Heliyon 10 (2024) e22615

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Distinct microbiome of tongue coating and gut in type 2 diabetes with yellow tongue coating

Yao Wang [a,1], Jiqing Li [b,1], Haiying Hu [c,1], Yalan Wu [a], Song Chen [d], Xiangrong Feng [a], Ting Wang [e], Yinrong Wang [b], Su Wu [b,**], Huanhuan Luo [a,f,*]

[a] School of Basic Medical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong Province, China
[b] Department of Endocrinology, Hainan Provincial Hospital of Traditional Chinese Medicine , Haikou, Hainan Province, China
[c] West China Hospital Sichuan University, Chengdu, Sichuan Province, China
[d] Science and Technology Innovation Center, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong Province, China
[e] Department of Emergency and Critical Care, Hainan Provincial Hospital of Traditional Chinese Medicine, Haikou, Hainan Province, China
[f] State Key Laboratory of Traditional Chinese Medicine Syndrome, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong Province, China

ARTICLE INFO

Keywords:
Tongue coating microbiome
Gut microbiome
Type 2 diabetes mellitus
Yellow tongue coating

ABSTRACT

The gut microbiome plays a critical role in the pathogenesis of type 2 diabetes mellitus (T2DM). However, the inconvenience of obtaining fecal samples hinders the clinical application of gut microbiome analysis. In this study, we hypothesized that tongue coating color is associated with the severity of T2DM. Therefore, we aimed to compare tongue coating, gut microbiomes, and various clinical parameters between patients with T2DM with yellow (YC) and non-yellow tongue coatings (NYC). Tongue coating and gut microbiomes of 27 patients with T2DM (13 with YC and 14 with NYC) were analyzed using 16S rDNA gene sequencing technology. Additionally, we measured glycated hemoglobin (HbA1c), random blood glucose (RBG), fasting blood glucose (FBG), postprandial blood glucose (PBG), insulin (INS), glucagon (GC), body mass index (BMI), and homeostasis model assessment of β-cell function (HOMA-β) levels for each patient. The correlation between tongue coating and the gut microbiomes was also analyzed. Our findings provide evidence that the levels of *Lactobacillus* spp. are significantly higher in both the tongue coating and the gut microbiomes of patients with YC. Additionally, we observed that elevated INS and GC levels, along with decreased BMI and HOMA-β levels, were indicative of a more severe condition in patients with T2DM with YC. Moreover, our results suggest that the composition of the tongue coating may reflect the presence of *Lactobacillus* spp. in the gut. These results provide insights regarding the potential relationship between tongue coating color, the gut microbiome, and T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by hyperglycemia [1]. It is caused by the dysfunction

* Corresponding author. School of Basic Medical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong Province, China.
** Corresponding author.
E-mail addresses: suwen67@163.com (S. Wu), avenluo@gzucm.edu.cn (H. Luo).
[1] These authors contributed equally: Yao Wang, Jiqing Li and Haiying Hu.

https://doi.org/10.1016/j.heliyon.2023.e22615
Received 4 November 2022; Received in revised form 8 November 2023; Accepted 15 November 2023
Available online 25 November 2023

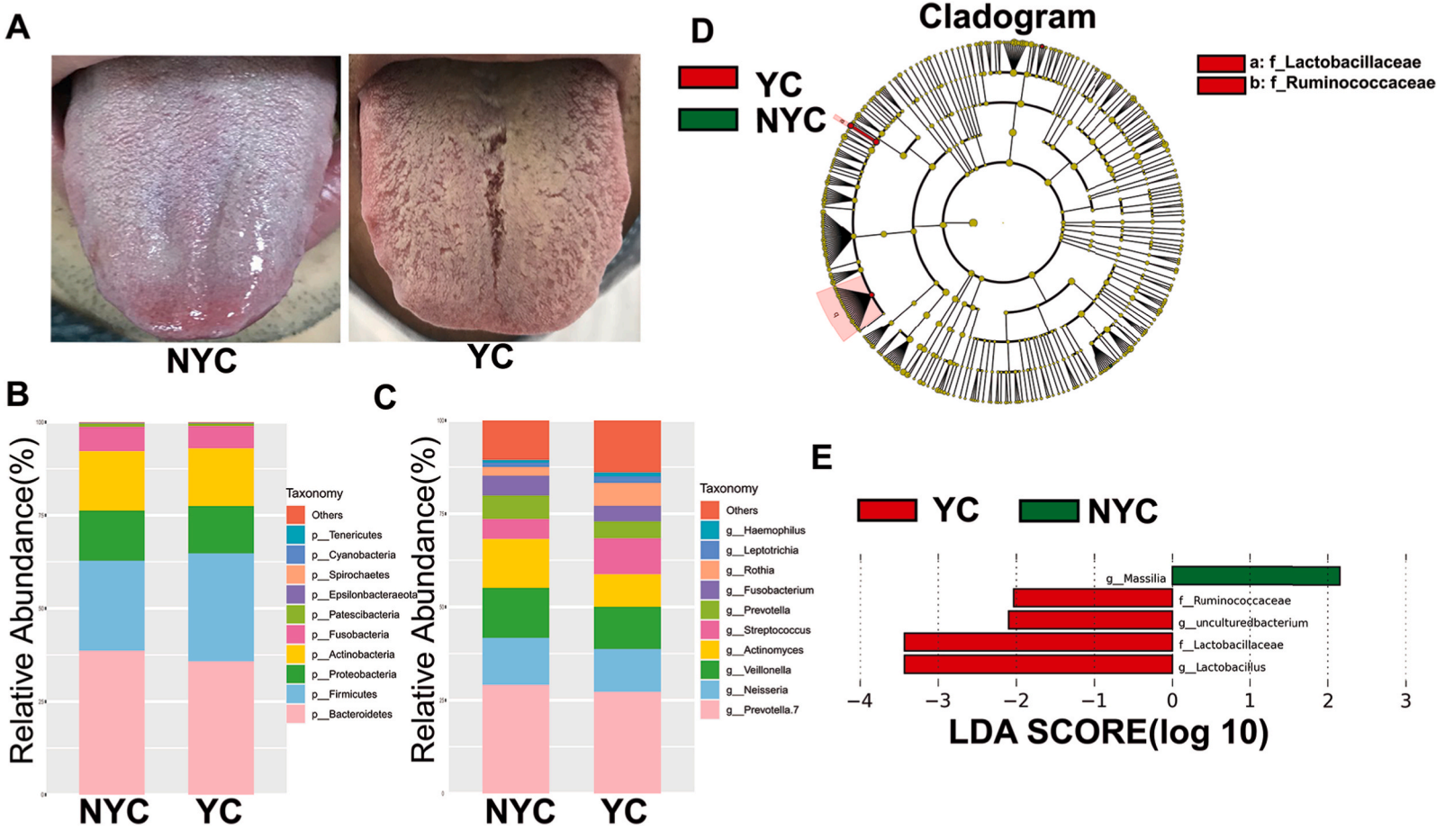

Fig. 1. Differences in tongue coating microbiome between patients with YC and NYC. A: Representative photographs showing YC and NYC in patients. B: Bar plots illustrating the taxonomic profiles of patients with NYC and YC at the phylum level. C: Bar plots displaying the taxonomic profiles of patients with NYC and YC at the genus level. D: Cladogram representing the enriched taxa in the tongue coating microbiome communities of the two groups. E: Histogram showing the LDA scores computed for different abundance levels between patients with NYC and YC.

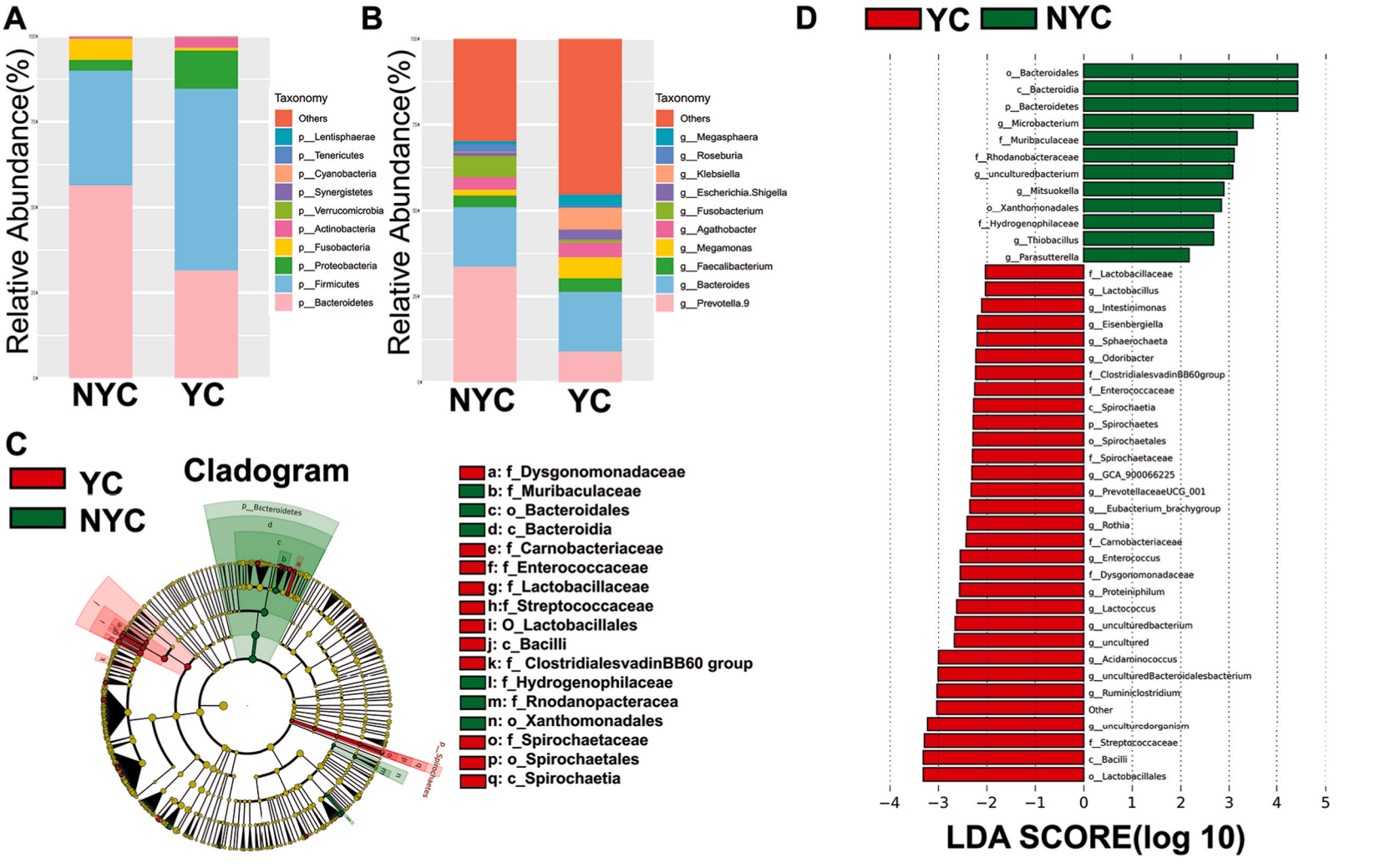

Fig. 2. Differences in gut microbiomes between patients with YC and NYC. Bacteria composition of the different communities at the (A) phylum and (B) genus levels in patients with NYC and YC. C: Cladogram generated using LEfSe analysis showing discriminative taxa in the feces of patients with NYC and YC. D: LDA scores of discriminative taxa shown in (C).

of islet β cells and insulin resistance (IR) [2]. Globally, an estimated 451 million individuals were affected by diabetes in 2017, and this number is projected to increase to 693 million by 2045 [3]. T2DM can lead to various complications including nephropathy [4], angiopathy [5], retinopathy [6], and peripheral neuropathy [7]. Early detection, diagnosis, and treatment are crucial for preventing these complications.

Tongue color, considered a sensitive noninvasive external sign, is important in Traditional Chinese Medicine (TCM), as it reflects the occurrence, development, and prognosis of diseases [8]. Tongue is a widely used diagnostic tool for TCM [9]. For instance, yellow tongue coating (YC) is traditionally associated with "damp heat" in the body. Tongue diagnosis is based on the TCM theory of the visceral picture, which suggests that all internal organs are directly or indirectly connected to the tongue through meridians. Therefore, abnormalities in tongue coating reflect imbalances within the entire human body, including the tongue coating microbiome [10]. Physiological functions and pathological changes in the internal organs are reflected in the tongue. Notably, the tongue condition of patients with acute stroke has been determined to be related to the clinical features [11]. The tongue dorsum, which consists of filiform, fungiform, annular, and foliate papillae, harbors a diverse bacterial community. In addition, the non-keratinized epithelium at the base of the tongue rapidly absorbs small molecules and interacts with the host [12]. The close proximity of the tongue to the tonsils allows compounds to be shed from the epithelial cells and the tongue-coating microbiome to be transported into the respiratory and digestive tracts. These unique characteristics increase the likelihood of oral-gut microbiome translocation and broaden the metabolic effects of the tongue-coating microbiome. Previous studies have identified correlations between tongue color and parameters such as sublingual vein width [13], female menstrual cycle [14], and various physiological indicators such as GLU, TCH, and HDL-C [15]. In our study, we observed a potential association between tongue color and tongue coating, as well as the gut microbiome.

The tongue-coating microbiome has been associated with chronic systemic diseases characterized by nutrient and metabolic disorders, such as gastritis and diabetes [16,17], as well as various types of cancer [18–20]. Given its association with chronic nonoral diseases, the tongue coating microbiome has the potential to serve as a marker of metabolic homeostasis and may be utilized as a diagnostic tool in the future. Metabolic disorders, which involve imbalances in the digestion and absorption of substances, play crucial roles in the pathogenesis of various diseases. Therefore, further research on the tongue coating microbiome, its relationship with metabolic diseases, and its role in metabolism is warranted. Overall, exploring the research directions of the tongue coating microbiome and its association with metabolic diseases will contribute to our understanding of this field and pave the way for potential diagnostic and therapeutic applications.

In the present study, we investigated the differences and relationships between the tongue coating microbiome, gut microbiome, and clinical indicators in patients with T2DM with YC and non-yellow tongue coatings (NYC). Our findings provide new insights and potential avenues for the future diagnosis of T2DM.

2. Results

2.1. Structural differences in tongue coating microbiome between patients with T2DM with YC and NYC

Tongue diagnosis is a unique method used in TCM. In this study, we aimed to investigate biodiversity differences in the tongue microbiome by grouping patients based on their tongue coating color. YC is a classic clinical sign used in TCM to assessing diabetes mellitus [21]. Several clinical reports have suggested that YC is a symptom of diabetes [22,23]. We enrolled 27 patients with T2DM, who were divided into two groups based on their tongue coating color: a NYC group with 14 patients and a YC group with 13 patients (Fig. 1A). The composition of the tongue microbiome at the phylum and genus levels is shown in Fig. 1B and C. At the family and genus levels, *Lactobacillus* spp. were more abundant in YC than in NYC (Fig. 1D and E).

2.2. Differences in gut microbiome structure between patients with YC and NYC

Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were the predominant phyla in both the gut- and tongue-coated microbiomes (Fig. 2A). At the genus level, there were differences between the predominant microbiomes of the tongue coating and the gut. Specifically, Prevotella.7, Neisseria, Veillonella, and Streptococcus were the predominant genera in the tongue-coating microbiome, whereas Bacteroides, Prevotella.9, Klebsiella, and Megamonas dominated the gut microbiome (Figs. 1C and 2B). Using LDA effect size (LEFSe) analysis, we determined that patients with YC had a higher abundance of *Lactobacillus* spp., *Intestinimonas* spp., *Eisenbergiella* spp., *Sphaerochaera* spp., *Odoribacter* spp., GCA_900066225, Prevotellaceae UCG _001, and Eubacterium-brachygroup at the genus level compared to patients with NYC (Fig. 2C and D). Moreover, we observed an increased abundance of Clostridiales vad in the BB60 group, Enterococcaceae, and Spirochaetales at the family level in patients with YC (Fig. 2C and D). These findings suggest that the tongue coating and gut microbiomes exhibit distinct microbial compositions in patients with different tongue coating colors.

2.3. Correlations between tongue coating and gut biomarkers and bio-clinical parameters

To assess the accuracy of differentiating the two groups of samples based on the relative abundance of tongue coating and gut microbial taxa, we used a receiver operating characteristic (ROC) curve, which is a common methodology for evaluating the classification performance of potential biomarkers (Fig. 3A and E). The area under the curve (AUC), which represents the true and false positive rates, was calculated to measure the performance of the potential classifier. The results demonstrated that *Lactobacillus* spp. from both the tongue coating and the gut could accurately differentiate between patients with NYC and those with YC (Fig. 3A and E).

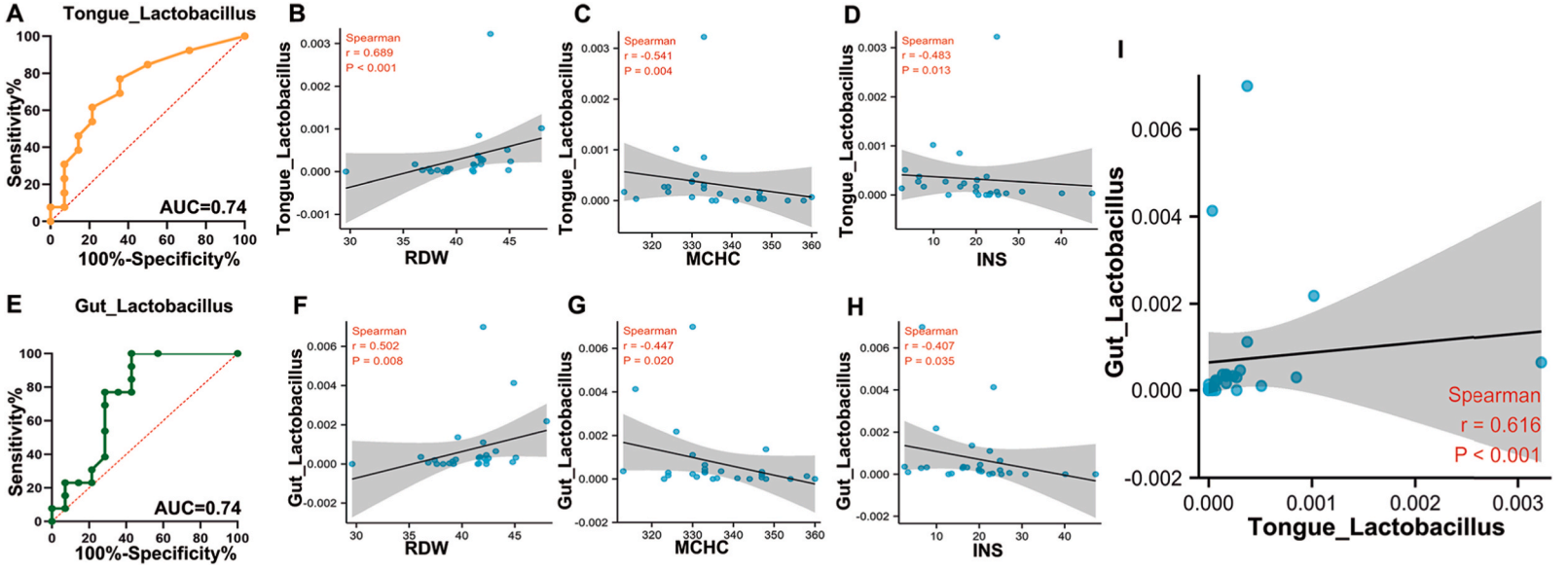

Fig. 3. A and E: ROC curve of the abundant tongue coating marker with the highest AUC. B and F: Positive correlation between tongue coating and gut *Lactobacillus* spp. abundance and RDW. C and G: Negative correlation between tongue coating and gut *Lactobacillus* spp. abundance and MCHC. D and H: Negative correlation between tongue coating and gut *Lactobacillus* spp. abundance and INS. I: Positive correlation between tongue coating and gut *Lactobacillus* spp. abundance.

Furthermore, we observed a positive correlation between the relative abundance of *Lactobacillus* spp. in the tongue coating and gut and the red cell volume distribution width (RDW) (Fig. 3B and F) and negative correlations with the mean erythrocyte hemoglobin concentration (MCHC) and insulin (INS) levels (Fig. 3C, D, G, and H). Additionally, a positive correlation existed between *Lactobacillus* spp. in the tongue coating and gut (Fig. 3I).

T2DM indicators were different between patients with YC and those with NYC We observed differences in T2DM indicators between the two groups. Specifically, we determined that although not statistically significant, glycated hemoglobin (HbA1c) and postprandial blood glucose (PBG) levels were higher in patients with YC than in those with NYC (Fig. 4A and D), whereas random blood glucose (RBG) and fasting blood glucose (FBG) levels were lower in patients with YC than in those with NYC (Fig. 4B and C). Notably, body mass index (BMI) was significantly lower in patients with YC than in those with NYC (Fig. 4E) ($P < 0.05$), which is associated with a later T2DM onset [24,25]. Moreover, INS and homeostasis model assessment of β-cell function (HOMA-β) levels were significantly lower in patients with YC than in those with NYC (Fig. 4F and G) ($P < 0.05$). The HOMA-β index is considered a good indicator for evaluating β-cell functionality, and a lower HOMA-β index is associated with poor prognosis [26,27]. Finally, we showed that glucagon (GC) levels were higher in patients with YC than in those with NYC (Fig. 4H, $P < 0.05$).

2.4. Changes in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and inflammation in patients with different tongue coating colors

We aimed to predict the functional capacity of the gut microbiome using KEGG pathways. Our analysis revealed that patients with YC exhibited lower expression of several KEGG pathways, including "Immune System," "Immune System Disease," "Circulatory System," "Energy Metabolism," "Metabolism of Cofactors and Vitamins," "Biosynthesis of other Secondary Metabolites," "Transport and Catabolism," ""Folding, Sorting, and Degradation," "Cell Growth and Death," "Replication and Repair," and "Nucleotide Metabolism," compared to patients with NYC (Fig. 5A). False-positive KEGG results are shown in Supplementary Material 1.

We hypothesized that changes in the immune system could signify the differences in inflammation between the two groups. Further analysis showed that patients with YC had higher leukocyte and lymphocyte counts than those with NYC (Fig. 5B, $P < 0.05$), indicating that patients with YC may have higher levels of inflammation than those with NYC.

3. Discussion

In this study, we performed a comprehensive comparative analysis of tongue coating and gut microbiomes in patients with T2DM. By leveraging the power of next-generation sequencing technology, we explored the correlations between microbiome composition and various indicators of T2DM. Our findings offer valuable insights and potential clinical tools for identifying the distinctive microbial

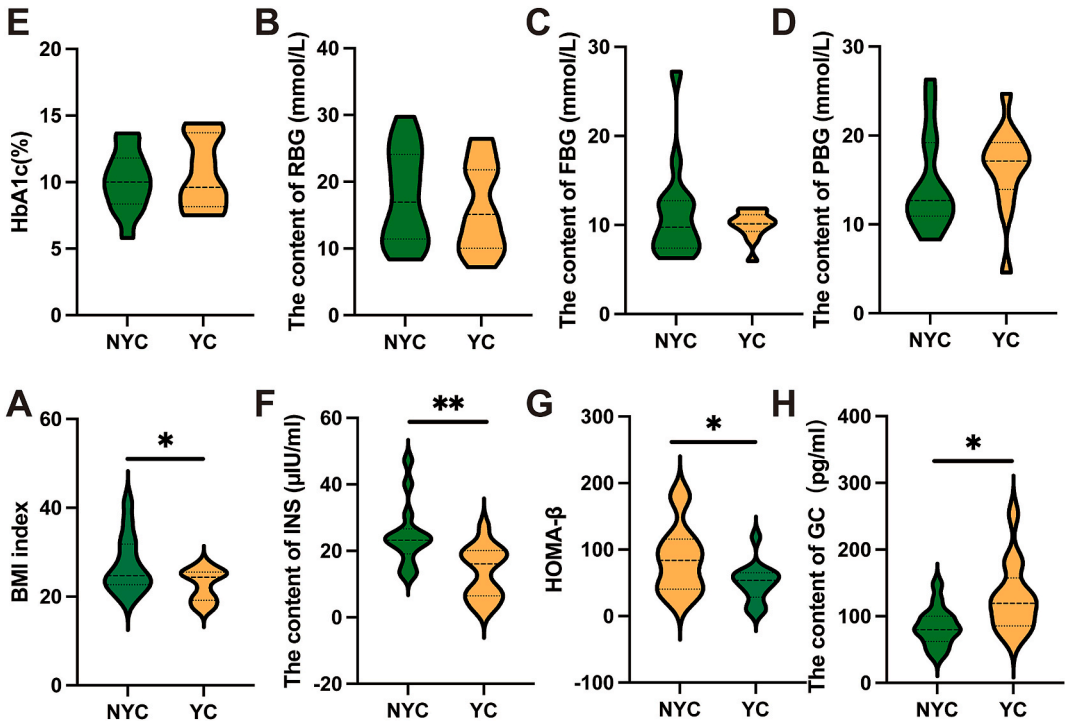

Fig. 4. Comparison of HbA1c (A), RBG (B), FBG (C), PBG (D), BMI (E), INS (F), HOMA-β (G), and GC (H) in patients with NYC and YC. *Statistical significance at $P < 0.05$; **Significantly different at $P < 0.01$.

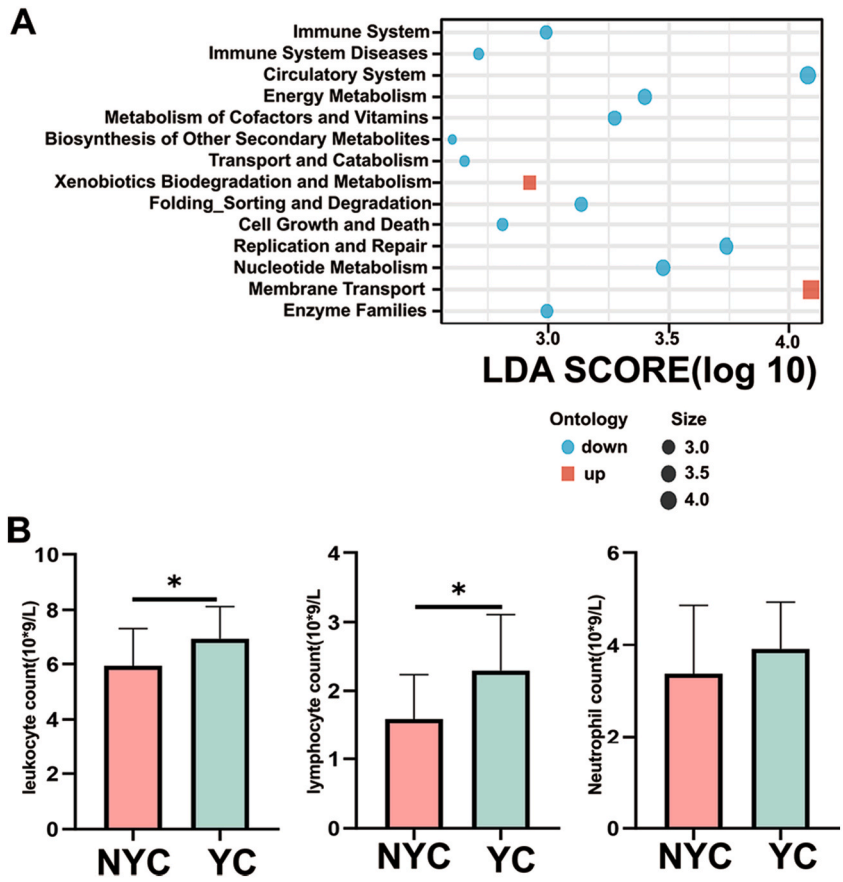

Fig. 5. A: The KEGG pathway. B: Comparison of leukocyte, lymphocyte, and neutrophil counts in patients with NYC and YC. *Statistical significance at $P < 0.05$.

signatures associated with T2DM.

Our findings revealed differences in both the composition of the tongue coating and the gut microbiome between patients with YC and NYC. The predominant microbial phyla shared by both sites were Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria; however, their distribution differed at each location. At the genus level, distinct differences were observed in the predominant microbiota of tongue coating and gut bacteria. Specifically, *Prevotella*, *Neisseria*, *Veillonella*, and *Streptococcus* were the major genera found in the tongue coating microbiome, whereas *Bacteroides*, *Prevotella*, *Klebsiella*, and *Megamonas* dominated the gut bacteria. Notably, Prevotella has been associated with improved glucose metabolism and insulin sensitivity due to its ability to ferment dietary fiber and produce propionate [28,29]. Additionally, *Prevotella* and *Bacteroides* have been shown to play key roles in the relationship between branched-chain amino acid (BCAA) biosynthesis and insulin resistance [30]. These studies highlight the interplay between specific bacteria and nutrients such as amino acids, showing the potential of targeting the microbiota as a preventive or therapeutic strategy [31]. These findings provide valuable insights into the distinct microbial profiles associated with tongue coating and gut microbiomes in patients with T2DM, and shed light on the potential metabolic implications of specific bacterial taxa. Further investigations into the functional roles of these microbial communities may contribute to the development of targeted interventions and therapeutic approaches for the management of T2DM.

The tongue coating microbiome is a complex and dynamic structure consisting of various bacterial entities, including single-layer sparse colonies, free-living bacteria, and bacteria attached to squamous epithelial cells [32]. This suggests that tongue dorsal epithelial cells are comprised of fast-shedding, sparse colony cells, and long-lived structures capable of forming larger biofilms. Extensive interspecies interactions occur between the microbiome and tongue papillae, which can be categorized as synergistic, signaling, or antagonistic [33,34]. Although different bacterial species and genera may have similar metabolic functions, functional redundancies are commonly observed [32]. The presence of diversity and appropriate redundancy in both the tongue coating and gut microbiome can enhance stability [35] and metabolic efficiency [36]. Both the tongue coating and gut microbiomes are closely associated with metabolic status [37], immune status, age [38], sex, genetic factors, environmental factors [39], antibiotic use, infant feeding status [40], and management of probiotics and prebiotics, which are closely related to diet [41,42]. Both tongue coating and gut microbiomes are dominated by anaerobic bacteria [40]. Some microbiomes may shift from the oral cavity to the gut [43] or interact with each other. First, the tongue coating microbiome can be transferred to the gut, causing fluctuations in the gut microbiome. Recently, it was shown

that 14 taxa from other host sites increased in the tongue coating and fecal samples from older adults, indicating that microbiomes from other parts of the host may migrate to the oral cavity [44]. This mechanism may involve a decline in gastrointestinal function or a decrease in gastric acid and bile secretion in older adults. The oral microbiome remains active and can reach the gut, whereas the tongue coating microbiome invades the gingival or tongue tissue, impairing the barrier function of the tongue epithelial mucosa. This can lead to a reduction in the levels of ligand proteins in the tongue [45] or Fap2-mediated blood diffusion to the gut [46,47]. Secondly, changes in the abundance of the tongue coating microbiome align with those of the gut microbiome in various diseases, and their abundance is influenced by the activation of immune receptors or abnormal hormone levels [48–50]. For instance, Veillonella spp. increase in both the oral cavity and gut of patients with autoimmune liver disease, and show a positive correlation [51]. The tongue and gut actively participate in digestion by reflexively stimulating the gastric system, pancreas, liver, and gallbladder [52]. Moreover, the microbiome can influence metabolism by interacting with taste receptors on the tongue and gut [53], which may be related to the specific effector mechanism of the oral-gut axis [12].

Tongue coating and gut microbiomes share dozens of genera [44], including *Lactococcus*, *Bilophila*, and *Akkermansia*. *Lactococcus* and *Lactobacillus* spp. are the common lactic acid bacteria. In our study, using LEFSe analysis, we observed that the abundance of *Lactobacillus* spp. was higher in the tongue coating and gut microbiome of YC than in NYC. These findings suggest a potential association between the presence of *Lactobacillus* spp. and the YC phenotype in patients with T2DM. It is worth noting that certain strains of *Lactobacillus* spp. may appear yellowish or reddish in color [54–56], which could explain the yellow coloration of the tongue coating in certain patients. *Lactobacillus* spp., a member of the genus Firmicutes, comprises over 100 species, approximately 30 % of which are found in the human gastrointestinal tract [57]. Owing to their protective characteristics such as enhanced intestinal barrier function and immunologic health, *Lactobacillus* strains are often utilized as probiotics [58]. Increased levels of *Lactobacillus* spp. have been reported in patients with T2DM; these strains activate the AMP-activated protein kinase (AMPK) pathway and regulate energy metabolism [59–61].

Our study also revealed a positive correlation between the presence of *Lactobacillus* spp. in both tongue coating and gut microbiomes and RDW, a parameter commonly included in complete blood count reports. High RDW values are associated with various acute and chronic cardiovascular diseases, such as acute coronary syndrome, peripheral artery disease, and ischemic cerebrovascular disease [62,63]. Moreover, high RDW values have been reported in individuals with diabetes [64] and reflect chronic inflammation and oxidative stress, both of which are associated with the pathogenesis of T2DM [65,66]. Several studies have shown that high RDW is an independent predictor of cardiovascular morbidity and mortality in the general population [67], chronic heart failure [68], and coronary heart disease [69]. These findings suggest that RDW is associated with high plasma glucose concentrations and could be a predictor of glucose metabolic disturbances [70].

The presence of *Lactobacillus* spp. in the tongue coating and gut was negatively correlated with MCHC and INS levels. A lower MCHC indicates increased afterload and higher demand for cardiac output, potentially leading to left ventricular wall thickening and left atrial enlargement [71]. Studies have reported an association between low MCHC levels and diastolic dysfunction parameters such as left atrial volume and transmitral flow in patients with chronic heart failure [72]. Moreover, low MCHC levels have been identified as a risk factor for mortality in patients undergoing maintenance hemodialysis [73] and those with acute heart failure [74]. Among individuals with chronic systolic heart failure, those with low MCHC and high RDW had the poorest prognosis [75]. Hence, it appears that patients with YC are more susceptible to developing cardiovascular complications associated with T2DM, which aligns with the lower “Circulatory System” KEGG result observed in these patients.

Our findings also indicate that patients with T2DM with YC had poorer health than those with normal tongue coating, as evidenced by BMI, HOMA-β, INS, and glucagon levels. Several studies have reported a positive association between YC and diabetes [23,76]. Additionally, tongue lesions are more prevalent among patients with T2DM than among healthy controls [77], and hyperglycemia has been identified as a risk factor for tongue lesions [78]. Furthermore, YC is a concomitant symptom of diabetes [22] and is associated with a high prevalence of diabetes mellitus [16]. These findings align with our results, which indicate that patients with YC had lower INS levels and BMI than those with NYC, whereas their glucagon levels were higher. Individuals with severe autoimmune diabetes and severe insulin-deficient diabetes typically have a low BMI [79].

Pancreatic islet β cells play a crucial role in the transport of insulin vesicles and promote insulin secretion by expressing the intracellular bacterial peptidoglycan receptor Nod1 to sense ligand signals from the intestinal bacteria [80]. nsulin is involved in the regulation of blood glucose homeostasis, which is crucial for energy metabolism [81]. In diabetes, insulin deficiency and hyperglycemia may inhibit immune responses [82]. Obese individuals have a higher proportion of Firmicutes in their intestines than healthy individuals [83]. Colonization of the gut microbiome is closely related to insulin resistance [84], which increases the body’s demand for insulin. Short-chain fatty acids derived from the intestinal bacteria have been found to promote insulin secretion by activating the vagus nerve [85]. Our study identified a low Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for energy metabolism in patients with YC, which may be due to their low insulin levels.

According to TCM, YC is associated with heat dampness syndrome, which involves inflammation [86,87]. We also found that leukocyte and lymphocyte counts were higher in patients with YC, consistent with a weakened immune response in patients with NYC, as suggested by KEGG pathway analysis. Inflammation is a key factor in the development of diabetic complications, and increased leukocyte activation plays an important role in patients with insulin resistance and T2DM [88,89]. Activated leukocytes contribute to the development of atherosclerosis [90] and microvascular diabetic complications [91,92], making patients with YC at a higher risk of cardiovascular complications of diabetes than patients with NYC. However, the sample size in our study was relatively small; thus, multicenter studies with larger sample sizes are needed to confirm our findings. Second, the 16S rDNA gene sequencing method used in this study did not provide a more in-depth and comprehensive species analysis. Therefore, further studies using metagenomic sequencing are needed for more detailed research.

Our study shows that microbial signatures in tongue coating can serve as potential diagnostic markers for patients exhibiting two different colors, and the composition of the gut microbiome can be reflected by the tongue coating microbiome. The importance of the gut microbiome in human health and disease is well recognized. Our study aimed to investigate the relationship between tongue coating and the gut microbiome in patients with T2DM. The microbiome may provide a new perspective for studying the mechanisms and treatments of T2DM. Our study co-analyzed tongue coating and gut microbiomes in patients with T2DM and revealed novel co-occurrence networks within and between microbial communities. Our findings provide a basis for future research on the use of tongue coating analysis to determine the severity of T2DM. Given the emerging knowledge on the role of the microbiome in human health and disease, we hope that these analyses will provide new insights into the development of microbial markers for the diagnosis of T2DM and guide treatment.

4. Conclusions

The conclusions of this study are as follows: (1) the tongue coating and gut microbiomes were different between patients with different colors of tongue coating; (2) tongue coating and gut microbiome *Lactobacillus* spp. could classify patients with YC and NYC; (3) the tongue coating and gut microbiome *Lactobacillus* spp. were positively associated with higher RDW and negatively associated with MCHC and INS; (4) the decreased INS and HOMA-β, increased GC may indicate that the condition of patients with T2DM with YC may be more severe than that of patients with NYC; (5) presence of *Lactobacillus* spp. in the tongue coating could reflect its presence in the gut.

5. Materials and methods

5.1. Ethics statement

The study has been registered in the Chinese Clinical Trial Registry with the registration number “ChiCTR1900023995.”

5.2. Study participants

Twenty-seven patients who were diagnosed with T2DM. Prior to the initiation of the study, the project protocol was approved by the Medical Ethics Committee of Hainan Traditional Chinese Medicine Hospital. The study was conducted in compliance with all relevant regulations and informed consent was obtained from all participants. All participants consented to the publication of images of their tongues.

The inclusion criteria were as follows: (1) individuals aged between 18 and 65 years; (2) participants who were diagnosed with T2DM, as defined by the criteria established by the American Diabetes Association [93]; (3) absence of any concurrent systemic or metabolic conditions apart from T2DM and no recent occurrence of infection within the preceding month; and (4) absence of dietary or medication regimens that could potentially affect glucose homeostasis, such as glucocorticoids or antibiotics, within the past 3 months.

Exclusion criteria were: (1) presence of clinically significant major systemic diseases, including malignancies; (2) clinical indication of hemoglobinopathies or anemia; (3) history of substance abuse or alcohol dependency; (4) occurrence of a major cardiovascular event within the preceding 6 months; (5) ongoing acute illnesses or current indications of acute or chronic inflammatory or infective diseases; (6) mental health conditions that hindered participants’ ability to comprehend the study’s nature, scope, and potential implications; (7) female gender.

The definitions of YC and NYC were confirmed by at least two doctors specializing in tongue diagnosis. These doctors independently evaluated a group of individuals with a clinically confirmed yellow tongue coating. In instances where there was a disagreement between the two doctors regarding the classification of the tongue coating, we would exclude the clinical sample from our study. All participants were then asked whether they usually brushed off their tongue coating (yes = no) and whether they consumed drinks that could influence their tongue coating (yes/no). Subsequently, participants were instructed to extend their tongues forward as far as possible for image capture. Representative images showing tongue color are shown in Fig. 1A. The tongue coating yellowness was assessed using the Commission Internationale de l’Eclairage $L^+a^+b^+$ (CIELAB) and $L + C\ ^+h^\circ$ (CIELCH) color models. These indices have been used for color segmentation in the diagnosis of tongue conditions using digital images [94,95]. In the CIELAB color model, L^+ represents lightness (0: black to 100: white), and a^+ ($+a^+$: redness to $-a^+$: greenness) and b^+ ($+b^+$: yellowness to $-b^+$: blueness) represent color-opponent dimensions [95,96]. Moreover, the CIELAB color model served as the foundation for calculating the hue-angle values, which were determined in relation to the *a*-axis [97]. The hue-angle value is utilized as a key parameter in the CIELCH color model. In this model, L^+ signifies the lightness (ranging from 0 for black to 100 for white), C^+ represents the chroma (ranging from 0 for completely unsaturated to 100 for notably high chroma), and h° represents the hue in degrees (or angles), ranging from 0° (red) to 90° (yellow), 180° (green), 270° (blue), and back to 0°. In our study, CIE b^+ and hue-angle values were used to quantify the degree of yellowness of tongue coatings. Using the RGB color model as a basis, CIELAB was used to capture measurements from all images encompassing the average red, green, and blue values from a 100-pixel area within a square situated along the center to the posterior of the tongue dorsum, where the distribution of the tongue coating is anticipated to be most prominent [98]. The formulation for computing the CIELAB and hue-angle values from the RGB color model is presented in Supplementary Material 2.

5.3. Sample collection

In the morning, before breakfast, the tongue coatings of all the participants were sampled between 6 a.m. and 9 a.m. Prior to sampling, the participants were instructed to rinse their mouths. They were asked to extend their tongues forward as far as possible. A glossopharyngeal swab was used to scrape the middle part of the tongue, and the collected tongue coating was stored in a test tube containing a DNA preservation solution. The test tubes were stored in a −80 °C refrigerator for preservation.

For fecal sample collection, participants collected samples using a stool collection device obtained from the Beijing Genomics Institution. The collected stool samples were stored in a DNA preservation solution and then kept in a −80 °C refrigerator for storage.

5.4. 16S rDNA gene sequencing

DNA extraction was performed using the TIANamp Stool DNA Kit (TIANGEN) in accordance with previously reported methods [99]. Briefly, the bead-beating method was used to collect the DNA dissolved in Tris-EDTA (TE) buffer after extraction using phenol and chloroform. The concentration and purity of the extracted DNA were assessed by 1 % agarose gel electrophoresis. Based on the concentration measurement, the DNA was diluted to a final concentration of 1 ng/μl using sterile water. Variable regions 3 and 4 (V3–V4) of the gene were amplified using the 341F and 806R primers (341: CCTAYGGGRBGCASCAG; 806: GGACTACNNGGGTATCTAAT) with barcoding [100,101]. Each polymerase chain reaction (PCR) was performed in a total volume of 30 μL, containing 15 μL of PhusionμHigh-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and approximately 10 ng of template DNA. The thermal cycling program consisted of an initial denaturation step at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s. The final extension step was performed at 72 °C for 5 min. An equal volume of 1X loading buffer (containing SYBR green) was mixed with the PCR products and subjected to electrophoresis on a 2 % agarose gel. Samples with a bright main band at 400–450 bp were selected for further experiments. The PCR products were mixed at equidensity ratios. PCR products were purified using a Qiagen Gel Extraction Kit (Qiagen). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina) following manufacturer's recommendations and index codes were added. Library quality was assessed using a Qubit@ 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina NovaSeq6000 platform and 250 bp paired-end reads were generated.

5.5. Data analysis

For data processing and filtering, raw sequencing data were initially subjected to preprocessed using a custom-developed program to ensure the generation of clean data. Subsequently, Fast Length Adjustment of Short reads (FLASH; v1.2.11) was used to merge pairs of reads derived from dual-end sequencing, thereby creating unified sequences based on their overlapping characteristics. This enables the extraction of tags corresponding to regions with pronounced variability. The clean tags were clustered into operational taxonomic units (OTUs). Subsequently, taxonomic classification of these OTUs was meticulously performed through annotation. Finally, the OTU species classification was refined by annotating the OTUs in preparation for the subsequent phase of analysis. Sequence analysis was performed using the UPARSE software package with UPARSE-OTU and UPARSE-OTUref algorithms. In-house Perl scripts were used to analyze alpha (within samples) and beta (among samples) diversity. Sequences with $\geq$97 % similarity were assigned to the same operational OTUs. We selected representative sequences for each OTU and used the RDP classifier to annotate the taxonomic information for each representative sequence. Linear discriminant analysis (LDA) was used to compare differences in the species diversity of different ecosystems among the samples. LEfSe analysis was used to discern distinct and abundant bacterial taxa among the different groups. Taxa with an LDA score greater than two were considered exclusively taken into consideration. Functional gene predictions were conducted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) method. This prediction was facilitated by employing the KEGG database, with high-quality sequences serving as input data.

ROC curves were generated using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). The default plugin of GraphPad Prism was used to perform statistical significance analysis of the AUCs for dysbiosis markers, and Spearman correlation analysis was performed using R software.

All clinical data were statistically analyzed using GraphPad Software. The data consisted of continuous variables following a completely randomized design. Prior to conducting statistical tests, the normality of the data was assessed using the Shapiro-Wilk test, and the homogeneity of variances was assessed using the Levene test. Statistical significance was set at $p < 0.05$. If the data were normally distributed and had homogeneous variances, two independent samples were compared using an independent samples *t*-test. If the data were normally distributed but had heterogeneous variances, two independent samples were compared using Welch's *t*-test. If the data did not follow a normal distribution, a non-parametric test, specifically the Mann-Whitney *U* test, was used.

Data availability statement

Data associated with the study are included in this published article. And the raw sequence reads for the 16S rDNA sequencing datasets obtained from both tongue coating and gut samples have been deposited in the NCBI Sequence Read Archive: PRJNA871372.

CRediT authorship contribution statement

Yao Wang: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Jiqing Li:** Investigation, Methodology. **Haiying Hu:** Methodology, Software, Supervision. **Yalan Wu:** Methodology, Software, Supervision. **Song Chen:** Investigation, Methodology. **Xiangrong Feng:** Investigation, Methodology. **Ting Wang:** Validation, Visualization. **Yinrong Wang:** Validation, Visualization. **Su Wu:** Conceptualization, Funding acquisition. **Huanhuan Luo:** Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to the Research Center of Integrative Medicine in School of Basic Medical Sciences of Guangzhou University of Chinese Medicine. The authors sincerely acknowledge the financial support received from the following organizations: the National Natural Science Foundation of China (82174244 and 81973720); the "Double First-Class" and High-level University Discipline Reserve Talent Cultivation Project of Guangzhou University of Chinese Medicine (grant 2021xk40); the "Double First-Class" and High-level University Discipline Collaborative Innovation Team Project of Guangzhou University of Chinese Medicine; the International Program for Postgraduates at Guangzhou University of Chinese Medicine. We gratefully appreciate the support provided by these funding sources. We are thankful to Shanghai Applied Protein Technology Co., Ltd., (Shanghai, China) for 16S rDNA sequencing analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22615.

References

[1] S. Chatterjee, K. Khunti, M.J. Davies, Type 2 diabetes, The lancet 389 (10085) (2017) 2239–2251.
[2] M.E. Cerf, Beta cell dysfunction and insulin resistance, Front. Endocrinol. 4 (2013) 37.
[3] N.H. Cho, et al., IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045, Diabetes Res. Clin. Pract. 138 (2018) 271–281.
[4] A. Mafi, et al., Metabolic and genetic response to probiotics supplementation in patients with diabetic nephropathy: a randomized, double-blind, placebo-controlled trial, Food Funct. 9 (9) (2018) 4763–4770.
[5] A. Nelaeva, K. IuV, E. Khobotova, Role of proinflammatory cytokines in the development and progression of micro-and macroangiopathies in patients with type 2 diabetes mellitus, Angiologiia i sosudistaia khirurgiia= Angiology and vascular surgery 19 (3) (2013) 9–13.
[6] H. Chen, et al., Enhanced expression of NLRP3 inflammasome-related inflammation in diabetic retinopathy, Investigative Ophthalmology & Visual Science 59 (2) (2018) 978–985.
[7] S. Liu, et al., Neutrophil-to-lymphocyte ratio is associated with diabetic peripheral neuropathy in type 2 diabetes patients, Diabetes Res. Clin. Pract. 130 (2017) 90–97.
[8] Y. Zhao, et al., Differences in metabolites of different tongue coatings in patients with chronic hepatitis B, Evid. base Compl. Alternative Med. 2013 (2013).
[9] R. Kanawong, et al., Automated tongue feature extraction for ZHENG classification in traditional Chinese medicine, Evid. base Compl. Alternative Med. 2012 (2012).
[10] B. Jiang, et al., Integrating next-generation sequencing and traditional tongue diagnosis to determine tongue coating microbiome, Sci. Rep. 2 (1) (2012) 1–15.
[11] M.-q. He, et al., Risk factors and clinical features in ischemic stroke patients with different tongue conditions, Chin. J. Integr. Med. (2016) 1–9.
[12] Y. Li, et al., Oral, tongue-coating microbiota, and metabolic disorders: a novel area of interactive research, Frontiers in Cardiovascular Medicine 8 (2021), 730203.
[13] A. Morita, et al., Combination image analysis of tongue color and sublingual vein improves the diagnostic accuracy of Oketsu (blood Stasis) in Kampo medicine, Front. Med. 8 (2021), 790542.
[14] S.F. Hsieh, L.L. Shen, S.Y. Su, Tongue color changes within a menstrual cycle in eumenorrheic women, J Tradit Complement Med 6 (3) (2016) 269–274.
[15] P. Liu, et al., Correlation between tongue manifestations and glucose, total cholesterol, and high-density lipoprotein cholesterol in patients with acute cerebral infarction, J. Tradit. Chin. Med. 34 (2) (2014) 162–165.
[16] K. Tomooka, et al., Yellow tongue coating is associated with diabetes mellitus among Japanese non-smoking men and women: the toon health study, J. Epidemiol. 28 (6) (2018) 287–291.
[17] P.-C. Hsu, et al., The tongue features associated with type 2 diabetes mellitus, Medicine 98 (19) (2019).
[18] S. Han, et al., Tongue images and tongue coating microbiome in patients with colorectal cancer, Microb. Pathog. 77 (2014) 1–6.
[19] J. Wu, et al., Tongue coating microbiota community and risk effect on gastric cancer, J. Cancer 9 (21) (2018) 4039.
[20] J. Cui, et al., Tongue coating microbiome as a potential biomarker for gastritis including precancerous cascade, Protein & cell 10 (7) (2019) 496–509.
[21] H. Zhang, et al., Study on the history of Traditional Chinese Medicine to treat diabetes, European Journal of Integrative Medicine 2 (1) (2010) 41–46.
[22] K. Su, et al., Retrospective study on Professor Zhongying Zhou's experience in Traditional Chinese Medicine treatment on diabetic nephropathy, J. Tradit. Chin. Med. 33 (2) (2013) 262–267.
[23] P.-Y. Liao, et al., Diabetes with pyogenic liver abscess—a perspective on tongue assessment in traditional Chinese medicine, Compl. Ther. Med. 22 (2) (2014) 341–348.
[24] M.A. Sabin, et al., Insulin and BMI as predictors of adult type 2 diabetes mellitus, Pediatrics 135 (1) (2015) e144–e151.
[25] C.G. Magnussen, et al., Pediatric metabolic syndrome predicts adulthood metabolic syndrome, subclinical atherosclerosis, and type 2 diabetes mellitus but is no better than body mass index alone: the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study, Circulation 122 (16) (2010) 1604–1611.
[26] M. Zhou, et al., Association between β-cell function estimated by HOMA-β and prognosis of non-diabetic patients with ischaemic stroke, Eur. J. Neurol. 25 (3) (2018) 549–555.

[27] V.J. Bermudez-Pirela, et al., Metformin plus low-dose glimeperide significantly improves homeostasis model assessment for insulin resistance (HOMAIR) and β-cell function (HOMAβ-cell) without hyperinsulinemia in patients with type 2 diabetes mellitus, Am. J. Therapeut. 14 (2) (2007) 194–202.
[28] P. Kovatcheva-Datchary, et al., Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of Prevotella, Cell Metabol. 22 (6) (2015) 971–982.
[29] F. De Vadder, et al., Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis, Cell Metabol. 24 (1) (2016) 151–157.
[30] H.K. Pedersen, et al., Human gut microbes impact host serum metabolome and insulin sensitivity, Nature 535 (7612) (2016) 376–381.
[31] P.D. Cani, Human gut microbiome: hopes, threats and promises, Gut 67 (9) (2018) 1716–1725.
[32] S.A. Wilbert, J.L.M. Welch, G.G. Borisy, Spatial ecology of the human tongue dorsum microbiome, Cell Rep. 30 (12) (2020) 4003–4015. e3.
[33] Y. Zhao, et al., Helicobacter pylori infection alters gastric and tongue coating microbial communities, Helicobacter 24 (2) (2019), e12567.
[34] S. Louca, et al., Function and functional redundancy in microbial systems, Nature ecology & evolution 2 (6) (2018) 936–943.
[35] J.E. Goldford, et al., Emergent simplicity in microbial community assembly, Science 361 (6401) (2018) 469–474.
[36] X. Yu, M.F. Polz, E.J. Alm, Interactions in self-assembled microbial communities saturate with diversity, ISME J. 13 (6) (2019) 1602–1617.
[37] T. Ogawa, et al., Composition of salivary microbiota in elderly subjects, Sci. Rep. 8 (1) (2018) 414.
[38] K. Anukam, N. Agbakoba, A comparative study of the oral microbiome compositions of healthy postmenopausal, premenopausal, and prepubertal Nigerian females, using 16s rrna metagenomics methods, Niger. J. Clin. Pract. 20 (10) (2017) 1250–1258.
[39] E.K. Costello, et al., Bacterial community variation in human body habitats across space and time, science 326 (5960) (2009) 1694–1697.
[40] T. Ding, P.D. Schloss, Dynamics and associations of microbial community types across the human body, Nature 509 (7500) (2014) 357–360.
[41] I. Kato, et al., Nutritional correlates of human oral microbiome, J. Am. Coll. Nutr. 36 (2) (2017) 88–98.
[42] G.R. Jaiswal, et al., Impact of bariatric surgery and diet modification on periodontal status: a six month cohort study, J. Clin. Diagn. Res.: J. Clin. Diagn. Res. 9 (9) (2015) ZC43.
[43] N. Segata, et al., Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples, Genome biology 13 (2012) 1–18.
[44] M. Iwauchi, et al., Relationship between oral and gut microbiota in elderly people, Immunity, inflammation and disease 7 (3) (2019) 229–236.
[45] W. Chen, et al., Grainyhead-like 2 (GRHL2) knockout abolishes oral cancer development through reciprocal regulation of the MAP kinase and TGF-β signaling pathways, Oncogenesis 7 (5) (2018) 38.
[46] L. Parhi, et al., Breast cancer colonization by Fusobacterium nucleatum accelerates tumor growth and metastatic progression, Nat. Commun. 11 (1) (2020) 3259.
[47] J. Abed, et al., Fap2 mediates Fusobacterium nucleatum colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc, Cell host & microbe 20 (2) (2016) 215–225.
[48] J.L. Round, S.K. Mazmanian, The gut microbiota shapes intestinal immune responses during health and disease, Nat. Rev. Immunol. 9 (5) (2009) 313–323.
[49] C. Zhu, et al., Roseburia intestinalis inhibits interleukin-17 excretion and promotes regulatory T cells differentiation in colitis, Mol. Med. Rep. 17 (6) (2018) 7567–7574.
[50] A.T. Vieira, et al., Influence of oral and gut microbiota in the health of menopausal women, Front. Microbiol. 8 (2017) 1884.
[51] C.-Y. Ko, et al., Analysis of oral microbiota in patients with obstructive sleep apnea-associated hypertension, Hypertens. Res. 42 (11) (2019) 1692–1700.
[52] M. Boland, Human digestion–a processing perspective, J. Sci. Food Agric. 96 (7) (2016) 2275–2283.
[53] A. Turner, et al., Intense sweeteners, taste receptors and the gut microbiome: a metabolic health perspective, Int. J. Environ. Res. Publ. Health 17 (11) (2020) 4094.
[54] J.R. Araújo, et al., Fermentation products of commensal bacteria alter enterocyte lipid metabolism, Cell host & microbe 27 (3) (2020) 358–375. e7.
[55] G. Reid, The scientific basis for probiotic strains of Lactobacillus, Appl. Environ. Microbiol. 65 (9) (1999) 3763–3766.
[56] D. Harty, et al., Pathogenic potential of lactobacilli, Int. J. Food Microbiol. 24 (1–2) (1994) 179–189.
[57] G.E. Felis, F. Dellaglio, Taxonomy of lactobacilli and bifidobacteria, Curr. Issues Intest. Microbiol. 8 (2) (2007) 44.
[58] J. Walter, Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research, Appl. Environ. Microbiol. 74 (16) (2008) 4985–4996.
[59] J. Qin, et al., A metagenome-wide association study of gut microbiota in type 2 diabetes, Nature 490 (7418) (2012) 55–60.
[60] F.H. Karlsson, et al., Gut metagenome in European women with normal, impaired and diabetic glucose control, Nature 498 (7452) (2013) 99–103.
[61] N. Larsen, et al., Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults, PLoS One 5 (2) (2010) e9085.
[62] W. Saliba, et al., The association between red cell distribution width and stroke in patients with atrial fibrillation, Am. J. Med. 128 (2) (2015) 192. e11–e192. e18.
[63] E. Danese, G. Lippi, M. Montagnana, Red blood cell distribution width and cardiovascular diseases, J. Thorac. Dis. 7 (10) (2015) E402.
[64] A.M. Nada, Red cell distribution width in type 2 diabetic patients, Diabetes, Metab. Syndrome Obes. Targets Ther. (2015) 525–533.
[65] O.A. Dada, et al., The relationship between red blood cell distribution width and blood pressure in patients with type 2 diabetes mellitus in Lagos, Nigeria, Hematol. Res. Rev. (2014) 185–189.
[66] G. Horta-Baas, M.d.S. Romero-Figueroa, Clinical utility of red blood cell distribution width in inflammatory and non-inflammatory joint diseases, International journal of rheumatic diseases 22 (1) (2019) 47–54.
[67] T.S. Perlstein, et al., Red blood cell distribution width and mortality risk in a community-based prospective cohort, Arch. Intern. Med. 169 (6) (2009) 588–594.
[68] G.M. Felker, et al., Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank, J. Am. Coll. Cardiol. 50 (1) (2007) 40–47.
[69] M. Tonelli, et al., Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease, Circulation 117 (2) (2008) 163–168.
[70] C. Tripolino, et al., Red blood cell distribution width predicts two-hours plasma glucose levels during OGTT, Clin. Hemorheol. Microcirc. 62 (1) (2016) 63–69.
[71] S. Yamaguchi, et al., Mean corpuscular hemoglobin concentration: an anemia parameter predicting cardiovascular disease in incident dialysis patients, J. Nephrol. 35 (2) (2022) 535–544.
[72] H. Kataoka, et al., Relationship between anaemia management at haemodialysis initiation and patient prognosis, Nephrology 20 (2015) 14–21.
[73] V. Ebrahimi, et al., Factors influencing survival time of hemodialysis patients; time to event analysis using parametric models: a cohort study, BMC Nephrol. 20 (1) (2019) 1–9.
[74] M. Kleber, et al., Relative hypochromia and mortality in acute heart failure, Int. J. Cardiol. 286 (2019) 104–110.
[75] C. Simbaqueba, et al., Prognostic implications of relative hypochromia in ambulatory patients with chronic systolic heart failure, Congest. Heart Fail. 19 (4) (2013) 180–185.
[76] D.N. Facciponte, et al., Identifying aerosolized cyanobacteria in the human respiratory tract: a proposed mechanism for cyanotoxin-associated diseases, Sci. Total Environ. 645 (2018) 1003–1013.
[77] A. de Souza Bastos, et al., Diabetes mellitus and oral mucosa alterations: prevalence and risk factors, Diabetes Res. Clin. Pract. 92 (1) (2011) 100–105.
[78] H.-L. Collin, et al., Oral symptoms and signs in elderly patients with type 2 diabetes mellitus: a focus on diabetic neuropathy, Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 90 (3) (2000) 299–305.
[79] E. Ahlqvist, et al., Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables, Lancet Diabetes Endocrinol. 6 (5) (2018) 361–369.
[80] Q. Zhang, et al., Intestinal lysozyme liberates Nod1 ligands from microbes to direct insulin trafficking in pancreatic beta cells, Cell Res. 29 (7) (2019) 516–532.
[81] Z. Huang, et al., Insulin and growth hormone balance: implications for obesity, Trends in Endocrinology & Metabolism 31 (9) (2020) 642–654.
[82] F.H. Tessaro, et al., Insulin influences LPS-Induced TNF-α and IL-6 release through distinct pathways in mouse macrophages from different compartments, Cell. Physiol. Biochem. 42 (5) (2017) 2093–2104.

[83] J. Dicksved, et al., Molecular analysis of the gut microbiota of identical twins with Crohn's disease, ISME J. 2 (7) (2008) 716–727.
[84] F. Bäckhed, et al., The gut microbiota as an environmental factor that regulates fat storage, Proc. Natl. Acad. Sci. USA 101 (44) (2004) 15718–15723.
[85] R.J. Perry, et al., Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome, Nature 534 (7606) (2016) 213–217.
[86] R. Li, et al., Imbalanced network biomarkers for traditional Chinese medicine Syndrome in gastritis patients, Sci. Rep. 3 (1) (2013) 1543.
[87] Y.-Y. Lu, et al., Serum cytokine profiling analysis for zheng differentiation in chronic hepatitis B, Chin. Med. 10 (1) (2015) 1–10.
[88] A. Van Oostrom, et al., Increased expression of activation markers on monocytes and neutrophils in type 2 diabetes, Neth. J. Med. 62 (9) (2004) 320–325.
[89] L. Fogelstrand, et al., Monocytic expression of CD14 and CD18, circulating adhesion molecules and inflammatory markers in women with diabetes mellitus and impaired glucose tolerance, Diabetologia 47 (2004) 1948–1952.
[90] S. De Servi, et al., Clinical and angiographic correlates of leukocyte activation in unstable angina, J. Am. Coll. Cardiol. 26 (5) (1995) 1146–1150.
[91] D. Min, et al., Mesangial cell-derived factors alter monocyte activation and function through inflammatory pathways: possible pathogenic role in diabetic nephropathy, Am. J. Physiol. Ren. Physiol. 297 (5) (2009) F1229–F1237.
[92] A.M. Joussen, et al., Leukocyte-mediated endothelial cell injury and death in the diabetic retina, Am. J. Pathol. 158 (1) (2001) 147–152.
[93] Diagnosis and classification of diabetes mellitus, Diabetes Care 33 (Suppl 1) (2010) S62–S69. Suppl 1.
[94] B. Zhang, et al., Tongue Color Analysis for Medical Application, vol. 2013, Evid Based Complement Alternat Med, 2013, 264742.
[95] W. Su, et al., Objectified study on tongue images of patients with lung cancer of different syndromes, Chin. J. Integr. Med. 17 (4) (2011) 272–276.
[96] H.H. Bosman, N. Petkov, M.F. Jonkman, Comparison of color representations for content-based image retrieval in dermatology, Skin Res. Technol. 16 (1) (2010) 109–113.
[97] Y. Xu, et al., [Chromaticity and optical spectrum colorimetry of the tongue color in different syndromes of primary hepatic carcinoma], Zhong Xi Yi Jie He Xue Bao 10 (11) (2012) 1263–1271.
[98] S. Mantilla Gómez, et al., Tongue coating and salivary bacterial counts in healthy/gingivitis subjects and periodontitis patients, J. Clin. Periodontol. 28 (10) (2001) 970–978.
[99] A. Salonen, et al., Comparative analysis of fecal DNA extraction methods with phylogenetic microarray: effective recovery of bacterial and archaeal DNA using mechanical cell lysis, J. Microbiol. Methods 81 (2) (2010) 127–134.
[100] X. Wang, et al., Microbiota dysbiosis in primary Sjögren's syndrome and the ameliorative effect of hydroxychloroquine, Cell Rep. 40 (11) (2022), 111352.
[101] G.C. Baker, J.J. Smith, D.A. Cowan, Review and re-analysis of domain-specific 16S primers, J. Microbiol. Methods 55 (3) (2003) 541–555.