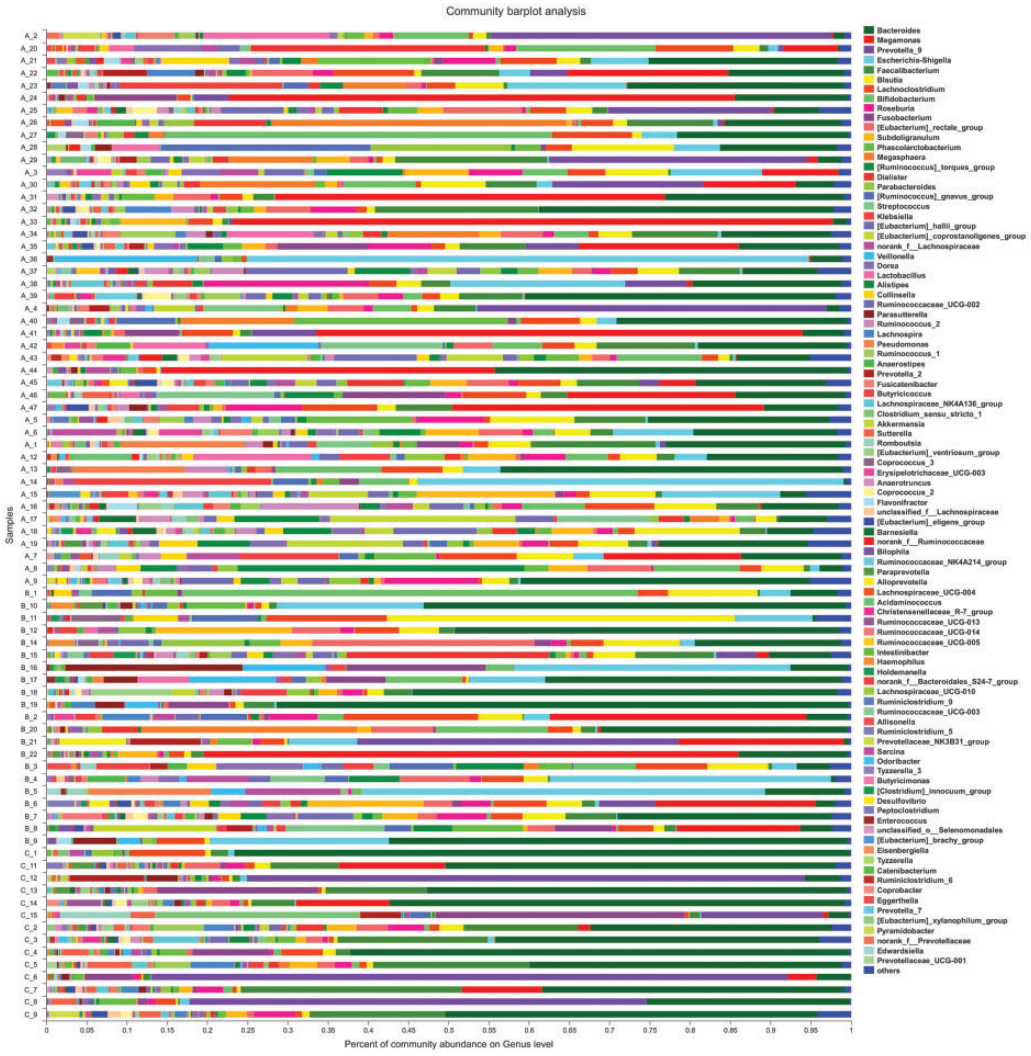

Figure 4. Microbial composition of different communities at the genus level. The relative read abundance of different microbial genera within the different communities is presented. Taxa with an abundance <1% are included as others. DPN, diabetes peripheral neuropathy group; DM, type 2 diabetes mellitus group; NC, normal control group.

significantly elevated in the DPN group, whereas that of *Escherichia-Shigella* ($P < 0.01$), *Lachnoclostridium* ($P < 0.05$), *Blautia* ($P < 0.001$), *Megasphaera* ($P < 0.01$), and *Ruminococcus torques* group ($P < 0.05$) was increased (Figure 5a, 5b).

Correlation between bacterial flora and clinical parameters

Spearman's correlation analysis was used to analyze the correlation of the top 50 most commonly detected species at the OTU level with the clinical parameters of patients

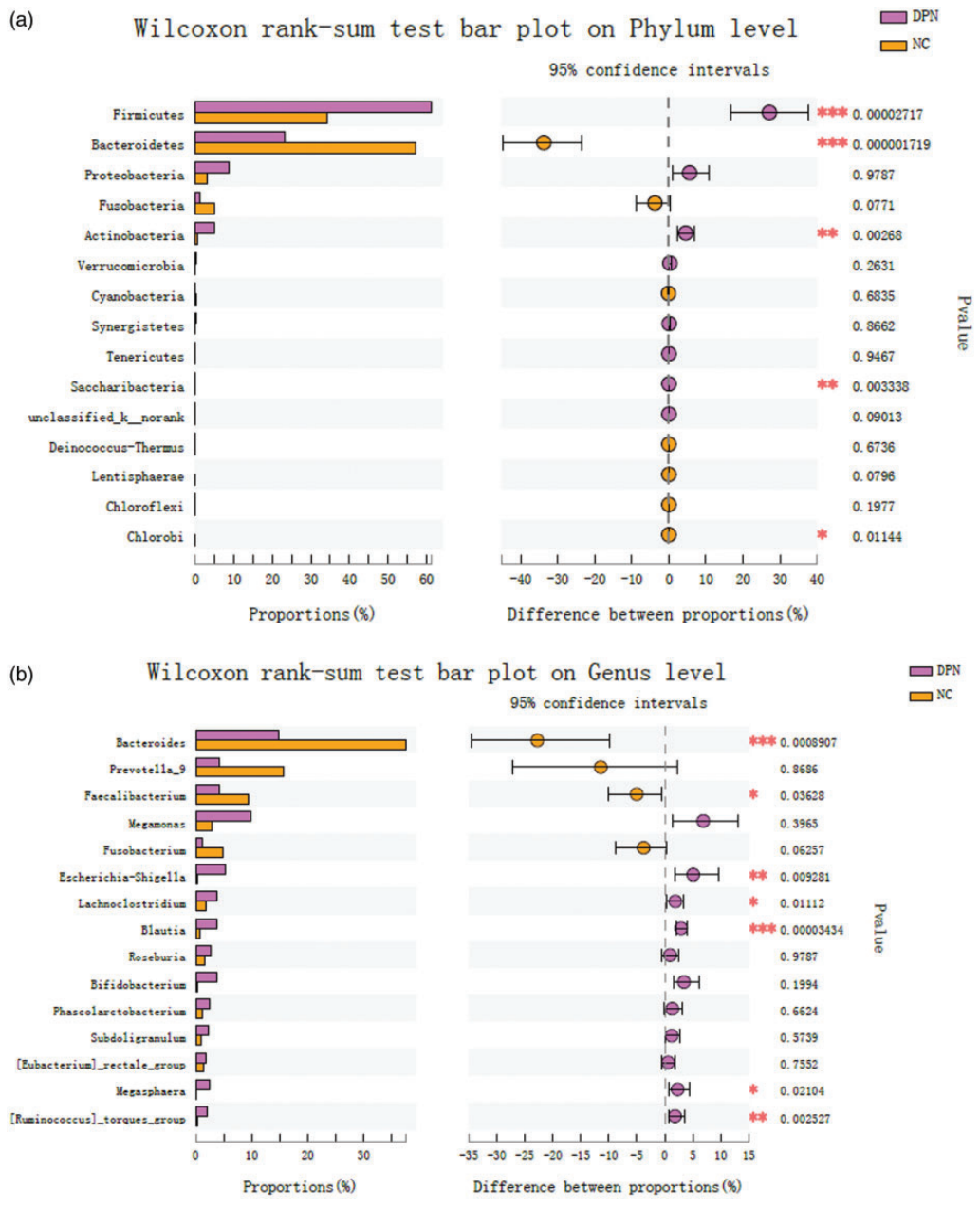

Figure 5. Analysis of microbial diversity among the groups. (a) Compared with the findings in the NC group, the richness of Firmicutes and Actinobacteria was higher in the DPN group increased, and that of Bacteroidetes was lower. (b) Compared with the findings in the NC group, the richness of *Bacteroides* and *Faecalibacterium* was significantly lower in the DPN group, and that of *Escherichia-Shigella*, *Lachnoclostridium*, *Blautia*, *Megasphaera*, and *Ruminococcus torques* group was increased. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. DPN, diabetes peripheral neuropathy group; DM, type 2 diabetes mellitus group; NC, normal control group.

in the DPN group. According to the heatmap, different clinical parameters exhibited particular relationships with particular genera. We found that HbA1c levels were negatively correlated with the presence of *Ruminococcus* 1 ($P < 0.05$), whereas FPG levels were positively correlated with the presence of *Bacteroides* ($P < 0.05$) and *Dialister* ($P < 0.01$). Concerning clinical parameters associated with serum lipid metabolism, TC levels were positively correlated with the presence of *Lachnoclostridium* and *Ruminococcus gnavus* group (both $P < 0.05$) and negatively correlated with the presence of *Alistipes* ($P < 0.05$) and *Barnesiella* ($P < 0.01$). In addition, TG levels were positively correlated with the presence of *Ruminococcus gnavus* group ($P < 0.01$) and *Klebsiella* ($P < 0.05$) and negatively correlated with the presence of *Ruminococcus* 2, *Butyricicoccus*, and Lachnospiraceae (all $P < 0.05$). Meanwhile, LDL-C levels were positively correlated with the presence of *Lachnoclostridium* ($P < 0.05$). Regarding the correlations between the inflammatory state and intestinal microbiota, we found that CRP levels were positively correlated with the presence of Parabacteroides ($P < 0.01$) and negatively correlated with the presence of *Butyricicoccus* and *Lachnospira* (both $P < 0.05$), whereas fasting C peptide levels were positively correlated with the presence of *Escherichia-Shigella* ($P < 0.05$) and negatively correlated with the presence of *Butyricicoccus* and *Coprococcus* 2 (both $P < 0.05$). Regarding the indications for insulin resistance, fasting insulin (FinS) and fasting plasma glucagon levels and HOMA-IR have great clinical significance. The results illustrated that FinS levels were negatively correlated with the presence of *Butyricicoccus* and Lachnospiraceae (both $P < 0.05$), fasting plasma glucagon levels was negatively correlated with the presence of *Anaerostipes* ($P < 0.05$), and HOMA-IR was positively correlated with the presence of *Megasphaera* ($P < 0.05$). Meanwhile, the presence of *Ruminococcus* 2, *Ruminococcus torques* group, and *Eubacterium rectale* group (all $P < 0.05$) was negatively correlated with BMI (Figure 6).

Correlation analysis between intestinal flora and bile acid

The presence of *Ruminococcus gnavus* group was positively correlated with glycine ursodeoxycholic acid (GUDCA) and tauroursodeoxycholic acid (TUDCA) levels (both $P < 0.001$). GUDCA levels also exhibited positive correlations with the presence of *Phascolarctobacterium* ($P < 0.001$) and Bacteroides ($P < 0.05$), and TUDCA levels also displayed positive correlations with *Parabacteroides* ($P < 0.001$) and *Blautia* ($P < 0.05$). In addition, both chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) levels were positively correlated with the presence of *Phascolarctobacterium* and *Coprococcus* 3. Meanwhile, we observed positive correlations between taurochenodeoxycholic acid levels and the presence of *Megamonas* ($P < 0.05$), between taurodeoxycholic acid levels and the presence of *Fusobacterium* ($P < 0.05$), and between lithocholic acid levels and the presence of *Bifidobacterium* ($P < 0.01$). Glycocholic acid and glycochenodeoxycholic acid (GCDCA) levels were positively correlated with the presence of *Coprococcus* 2 ($P < 0.001$ and $P < 0.05$, respectively) and *Prevotella* 9 (both $P < 0.05$), and GCDCA levels additionally had a positive correlation with the presence of *Phascolarctobacterium* (Figure 7).

Discussion

The intestinal tract hosts approximately 1×10^{14} microorganisms, including more than 1000 species of bacteria. The amount of genetic information encoded in the

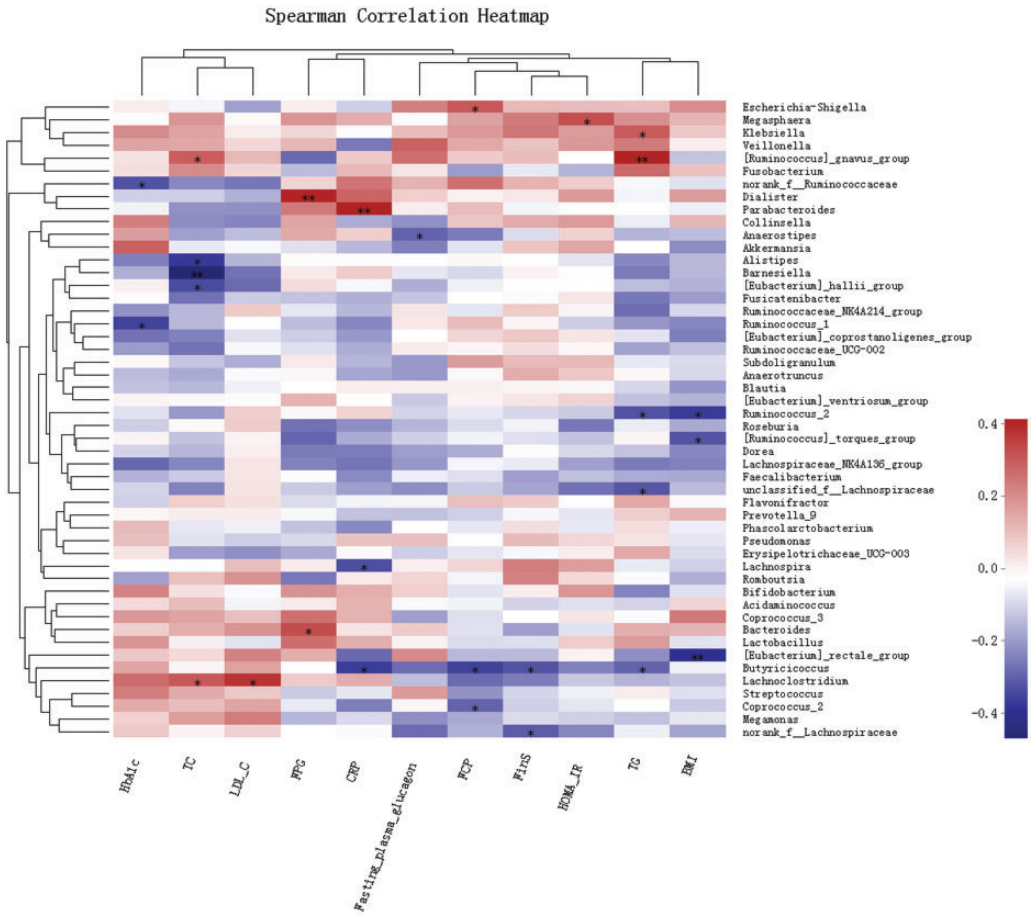

Figure 6. Correlations between bacterial flora and clinical parameters. Heatmap analyses of the correlations between intestinal flora and blood parameters at the genus level were conducted using Spearman's correlation analyses. TG, triglyceride; FCP, Fasting C peptide; FinS, fasting insulin; HOMA-IR, homeostasis model assessment insulin resistance index; TC, total cholesterol; CRP, C-reactive protein; LDL-C, low-density lipoprotein cholesterol; BMI, body mass index; FPG, fasting plasma glucose. Note: The X-axis and Y-axis are the clinical parameters and intestinal flora, respectively, and the correlation coefficient (R) and *P* values were calculated. The results for R are shown in different colors in the figure. Values for which $P < 0.05$ are marked by an asterisk, and the right legend presents the color interval with different values of R. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

microbiome is 300-fold larger than that encoded by the human genome.[14] According to alpha diversity analysis, DPN can result in more severe disruption of microbiota community richness than type 2 diabetes alone. At the same time, although the bacterial abundance was higher in the NC group than in the DM group, the difference was not statistically significant. As assessed using the Shannon entropy index, the microbial diversity of the DPN group significantly differed from that of the DM group. In addition, patients with peripheral neuropathy associated with type 2 diabetes had greater intestinal flora abundance than healthy people, and the

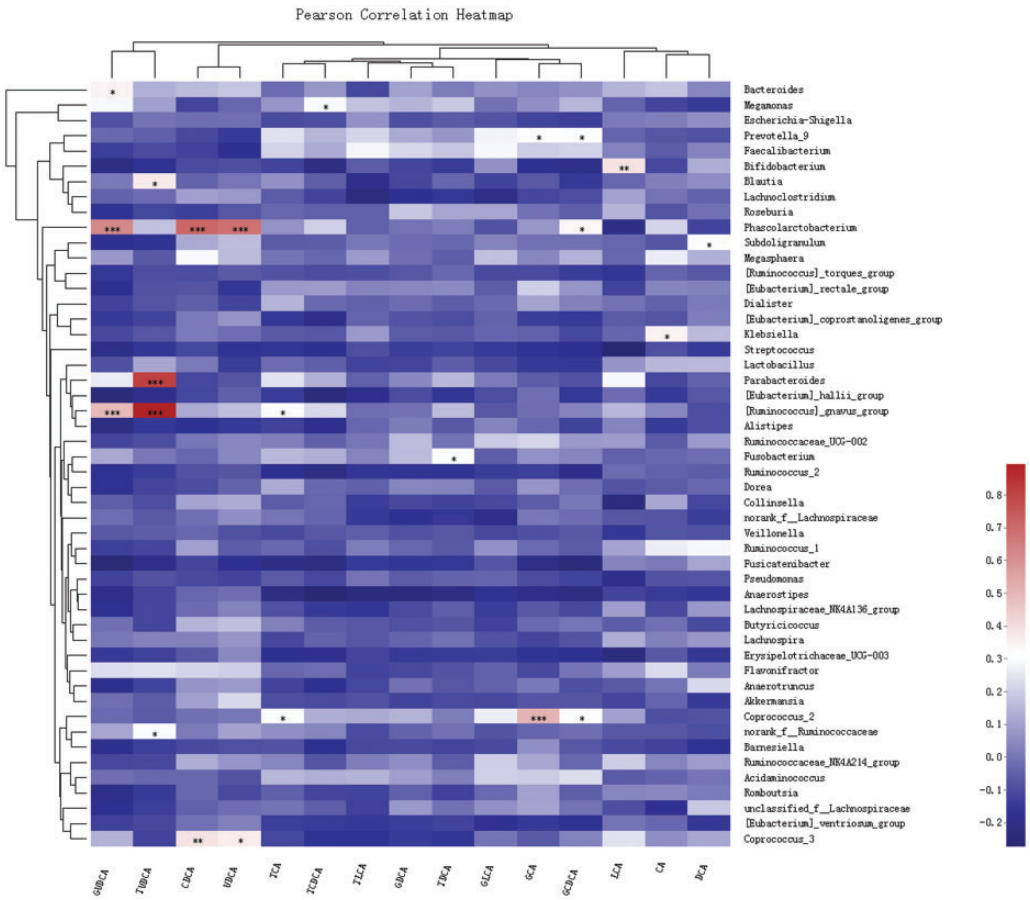

Figure 7. Correlation analysis between intestinal flora and bile acids. CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; GCA, glycocholic acid; GLCA, glycosidic acid; GDCA: glycodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TCA, taurocholic acid; TLCA, taurolithocholic acid; TDCA, taurodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; TUDCA, tauroursodeoxycholic acid. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

long-term use of metformin may explain the difference.

Microorganisms colonize the human intestine, in which in 99% of the bacteria represent five main phyla: Firmicutes, Bacteroidetes, Actinomycetes, verrucous microflora, and Proteobacteria. The current study found that the most dominant microbial taxa in the three groups were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria. Bacteroidetes was the most common phylum in the NC group (57.18%), and this phylum is associated with human nutritional metabolism. The proportion of Firmicutes was highest in both the DM (50.43%) and DPN groups (61.62%), and these bacteria are associated with metabolic diseases such as diabetes. In addition, Proteobacteria accounted for 8.67% of bacteria in the DPN group and 14.07% of those in the DM group. Actinobacteria

accounted for 5.09% of bacteria in the DPN group and 5.98% of bacteria in the DM group, and both proportions were significantly higher than those in the NC group. Of note, the five most prevalent taxa in the DPN group were Bacteroidetes (14.76%), *Megamonas* (9.79%), *Escherichia-Shigella* (5.36%), *Prevotella* 9 (4.31%), and *Faecalibacterium* (4.29%). However, the significance of these differences has not been clarified, and further analysis is warranted to fill this glaring gap in knowledge.

Patients with type 2 diabetes often have varying degrees of insulin resistance, and insulin resistance is associated with higher levels of inflammation and changes in lipid metabolism.[15–17] Bifidobacteria and *Akkermansia* detected in patients with type 2 diabetes produce short-chain fatty acids, which are inversely associated with low-grade inflammation and insulin resistance.[18] The current study found that the richness of *Megasphaera* was higher in the DPN group than in the NC group, and HOMA-IR was positively correlated with the presence of *Megasphaera* (coccidia) in the DPN group, further confirming that the occurrence of insulin resistance plays an important role in DPN associated with type 2 diabetes.

It has been proven that *Parabacteroides* species play a positive role in the regulation of metabolic diseases, especially those associated with glycolipid metabolism. In both genetically and diet-induced obese mouse models, oral *Parabacteroides* species can improve metabolism, control body weight, reduce hyperglycemia, and reverse fatty liver.[19] In addition, low-grade inflammation is a hallmark of metabolic diseases, and CRP is a sensitive indicator of inflammation. In previous studies, patients with type 2 diabetes tend to display intestinal flora imbalance, increased pathogenic bacteria counts, and potentially elevated pro-inflammatory factors. In addition, the correlation analysis in this study revealed that CRP levels in patients with DPN were positively correlated with the presence of *Parabacteroides*. Hence, we postulated that patients with DPN, a complication of diabetes, may be affected by CRP levels and the abundance of *Parabacteroides*, which may be related to insulin resistance and dyslipidemia.

Parabacteroides species can also convert bile acids, thereby increasing the levels of secondary bile acids (LCA), TUDCA, and UDCA, as well as produce succinic acid.[19] In this study, we further analyzed patients with DPN and found that the presence of *Parabacteroides* was positively correlated CRP and TUDCA levels. TUDCA is an FXR antagonist. Through FXR and TGR5, bile acids acquire the ability to regulate glycolipid metabolism. This may explain the elevated blood levels of bile acids, especially taurocholic acid, in patients with diabetes mellitus after gastric bypass, and the correlation between bovine sulfonate bile acid and clinical parameters related to diabetes, including FBG levels, insulin sensitivity and weight.[20] These findings provide new insights into the underlying mechanisms of hypoglycemic agents. It has been proven that acarbose can increase the relative richness of lactic acid bacteria and bifidobacteria in the intestinal flora and reduce the levels of spoilage bacteria (such as *Clostridium* and *Bacteroides*), thereby affecting the composition of bile acids, regulating metabolism, and improving insulin resistance.[21] Therefore, these findings are sufficient to conclude that intestinal flora and bile acid metabolism have interdependent and mutually causal relationships.[22] The current study found that some treatments for diabetes have important effects on the intestinal flora. Forslund[23] reported that metformin significantly influenced the intestinal microbiome to enhance the production of butyrate and propionate and simultaneously improved the patient's ability to catabolize multiple amino acids (such

as glycine and tryptophan). This effect of metformin may be attributable to increased growth of *Akkermansia*.[24] In addition, as all patients in the DPN and DM groups used metformin, *Bifidobacterium* counts were higher in these groups than in the NC group. These findings provide insights into the pathogenesis of insulin resistance, and they could facilitate the development of clinical strategies against type 2 diabetes-linked diseases. Studies indicated that standardized fecal bacteria transplantation can improve insulin resistance and insulin sensitivity by regulating intestinal flora and restoring the normal microecology of the intestines.[25]

In summary, the intestinal flora of patients with type 2 diabetes and peripheral neuropathy associated with type 2 diabetes have obvious structural and richness disorders compared with the observations in normal healthy people, including relative increases in the abundance of conditional pathogens. Changes in *Parabacteroides* counts and TUDCA levels, which could lead to insulin resistance and dyslipidemia, may play an essential role in the development of peripheral neuropathy in type 2 diabetes.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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