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ORIGINAL ARTICLE

Understanding the gut–kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition

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Abstract

Aims Type 2 diabetes mellitus (T_2DM) has a rising prevalence and gut microbiota involvement is increasingly recognized. Diabetic nephropathy (DN) is a major complication of T_2DM. The aim of the study was to understand the gut–kidney axis by an analysis of gut microbiota composition among biopsy-proven DN, T_2DM without kidney disease, and healthy control.

Methods Fecal samples were collected from 14 DNs, 14 age/gender-matched T_2DMs without renal diseases (DM), 14 age and gender-matched healthy controls (HC) and household contacts (HH) of DM group. The microbiota composition was analyzed by 16sRNA microbial profiling approach.

Results Substantial differences were found in the richness of gut microbiota and the variation of bacteria population in DM compared to HC, and DN compared to DM, respectively. DM could be accurately distinguished from age/gender-matched healthy controls by the variable of genus *g_Prevotella_9* (AUC = 0.9), and DN patients could be accurately distinguished from age/gender-matched DM by the variables of two genera (*g_Escherichia-Shigella* and *g_Prevotella_9*, AUC = 0.86). The microbiota composition of HH group was close to that of HC group, and was different from DM group. Under the same diet, DM could be more accurately detected by the same genus (*g_Prevotella_9*, AUC = 0.92).

Conclusion Gut microbiota composition was explored to be related to the occurrence of biopsy-proven DN from DM. DM could be distinguished from HC by detecting *g_Prevotella_9* level in feces, while DN was different from DM by the variables of *g_Escherichia-Shigella* and *g_Prevotella_9*, which potentially contributed to the physiopathological diagnosis of DN from DM.

Keywords Gut–kidney axis · Gut microbiota · Diabetic nephropathy · Type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T_2DM) is a disorder of elevated blood glucose levels (hyperglycaemia) primarily due to insulin resistance, inadequate insulin resistance or inadequate insulin secretion, with the rising global prevalence [1–3]. Genetic and environmental risk factors are known, the latter including dietary habits and sedentary lifestyle [4], and gut microbiota involvement is increasingly recognized [5–7].

Although findings diverged between studies [8], e.g., one study [6] reported several *Clostridium* species enriched in T_2DM whereas another [5] instead reported enrichment of several *Lactobacilli*. It was generally accepted that gut microbiota played a crucial role in gastro-intestinal mucosa permeability and regulated the

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fermentation and absorption of dietary polysaccharides, which was key in the regulation of fat accumulation and occurrence of T_2DM [9].

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes mellitus and is the most common cause of end-stage renal diseases (ESRD) worldwide [10–12]. Kidney disease develops in approximately 35% of patients with T_2DM [13] and is associated with the increased mortality [14]. The definition of DN is generally based on the changes in albuminuria and glomerular filtration rate (GFR), which does not exclude non-diabetes causes of renal disease in patients with diabetes mellitus, unless confirmed histologically [15]. Although endotoxin, protein and metabolites [16, 17] derived from gut microbiota has been proved to be involved in kidney diseases and predicting biomarkers were also found in several diseases [18, 19], the association with DN and gut microbiota remained unclear.

The aim of our study was to explore the possible differences in gut microbiota composition among DN, T_2DM without kidney diseases and healthy controls, and to assess if diet overweighs diseases in gut microbiota composition, since the human gut microbiota is a complex ecosystem where diet plays a vital role [20].

Methods

Study participants

At West China Hospital, Chengdu, Sichuan, China, we enrolled 14 T_2DM patients with DN confirmed by renal biopsy from March, 2017 to January, 2018, with estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m^2 and urine albumin creatinine ratio (UACR) ≥ 30 mg/g [21] as group DN. We also enrolled 14 T_2DM patients without any renal disease (UACR < 30 mg/g and eGFR > 60 mL/min/1.73 m^2) [21, 22], matched for gender and age, as group DM. We included two control groups composed of gender and age-matched healthy subjects (HC group), as well as household contacts on the same diet of each patient on group DM (HH group). Each group consisted of 14 subjects.

Excluded from all groups were subjects under antibiotic treatment in the 30 days before the evaluation, with gastro-intestinal or systemic diseases known to affect gut composition, following restricted dietary regimens or taking prebiotics/probiotics.

All the participants gave their written informed consent for participation in the study. The study protocol was approved by Biomedical Ethics Committee of West China Hospital of Sichuan University (No. 2016-273). All the procedures followed the Declaration of Helsinki principles.

Stool sample collection

Stool samples freshly collected from each participant were immediately frozen and stored at − 80 °C until analysis [23, 24].

DNA extraction and PCR amplification

Microbial deoxyribonucleic acid (DNA) was extracted from 55 samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3–V4 hypervariable regions of the bacteria 16S ribosomal ribonucleic acid (16S rRNA) gene were amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by thermocycler polymerase chain reaction (PCR) system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20-μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM deoxynucleotide triphosphates (dNTPs), 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Processing of sequencing data

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing two nucleotides mismatching and reads containing ambiguous bases

were removed. (iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence.

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by remote desktop protocol (RDP) Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.

By pyrosequencing, we generated a dataset consisting of 2,170,497 high-quality 16S rRNA gene sequences with an average of 39463.58 sequences obtained for each of the samples (Supplementary Table S1).

Statistical analysis

All statistical analyses were made in the R software. We performed the PERMANOVA [25] (permutational multivariate analysis of variance) analysis using implemented method in R package "vegan", and the permuted *P* value was obtained by 10,000 times permutations. Principal component analysis (PCA) was performed in R using the ade4 [26] package. Differential abundance of genus was tested by Wilcoxon rank sum test. The correlation between biochemical indicators and various microbes were calculated by Spearman rank correlation coefficient and visualized by heatmap in R using the "heatmap" package.

Results

General characteristics of all participants

We included 14 DN patients confirmed by renal biopsy with a median age of 52.93 years. All DN patients were with eGFR ≥ 60 mL/min/1.73 m^2 and UACR ≥ 30 mg/g. Baseline characteristics of the DN, DM and two control groups (unrelated age-matched healthy subjects and household contacts on the same diet of DM group) are summarized in Table 1.

The analysis of gut microbiota composition among DN, DM, and HC

A total of 793 OTUs were obtained at a 97% homology cutoff. The HC group showed the largest amount of OTUs. As exhibited in the Venn diagram (Fig. 1a), the number of OTUs in common between HC and DM groups was 493, while DM and DN groups had 455 OTUs in common. The DM group had 26 specific OTUs and the DN group had 24 specific OTUs which another two groups did not share (Fig. 1b, c). Analyzed from the ternary analysis (Fig. 1d), the higher counts of *Firmicutes* were found in the fecal samples of DM, while DN patients had the higher amounts of *Proteobacteria*, compared to HC and DM groups.

Sobs, chao, ace indices on the OTU profile were used to estimate community richness and shannon, simpson indices were used to estimate community diversity (Table 2). Although the differences of community diversity were not significant (shannon $P = 0.1031$ HC vs. DM, $P = 0.07296$

Table 1 Baseline characteristics of participants

	DN ($n = 14$)	DM ($n = 14$)	HC ($n = 14$)	HH ($n = 14$)	*P* value
Age, years	52.93 ± 9.98	53.29 ± 9.00	52.86 ± 9.91	44.29 ± 17.31	0.149
Gender, female/male	5/9	5/9	5/9	10/4	0.144
Body mass index, kg/m^2	27.47 ± 3.63	25.01 ± 2.00	24.47 ± 2.47	25.33 ± 4.19	0.08
Course of T2DM, years	10.29 ± 5.36	3 (3, 7.25)	–	–	–
Smoking history, yes/no	6/8	7/7	5/9	4/10	0.683
Hypertension, yes/no	14/0	6/8	2/12	1/13	< 0.001
Stroke history, yes/no	3/11	1/13	0/14	0/14	0.091
Heart diseases history, yes/no	2/12	3/11	0/14	0/14	0.115
Fasting blood glucose, mmol/L	8.86 ± 4.63	8.18 ± 3.89	4.9 ± 0.81	5.95 ± 2.15	0.006
HbA1c, %	7.73 ± 1.47	7.36 ± 1.89	5.86 ± 0.26	5.91 ± 1.24	< 0.001
Serum creatinine, μmol/L	73.93 ± 20.2	73.71 ± 14.08	73.07 ± 10	60.14 ± 8.75	0.03
UACR, mg/g	2280.84 ± 2116.68	12.09 ± 8.56	10.49 ± 8.65	19.50 ± 6.90	< 0.001
eGFR, ml/min/1.73 m^2	93.26 ± 17.01	93.48 ± 13.02	96.01 ± 9.29	106.92 ± 16.05	0.173

One-way ANOVA was used to compare continuous variables (age, body mass index, fasting blood glucose, HbA1c, serum creatinine, UACR, eGFR) and Chi-square was used to compare categorical variables (gender, smoking/stroke/heart diseases history, hypertension)

DN diabetic nephropathy, *DM* type 2 diabetes mellitus without renal diseases, *HC* healthy controls, *HH* households, *T2DM* type 2 diabetes mellitus, *HbA1c* glycosylated hemoglobin, *UACR* urine albumin creatinine ratio, *eGFR* estimated glomerular filtration rate

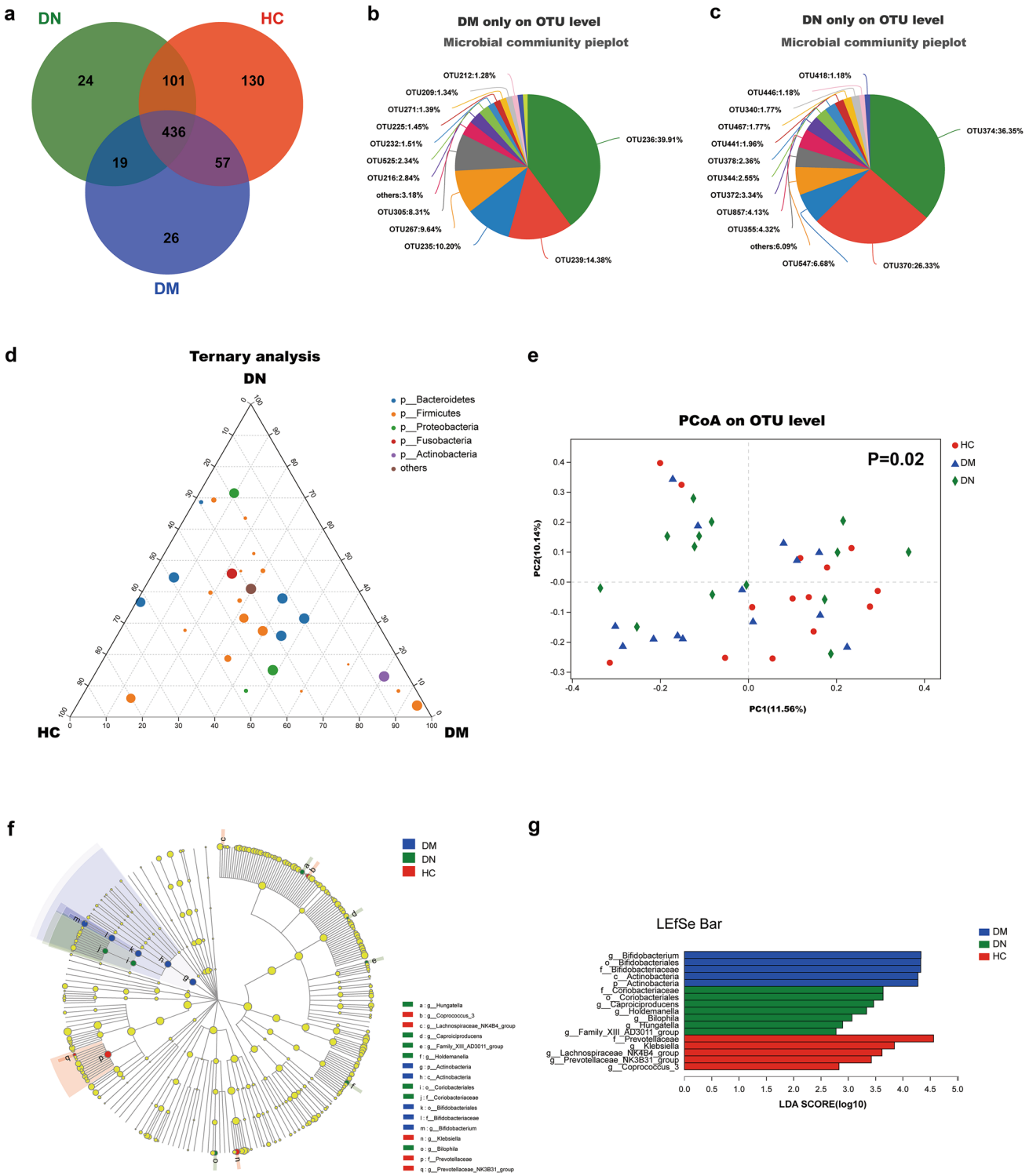


Fig. 1 Gut microbiota compositions differed among DN, DM, HC groups. **a** Venn diagram of DN, DM, and HC; **b** OTUs specifically in DM; **c** OTUs specifically in DN; **d** Ternary analysis of DN, DM, and HC; **e** PCoA of DN, DM, and HC; **f, g** LDA and LEfSe of DN, DM and HC. *DN* diabetic nephropathy, *DM* type 2 diabetes mellitus without renal diseases, *HC* healthy controls, *OTUs* operational taxonomic units, *LDA and LEfSe* linear discriminate analysis and effect size

Table 2 α-Diversity among DN, DM and HC

Estimators	DN-mean	DN-SD	HC-mean	HC-SD	DM-mean	DM-SD	*P* value (DM–DN)	*Q* value (DM–DN)	*P* value (DM–HC)	*Q* value (DM–HC)
Sobs	215.71	58.157	258.79	64.351	161.29	61.269	0.02328	0.08094	0.0003547	0.001064
Shannon	3.3128	0.42657	3.3037	0.55558	2.8845	0.74377	0.07296	0.08802	0.1031	0.1547
Simpson	0.10041	0.050548	0.094649	0.064882	0.14299	0.10684	0.1893	0.1893	0.1598	0.1808
Ace	251.64	62.925	312.93	72.678	209.69	55.794	0.07335	0.08802	0.0002656	0.001064
Chao	256.02	69.988	310.63	77.74	208.33	58.897	0.06195	0.08802	0.0005692	0.001138

DN diabetic nephropathy, *DM* type 2 diabetes mellitus without renal diseases, *HC* healthy controls

Table 3 *P* values of one-way ANOVA analysis among DN, DM, and HC at the genus level

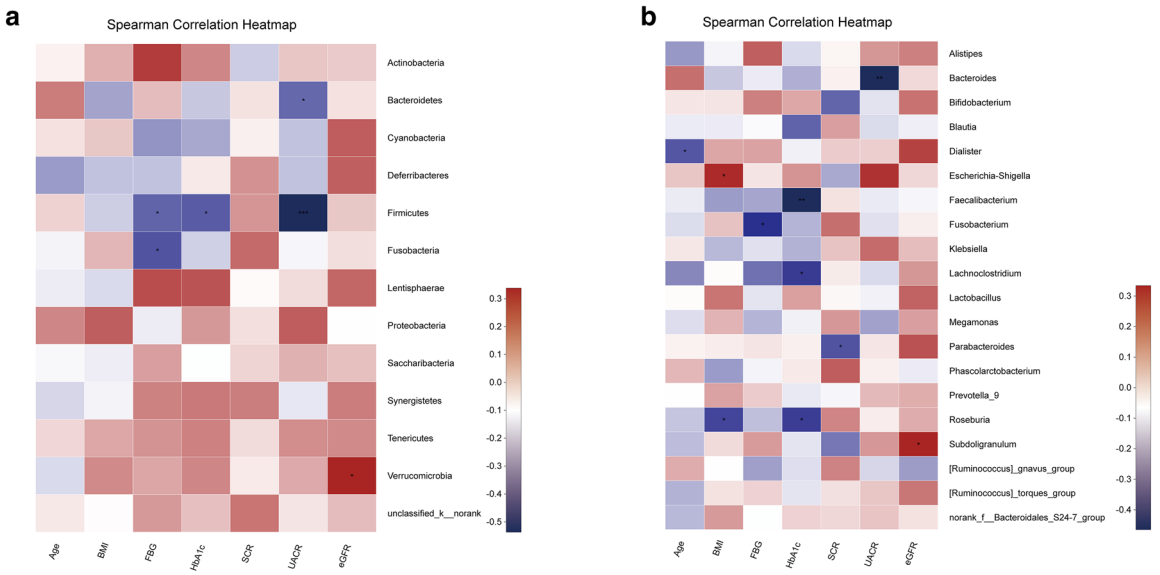
Species name	*P* value
g_Bacteroides	0.127
g_Faecalibacterium	0.9158
g_Roseburia	0.1523
g_Escherichia-Shigella	0.02582
g__Megamonas	0.2597
g_Phascolarctobacterium	0.8874
g_Prevotella_9	0.01152
g_Blautia	0.291
g_Lachnoclostridium	0.8909
g_Lactobacillus	0.5012
g_Bifidobacterium	0.05834
g_Fusobacterium	0.8414
g_Alistipes	0.2899
g_Subdoligranulum	0.2026
g_Dialister	0.1397

DM vs. DN), different richness of OTUs were found in DM and DN patients (sobs $P = 0.0003547$ HC vs. DM, $P = 0.02328$ DM vs. DN).

Principal co-ordinates (PCoA) based on Bray–Curtis dissimilarity at OTU level exhibited that the microbiota composition of HC, DM, and DN patients were significantly different in Fig. 1e ($P = 0.02$).

To determine different taxa from phylum to genus level among three groups, the linear discriminate analysis (LDA) effect size (LEfSe) algorithm was used. The LEfSe showed one class, one family, one genus increased in DM patients and one family, five genera increased in DN patients (Fig. 1f). At the phylum to family level, the most differentially abundant bacteria in healthy subjects was *Prevotellaceae*. *Actinobacteria*, and *Bifidobacteriaceae* were overrepresented in DM group. *Coriobacteriaceae* was differentially enriched in DN subjects (Fig. 1g). The results of one-way ANOVA analysis indicated that at the genus level, *Escherichia-Shigella* was significantly increased while *Prevotella_9* significantly decreased in DN group (Table 3).

Correlations between the differences of gut microbiota and clinical biomarkers were further evaluated by Pearson correlation analysis. At phylum level, *Firmicutes* showed strongly negative correlations with fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), and urine albumin creatinine ratio (UACR). *Fusobacteria* also showed negative correlation with FBG level. *Bacteroidetes* had a negative correlation with UACR while *Verrucomicrobia* showed a significantly positive correlation with estimated glomerular filtration rate (eGFR) (Fig. 2a, Supplementary Table S2). Down to genus level, *Faecalibacterium, Lachnoclostridium*, and *Roseburia* showed remarkedly negative correlations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Spearman Correlation Heatmap
Actinobacteria
Bacteroidetes
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Escherichia-Shigella
Faecalibacterium
Fusobacterium
Klebsiella
Lachnoclostridium
Lactobacillus
Megamonas
Parabacteroides
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Prevotella_9
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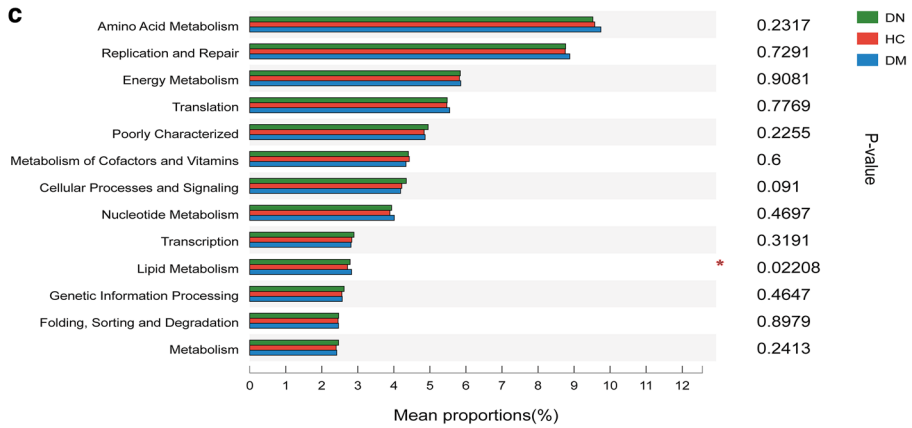
c
Amino Acid Metabolism
Replication and Repair
Energy Metabolism
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Poorly Characterized
Metabolism of Cofactors and Vitamins
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Transcription
Lipid Metabolism
Genetic Information Processing
Folding, Sorting and Degradation
Metabolism
0.2317
0.7291
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DN
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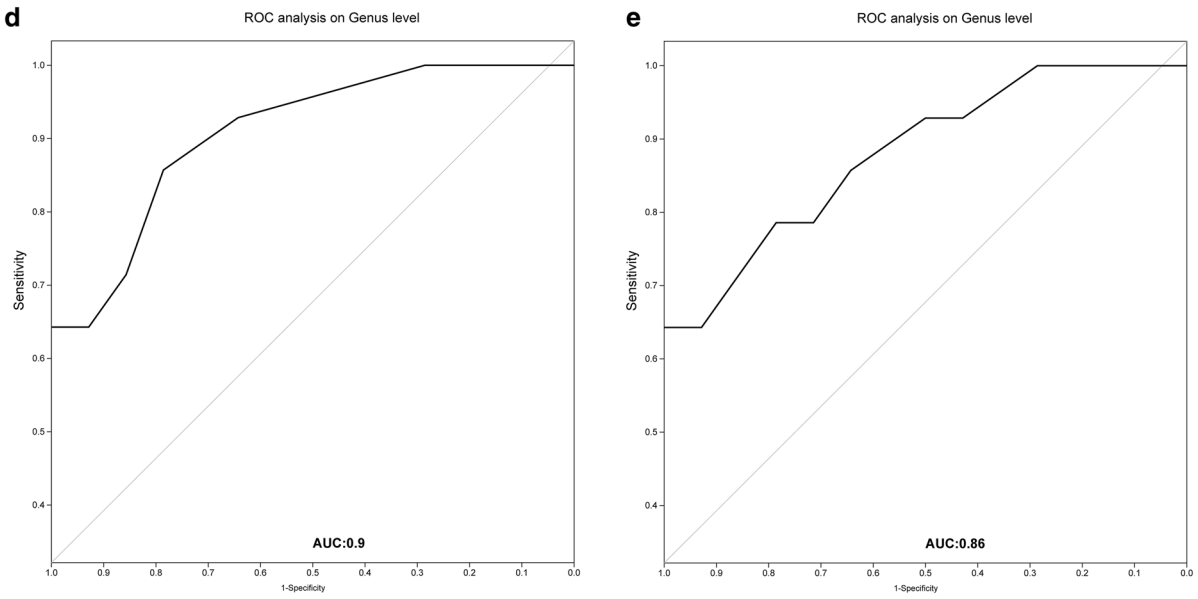
Mean proportions(%)


d
ROC analysis on Genus level
Sensitivity
1-Specificity
AUC:0.9
e
ROC analysis on Genus level
Sensitivity
1-Specificity
AUC:0.86

◂**Fig. 2** Classifications to identify DN and DM. **a** Heatmap showing correlations between phyla and biochemical indicators; **b** Heatmap showing correlations between genera and biochemical indicators; **c** Metagenome prediction among DN, DM, and HC; **d** ROC curve classifying DM from HC, based on *g_Prevotella_9*; **e** ROC curve classifying DN from DM, based on *g_Escherichia-Shigella* and *g_Prevotella_9*. *DN* diabetic nephropathy, *DM* type 2 diabetes mellitus without renal diseases, *HC* healthy controls, *ROC* receiver operating characteristic, *BMI* body mass index, *FBG* fasting blood glucose, *HbA1c* glycosylated hemoglobin, *SCR* serum creatinine, *UACR* urine albumin creatinine ratio, *eGFR* estimated glomerular filtration rate

with HbA1c. *Parabacteroides* exhibited a negative correlation with serum creatinine (SCR). Negative correlations also existed between *Dialister* and age, *Fusobacterium* and FBG, *Bacteroides* and UACR. The *Escherichia-Shigella* showed a positive correlation with BMI and *Subdoligranulum* had a positive correlation with eGFR (Fig. 2b, Supplementary Table S3). From the metagenome prediction on the basis of PICRUS analysis, abundant bacterial functions related to lipid metabolism were observed among DN, DM, and controls ($P=0.02208$) (Fig. 2c).

To illustrate the microbial signature of DM and DN and further explore the potential of gut microbiome in DN identification, the receiver operating characteristic (ROC) curves for classifying DM from HC, DN from DM patients were developed. We could detect DM individuals accurately based on only one genus (*g_Prevotella_9*) on the genus level, as indicated by the area under the receiver operating curve (AUC) of up to 0.9 (Fig. 2d). Similarly, two genera (*g_Escherichia-Shigella* and *g_Prevotella_9*) richness were effective to classify DN samples from DM subjects, showing an AUC of 0.86 (Fig. 2e).

The analysis of gut microbiota composition among DM, households (HH), and age/gender-matched healthy controls (HC)

As patients with DM are subjected to substantial dietary restrictions and other therapeutic interventions, it is difficult to elucidate the impact of disease itself on the microbial composition. Therefore, we included another control group of households contact sharing the dietary habits of the DM patients, as well as an age and gender-matched healthy control group. In addition, there were neither significant between-group differences in age, gender, BMI nor eGFR.

Totally, 901 OTUs were obtained at a 97% homology cutoff. The DM group had 538 OTUs, which was the smallest amount among three groups (Fig. 3a, b). As showed in the Ternary analysis (Fig. 3c), higher counts of *Lactobacillales* were found in the fecal samples of DM patients. Differences of community diversity were not significant (shannon $P=0.103$ HC vs. DM, $P=0.1391$ HH vs. DM). However, significantly reduced richness of OTUs existed in DM patients (sobs $P=0.0006038$ HC vs. DM, $P=0.03566$ HH vs. DM) (Table 4). PCoA based on Bray–Curtis dissimilarity at OTU level showed that the microbiota composition of DM, HH, HC groups were different significantly ($P=0.033$) (Fig. 3d). As showed in the hierarchical clustering tree on OTU level (Fig. 3e), microbiota composition of HH group is close to HC group, still different from DM group.

To figure out different taxa from phylum to genus level, the LDA and LefSe algorithm was used (Fig. 3f, g). One-way ANOVA bar plot showed that at the genus level, *Bacteroides* and *Alistipes* significantly increased while *Prevotella_9* significantly decreased in DM group (Fig. 3h).

Correlations between differences of gut microbiota and clinical biomarkers were analyzed using the Pearson correlation analysis. At phylum level, *Actinobacteria* showed strongly positive correlations with FBG and HbA1c and *Deferribacteres* showed a negative correlation with age (Fig. 4a, Supplementary Table S4). Down to genus level, *Prevotella_9* showed significantly negative correlations with HbA1c and SCR while *Bifidobacterium* showed a positive correlation with HbA1c and FBG (Fig. 4b, Supplementary Table S5).

To exploit if the potential of gut microbiome in DM identification could go even better under the same diets, the receiver operating characteristic (ROC) curve for classifying DM from HH was developed. We could detect DM individuals accurately based on only one genus (*g_Prevotella_9*) on the genus level, as indicated by the area under the receiver operating curve (AUC) of up to 0.92 (Fig. 4c), which was a little higher than the AUC between DM and HC groups mentioned before.

Discussion

In the study, we demonstrated the substantial differences in the richness of gut microbiota and the variation of bacteria population both in DM compared to HC, and DN compared to DM. Importantly, DM could be accurately distinguished from the age and gender-matched healthy controls by variables of *g_Prevotella_9* (AUC = 0.9) and DN could be accurately distinguished from age and gender-matched DM patients by variables of *g_Escherichia-Shigella* and *g_Prevotella_9* (AUC = 0.86). As DM and DN not only imply the disease itself, but goes along with the restrictions in diet, additional analysis to elucidate the differential impact of diet was performed. DM patients were compared with both age and gender-matched healthy controls and household contacts on the same diet. Interestingly, gut microbiota composition of HH group was close to HC group, and was still different from DM group, indicating the disease itself weighed more than dietary habits. Nevertheless, DM could be more

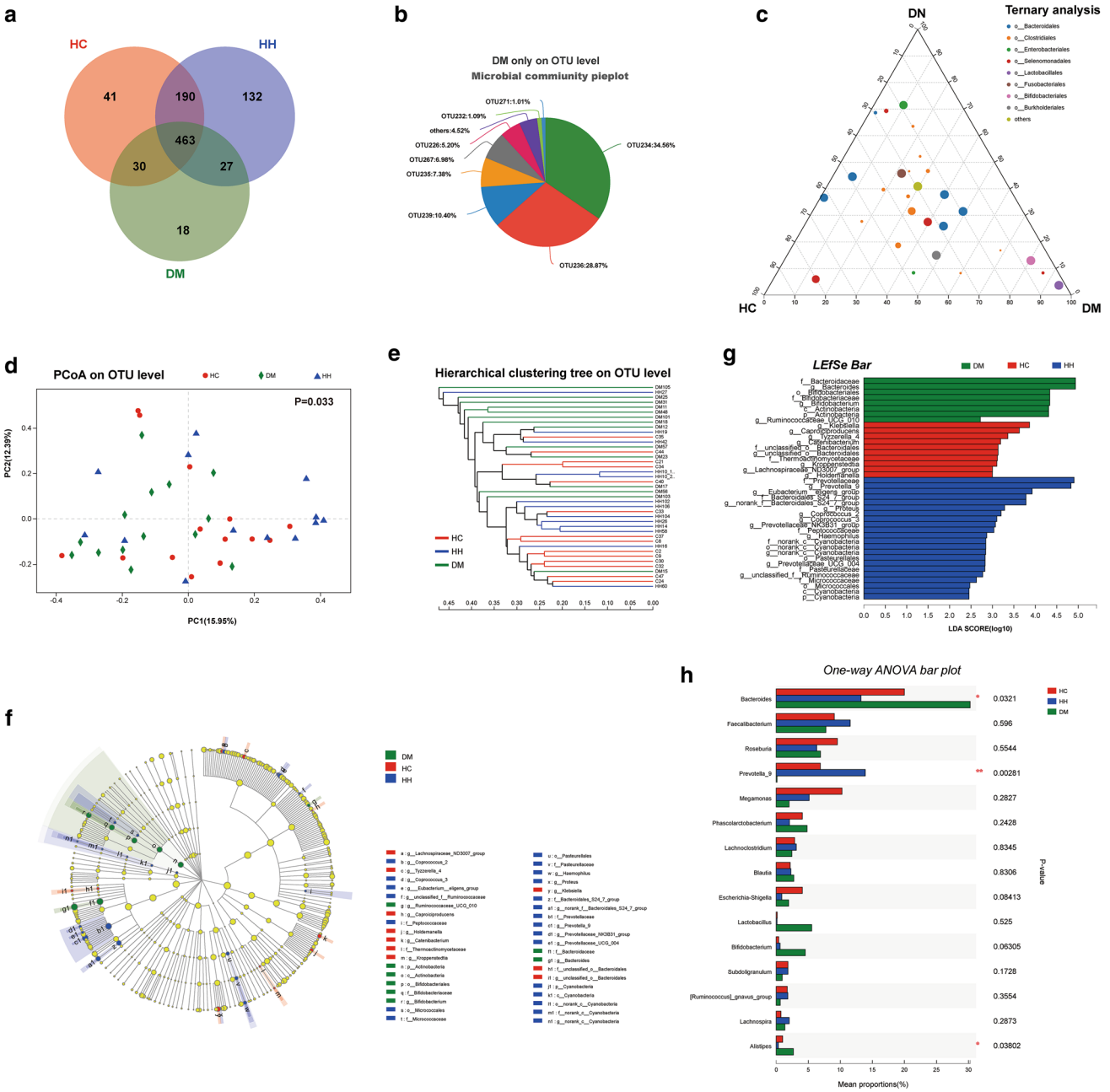


Fig. 3 Gut microbiota among DM, HH, HC groups. **a** Venn diagram of DM, HH, and HC; **b** OTUs specifically in DM; **c** Ternary analysis of DM, HH, and HC; **d** PCoA of DM, HH, and HC; **e** Hierarchical clustering tree of DM, HH, and HC on OTU level; **f, g** LDA and LEfSe of DM, HH, and HC; **h** one-way ANOVA bar of DM, HH, and HC on genus level. *DM* type 2 diabetes mellitus without renal diseases, *HH* households, *HC* healthy controls, *OTUs* operational taxonomic units, *LDA and LEfSe* linear discriminate analysis and effect size

accurately detected by the same genus (*g_Prevotella_9*, AUC = 0.92) under the same diet.

Various extra-intestinal non-communicable diseases are associated with gut microbiota through intestinal immunity [27–29]. T_2DM was proved to be associated with a decrease in genera containing known butyrate producers [5, 6, 30]. De Angelis et al. [31] found that the composition of the main bavterial phla (*Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria*) significantly differed in the fecal microbiota of immunoglobulin A nephropathy (IgAN) patients and healthy controls. Jiang et al. [32] found that *Prevotella* was enriched in healthy controls, and *Bacteroidetes* in CKD patients. In Forslund et al's study, a validated food frequency questionnaire (FFQ) was used to obtain dietary

habits. All food items in the FFQ were linked to food items in the Danish Food Composition Databank. When evaluating the dietary data the consumed quantity was determined by multiplying portion size by the corresponding consumption frequency reported. Estimation of daily intake of macro- and micronutrients was calculated by the software FoodCalc. They proved the influence of diet could be excluded, which was similar to our findings [30]. However, fecal metabolite profiles of household contacts closely resemble those of patients on hemodialysis in Poesen et al's study [33]. It's worth mentioning that they studied metabolites profiles in fecal samples, probably more reflecting distal colonic microbial metabolism than that of the entire colon, let alone gut microbiota composition.

Overall, metabolites produced by intestinal microbes play a direct role in health and disease [34]. As shown by metagenomic studies, the intestinal microbiota was directly involved in the metabolism of proteins, free amino acids (FAA), and carbohydrates [29]. Previously, serum and urine samples of DM and DN had altered level of some metabolites, including an increase of FAA [35–37]. An alteration of the fecal microbiota composition, which was related to an increased level of FAA, was described in another disease [38]. Esterification reactions at the colon level were considered as a microbial strategy to remove toxic compounds [39]. The conversion of fatty acids into ketones and glucose increased in the hepatic cells to supply energy for peripheral cells, which were unable to transport glucose in the absence of insulin [40]. Those all partially explained how gut microbiota was associated with the occurrence of DM and the progression to DN.

Our findings demonstrated that healthy controls had more *Prevotella* in gut microbiota, compared with DM and DN patients. *Prevotella* could produce short-chain fatty acids (SCFA), which were proved to be able to reduce inflammation in acute kidney injury (AKI) [41]. HC and DM groups possessed *Firmicutes* than DN group. *Firmicutes* are typically producing butyrate bacteria [42], which is involved in the adjustment of body reaction to inflammation [43]. *Bacteroides*, more enriched in DM patients than HC and HH groups, synthesized lipopolysaccharide (LPS) [44]. *Proteobacteria*, which was more enriched in DN patients, is also effective in increasing the LPS level in circulation [45, 46]. LPS may accelerate the activation of neutrophils and macrophages/monocytes, further leading to persistent inflammation. The presence of inflammation magnifies the risk of poor outcome and is a risk factor of cardiovascular diseases (CVD) [47]. We proved that DN patients had significantly higher amount of *Eschericha-Shigella*. The increased *Eschericha-Shigella* could exacerbate gut leakiness by penetrating intestinal epithelial barrier [48], produce ethanol [49], which enters the liver through blood circulation and causes disordered fatty acid metabolism [50].

Table 4 α-Diversity among DM, HH, and HC

Estimators	HC-mean	HC-SD	DM-mean	DM-SD	HH-mean	HH-SD	*P* value (HC–DM)	*Q* value (HC–DM)	*P* value (DM–HH)	*Q* value (DM–HH)
Sobs	243.57	56.957	154.93	63.104	228	104.36	0.0006038	0.001299	0.03566	0.05944
Shannon	3.303	0.5544	2.8836	0.74494	3.2558	0.48204	0.103	0.1288	0.1391	0.1575
Simpson	0.094517	0.06459	0.14309	0.10701	0.093813	0.060448	0.1579	0.1579	0.1575	0.1575
Ace	298.19	61.575	199.09	68.52	272.27	100.32	0.0004376	0.001299	0.0351	0.05944
Chao	296.31	65.133	199.07	70.071	275.01	101.61	0.0007796	0.001299	0.03173	0.05944

DM type 2 diabetes mellitus without renal diseases, *HH* households, *HC* healthy controls

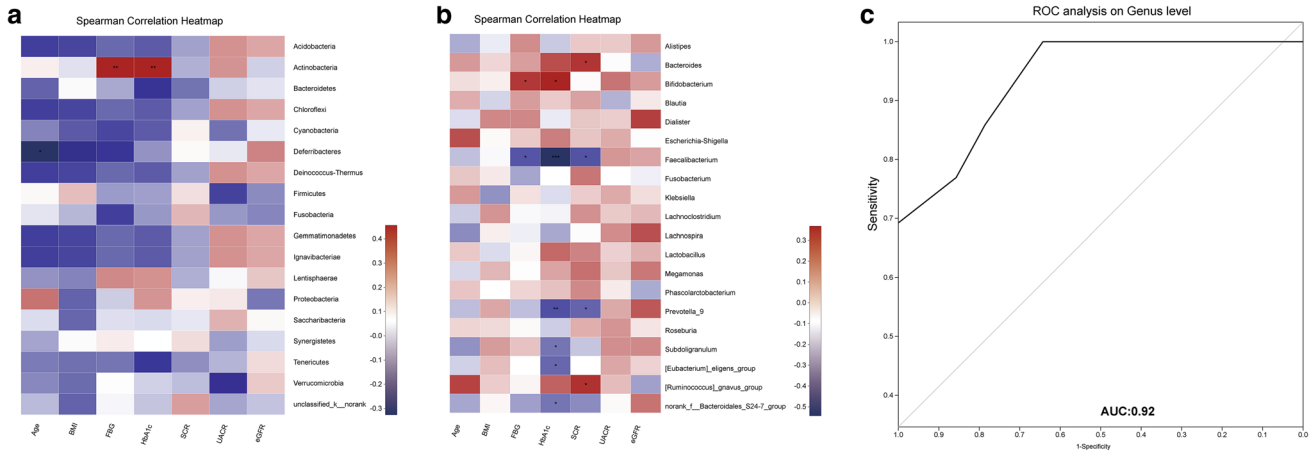


Fig. 4 Classifications to identify DM from HH. **a** Heatmap showing correlations between phyla and biochemical indicators; **b** heatmap showing correlations between genera and biochemical indicators; **c** ROC curve classifying DM from HH, based on *g_Prevotella_9*. *DM* type 2 diabetes mellitus without renal diseases, *HH* households, *HC* healthy controls, *ROC* receiver operating characteristic, *BMI* body mass index, *FBG* fasting blood glucose, *HbA1c* glycosylated hemoglobin, *SCR* serum creatinine, *UACR* urine albumin creatinine ratio, *eGFR* estimated glomerular filtration rate

To our knowledge, this is the first study indicating a direct association between gut microbiota and DN. All the enrolled DN patients had eGFR > 60 mL/min/1.73 m^2 and UACR ≥ 30 mg/g and whose diagnosis was confirmed by renal biopsy, aiming to exclude the impact of eGFR on gut microbiota [20, 32, 51]. Age and gender-matched HC and DM groups were enrolled to minimize those confounding factors. Additionally, we compared DM patients with both age and gender-matched healthy controls and household contacts on the same diet, to evaluate if diets exerted all-important impact on microbiota composition. However, there are limitations to our study. Although household contacts were selected based on a statement of same dietary habits, we did not formally include dietary assessments. Therefore, minor dietary differences between DM patients and HH contacts cannot be excluded but would probably not substantially alter the abovementioned conclusions. Additionally, correlations between gut microbiota composition and lifestyles or drug therapy need to be explored in further studies. Moreover, our study population consists of patients of Chinese origin. Care must be taken to extrapolate our data to other patient populations.

The results of our study demonstrated that DM could be distinguished relatively accurately from HC and DN from DM, by detecting only one or two genera in feces. Cai et al. [18] previously applied an excellent statistical variable selection procedure screening to gene files that obtained from metagenome sequencing, and 48/24 meta-markers were selected in Chinese/European cohorts as predictors with 0.97/0.99 accuracy in AUC. These results indicated the power and utility of data mining technologies within the large-scale and ultra-high dimensional genomic-related dataset for diagnostic and predictive markers identifying. Thus, further studies are still required to establish the sole effect of gut microbiota on DN progression and data mining technologies could be further applied for identifying diagnostic and predictive markers.

In summary, we for the first time explored the gut microbiota composition related to the occurrence of biopsy-proven DN from DM in the study. The results highlighted that DM could be accurately distinguished from HC or DN from DM, by detecting only one or two genera in feces, respectively. The analysis of selected gut microbiota level is a potential approach toward the physiopathological diagnosis of DN.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval was approved by Biomedical Ethics Committee of West China Hospital of Sichuan University (No. 2016-273). All the procedures followed the Declaration of Helsinki principles.

Informed consent All participants provided written informed consent before enrolment in the study.

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