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**Original Article**

# Characterization of lingual microbiota in pediatric geographic tongue

Yi You[1], Yuan He[2], Peicheng Huang[1]

[1]*Department of Stomatology, Hunan Children's Hospital, Hunan;* [2]*Hunan Key Laboratory of Oral Health Research & Hunan 3D Printing Engineering Research Center of Oral Care & Hunan Clinical Research Center of Oral Major Diseases and Oral Health & Academician Workstation for Oral-maxilofacial and Regenerative Medicine & Xiangya Stomatological Hospital & Xiangya School of Stomatology, Central South University, China.*

## ABSTRACT

**Background.** Geographic tongue is an oral mucosal lesion affecting the tongue. The association between geographic tongue and the mucosal microbiota in children remains unclear.

**Method.** To characterize the feature of lingual microbiota in pediatric geographic tongue, lingual swabs were collected from lesion sites and healthy sites of 25 patients with geographic tongue (14 males and 11 females; age 5.21 ±2.94 years) and 19 controls (10 males and 9 females; age 5.31±2.82 years). DNA was extracted and the 16S rRNA was amplificated, sequenced and analyzed.

**Results.** The lingual microbiota composition was significantly different between children with geographic tongue and the healthy cohort; *Streptobacillus* was reduced in geographic tongue, while *Catonella, Bacillus* and *Oribacterium* were overrepresented. When the lesions and the normal mucosa were compared, an increased abundance of *Prevotella oris* was observed.

**Conclusion.** Our results provided new insight into the association between oral microbiota and pediatric geographic tongue.

**Key words:** geographic tongue, children, microbiota, 16S rRNA.

Geographic tongue (GT), also known as benign migratory glossitis, is a common inflammatory condition that affects the mucous membranes of the tongue.[1] It manifests as irregular, map-like patches on the tongue's surface with papillary atrophy, surrounded by a whitish peripheral zone. These patches may change in shape, size, and location over time. Generally, GT is asymptomatic, while in some cases, symptoms such as soreness, sensitivity, and burning sensations, are triggered by acidic drinks and spicy foods.[2] Epidemiological studies have reported varying prevalence rates, ranging from 1% to 3% in the general population[3], with a slightly higher incidence in women compared to men[4], while in the pediatric population it ranges from 0.37% to 14.3%.[1]

The pathogenesis of geographic tongue involves complex interactions between genetic and environmental factors.[5] Genetic predisposition plays a significant role, as the condition often demonstrates a familial tendency.[6] Mutations in genes involved in immune regulation and epithelial cell function, such as *IL36RN*[7] and human leukocyte antigen (HLA) genes[8], have been implicated in GT development. Association with other conditions like psoriasis[6] and juvenile diabetes[9] has also been observed.

Environmental factors, including stress, hormonal changes, allergic reactions, certain dietary factors, and oral microbiota, may contribute to GT development. Papillary atrophy, a structural change in the tongue associated with GT, may alter the bacterial

✉ Yi You
yuyudany@sina.com

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community, leading to inflammation. Elevated levels of inflammatory mediators like interleukin (IL)-8 and calprotectin in GT suggest a reciprocal relationship between microbiota dysbiosis and inflammation.[10,11]

The presence of fungi had been reported to be associated with GT.[12,13] The development of Next-generation sequencing (NGS) allowed the study of microbial communities.[14] Recent research has highlighted the role of oral microbiota in oral mucosal lesions like oral cancer[15], oral submucous fibrosis[16], oral leukoplakia[17] and oral lichen planus.[18] Studies on the lingual microbiota profiles of adults with GT identified *Microbacterium*, *Leptospira*, *Methylotenera*, and *Lactococcus* as bacteria associated with GT lesion sites.[19]

The oral microbiome undergoes changes with age, and microbial profiles in children differ from those of other age groups.[20] Bacterial diversity and richness increase during early childhood.[21] These findings suggest that the oral microbiota in children has distinct characteristics. However, the association between oral microbiota and GT in children remains unclear.

Our study characterizes the microbiota profiles of children with GT at the lesion and surrounding healthy sites. By comparing these profiles to the bacterial community of healthy control children, we aim to shed light on the role of microbiota in pediatric GT.

## Materials and Methods

### *Ethics approval and consent to participate*

The study was examined and given approval by the Ethics Committee of Hunan Children's Hospital. All the methods were carried out in line with relevant guidelines and regulations. The participants provided informed consent prior to taking part in the study.

### *Patients and sample collection*

Children (n=25) with only primary untreated GT between 2 and 10 years of age fulfilling diagnostic criteria were recruited at the Hunan Children's Hospital. Healthy controls (n=19) were also recruited from Hunan Children's Hospital. All individuals were free of antibiotic therapy for a month prior to the study. Specimens of GT lesion and normal mucosa on the opposite side from the GT participant were collected unstimulated by swab in accordance with the Manual of Procedures for Human Microbiome Project. This was done at least 1 hour after eating or drinking. If the patient had eaten within one hour, mouth rinsing was done and then a wait of 10 minutes was observed before sample collection. Swabs were collected in 1.5 mL tubes and stored at −80 °C before use.

### *DNA extraction*

Microbial DNA was extracted from each sample using the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instruction. The DNA concentrations were measured by using the Qubit quantification system (Thermo Scientific, Wilmington, DE, US). The extracted DNA was stored at -20 °C until use.

### *16S gene amplicon sequencing*

The 16S ribosomal RNA (rRNA) gene amplification procedure included two polymerase chain reaction (PCR) steps. Briefly, For the first PCR reaction, the V3-V4 hypervariable region of the 16S rRNA gene was amplified using primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC).

DNA was amplified in 96-well plates with a reaction mixture of 1X KAPA HiFi Hot start Ready Mix, 0.1μM primer 341 F, 0.1 μM primer 805 R, and 12.5 ng template DNA, totaling a total volume of 50 μL per sample. Reactions were conducted in a T100 PCR thermocycle (BIO-RAD) according to the following cycling program: 3 minutes of denaturation at 94 °C, followed by 18 cycles of 30 seconds at 94 °C (denaturing), 30 seconds at 55 °C (annealing), and 30 seconds at 72 °C (elongation), with a final

extension at 72 °C for 5 minutes. Subsequently, the amplified products were examined by 2% agarose gel electrophoresis and quantified by the Qubit quantification system (Thermo Scientific, Wilmington, DE, US). In the second PCR step, sequencing primers and adaptors were added to the amplicon products. Specifically, 2 μL of the diluted amplicons were mixed with a reaction solution consisting of 1×KAPA HiFi Hotstart ReadyMix, 0.5μM fusion forward and 0.5μM fusion reverse primer, and 30 ng Meta-gDNA (total volume 50 μL). The PCR was run following the previous cycling program except with a cycling number of 12. The amplification products were purified with AMPure XP Beads (Beckman Coulter Genomics, MA, USA) according to the manufacturer's instructions and quantified as described earlier. Equimolar amounts of the amplification products were pooled into a single tube, and the concentration of the pooled libraries was measured by the Qubit quantification system. 2 × 250 bp paired-end sequencing with dual-index reads were carried out at MiSeq Reagent Kits v2 (Illumina Inc.) on the Illuimina MiSeq System (Illumina Inc., CA, USA).

### *Data processing*

Fastq-files were demultiplexed by the MiSeq Controller Software (Illumina Inc.). Then the amplification primers, diversity spacers, and sequencing adapters, merge-paired were trimmed and the low quality reads were filtered by USEARCH. Operational taxonomic unit (OTU)s equaling or above 97% were clustered by UPARSE. The RDP classifier were used for assignment of taxonomy of the OTUs and alignment for the sequences\. The OTUs were analyzed by phylogenetic and OTU methods in the Quantitative Insights into Microbial Ecology (QIIME) software version 2. $\alpha$-diversity (Observed OTU number, Shannon index, Simpson index, Chao1 index, observed species, ACE index) and β-diversity (Unweight UniFrac distances and Weight UniFrac distances) were calculated based on the rarefied OTU counts. Function prediction was performed by PICRUSt2, and differentially abundant function predictions were identified by STAMP.

### *Statistics and data analysis*

Differential $\alpha$-diversity analysis was performed utilizing the Wilcoxon rank-sum or Kruskal-Wallis test. The software linear discriminant analysis (LDA) Effect Size (LEfSe) was used to identify taxa characterizing the differences between conditions.

## Results

### *Characteristics of the bacterial communities*

To characterize the oral microbiome community in GT, oral swapping specimens were collected from GT children (n= 25) and healthy controls (n= 19) (Table I). Samples were sequenced on an Illumina Miseq system, a total of $5.98\times10^6$ raw sequences were obtained. Samples with a low number of combined paired reads (<10000) were excluded in the following analysis. According to the rarefaction data (Fig. S1), subsets of 10000 reads (this quantity was sufficient to identify

**Table I.** Baseline characteristics of the cohort.

| | Heathy control | Geographic tongue | P value |
|---|---|---|---|
| Age | 5.31±2.82 | 5.21 ±2.94 | 0.9 |
| Female / Male | 9 / 10 | 11 / 14 | 1 |
| Raw reads | 91403.21±3364.51 | GT-H: 83715.2±11027.8 | |
| | | GT-L: 86048.88±8133.74 | <0.01 |
| Filtered reads | 75787.32±3511.34 | GT-H: 70408.4±9561.95 | |
| | | GT-L: 71325.76±6551.8 | <0.01 |

GT-H, Healthy tongue area of geographic tongue patients; GT-L, Lesions of geographic tongue patients.

most of the bacterial community members as the rarefaction curve of the observed OTUs reached a plateau at this point) were picked randomly to normalize sequencing depth for subsequent community composition analysis. The clustering of the picked reads with a 97% sequence identity led to 2985 unique OTUs in the datasets, which were classified into 17 distinct bacterial phyla, 69 distinct bacterial families, and 123 distinct bacterial genera (Table S1). Nearly half of the OTUs were shared by the groups (Fig. 1A), and the counts of OTUs mapped to different levels of taxon were comparable (Fig. 1B). At the phylum, bacteria from *Firmicutes* (44.7%), followed by Proteobacteria (26.4%), Bacteroidetes (14.9%), Actinobacteria (8.3%) and *Fusobacteria* (5.3%) dominated (Fig. S2A). For genera, *Streptococcus* (23.6%), *Neisseria* (12.9%), *Haemophilus* (11.5%), *Veillonella* (9.9%), *Rothia* (5.7%) and *Granulicatella* (4.2%) compromised the most predominant genus, and these predominant taxa show no difference between groups. (Fig. S2B).

### *Richness and diversity of the microbiota comparison by groups*

Alpha diversity indices did not differ by gender or age. No significant difference was observed between healthy controls with normal lingual mucosa in GT patients or between normal mucosa with GT lesions (Fig. S3).

The samples in GT groups seemed to cluster together based on OTU abundance (Fig. S4). And a scatter plot based on principal coordinate analysis (PCoA) or non-metric multidimensional scaling (NMDS) used weighted or unweighted Unifrac distance was utilized to reveal bacterial community composition. Cluster of samples of different conditions was observed, especially at weighted Unifrac distance (Fig. S5B, D). A minor shift of bacterial community was found from normal mucosa or lesions in GT patients to healthy controls (Fig. S5). According to multi-response permutation procedures (MRPPs, Adonis, Anosim), the differences between groups was statistically significant, even though they seem to be small (Table S2). Partial Least Squares Discriminant Analysis (PLS-DA) could separate those samples of different conditions (Fig. S6).

### *Differences in the compositions of the microbiota in GT patients and healthy controls*

Then the bacterial communities were analyzed at different taxonomic levels by LEfSe based on taxonomy data. Compared to healthy controls, *Streptobacillus* was depleted in the normal lingual mucosa in GT patients, while *Catonella, Oribacterium, Bacillus, Prevotella scopos* and *Prevotella nanceiensis* were overrepresented (Fig. 2A, 2B, Table S3), *Prevotella oulorum* was underrepresented, while the abundance of *Bacillus* was increased in GT lesion compared to

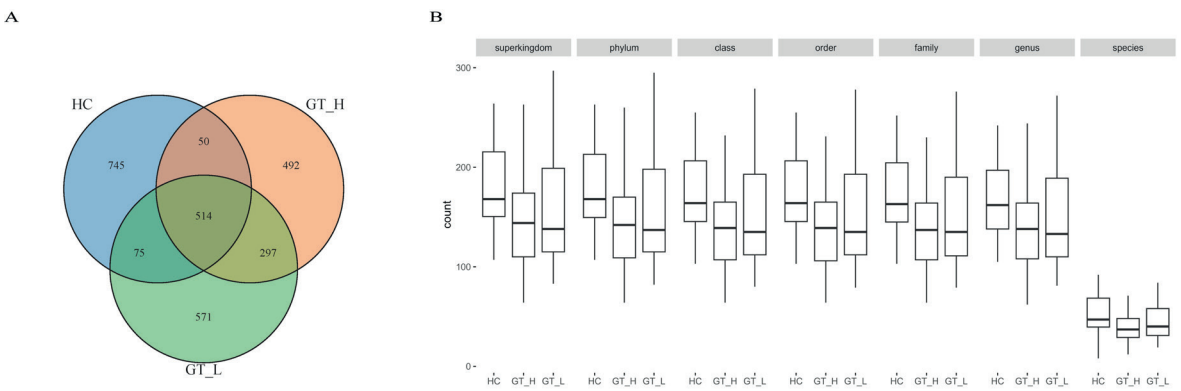


**Fig. 1.** The distribution of OTUs detected. A: Venn diagram showed the overlap of OTUs in different groups. B: Number of taxa at different levels assigned by OTUs. HC indicated healthy control, GT-H indicated the normal lingual area in geographic tongue patients, GT-L indicated the lesion in geographic tongue patients. OTU: operational taxonomic unit.

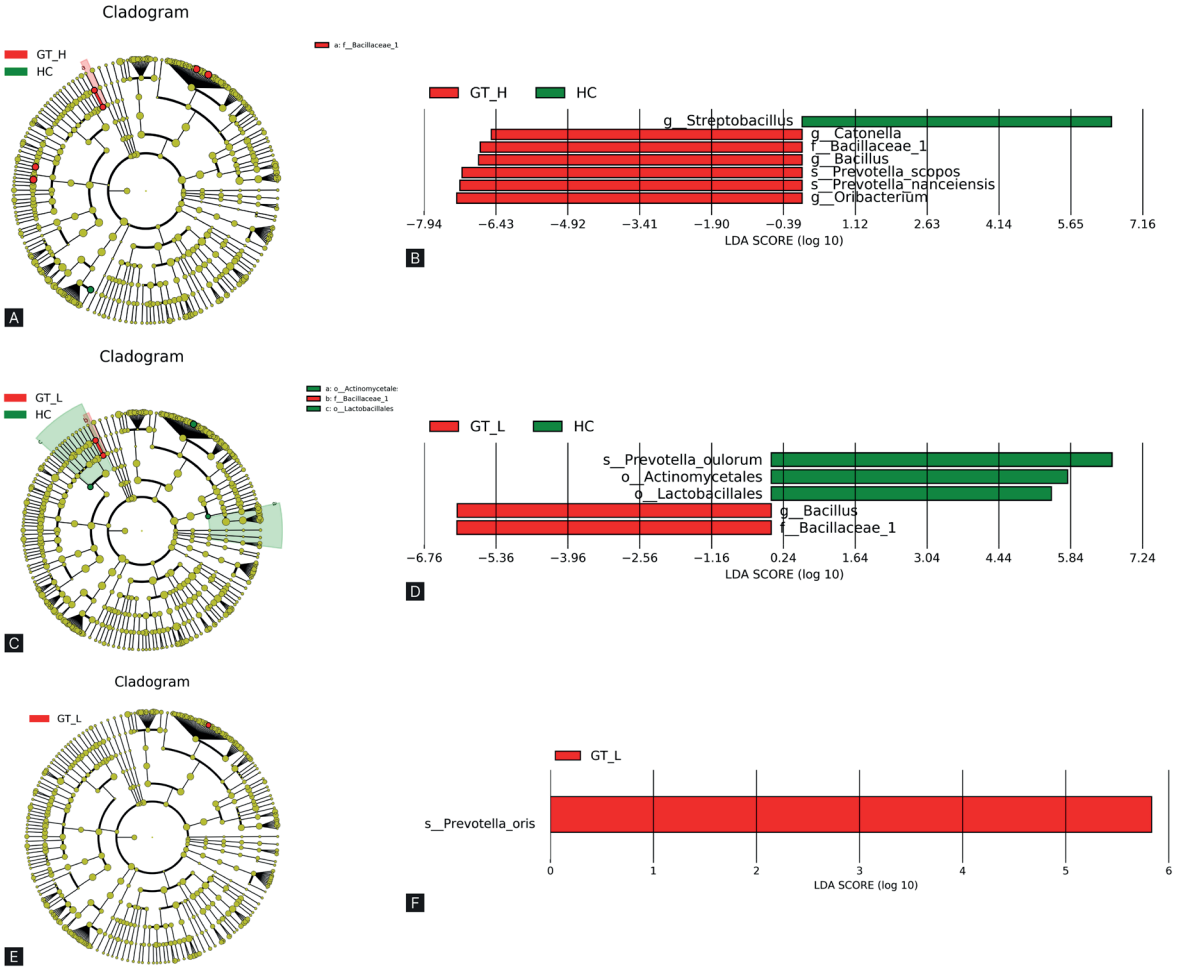


**Fig. 2.** The differentially enriched bacteria in different groups were determined by linear discriminant analysis (LDA) effect size (LEfSe) analysis. A: Cladogram using the LEfSe method indicating the phylogenetic distribution of microbiota exhibited different abundance in the tongues of heathy controls (HC) and geographic tongue patients (GT-H). B: Histogram of the LDA scores was calculated for the selected taxa which showed the significant bacterial difference in heathy controls and geographic tongue patients. C: Cladogram showed the phylogenetic distribution of microbiota exhibited different abundance at normal lingual site (GT-H) or lesion (GT-L) in geographic tongue patients. D: Histogram of the LDA scores for the selected taxa which showed the significant bacterial difference at healthy lingual or lesion in geographic tongue patients.

healthy controls (Fig. 2C, 2D, Table S3). When the normal mucosa was compared to lesions, increased abundance of *Prevotella oris* was observed (Fig. 2E, 2F, Table S3).

PICRUSt2 was utilized for function prediction, and differentially abundant KEGG pathways were observed, microbiota at normal mucosa in GT had increased function of phosphonate and phosphonate metabolism, apoptosis, glycosaminoglycan degradation, and Vibrio cholerae infection, while decreasing function of synthesis and degradation of ketone bodies (Fig. 3A). Compared to normal mucosa, the bacteria at the lesion had decreased linoleic acid metabolism (Fig. 3B), and the bacteria communities at the lesion had increased function of *Vibrio cholerae* infection, endocytosis, while reduced valine, leucine and isoleucine degradation and synthesis and degradation of ketone bodies was revealed (Fig. 3C). Distinct function profiles of COG (Fig. S7), KO (Fig. S8), and METACYC (Fig. S9) were also observed.

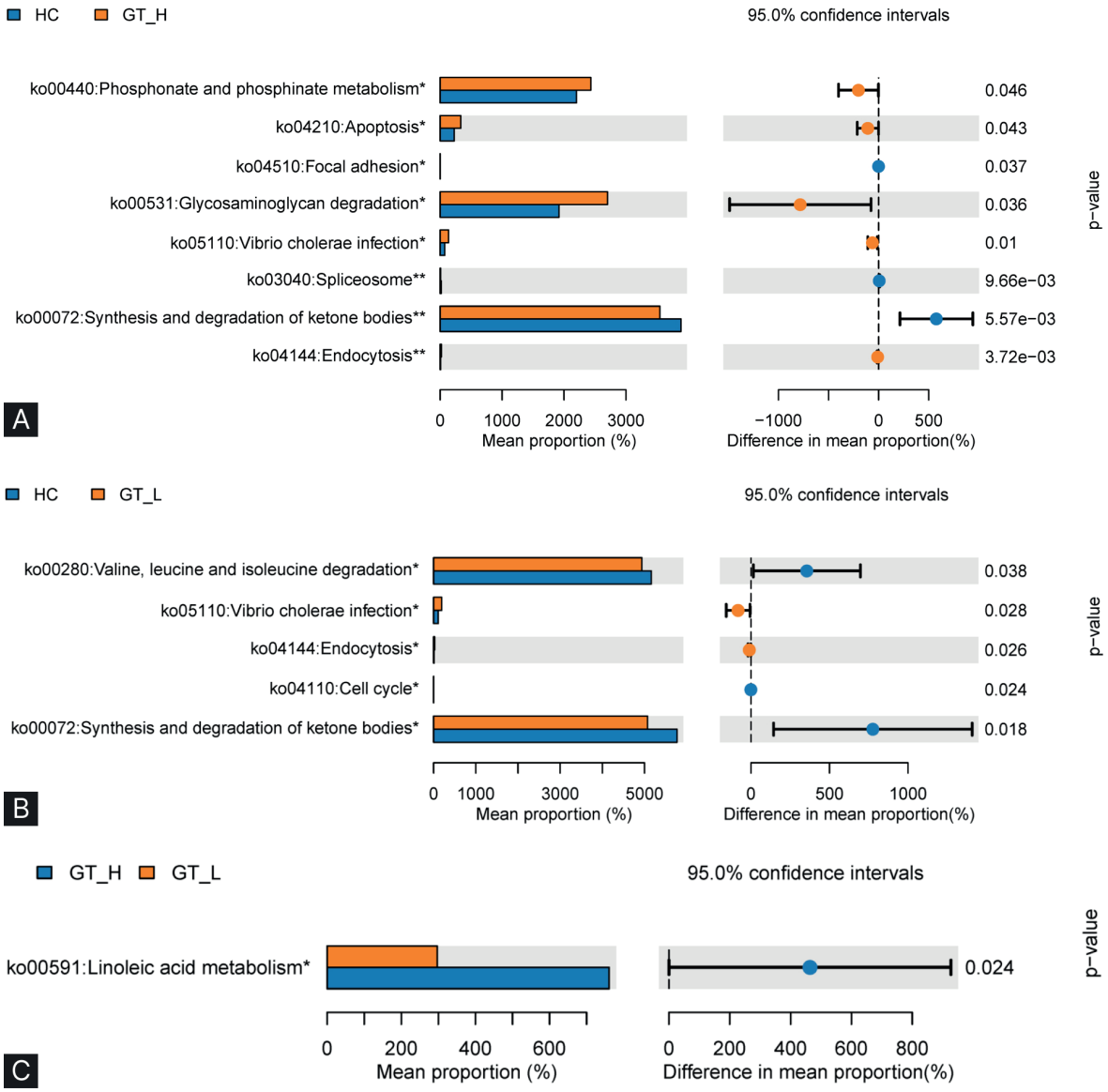


**Fig. 3.** The differentially enriched KEGG pathway predicted by PICRUSt2 was determined by STAMP. A: Differentially enriched KEGG pathway at lingual in heathy controls (HC) and geographic tongue patients (GT-H). B: Differentially enriched KEGG pathway at normal lingual (GT-H) or lesion (GT-L) sites in geographic tongue patients.

## Discussion

We characterized the bacterial community of the GT lesion and normal mucosa in children, revealing an association between GT and dysbiosis of the lingual microbiota. These findings suggest that the oral microbiome may play a role in GT pathogenesis.

The normal tongue structure, characterized by papillae, provides a niche for the colonization of oral microbiota. One of the most striking features of GT is the loss of filiform papillae[22], potentially leading to the loss of bacterial niches and resulting in microbiota dysbiosis. A reciprocal cause-and-effect relationship between dysbiosis and inflammation is

possible[23], with inflammation fueling the selective growth of dysbiotic communities and dysbiosis exacerbating inflammation.[24-26]

The oral microbiome develops during early childhood and undergoes dynamic changes over time. Species richness increases, and the relative abundance of dominant bacteria changes with age[21], resulting in distinct microbiota patterns between adults and children.[27] Therefore, the microbiota of children may exhibit unique features under pathological conditions compared to adults.

In previous studies of adult GT patients, Shannon and Simpson diversity indices were found to be increased in the healthy mucosa of GT patients compared to GT lesions or healthy controls.[19] However, in our cohort, no significant difference in alpha diversity was observed, although the average values of observed species, Chao1, and ACE indices were decreased in the GT lesion and nearby healthy mucosa, while the Shannon and Simpson indices showed no such trend. This discrepancy may be attributed to the distinct characteristics of the children's microbiota. Based on PCoA or NMDS of beta diversity, a shift between healthy controls, healthy mucosa, and GT lesions was observed, indicating alterations in bacterial communities. Nevertheless, the high variability of tongue microbiota resulted in scattered subject distribution.

The abundance of *Catonella*, *Oribacterium*, *Bacillus*, *Prevotella scopos* and *Prevotella nanceiensis* was elevated in the normal mucosa of GT patients compared to healthy controls. Higher relative abundance of *Catonella* in subgingival dental plaque has been associated with periodontitis[28,29], and Catonella species have been implicated in endodontic infections and oral cancer.[30] *Oribacterium* has been found to be enriched in proliferative verrucous leukoplakia (PVL) patients and may serve as a biomarker for PVL.[31] Significantly higher abundances of *Oribacterium* were observed in oral rinse samples of oral cavity cancer (OCC) and oropharyngeal cancers.[32] *Bacillus* was strongly associated with chronic erosive gastritis (CEG) patients with typical yellow tongue coating (YTC).[33] Children of parents with periodontitis had over 6- and 4-fold increases in *Prevotella scopos*.[34] *Prevotella nanceiensis* was more abundant in children with Henoch-Schönlein purpura and positively correlated with the amount of IgA.[35] Thus, increased abundance of these taxa may be associated with inflammatory responses.

Additionally, we found *Prevotella oris* to be overrepresented in GT lesions. *Prevotella oris* has been detected at high abundance in bronchoalveolar lavage fluid samples from some cystic fibrosis individuals[36] and is considered a likely pathogen capable of causing lung infections.[37] However, its association with pathological status remains unclear.

It is noteworthy that the differentially abundant taxa differ from those reported in previous studies of adult GT patients, as the oral bacterial profiles in children are under development and influenced by diet and living habits. Upon reviewing the dietary habits of the study group, it was observed that a subset of children (3 out of 25 in the patient group and 2 out of 19 in the control group) had a history of consuming chili peppers. However, the specific quantities consumed were not documented. It was reported that chili peppers can induce changes in the gut microbiota[38], yet our understanding of their impact on oral microbiota remains limited. Unfortunately, the precise meal plans for the cohort were not accessible. Nonetheless, it was noted that rice and rice noodles were the primary dietary staples, accompanied by common dishes such as eggs, fried potatoes, tofu, dried bean curd, boiled fish, Kung Pao chicken, stir-fried lettuce stem slices, and Chinese sponge gourd. Further research is warranted to elucidate the intricate relationship between diet and oral microbiota. Functional prediction using PICRUSt2 indicated alterations in the overall metabolism of microbiota in GT patients and GT lesions, which could act as a mediator between the bacteria and inflammation.

Our study demonstrates an association between microbiota imbalance and GT in children. Several taxa showed differential abundance between normal mucosa in GT patients and healthy controls, as well as between GT lesions and normal mucosa in GT patients. Establishing the reciprocal relationship between microbiota dysbiosis and inflammation requires further investigation.

## Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.4638

## Ethical approval

This study was approved by the Medical Ethics Committee of Hunan Children's Hospital (No. KY-2021-57).

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: YY; data collection: YY, PH; analysis and interpretation of results: YY, YH; draft manuscript preparation: YY. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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