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Differences in oral microbiota associated with type 2 diabetes mellitus between the Dai and Han populations

Lingtong Tang[a,b], Keke Ding[c], Mengci Li[c], Xiaowen Chao[c], Tao Sun[c], Yuhuai Guo[c], Xufei Peng[c], Wei Jia[c,d], Tianlu Chen[c], Guoxiang Xie[e] and Lei Feng[b]

[a]Department of Clinical Laboratory, The People's Hospital of Gao County, Yibin, Sichuan, China; [b]Department of Clinical Laboratory, Yan'an Hospital Affiliated to Kunming Medical University, Kunming, China; [c]Center for Translational Medicine and Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China; [d]Department of Pharmacology and Pharmacy, University of Hong Kong, Hong Kong, China; [e]Human Metabolomics Institute Inc, Shenzhen, China

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) development is closely linked to microbiota, influenced by geography, ethnicity, gender, and age. While the relationship between oral microbiota and T2DM has been explored, specific microbiota associated with T2DM in the Dai and Han populations remains unclear. This study aims to compare oral microbiota differences and identify keystone species between these populations, both with and without T2DM.
Methods: We recruited 28 han participants (6 healthy children, 10 healthy adults, 12 adults with T2DM) and 34 Dai participants (11 healthy children, 10 healthy adults, 13 adults with T2DM). Blood samples were collected for biochemical analysis, and saliva samples underwent DNA extraction and 16S rRNA sequencing.
Results: Age significantly influenced oral microbiota differences between the Dai and Han populations, overshadowing the effects of diabetes. In the Dai population with T2DM, notable increases in *Alistipes putredinis*, *Lactobacillus spp.*, *Faecalibacterium prausnitzii*, and *Akkermansia muciniphila* were observed compared to the Han population. Keystone genera differed, with *Fusibacter* central to the Dai population's microbial network, while the Han network was more scattered.
Conclusion: This is the first comparative analysis of oral microbiota in the Dai and Han populations with T2DM, highlighting age and ethnicity's influence on microbial composition.

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Oral microbiota; type 2 diabetes mellitus; Dai population; Han population; ethnic

Introduction

In 2021, the global population of individuals with diabetes was estimated at 529 million, with a worldwide age-standardized total prevalence of diabetes reaching 6.1%. T2DM accounted for 90% of these cases [1,2]. By 2050, the number of people with diabetes is projected to exceed over 131 billion, posing significant social, financial, and health system burdens globally [3].

The influence of genetic and environmental factors on T2DM is well established [4]. Many studies analyzing the microbiota in relation to T2DM focus primarily on the gut; however, research on the oral microbiota, which is the first microbial community encountered during food intake, is relatively scarce. The oral microbiota plays a critical role in the digestive tract and may influence systemic health, including metabolic diseases like T2DM. Li et al. [5] reported that T2DM patients exhibited specific changes in their oral microbiota, such as an enrichment of periodontal pathogens (e.g. *Porphyromonas gingivalis* and *Prevotella melanogenica*), even in the absence of oral diseases. Notably, caries-associated pathogens, such as *Streptococcus mutans*, did not show significant differences between T2DM patients and healthy controls. At the phylum level, T2DM patients demonstrate an increased abundance of Firmicutes, Synergistetes, Actinobacteria, and Cyanobacteria, along with a decrease in Bacteroidetes. At the genus level, there are elevated levels of Rothia, Faecalibacterium, and Selenomonas, accompanied by reduced levels of Prevotella and Porphyromona [6]. T2DM is also associated with a higher Firmicutes/Bacteroidetes ratio and an increase in genera such as Neisseria

CONTACT Tianlu Chen chentianlu@sjtu.edu.cn Center for Translational Medicine and Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Sixth People's Hospital Affiliated to Shanghai jiao Tong University School of Medicine, 600 Yishan Road, Shanghai 200233, China; Guoxiang Xie xieguoxiang@hmibiotech.com Human Metabolomics Institute, Inc., 6 Qinglong Road, Longhua District, Shenzhen 518109, China; Lei Feng fngj2004@163.com Department of Clinical Laboratory, Yan'an Hospital Affiliated to Kunming Medical University, 245 Renmin East Road, Kunming 650051, China

[*] These authors contributed equally to this work: Lingtong Tang, Keke Ding, Mengci Li

and Streptococcus [7]. Changes in microbiota in response to treatments, such as increased Prevotella and Veillonella linked to short-chain fatty acids (SCFAs), further highlight the dynamic interaction between oral microbiota and T2DM [8,9]. Alterations in inflammatory pathways, including the NLRP3 inflammasome, connect imbalances in oral microbiota to increased inflammatory mediators in T2DM [10]. Interestingly, some studies have found no significant differences in microbiota profiles between medicated T2DM patients and controls, suggesting that lifestyle or diet may influence these changes more than the disease itself [11]. The interaction between oral and intestinal microbiota also reveals shared markers, such as Actinobacteria and Blautia, and highlights pathways like glycine betaine degradation that are upregulated in T2DM [12]. These findings illustrate the intricate relationship between oral microbiota, inflammation, and metabolic dysregulation, providing new insights into the systemic impact of microbial communities in T2DM.

The interaction between microbiota and T2DM is further complicated by ethnic differences. A study on the prevalence and ethnic patterns of diabetes and prediabetes in China revealed that, compared to the Han population, the crude prevalence of total diabetes was lower in major ethnic groups (Tibetan, Zhuang, Uygur, Muslim), except for the Manchu. The crude diabetes prevalence of Han participants was 14.7%, while it was 4.3%, 12.0%, 12.2%, and 10.6% among Tibetan, Zhuang, Uygur, and Muslim participants, respectively [13]. The Dai is a distinct ethnic minority in Yunnan, China, with a unique genetic background, lifestyle, and geographic environment compared to the Han population. Significant differences exist among different ethnic groups in terms of genetic background, culture, socioeconomic level, climate and geographical characteristics of residence, lifestyle, and dietary patterns [14]. Based on these, we hypothesize that there may be some underlying variations in the microbiota of these two populations with and without T2DM.

We previously explored the differences in gut microbiota between the Dai and Han populations [15], and other studies explored the relationship between oral microbiota and T2DM, the specific microbiota associated with T2DM in the Dai and Han populations remains unclear. This study aims to compare the differences and identify the keystone species in oral microbiota between the Dai and Han populations, stratified by T2DM status. Our findings may contribute to a deeper understanding of the interplay between ethnicity, age, oral microbiota, and T2DM, offering insights that may inform personalized interventions for diabetes management.

Materials And methods

Subject recruitment

Healthy kids, healthy adults, and adult T2DM patients both in Dai and Han populations residing in the same locality were recruited from the Sixth Affiliated Hospital of Kunming Medical University (Yunnan, China) (Figure 1). With an estimated effect size of 1.3 for alterations of microbial variables, to have at least 80% power to detect the statistically significant difference of microbes between 2 groups using an independent two-sided t-test at the significant level of 0.05, each group will have 11 samples. The inclusion criteria of adult T2DM patients were [16]: (1) FBG ≥7.0 mmol/L or HbA1c ≥ 6.5%; (2) newly diagnosed patients without medication. The exclusion criteria were: (1) Subjects who had used antibiotics within the past 3 months; (2) were pregnant or breastfeeding; (3) had inflammatory bowel disease. All participants filled out a research questionnaire regarding personal information while donating blood and saliva samples. The ethics committee of the Sixth Affiliated Hospital of Kunming Medical University (2023-kmykdx6f-66) approved this study, following the principles of the Declaration of Helsinki. All participants or their caregivers gave written informed consent. All relevant ethical regulations were followed during the study.

Sample collection

All participants provided fasting venous blood and saliva samples in the morning. Serum samples were centrifugated at 4°C with 3000 rpm for 10 min and then were promptly extracted and packaged. The storage temperature was −80°C for all blood and serum samples until further biochemical analysis. Prior to saliva sampling, participants rinsed their mouths with water to minimize contamination. Subjects were refrained from eating, drinking, smoking or oral hygiene procedures for at least 1.5 hour before the collection. Non-stimulated saliva (2–3 mL) was collected in sterile EP tubes and were stored at −80°C for subsequent experiments.

Genomic DNA extraction and 16S rRNA gene sequencing

The OMEGA Soil DNA Kit (M5635–02) from Omega Bio-Tek (Norcross, GA, United States) was used to extract genomic DNA samples, following the manufacturer's instructions. Extracted DNA was quantitatively and qualitatively assessed by NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. After preparation, DNA samples were stored at −20°C.

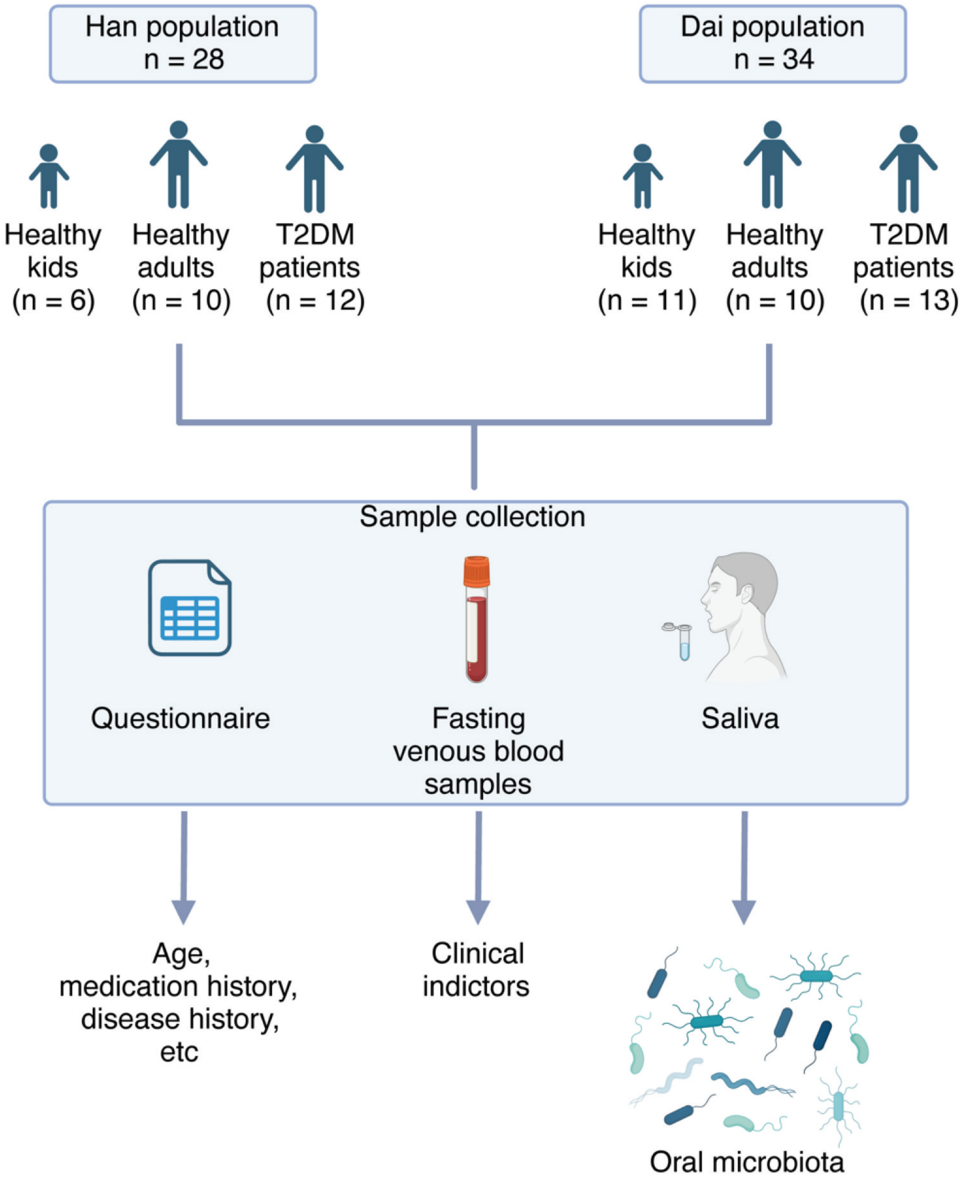

Figure 1. Study design and flow diagram.

The polymerase chain reaction (PCR) was used to amplify the V3-V4 region of the 16S rRNA gene. Primers 338F (5'ACTCCTACGGGAGGCAGCA-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') were used. Thermal cycling conditions consisted of an initial denaturation at 98°C for 5 min, followed by 25 cycles of denaturation at 98°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 5 min. PCR amplicons were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, United States). Amplicons were then pooled equally and sequenced on the Illumina NovaSeq platform using the NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Sequence analyses

Microbiome bioinformatics was performed using QIIME2 2019.4 with minor modifications. The official tutorials are available at https://docs.qiime2.org/2019.4/tutorials/. The raw sequence data was demultiplexed using the demux plugin, followed by the trimming of the primers using the cutadapt plugin. The DADA2 plugin was used for quality filtering, denoising, merging, and chimera removal. Amplicon sequence variants that were not singletons were aligned using MAFFT and a phylogenetic tree was constructed using FastTree2. The classify-sklearn naive Bayes taxonomy classifier within the feature-classifier plugin was used for taxonomic assignments.

Keystoneness calculation

In microbial communities, certain key species play critical roles in shaping the structure and function of the entire community. Understanding and studying keystone species in microbiota is crucial for comprehending the developing and maintenance of personal health and disease. We calculated the mean keystone values for oral microbiota in different populations, using a data-driven keystone species identification (DKI) based on deep learning method reported by Wang et al.[17].

Statistical and bioinformatic analysis

Continuous data were expressed as mean ± standard deviation. Statistical significance was determined using either the Student's t-test or the Mann-Whitney U test for two-group comparisons. Fisher's exact test was used to compare categorical variables.

To evaluate differences among microbial communities, we conducted α diversity analysis using metrics such as Chao1, Simpson, Shannon, Pielou_e, Observed_species, and Faith_pd, and β-diversity analyses using Principal Coordinate Analysis (PCoA) were performed to assess differences between microbial communities. Non-parametric Kruskal–Wallis tests were used to determine overall differences. Pairwise comparisons between groups were made using Dunn's multiple comparison post-hoc test to assess between-group variances.

A comparison of oral microbiota between the Dai and Han populations was performed using linear discriminant analysis effect size (LEfSe) to identify key taxa responsible for the observed differences. Spearman's rank correlation analysis, adjusted for age and gender, was then employed to assess the relationship between oral microbiota and clinical indicators, as well as the correlation between the top 20 keystone genera and clinical indices.

R 4.3.1, IBM SPSS Statistics 26.0, and Python 3.11.5 were used for statistical analyses. The R package and GraphPad Prism v9.0 were used to generate graphs. All statistical tests were two-tailed, and a significance level of $p < 0.05$ was considered statistically significant.

Results

Population characteristics

A total of 28 subjects from the Han population (healthy kids, $n = 6$; healthy adults, $n = 10$; and T2DM patients, $n = 12$) and a total of 34 subjects from the Dai population (healthy kids, $n = 11$; healthy adults, $n = 10$; and T2DM patients, $n = 13$) were enrolled in this study (Figure 1). Subject demographics and blood biochemicals are shown in Table 1. In the Han population, the levels of total bilirubin (TBiL), direct bilirubin (DBil), indirect bilirubin (IBiL), fasting blood glucose (FBG), and Hemoglobin A1c (HbA1c) were significantly higher in T2DM patients compared to healthy adults, while

Table 1. Participant demographics and blood biochemical parameters.

	Han ethnicity			Dai ethnicity		
Parameter	Healthy kids, $n = 6$	Healthy adults, $n = 10$	T2DM patients, $n = 12$	Healthy kids, $n = 11$	Healthy adults, $n = 10$	T2DM patients, $n = 13$
Sex (male/female)	2/4	3/7	8/4	4/7	5/5	10/3
Age in yr	5 ± 2.1[a]	52.3 ± 6.27	54 ± 12.05	5.55 ± 1.13[c]	45.7 ± 14.66	55.62 ± 8.75[c]
WBC as 10^9/L	6.95 ± 1.11	6.07 ± 1.27	6.42 ± 1.78	7.8 ± 1.16	6.79 ± 1.43	7.09 ± 2
RBC as 10^{12}/L	4.89 ± 0.27	5.14 ± 0.59	4.73 ± 0.62	5 ± 0.52	5.09 ± 0.65	5.1 ± 0.57
HGB in g/L	138 ± 9.78	150.3 ± 17.26	151.17 ± 18.62	127.91 ± 9.15	138.5 ± 13.83	140.38 ± 19.45
PLT as 10^9/L	362.5 ± 118.44[a]	239.6 ± 74.44	184.17 ± 42.95[a]	379.45 ± 133.84[c]	283.6 ± 71.02	255.38 ± 71.32[d]
TBil in µmol/L	6.07 ± 4.48	7.07 ± 1.76	9.33 ± 2.53[a]	5.42 ± 2.5	8.71 ± 5.08	6.19 ± 1.9[d]
DBil in µmol/L	2.92 ± 2.34[a]	3.96 ± 0.89	6.32 ± 2.78[a]	2.95 ± 1.45	3.96 ± 2.05	3.35 ± 0.98[d]
IBil in µmol/L	3.15 ± 2.28	3.11 ± 0.9	6.35 ± 4.53[a]	2.46 ± 1.42[c]	4.75 ± 3.05	2.85 ± 1.28[d]
ALT in U/L	15.83 ± 14.85	25 ± 11.54	29.5 ± 25.94	13.09 ± 14.33	25.5 ± 15.68	27.62 ± 16.9
AST in U/L	31.5 ± 7.18	24.1 ± 8.58	27.42 ± 24.41	27.36 ± 6.12	25.1 ± 10.92	23.23 ± 8.63
GGT in U/L	9.67 ± 2.94[a]	41.2 ± 28.28	24.9 ± 14.98	13 ± 7.91[c]	34.43 ± 19.43	39.92 ± 22.28
TBA in µmol/L	3.6 ± 1.8	4.15 ± 3.03	3.99 ± 1.68	3.49 ± 1.48	4.42 ± 3.58	3.82 ± 1.67
BUN in mmol/L	3.68 ± 0.82[a]	5.32 ± 1.41	4.4 ± 1.75	3.93 ± 1.04	5.12 ± 1.51	5.22 ± 2.09
Cr in µmol/L	32.17 ± 7.6[a]	70.9 ± 7.8	69.75 ± 19.96	32.36 ± 3.98[c]	68.2 ± 17.69	83.54 ± 29.78
UA in µmol/L	268 ± 32.12[a]	389.7 ± 65.17	353.58 ± 71.91	253.73 ± 61.45[c]	341.9 ± 80.54	344.54 ± 99.81
TC in mmol/L	4.01 ± 0.88[a]	5.19 ± 0.55	3.84 ± 1.42[a]	3.95 ± 0.55[c]	5.51 ± 1.04	4.83 ± 1.21[d]
TG in mmol/L	0.97 ± 0.24[a]	1.89 ± 0.56	2.3 ± 1.5	1.07 ± 0.45[c]	3.9 ± 4.1	2.43 ± 1.84
HDL-C in mmol/L	1.48 ± 0.34	1.32 ± 0.26	1.04 ± 0.31[a]	1.36 ± 0.4	1.23 ± 0.28	1.26 ± 0.38
LDL-C in mmol/L	2.16 ± 0.63[a]	3.12 ± 0.58	1.85 ± 0.69[a]	2.23 ± 0.43	2.98 ± 1.23	2.76 ± 1.01[d]
APO-A1 in g/L	1.75 ± 0.36	1.8 ± 0.27	1.37 ± 0.36[a]	1.6 ± 0.35	1.83 ± 0.23	1.76 ± 0.37[d]
APO-B in g/L	0.72 ± 0.19[a]	1.03 ± 0.14	0.69 ± 0.17[a]	0.67 ± 0.1[c]	0.98 ± 0.24	0.93 ± 0.28[d]
FBG in mmol/L	3.23 ± 0.99[a]	5.36 ± 1.24	9.61 ± 5.76[a]	4.51 ± 0.28[c,e]	5.62 ± 1.36	8.11 ± 2.65[c]
HbA1c as %	5.23 ± 0.15[a]	5.8 ± 0.57	8.41 ± 2.52[a]	5.22 ± 0.34[c]	5.58 ± 0.19	7.34 ± 0.82[c]
hs-CRP in mg/L	2.84 ± 6.66	2.02 ± 3.06	3.34 ± 5	0.44 ± 0.48[c]	1.84 ± 1.57	1.74 ± 1.45
Hcy in µmol/L	14.2 ± 3.12	15.65 ± 3.06	15.09 ± 9.6	11.47 ± 1.47	13.59 ± 3.57	16.55 ± 3.36

[a] Significantly differences from healthy adults from the Han ethnicity at $p < 0.05$.

[b] Significantly differences between healthy adults from the Han ethnicity and healthy adults from the Dai ethnicity at $p < 0.05$.

[c] Significantly differences from healthy adults from the Dai ethnicity at $p < 0.05$.

[d] Significantly differences between adult T2DM patients from the Han ethnicity and adult T2DM patients from the Dai ethnicity at $p < 0.05$.

[e] Significantly differences between healthy kids from the Han ethnicity and healthy kids from the Dai ethnicity at $p < 0.05$.

the levels of Platelet (PLT), High-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), Apolipoprotein-A1 (APO-A1) and Apolipoprotein-B (APO-B) were significantly lower. In the Dai population, while an elevated FBG and HbA1c in T2DM patients versus healthy adults was observed, while there were no significant differences in other indicators, which indicated that there was less difference in clinical indicators between Dai healthy population and T2DM patients than it was in the Han population. As for the differences between the Han and Dai individuals, no significant differences were observed between healthy adults in the two populations. We also observed increased levels of PLT, LDL-C, APO-A1, and APO-B, and decreased levels of TBiL, DBiL, and IBiL in T2DM patients from the Dai population, compared to Han T2DM patients. However, interestingly, the level of FBG was observed to be higher in Dai healthy children compared to Han healthy children, but the difference was disappeared in adults of the Dai and Han populations.

Overview of oral microbiota in Dai and Han populations

To observe the whole differences in oral microbial community composition in these two nationalities, a stacked column chart, analysis of α diversity and PCoA were performed. Figure 2(a) showed differences in the horizontal composition of different phyla with the proportion of Firmicutes in each group ranked the highest. Differences were also observed at the genus level, it was demonstrated that *Streptococcus* has the highest proportion of bacterial communities in each group (Figure 2(b)).

α diversity revealed a significantly lower diversity (the index of Chao1, Simpson, Shannon, Pielou_e, Observed_species, and Faith_pd) for the Dai children than other groups. Besides, Dai children's α Diversity was also lower than that of Han Chinese children (Figure 2(c)). PCoA plots based on Bray-Curtis dissimilarity for the oral microbiota in the Han and Dai populations indicated a clear distinction in the oral microbiota between healthy adults and healthy children, not only in the Dai nationality but also in the Han population (Figure 2(d,e)). When comparing healthy adults with T2DM patients, the observed differences were less significant than those attributed to age discrepancies, suggesting that the impact of T2DM on these differences may diminish over time. Between the Dai and Han populations, three paired groups (Dai healthy kids VS Han healthy kids; Dai healthy adults VS Han healthy adults; Dai T2DM patients VS Han T2DM patients) were compared (Figure 2(f)). The result showed the first two pairs had considerable differences, but not in the third group (Dai T2DM patients VS Han T2DM patients).

Crucial oral microbiome in the Dai and Han populations during the transition from childhood to adulthood, and in the development of T2DM

To investigate differences in the oral microbiota between the Dai and Han populations, we conducted LEfSe analysis (with an LDA score cut off > 2.0) to identify the key taxa responsible for the variations in the compositions of the oral microbiota between the two populations. Figures 3(a) and Figures 3(b) demonstrated differential bacteria in the comparison of distinct groups in the Dai and Han populations, respectively. We found that the number of differential bacteria between healthy children and healthy adults was more than that between healthy adults and those with T2DM in both the Dai and Han populations, which indicated that the difference caused by age was greater than that caused by disease. Next, we also compared three groups (Dai healthy kids VS Han healthy kids; Dai healthy adults VS Han healthy adults; Dai T2DM patients VS Han T2DM patients) in the Dai and Han populations (Figure 3(c)). The distinct bacteria between Han and Dai healthy adults were greater than that between Han and Dai healthy children. It could be observed that the number of differential bacteria decreased after getting T2DM in the case of comparing two populations. This is consistent with the trends observed in the PCoA plots. We also found that the differential bacteria in individuals with T2DM differed completely between the Han and Dai populations, compared to healthy adults (Figures 3(a,b)). Specifically, in the Dai population with T2DM, increased levels of *Alistipes putredinis* (*A. putredinis*), *Lactobacillus spp.*, *Faecalibacterium prausnitzii* (*F. prausnitzii*), *Faecalibacterium spp.*, and *Akkermansia muciniphila* (*A. muciniphila*) were observed, in contrast to Han population with T2DM (Figure 3(c)).

Associations between oral microbiota and clinical data in the Han and Dai populations

Correlation analysis using Spearman's rank correlation coefficient was performed to examine the associations between clinical indicators and the genera of the Dai and Han populations (Figure 4). Blood glucose-related indicators, such as FBG and HbA1c, were more positively correlated with genera, this phenomenon could be seen in both groups (Figures 4(a,b)). In the Han population, blood lipid and renal function indicators could be seen to be negatively correlated with genera (Figure 4(b)). We conducted the proportion of significant P-values for further comparison. In the

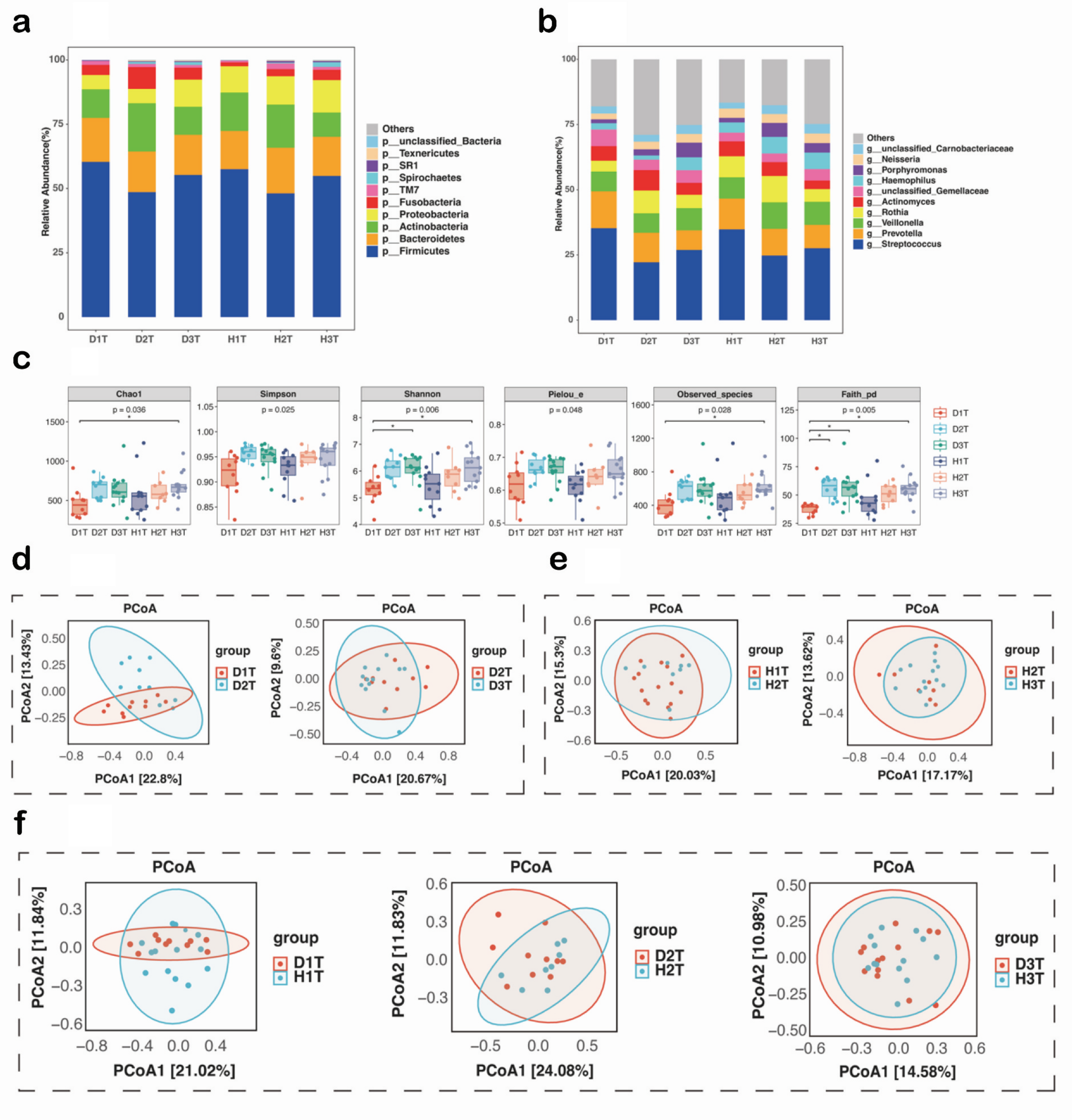

Figure 2.Overview of oral microbiota in the Dai and Han populations. a-b: Stacked column chart of six groups. a, at the phylum level. b, at the genus level. c: Analysis of alpha diversity in the oral microbiota. *$p < 0.05$. d-f: Principal coordinate analysis (PCoA) of oral microbiota composition. The ellipse represents the 95% confidence interval. d: in dai population. e: in Han population. f: between the Dai and Han populations.

D1T: Dai healthy kids; D2T: Dai healthy adults; D3T: Adult T2DM patients in the Dai population

H1T: Han healthy kids; H2T: Han healthy adults; H3T: Adult T2DM patients in the Han population

Dai population, compared to the Han population, it could be seen that a higher proportion of PLT is negatively correlated with genera, while a higher proportion of hemoglobin and Alanine transaminase (ALT) are positively correlated with genera (Figure 4(c)). In the Han population, we could observe a higher proportion of hemoglobin, urea, HDL-C, and homocysteine are negatively correlated with genera, while a higher proportion of TBiL, DBiL, Triglyceride (TG), FBG, and HbA1c are positively correlated with genera (Figure 4(d)). The proportion of positive correlation between blood glucose-related indicators and microbiota was higher in the Han population.

Keystone genera in the Han and Dai populations

Table 2 lists the top 20 keystone genera except for undefined bacteria. *Dehalobacterium* ranked first in the Dai population, while in the Han population, *Streptococcus* ranked first. The intersection number of the top 20 keystone genera between the two populations is 2, which was *Streptococcus* and *Thermus* (Table 2, Figure 5(a)).

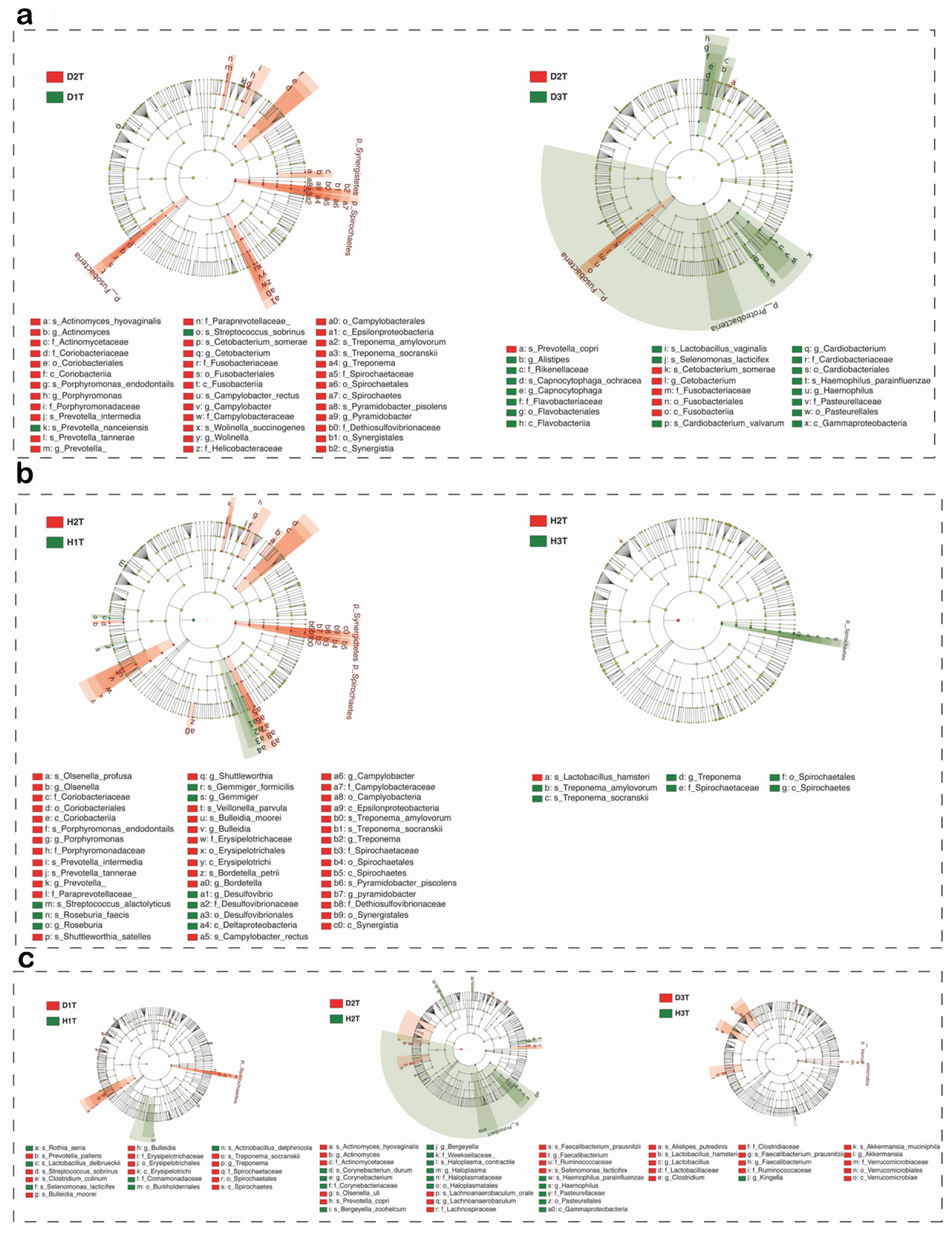

Figure 3. Comparison of differences in oral microbiota between the Dai and Han populations. a-c: Cladogram generated from the Linear discriminant analysis Effect Size analysis (LEfSe). a: in the Dai population. b: in the Han population. c: between the Dai and Han populations.

D1T: Dai healthy kids; D2T: Dai healthy adults; D3T: Adult T2DM patients in the Dai population

H1T: Han healthy kids; H2T: Han healthy adults; H3T: Adult T2DM patients in the Han population

Then, we used these top 20 keystone genera to calculate the relationship between them and clinical indicators in the Dai and Han populations, separately. In the Dai population, *Fusibacter* displayed a relatively central position as it had the most surrounding connections (Figure 5(b)). *Fusibacter* could be observed to have a negative relationship with Total cholesterol (TC), APO-B,

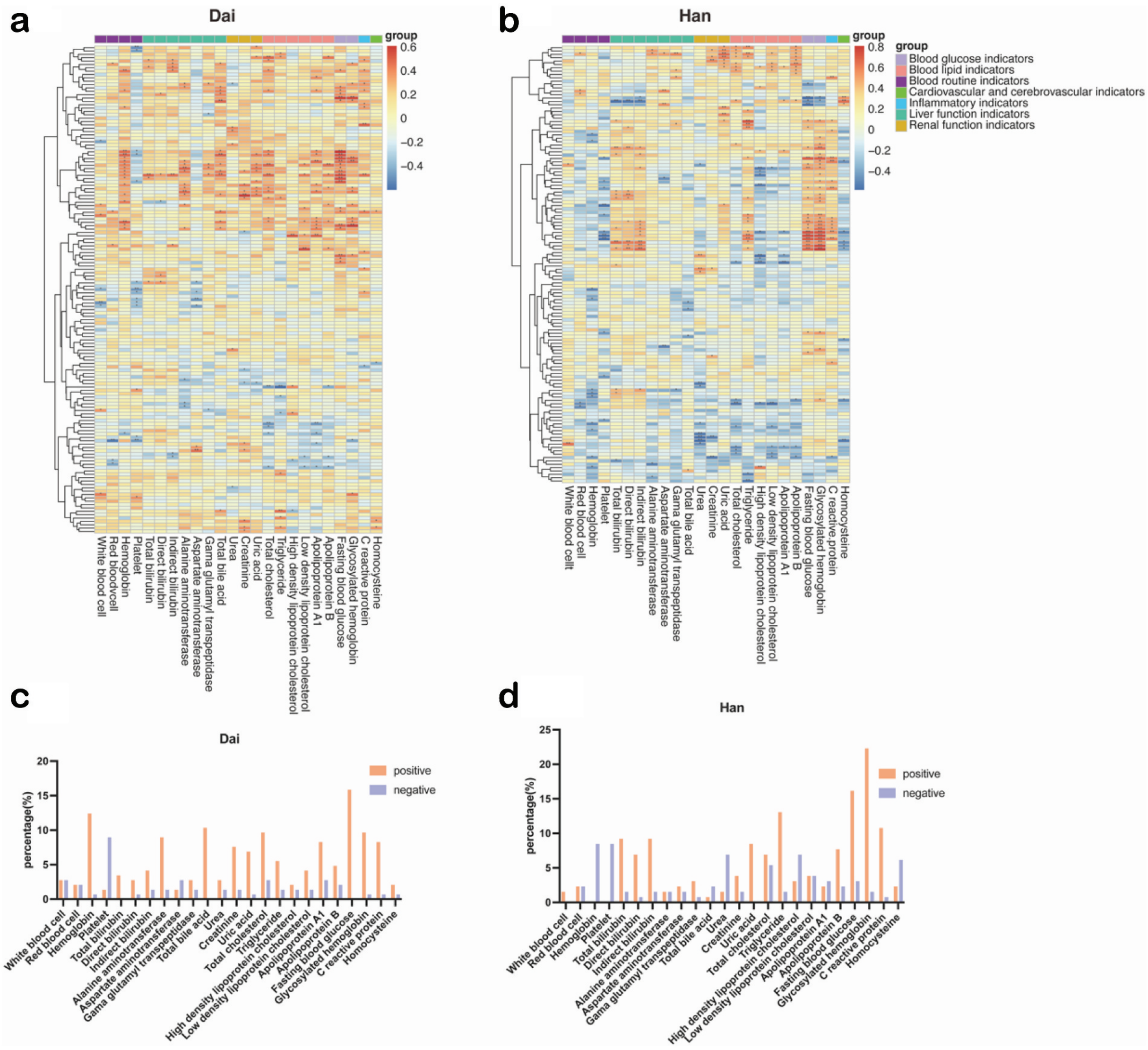

Figure 4.The correlation between genus and clinical indicators. a: Heat map illustrating the correlation between genus and clinical indicators in the Dai population. b: Heat map depicting the correlation between genus and clinical indicators in the Han population. c: The proportion of significant ($p < 0.05$) positive and negative correlations in the Dai population, which is based on a total sample size of 145. d: The proportion of significant ($p < 0.05$) positive and negative correlations in the Han population, which is based on a total sample size of 130. Before calculating the correlation, bacteria with more than 80% zero abundance were excluded. Spearman's rank correlation coefficient (R) and associated probability (p) were employed to evaluate statistical significance. Negative correlations are depicted by blue grids, while positive correlations are depicted by red grids. The intensity of color reflects the magnitude of the R-value, indicating the strength of association between microbiota and clinical indicators, with significant p-values emphasized. $^{*}p < 0.05$, $^{**}p < 0.01$; $^{***}p < 0.001$.

IBiL, and LDL-C; and *Fusibacter* was positively correlated with White blood cell (WBC), Creatinine (Cr) and Aspartate aminotransferase (AST). The network diagram of the Han group was more scattered than that of the Dai population (Figure 5(c)). In the Han population, *Symbiobacterium* and *Desulfomicrobium* showed relatively great importance. As for the blood glucose-related indicators like FBG, it was positively correlated with *Exiguobacterium*, *Thermus*, and *Brevibacterium* in the Dai population. However, FBG positively related to *Desulfomicrobium* in the Han population.

Discussion

In this study, we compared the oral microbiota between the Dai and Han populations as healthy children transitioned to healthy adults and developed T2DM. Our findings revealed notable differences in the oral microbiota between these two populations, particularly among individuals with T2DM and the impact of age on oral microbiota differences outweighed that of diabetes.

In line with previous studies, we observed significant changes in lipid profiles in T2DM patients [18,19]. Specifically, the Han T2DM patients showed decreased HDL-C and increased LDL-C compared to

Table 2. Top 20 keystone genera in the Dai and Han populations.

Dai		Han	
Taxa	str_pred_mean	Taxa	str_pred_mean
Dehalobacterium	0.0604	*Streptococcus*	0.0737
Clostridium	0.0514	*Symbiobacterium*	0.0495
Flavisolibacter	0.0506	*Pseudanabaena*	0.0455
Pelagicoccus	0.0497	*Glycomyces*	0.0454
p-75-a5	0.0436	*Acidovorax*	0.0434
Pseudoalteromonas	0.0434	*Thermus*	0.0408
Streptococcus	0.0424	*Clostridium*	0.0401
Rubrobacter	0.0395	*Sphingobacterium*	0.0400
Salinimicrobium	0.0385	*Brachymonas*	0.0399
Nitrospira	0.0277	*Lactococcus*	0.0387
Thermus	0.0272	*Actinomadura*	0.0383
Exiguobacterium	0.0266	*Salegentibacter*	0.0367
Brevibacterium	0.0265	*Thiobacillus*	0.0366
Eggerthella	0.0260	*Peptoniphilus*	0.0350
Alloiococcus	0.0229	*Enterococcus*	0.0350
Craurococcus	0.0220	*Desulfomicrobium*	0.0337
Pseudochrobactrum	0.0217	*Mycobacterium*	0.0327
Propionivibrio	0.0207	*Actinokineospora*	0.0314
Fusibacter	0.0205	*Sediminibacterium*	0.0312
Williamsia	0.0192	*Massilia*	0.0310

The Str_pred_mean was computed using the data-driven Keystone Identification (DKI) method, representing the average value for each bacterial genus. After excluding undefined bacterial genera, the top 20 genera, sorted by Str_pred_mean, are listed in the table.

healthy controls, consistent with the findings of previous study [20]. However, in contrast, Dai T2DM patients exhibited increased LDL-C and APO-A1, suggesting that population-specific genetic factors, such as the APOE gene polymorphisms, may contribute to these differences [21]. Notably, the clinical divergence between Dai healthy adults and T2DM patients was less pronounced than in the Han population, suggesting a unique pattern of diabetes progression in the Dai group.

Previous studies have shown that T2DM is significantly associated with intestinal microbiota composition, but fewer studies have explored the impact of diabetes on oral microbiota. Most research on oral microbiota in diabetes focuses on periodontal disease, with some suggesting no significant effect of diabetes on oral microbiota [22]. Our study addresses this gap, finding increased levels of *Capnocytophaga ochracea* (*C. ochracea*), *Capnocytophaga spp.*, *Haemophilus parainfluenzae* (*H. parainfluenzae*), and *Haemophilus spp.* in the oral microbiota of Dai T2DM patients compared to healthy adults. Previous studies also report higher prevalence of *C. ochracea* in diabetic patients [23], and its association with periodontal lesions in adolescents and insulin-dependent diabetes mellitus patients [24]. Interestingly, *H. parainfluenzae* and *Haemophilus spp.* are more commonly found in individuals with normal blood glucose [25,26], differing from our findings. In the Han population, we observed increased *Treponema socranskii* (*T. socranskii*), *Treponema amylovorum* (*T. amylovorum*), and *Treponema spp.* in T2DM patients, which are linked to periodontal disease [27].

The Dai population is one of the major minority ethnic groups in China, with a population of approximately 1.2 million. They mainly reside in Xishuangbanna and Dehong Autonomous Prefectures in Yunnan Province, Southwest China [28]. Previous studies have shown that there are differences in the composition of gut microbiota among different ethnic groups [29,30]. Deschasaux et al. [29] conducted a fecal 16S ribosomal RNA gene sequencing study of 2,084 healthy individuals in an Urban Setting (HELIUS) research and found that individuals living in the same city tended to share similar gut microbiota characteristics with others of the same ethnic background. In China, previous research on the differences in gut microbiota composition among different ethnic groups has mainly focused on the Han and Tibetan populations [31], with less research on the differences between the Han and Dai populations. Sun et al. [32] analyzed and compared the fecal mycobiome of 942 healthy individuals from different geographical regions in China (Hong Kong and Yunnan), covering six populations: Han, Tibetan, Bai, Hani, Dai, and Miao (including urban and rural residents of each population).

Our previous study added to the evidence of differences in the gut microbiota between the Han and Dai populations [15]. However, previous studies only investigated fecal samples. To further elucidate the differences in oral microbiota between these two populations, we analyzed their saliva samples and obtained the differential oral microbiota between the Han and Dai populations.

We found that compared to the Han population with T2DM, the Dai population with T2DM showed increased levels of *A. putredinis*, *Lactobacillus spp.*, *F. prausnitzii*, *Faecalibacterium spp.*, and *A. muciniphila*. *Lactobacillus* was found to be positively associated with FBG, HbA1c, and homeostatic assessment of insulin resistance (HOMA-IR) [33]. However, other microbiota, including *A. putredinis*,

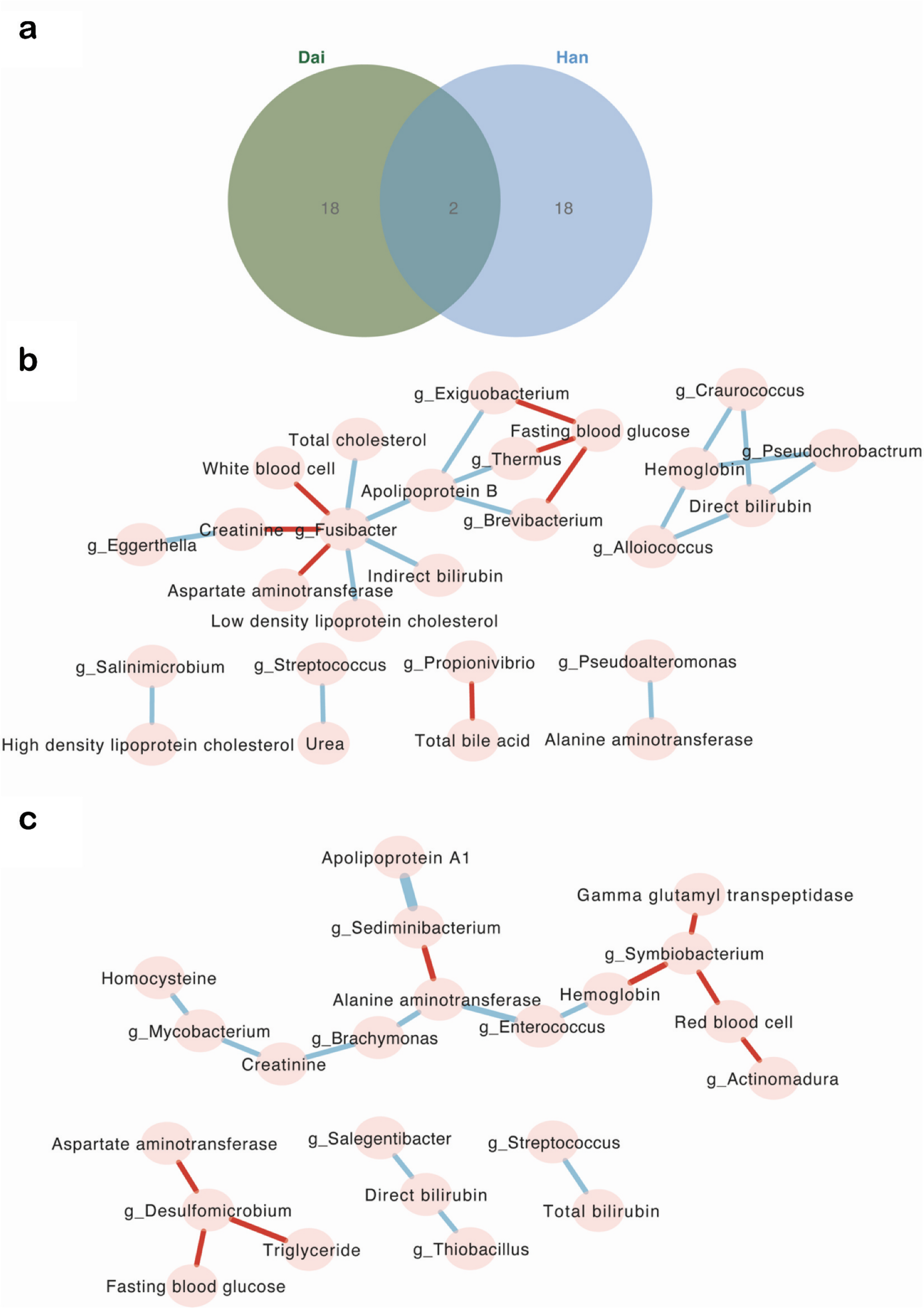

Figure 5. The relationship between the top 20 keystone genus and clinical indicators in the Dai and Han populations. a: Venn diagram illustrating the unique and shared top 20 keystone genera in the Dai and Han populations. b-c: Network diagrams depicting the top 20 keystone genera and their associations with clinical indicators ($p < 0.05$). b: in the Dai population. c: in the Han population. Nodes represent keystone genera or clinical indicators, and the connections between nodes indicate a correlation between two nodes. Red lines denote positive correlations, while blue lines indicate negative correlations. The thickness of connecting lines reflects the strength of the correlation coefficient. Nodes with more connections are closely associated with keystone bacteria or clinical indicators.

Faecalibacterium, and *Akkermansia*, were negatively correlated with the risk of developing T2DM [34–36]. Additionally, a decrease in the abundance of *A. muciniphila* was found to impair insulin secretion and disrupt glucose homeostasis in lean individuals with T2DM [37]. In a randomized, double-blind, placebo-controlled clinical trial, pasteurized *A. muciniphila* was found to improve insulin sensitivity, reduce insulin levels, and lower plasma total cholesterol compared to placebo [38]. *F. prausnitzii*, a bacterium that produces butyrate, plays a crucial role in diabetes. Transplantation of *F. prausnitzii* has been used as an intervention strategy to address dysbiosis in the gut microbiota, which is associated with inflammation preceding autoimmune diseases and diabetes [39]. These bacteria, which may have a protective effect against diabetes, could potentially explain the lower levels of FBG and HbA1c observed in individuals with T2DM from the Dai population compared to Han population, although these differences were not statistically significant.

For a more comprehensive understanding of the oral microbiota of these two populations, in addition to comparing the T2DM groups, we also compared the children's groups and healthy adult groups of Dai and Han populations. Interestingly, we found that the differential taxa were fewer in the children's groups of both populations compared to the adult groups. Furthermore, when T2DM was present, the differential taxa were even fewer, with PCoA curves approaching overlap. Compared to Han children, Dai children exhibited higher levels of *Prevotella pallens* (*P. pallens*) and *Streptococcus sobrinus* (*S. sobrinus*) in their oral microbiota, which have been associated with toddler halitosis and dental caries respectively [40–42]. Additionally, *Clostridium colinum* (*C. colinum*) was found to be elevated in Dai children, with this bacterium being higher in the GDM group ($p < 0.05$) and potentially serving as a marker of obesity [43]. Conversely, among Han kids, we observed an increase in *Rothia aeria* (*R. aeria*) and *Lactobacillus delbrueckii* (*L. delbrueckii*). A previous study found that feeding STZ-induced diabetic mice with *L. delbrueckii* subsp. lactis PTCC1057 resulted in decreased FBG and fetuin-A levels and an increase in serum sestrin 3 levels [44]. This may explain why the FBG levels of Han kids in our study were lower than those of Dai kids, despite the Dai population exhibiting lower levels during the diabetic stage. The diversity of differential taxa between adult individuals of the two populations is likely greater compared to children, possibly due to increased divergence in dietary habits and other factors after reaching adulthood. In our results, we found that both in the Dai or Han populations, the diversity of children's oral microbiota was lower compared to that of adults. Previous research has indicated that as infants grow, oral bacterial diversity and richness continue to increase over time [45]. Dzidic et al. [46] examined the temporal evolution and maturation of the oral microbial ecosystem in early infancy and childhood by sequencing the V3–V4 hypervariable regions of the 16S rRNA gene from a longitudinally collected oral sample set involving 90 children. The oral microbial community continues to develop with the eruption of primary teeth in early infancy and the establishment of permanent dentition in children, evolving into a complex and diverse microbiome. A complex interplay occurs between the establishment and development of the neonate's immunity and early microbial acquisition [47,48]. The oral taxonomic characteristics are associated with various disease statuses, including early childhood caries, celiac disease, autism, and pediatric appendicitis. Docktor et al. [49] examined the oral microbiome (swab samples taken from the tongue and buccal mucosa) of a total of 114 children with celiac disease (CD), ulcerative colitis (UC), and healthy controls, and found an overall decrease in alpha diversity of the oral bacterial community in children with CD compared to healthy controls.

Compared to healthy adults of Han individuals, healthy adults of Dai population exhibited increased levels of *Actinomyces*, *P. copri*, *F. prausnitzii*, and *Faecalibacterium*. A Mendelian randomization study identified the genus *Actinomyces* as a risk factor for T2DM [50]. However, another study reported a 66% decreased risk of diabetes associated with the genus *Actinomyces*, with a p-value of 8.9×10^{-3} [51]. The remaining three differential bacteria were considered beneficial for diabetes in previous studies [52,53]. Short-chain fatty acid-producing genera, including *Faecalibacterium* and *Prevotella*, as well as the species *F. prausnitzii* and *P. copri* [54], were significantly reduced in the GDM group. Some studies have highlighted the special role of bacteria from the genus *Faecalibacterium* (especially *F. prausnitzii*) in maintaining intestinal wall integrity.

To further understand the composition of oral microbiota in the Han and Dai populations and identify key genera, we employed deep learning to search for keystone genera and constructed correlation networks between them and clinical indicators. After excluding undefined bacterial genera, the top 20 genera both in the Dai and Han populations, were listed in Table 2. We discovered that among the keystone genera in both populations, two overlapping genera were identified: *Clostridium* and *Streptococcus*. In the top 20 keystone genera among the Dai population, *Streptococcus*, *Rubeobacter* showed negative correlations with diabetes [55], *Brevibacterium* showed a positive correlation with diabete [56]. Several keystone genera were associated with inflammatory responses, such as *Dehalobacterium*, *Desulfovibrio*, *Streptococcus*, *p-75-a5*, and *Eggerthella* [57,58]. In the top 20 keystone genera among the Han population, notable differences from the Dai population were observed. *Lactococcus* showed a negative correlation with diabetes [59], while *Enterococcus* and *Sediminibacterium* showed positive correlations with diabetes [60,61].

Our study is the first to compare the differences between the Dai and Han oral microbiota, and for the first time to find the keystone genera of the Dai and

Han populations by the method of deep learning. The composition of the microbiota is influenced by various factors, including diet, environment, and genetic background [62]. Our study involves two populations (Han and Dai) with different genetic backgrounds, lifestyles, and environmental factors, indicating that the results may have high generalizability within specific populations. Although the results of our study may not be fully applicable to other ethnic groups or populations from different regions, our findings contribute to a better understanding of the relationship between microbial communities and the incidence of T2DM in certain ethnic populations. These microbial differences indicate that the microbiota may play an important role in the onset and progression of diabetes. The identification of specific microorganisms (such as *A. muciniphila* and *F. prausnitzii*) associated with T2DM indicates that these microorganisms could serve as potential targets for diagnosis [63], prevention and treatment. In addition, based on the microbiota characteristics of different ethnic groups, future diabetes management may need to be more personalized. For example, interventions targeting specific microbiota, such as probiotics or oral hygiene interventions, may have varying effects among diabetes patients from different ethnic backgrounds and with specific lifestyles and dietary habits. Therefore, combining the results of this study, it may be worth considering the development of personalized treatment strategies suitable for different ethnic groups to improve treatment outcomes and patients' quality of life. Some limitations of this study should be considered: Firstly, the sample size of this study is relatively small, the study provides preliminary evidence of differences in oral microbiota between different ethnic groups, suggesting that future research should validate these findings in larger samples. Secondly, the influence of confounding variables such as smoking, diet, and gender was not taken into account. Thirdly, this study is a cross-sectional study, and it would be better to have longitudinal data of both Dai and Han populations from childhood to adulthood to demonstrate the changes in gut microbiota at different time points and after disease onset.

Conclusion

These findings indicate significant differences in oral microbiota among different populations, highlighting the complexity of the microbiota and providing clues for our future research on specific microbial targets. Additionally, the distinct differences in the top 20 genera also demonstrate varied disease associations, partially explaining the differences in the prevalence of diabetes between the Han and Dai populations, and opening up new avenues for exploring ethnic disparities in diabetes.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Author contributions

Lei Feng, Guoxiang Xie, and Tianlu Chen conceived the design and led the team; Lintong tang and Lei Feng contributed to recruiting patients and collected the data; Tao Sun, Yuhuai Guo, and Xufei Peng preprocessed the data; Keke Ding, Mengci Li, and Xiaowen Chao analyzed the data; Keke Ding and Mengci Li drafted the manuscript; Wei Jia, Tianlu Chen, Guoxiang Xie and Lei Feng critically reviewed and revised the manuscript.

Ethical approval

This study was reviewed and approved by the Ethics Committee of the Sixth Affiliated Hospital of Kunming Medical University (2023-kmykdx6f-66). Before study enrollment, informed written consent was obtained from all participants or their legal guardians.

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