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Correlations between gastrointestinal and oral microbiota in children with cerebral palsy and epilepsy

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We here studied the correlation between gut and oral microbiota in children with cerebral palsy and Epilepsy (CPE). We enrolled 27 children with this condition from the social welfare center of Longgang District, collected their oral plaque and stool samples, and analyzed their gut microbiota (GM) and oral microbiota (OM) through 16S rRNA gene sequencing. Taxonomical annotation revealed that the levels of Firmicutes and Bacteroides in the oral cavity were significantly lower in CPE children than in healthy children, whereas the abundance of Actinomycetes increased significantly in CPE children. In addition, *Prevotella*, *Fusobacterium*, and *Neisseria* were the top three abundant genera, representing 15.49%, 9.34%, and 7.68% of the OM and suggesting potential correlations with caries, periodontitis, and malnutrition. For the GM, *Bifidobacterium*, *Bacteroides*, and *Prevotella* were the top three abundant genera in CPE children and probably contributed to the development of chronic inflammation and malnutrition. Furthermore, the OM and GM correlated with each other closely, and the bacterial components of these microbiota in CPE children were remarkably different from those in healthy children, such as *Bifidobacterium*, *Fusobacterium*, *Bacteroides*, and *Neisseria*. Conclusively, dysbiotic OM can translocate to the intestinal tract and induce GM dysbiosis, suggesting the consistency between OM and GM variations. Altered oral and gut microbial structures have potential impacts on the occurrence of clinical diseases such as periodontitis, caries, and malnutrition.

KEYWORDS

cerebral palsy, epilepsy, oral microbiota, gut microbiota, malnutrition

Introduction

Gut and oral microbiota are the two most abundant microcommunities inhabiting humans, and their impacts on human health are significant. Oral microbiota (OM) are translocated to the gut *via* sputum swallowing (1), and each adult can swallow 600 ml sputum each day, which contains 10⁹/ml bacterial cells. Similarly, 45% of gut microbiota (GM) are also found among the oral microbiota (OM) (1). As previously reported, the levels of periodontitis-associated *Porphyromonas* and *Fusobacterium*

nucleatum increased in the GM of liver cirrhosis and colorectal cancer patients, and these bacteria regulated the expression of specific proteins that promote the incidence of these medical conditions (2). In addition, periodontitis and dysbiosis of OM are commonly found to be correlated with inflammatory bowel disease (IBD) (3), and gingival microbiota in liver cirrhosis differed from typical periodontitis-associated microbes (4).

Children with cerebral palsy and epilepsy (CPE) usually have oral health problems, such as periodontitis and dental caries (5, 6). Additionally, increased *F. nucleatum* and *Porphyromonas gingivalis* (7, 8) in the oral cavity can induce GM dysbiosis, thereby causing gastrointestinal dysfunctions such as constipation (9–11). Clinical studies have observed higher incidence of GM dysbiosis, dental caries, and periodontitis in CPE children than in healthy children, partly owing to a decreased level of specific microbes that improve iron, zinc, calcium, and vitamin D synthesis as well as amino acid absorption to prevent dental caries development (12). Our previous report documented the association of disordered GM in CPE children (13) with malnutrition (14). Moreover, the components of GM in CPE children were affected by diets, and gastrointestinal dysfunction was prevalent in children consuming liquid food (15). Although previous studies have explored the impact of OM and GM on human health, the association between OM and GM as well as the combinational effect of the OM and GM on host health require further investigation.

This study recruited 27 children with CPE to analyze oral and gut microbial structures. Clinical phenotypes were also included to understand the correlation of the OM and GM with periodontitis, dental caries, and malnutrition.

Materials and methods

Study design and subject enrollment

We here enrolled 27 CPE children (age: 4–14 years) who received liquid food from the social welfare center of Longgang District. The recruited children had no inherited metabolic diseases, gastrointestinal dysplasia, long-term parenteral nutrition, and antibiotic or probiotic exposure 4 weeks before enrollment. Guardians of the enrolled children provided informed consent. Of the 27 children, children had malnutrition, 26 (96.3%) had periodontitis, 22 (81.48%) had dental caries, and 11 (40.74%) had intractable constipation (**Supplementary Table S1**).

Sample collection

Oral sampling was performed for children who had not consumed food for at least 2 h. After gargling with sterile

saline, two dentists from our hospital collected subgingival microbial samples for the first deciduous or permanent molar at the upper right and stored the samples in buffer (MoBio). Regarding the feces sample, enema was applied if children had not defecated, and then, a caregiver used sterile swabs to collect feces (approximately 5 g). All microbial samples were stored at -80°C within 30 min after sampling.

DNA extraction and sequencing

The bacterial DNA was extracted from the stool and oral samples by using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, USA) and was analyzed through 1% gel electrophoresis. The eligible DNA was amplified with 16S rRNA V3–V4 primers (341-FR: CCTACGGGRBGCASCAG, 806-RR: GGACTACNNGGGTATCTAAT) by using the TruSeq DNA PCR-Free kit (Illumina, San Diego, CA, USA). The qualified amplicon library was prepared for high-throughput sequencing with 250-bp paired-end reads using the Miseq platform (Illumina, USA).

Data processing and bioinformatics analysis

The sequenced raw data were filtered using trimmomatic (v0.39) with parameters (LEADING:3 TRAILING:3 SLIDINGWINDOW:30:15 MINLEN:150). Then, the high-quality paired-end reads were assembled using FLASH software (v1.2.11). USEARCH was applied to cluster the connected sequences to form operational taxonomic units (OTUs). Subsequently, the representative OTUs were taxonomized using the RDP classifier against the Greengenes database (v13.5). Finally, we produced bacterial community composition profiles at the phylum, class, order, family, genus, and species levels.

Statistical analysis

The principal component analysis method (ade4 package) was applied to the bacterial relative abundance profile at the genus level, and then, the top two principal components were selected to perform a scatter plot and distinguish samples from different groups. Permutational multivariate analysis of variance (PERMANOVA, vegan package) was used to assess the impact of phenotypes on the microbiota. Significant statistical differences were selected when P was below 0.05. The Pearson's correlation method was applied to analyze the associations between OM and clinical indicators, GM and clinical indicators, and OM and GM. The absolute value of correlation $P < 0.05$ was considered as a significant correlation.

All the data analysis and visualization in this study were conducted using R software (v3.3.3) and the ggplot2 package.

Results

Characteristics of OM and GM in CPE children at the phylum level

The top five phyla in the GM were Firmicutes (averaged 32.689%), Bacteroidetes (28.869%), Actinobacteria (27.547%), Proteobacteria (4.698%), and Fusobacteria (1.903%) (Table 1). On the other hand, the abundance of Bacteroidetes (27.808%), Proteobacteria (23.655%), Firmicutes (15.681%), Actinobacteria (13.186%), and Fusobacteria (10.905%) was the highest in the OM (Table 2).

Characteristics of OM and GM in CPE children at the genus level

We selected the top 15 genera in the OM and GM to perform a comparative analysis. Bacterial composition is different between the OM and GM (Table 2). *Bifidobacterium*, *Prevotella*, and *Streptococcus* were identified in both OM and GM. The levels of *Bifidobacterium*, *Bacteroidetes*, and *Prevotella* were the highest in the GM, but the top three genera in the OM were *Prevotella*, *Fusobacterium*, and *Neisseria*.

Relations between the OM/GM and clinical phenotypes

We identified a positive correlation between the defecation frequency and the levels of *Solobacterium* ($r=0.662$, $P=0.000$), *Lachnoanaerobaculum* ($r=0.522$, $P=0.005$), *Corynebacterium* ($r=0.408$, $P=0.035$), and *Veillonella* ($r=0.390$, $P=0.044$) in the OM (Figure 1). *Actinomyces* ($r=-0.413$, $P=0.032$), *Corynebacterium* ($r=-0.492$, $P=0.009$), *Leptotrichia* ($r=-0.524$, $P=0.005$), and *Veillonella* ($r=-$

-0.385 , $P=0.047$) in the OM correlated negatively with the spasm frequency (Figure 1). Regarding the GM, the defecation frequency associated positively with *Alloprevotella* ($r=0.396$, $P=0.041$) and *Blautia* ($r=0.402$, $P=0.038$), but negatively with *Alistipes* ($r=-0.488$, $P=0.010$) and *Clostridium_XVIII* ($r=-0.424$, $P=0.027$) (Figure 2). In addition, the spasm frequency was positively associated with *Senegalimassilia* ($r=0.724$, $P=0.000$), *Staphylococcus* ($r=0.698$, $P=0.000$), *Actinomyces* ($r=0.521$, $P=0.005$), and *Bacillus* ($r=0.515$, $P=0.006$), but negatively associated with *Sutterella* ($r=-0.411$, $P=0.033$) and *Victivallis* ($r=-0.475$, $P=0.012$) (Figure 2). These findings suggest the significant association of defecation and spasm frequencies with the OM and GM; however, the underlying mechanisms should be further explored.

Association between OM and GM in the CPE children

Given the significant correlation between the defecation/spasm frequency and OM/GM, we further conducted association analysis for the OM and GM. Based on Spearman's coefficient ($P<0.05$), we found that oral *Capnocytophaga* was positively correlated with intestinal *Christensenella* ($r=0.847$), *Alistipes* ($r=0.850$), and *Clostridium-IV* ($r=0.837$) (Figure 2). A positive correlation was also identified between oral *Campylobacter* and intestinal *Lachnospiraceae-incertae-sedis* ($r=0.837$), between oral *Actinomyces* and intestinal *Phascolarctobacterium* ($r=0.842$) and *Alistipes* ($r=0.834$), and between oral *Treponema* and intestinal *Clostridium-XIVa* ($r=0.845$), *Parabacteroides* ($r=0.849$), and *Alistipes* ($r=0.851$).

Discussion

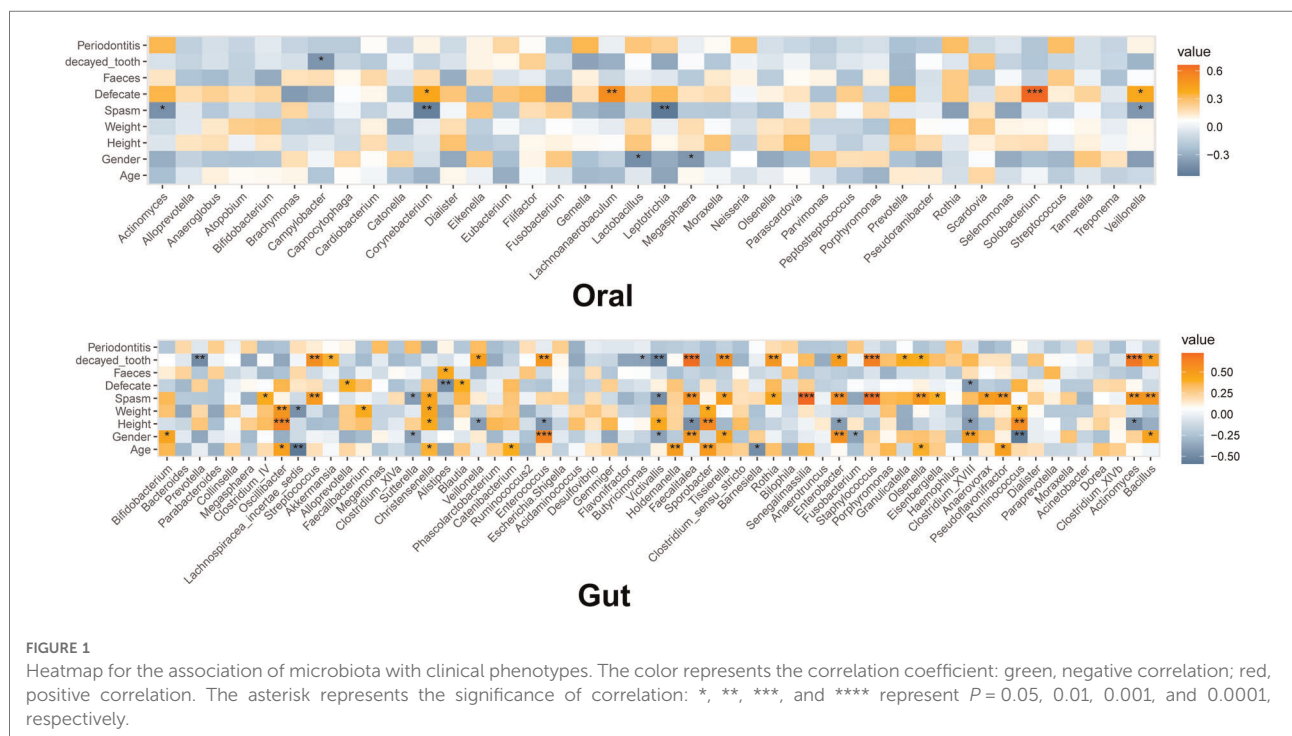
Microbial components in the OM and GM are in dynamic homeostasis, which is maintained by various factors such as bile acids in the gut lumen (16–18). Although millions of microbial cells can be swallowed from the oral cavity into the gastrointestinal tract, the stomach and small intestine function as barriers for these OM and GM because 99% of swallowed microbes are killed at these two sites. Schmidt et al. (19) observed that microbial strains in the gut are translocated from the oral cavity, and a correlation analysis found the oral–gut transmission of opportunistic pathogenic agents as well as dental caries/periodontitis-associated pathogens. For instance, sputum-derived *Klebsiella* in IBD patients induced intestinal inflammation in gnotobiotic mice, suggesting the role played by microbial transmission in etiology (20). The aforementioned findings implicated the translocation of oral

TABLE 1 Top five abundant phyla in oral and gut microbiota.

Gut microbiota			Oral microbiota		
Phylum	Mean (%)	SD (%)	Phylum	Mean (%)	SD (%)
Firmicutes	32.689	13.083	Bacteroidetes	27.808	9.716
Bacteroidetes	28.869	20.77	Proteobacteria	23.655	20.185
Actinobacteria	27.547	19.026	Firmicutes	15.681	10.388
Proteobacteria	4.698	3.689	Actinobacteria	13.186	11.459
Fusobacteria	1.903	2.422	Fusobacteria	10.905	8.052

TABLE 2 Top 15 abundant genera in oral and gut microbiota.

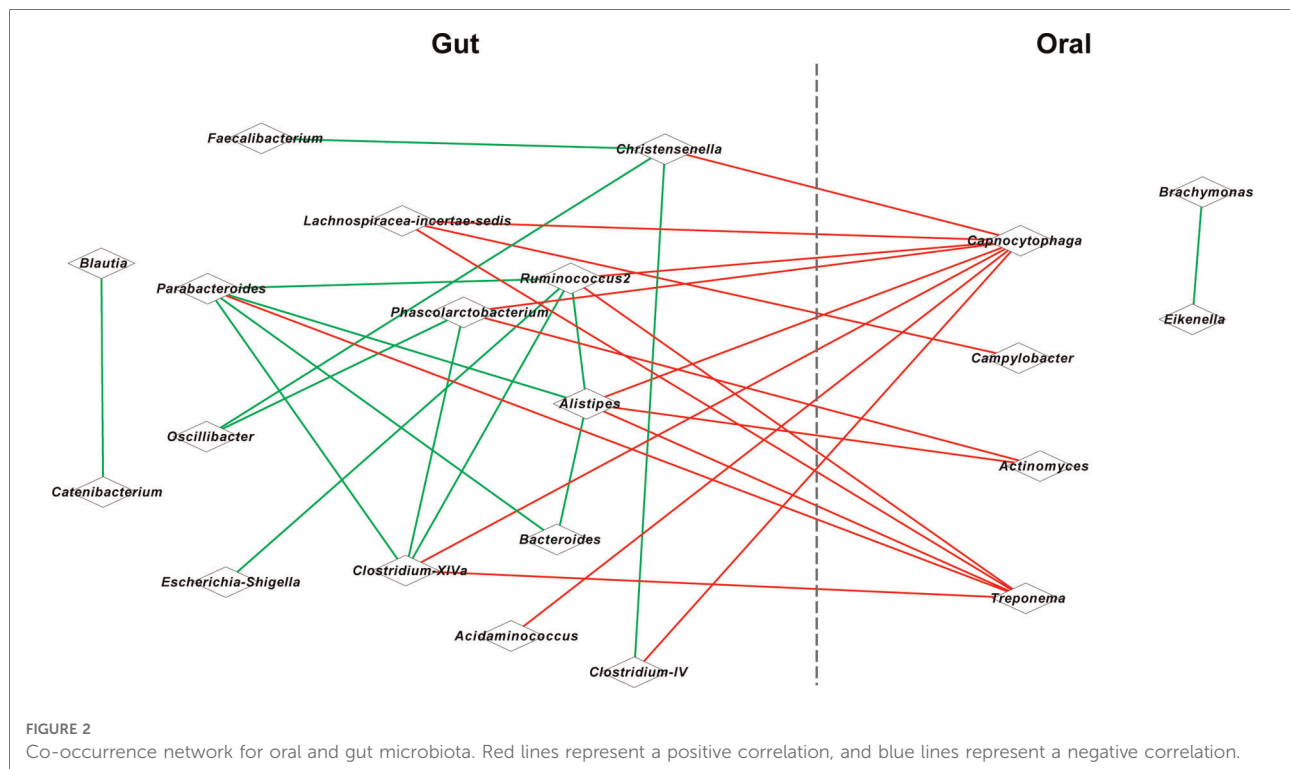
Gut microbiota			Oral microbiota		
Genera	Average (%)	SD (%)	Genera	Average (%)	SD (%)
<i>Bifidobacterium</i>	21.73	16.507	<i>Prevotella</i>	15.49	12.253
<i>Bacteroides</i>	9.991	10.265	<i>Fusobacterium</i>	9.34	7.831
<i>Prevotella</i>	8.718	14.52	<i>Neisseria</i>	7.68	11.812
<i>Parabacteroides</i>	4.7	4.989	<i>Actinomyces</i>	5.494	5.84
<i>Collinsella</i>	4.631	4.213	<i>Brachymonas</i>	3.881	6.797
<i>Megasphaera</i>	2.992	3.79	<i>Streptococcus</i>	3.408	4.339
<i>Clostridium_IV</i>	2.633	4.351	<i>Porphyromonas</i>	3.082	3.226
<i>Oscillibacter</i>	2.317	2.11	<i>Capnocytophaga</i>	3.005	4.481
<i>Lachnospiracea_incertae_sedis</i>	2.215	2.705	<i>Corynebacterium</i>	2.509	4.674
<i>Streptococcus</i>	2.09	3.337	<i>Campylobacter</i>	2.09	2.005
<i>Akkermansia</i>	1.896	2.422	<i>Veillonella</i>	1.669	2.424
<i>Alloprevotella</i>	1.837	5.099	<i>Rothia</i>	1.629	4.206
<i>Faecalibacterium</i>	1.802	2.757	<i>Bifidobacterium</i>	1.445	3.523
<i>Megamonas</i>	1.525	4.538	<i>Leptotrichia</i>	1.401	4.078
<i>Clostridium_XIVa</i>	1.396	1.055	<i>Treponema</i>	1.398	2.831



microbes to the gut, which thus can impact the functional network of the GM.

Increasing evidence has demonstrated the association of OM with dental health problems such as periodontitis and dental caries (21–23). Clinical studies have indicated that 86% of children with cerebral palsy (CP) had mild-to-moderate gingivitis (24) and 52.5%–81% had dental caries (25–27).

Similarly, we found 96.3% incidence of periodontitis and 81.48% incidence of gingivitis in these children, which can be partly explained by their impaired oral functions, such as occlusion and swallow (25–27). Studies have observed the association of oral health with OM, including *Porphyromonas*, *Treponema*, *F. nucleatum*, *Pseudoramibacter*, *Streptococcus*, *Prevotella* as well as lactic acid-producing bacteria (7, 8, 28–



34). Liu et al. found that the core caries-associated microbiota in CP children included *Prevotella*, *Alloprevotella*, *Actinomyces*, *Catonella*, and *Streptobacillus*, while *Capnocytophaga* and *Campylobacter* were dental health-associated microbiota in these children (35). In line with other studies, the present study identified the accumulation of *Prevotella*, *Fusobacterium*, *Neisseria*, *Actinomyces*, *Brachymonas*, *Streptococcus*, *Porphyromonas*, *Capnocytophaga*, and *Campylobacter* in the OM of CPE children (7, 8, 26, 27, 35).

Our studies have found that the abundance of *Bacteroides*, *Faecalibacterium*, *Lactobacillus*, *Ruminococcus*, *Roseburia* and other beneficial bacteria is significantly reduced in the gut of CP children, whereas the abundance of harmful bacteria such as *Streptococcus*, *Collinsella*, *Alistipes*, *Eggerthella*, *Enterococcus*, and *Veillonella* is significantly increased (13, 15). Neurodegenerative diseases were mainly attributed to *Streptococcus* (13). Moreover, we found that the abundance of *Bifidobacterium* was significantly increased in CP children who consumed liquid food, while the abundance of *Prevotella* increased significantly in CP children who ate normal food (15). Given the strong proof for the association of malnutrition with GM (36), this study also explored GM structures for enrolled children who had malnutrition (40.7% had intractable constipation). As previously reported (13, 15), in this study, we observed high levels of pathogens in the recruited children, including *Collinsella*, *Enterococcus*, and *Streptococcus*, and the levels of beneficial microbes such as

Bacteroides, *Prevotella*, *Roseburia*, and *Lactobacillus* were depleted (37, 38). These beneficial microbes can produce short-chain fatty acids that can function as nutrients, protect gut barriers, and alleviate inflammation (39). Therefore, this is the rationale to speculate the role of GM dysbiosis in malnutrition in our study.

In addition, we found that oral disease-associated microbial components are significantly associated with the gut components (7, 8, 25–28, 32–34), which correlated with the protection offered by gut barrier and mitigation of intestinal inflammation (15, 40–42). This suggested the translocation of disordered OM to the gut, which modulate the gut microenvironment and health.

In general, oral microbes are among the sources of GM, and the oral–gut transmission plays a major role in gastrointestinal health (19). As our previous study presented results similar to those of this study, we suspected that disordered OM used various mechanisms to affect gut microenvironments; gut dysfunction can impact gastrointestinal health and disease development. Our findings suggested the potential of probiotics/prebiotics intervention in modulating the OM and GM as well as improving health. However, the lack of healthy children and a small sample size limited the exploration of the association of OM/GM with the health of CPE children. Longitudinal data will also improve the understanding of mechanisms for the correlation between OM/GM and health.

Data availability statement

The dataset generated for this study is accessible from the NCBI sequence Archive (SRA) database with project number PRJNA853735.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of Longgang District Maternity and Child Healthcare Hospital of Shenzhen city with the registration number of LGFYXLL-024. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

CH and FW managed the project. CC, YP and NZ collected fecal samples and patients' information. CH and ZY prepared the DNA. CC and ZY were responsible for bioinformatics analysis in this work. JY and FW optimized the graph and statistical analysis. CH and FW explained the analysis results and wrote a paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fped.2022.988601/full#supplementary-material>.

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