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Associations of the Oral Microbiota with Obesity and Menarche in Inner City Girls

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Abstract

Objective: Alterations of the oral microbiome have been associated with obesity, possibly based on inflammatory processes mediated by bacteria. Specific bacterial strains have been associated with obesity and periodontal disease. Little is known about the oral microbiome in children. Understanding the relationship between oral health and childhood growth could help identify preventable factors contributing to obesity and related conditions, including onset of menarche which is associated with obesity.

Methods: In this pilot study, we investigated the saliva microbiome among 25 girls 7–15 years old (mean 11.1) and their mothers in an inner city dental clinic in New York City. The main outcome measures were body size, presence or absence of menarche and dental practices. We examined associations of microbiome richness, diversity, and relative abundance with pubertal and demographic factors and oral health.

Results: Girls had good dental health and a typical rich oral microbiome, based on the Shannon Index of all species detected. Older girls flossed more often and younger girls had more frequent dental check–ups. Microbiome richness among girls was similar to their mothers', but diversity was greater among mothers than girls. Richness was reduced among mothers with gum bleeding, flossing and increased teeth brushing. Overweight girls had greater diversity and less richness than normal weight girls. Certain bacterial species differed in abundance with respect to whether girls had reached menarche (*Flavobacteria, Actinobacteria*), overweight (*Megasphaera,*

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Conflict of Interest

The authors declare no conflict of interest.

Lactorbacillales, Lactobacillus) and gingivitis in the girls (*Scardovia, Bifidobacteriales, Gemellaceae*).

Conclusions: Differences found in specific bacteria in the oral microbiome were related to body size and menarche. With increasing interest on studying microbiome variability related to the multifactorial etiology of obesity in children, saliva is capable of providing clinically informative markers of this and related conditions.

Keywords

Oral microbiome; Diversity; Richness; Dental health; Puberty; Obesity

Introduction

Increasing evidence supports associations of the oral microbiome with several human health conditions, such as cardiovascular diseases [1] and obesity [2–4]. Specific bacteria and low overall diversity of oral microbial organisms have been linked to common oral diseases, dental caries and periodontal disease [5]. It is thought that the microbiome may act through inflammation and immune response, as both conditions are related to obesity as well as dental disorders of periodontitis, caries, and plaque [6,7]. Several studies in both animals and humans have found differences of important bacterial species in the gut microbiota between obese and normal weight children and adults [8–10]. For the oral microbiome, less is known about its relationship with obesity, although specific bacteria have also been found to vary with obesity in both adolescents and adults [4,11]. The composition of the oral microbiome is affected by the endogenous hormone milieu [12]; after the onset of puberty there appears to be a shift in the composition and abundance of certain bacteria of the oral microbiome including black pigmented bacteroides, especially *B. intermedius* [13,14] Obesity is a major risk factor for early puberty [15], yet the relationship among obesity, puberty and oral microbiome abundance and diversity remains unclear.

Imbalance of the gut microbiota has been suggested as an important component in the pathogenesis of obesity [8–10]. Thus, body mass index (BMI) differed significantly with respect to the proportion of Campylobacter rectus and Neisseria mucosa, Tannerella forsythia in the subgingival biofilm with greater proportions among obese than normal weight adults and adolescents [4,10,11,16].

Differences are mostly detected at the phylum level, particularly *firmicutes* and *bacteriodes.* Variations at the genus level have been less informative [17]. Given these identified associations of obesity with gut microbiome, and the inflammatory changes in the oral cavity seen in obesity, it may be useful to investigate the dominant bacterial phyla (i.e., *firmicutes, bacteriodes*) of the mouth, as the saliva microbiome is easily accessible.

Based on these lines of evidence, we hypothesized that oral microbiota in adolescent girls would vary in diversity and richness by age at menarche and body size. We undertook an exploratory study in an inner city dental clinic to investigate oral microbiome variability in girls and their mothers. The focus on girls was motivated by our ongoing research on environment exposures and girls' pubertal development.

Materials and Methods

Study population

Potential study participants were identified at the Dental Clinic at the Mount Sinai Hospital Department of Dentistry. Initial contact was by telephone or in the waiting room on the day of their dental appointment. The project was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai. Guardians provided informed consent and verified child assent. Girls and their female guardian were recruited as study participants.

We recruited mothers because family members are known to share microbiota, and in particular the mother is the primary source of her children's oral microbial profile [18–20]. To be eligible for participation, girls had to be between 10 and 17 years of age, have a parent accompany them at the dental visit and have no medical conditions that would affect their dental health or immune system (e.g., cancer).

We identified 74 potentially eligible girls and after initial screening, 6 were found to be ineligible, 34 did not show up for their appointment, and 9 were not interested in participating.

The final study population included 25 eligible girls and 25 guardians; four girls were accompanied by either their father (n=2) or their female legal guardian (n=2) who was not the biological mother while the remaining 21 girls were accompanied by their biological mother. Interviews were conducted in person in either English or Spanish with girls (n=25) and their female guardians (n=23). Height and weight were measured for both girls and their biological mothers using calibrated scales and stadiometers using a standard protocol for children as described previously [15].

Questionnaire and anthropometrics

The female guardian and all girls completed a brief questionnaire to ascertain demographics, dental practices and dental health, including presence of gum disease, bleeding when brushing and the presence of menarche and certain medical conditions. Guardians identified the girls as black, white, Asian, Hispanic or other. Socioeconomic status was represented by the highest attained education level of the primary caregiver (≤high school diploma *vs.* some college or greater). Girls were asked whether they had had their first menstrual period. BMI was calculated as weight in kg/height (in cm)-squared; girls were classified as normal weight ($<85^{th}$ national percentile, age- and sex-specific) or overweight ($\geq 85^{th}$ percentile) based on CDC growth charts [21].

Dental exam

Dental health and habits were assessed using standardized questions [22]. An abbreviated dental exam was performed on girls by a dentist in the clinic using a dental probe and mouth mirror. The exam consisted of counting the number of decayed, missing and filled permanent teeth in a child's mouth (DMFT index), and an assessment of gingival health in accordance with the World Health Organization recommendations (www.who.int/oral_health/publications/en/). Caries and existing restorations were cross-checked after the

exam with bitewing and panoramic radiography from their Mount Sinai Clinic dental record. Missing teeth due to caries were assigned when clinic records indicated removal and congenitally missing teeth were excluded from the count. For statistical analysis, gingival inflammation was categorized as healthy, gingivitis, or periodontal disease by visual examination. Gingival status of guardians was self-reported.

Saliva microbiome profiling

The oral microbiome analyses were restricted to girls (n=25) and their biological mothers (n=21). A saliva sample (~2 mL) was collected before the dental exam using the OMNIgene•DISCOVER saliva sample collection kit (DNA Genotek Inc., Canada) following manufacturer's instruction. Samples and microbial DNA from this kit are stable for long-term room temperature storage. Total microbial DNA from saliva was extracted using Qiagen blood mini kit (Qiagen, Germantown, MD, USA) and stored at −20°C.

We characterized the oral microbiome by standard taxonomic classification criteria using 16S rRNA gene sequences to group "species-level" phylotypes and to further classify bacteria within phylum-to-genus levels. We conducted 16s sequencing of extracted microbial DNA on 2 × 250 pair-end MiSeq sequencing platform. Phylogenetically informative v3-v4 regions of bacterial 16S ribosomal RNA (rRNA) genes were amplified using universal primer 347F/803R from 46 saliva DNA samples (25 girls and 21 biological mothers).

The PCR amplicons contain a unique dual 6-mer barcode combination attached on the 5'ends of both forward and reverse PCR primers for each sample. The primers were synthesized by IDT (Integrate DNA technology, IA); sequences are provided in Table 1.

The amplicons were pooled with equal molarity and submitted for Illumina Miseq 2 × 300 pair-end sequencing at high depth. The paired sequence readings were merged and filtered by size (>400 bp) and quality score (>Q30) using CLC genomics workbench version 6. The processed readings were further split by dual barcode for each sample and assigned taxonomic classification using QIIME pipeline 1.7.0 [22]. Triplicate measurements of two samples and duplicate measurements of one sample were made to verify the sequencing reproducibility. The 16S sequencing yielded 7,818,631 merged pair-end reads, of which 5,398,842 passed standard size and quality requirements.

After sorting the final readings to obtain 46 individual results, we obtained on average 63,235 reads (range, 659 to 143,605) per sample. We further assigned taxonomic classification to the filtered sequence reads and determined the saliva microbiome composition. After processing, QIIME provided OTU tables containing the microbiome composition and abundance for each individual sample.

Data analysis

Our approach was to first characterize the oral microbiome of the overall population and then compare mothers to daughters. Then we used similar approaches to assess differences between normal weight and overweight girls, our main outcome, as well as other characteristics. Several approaches were used to characterize the oral microbiome.

Two of the measures used to compare the oral microbiome between individuals are richness and diversity. Richness quantifies how many different types of species are detected in a sample (person), but does not account for abundance. We measured richness of the bacterial community, also called genus alpha-diversity, within each saliva sample, using the Shannon Index [23,24].

Diversity of microbes within a given sample (person) can be defined as the number and the abundance of distinct types of organisms. We summarized the relative abundance of the saliva oral microbiome by genus and species in all subjects combined. Student's t-test was used to assess differences in the Shannon Index by various characteristics and for the *firmicutes-to-bacteriodetes* ratio by normal and overweight status. Next, the beta-diversity, which is the overall structural difference between individual microbiomes at the genus level, was assessed using the weighted Unifrac distance matrices, a phylogeny-based distance metric ranging from 0 (identical bacterial communities) to 1 (totally different) that was visualized by non-metric multiple dimensional scaling (nMDS) method.

Smaller distances indicate a higher degree of similarity among taxa and their relative abundances. The PerMANOVA test [25,26] with the maximum number of permutations=999, was performed using the [Adonis] function of the R package vegan 2.0–5 [27] to test how the beta-diversity varied by different characteristics including race, BMI, menarcheal status, dental practices (flossing, brushing), DMFT index (none *vs.* 1+) and gingival status.

We used the linear discriminant analysis effect size (LEfSe) method [28] to compare the microbiome composition and abundance at various taxonomic ranks from phylum to genus. Finally taxa features that differed by characteristics were selected using the random forest algorithm in Rpackage rfPermute and were confirmed by Boruta feature selection (R package Boruta).

We considered comparisons with $p<0.05$ to be significant and those where $0.05 \leq p<0.1$ to have borderline significance, so that potentially with more subjects the association would approach significance.

Results

Population characteristics

We obtained demographic and dental information on 23 female mothers or guardians (21 biologic and 2 female guardians) and daughters in this pilot study, including 21 biological mother/daughter pairs. The mean age of the girls was 11.1 (SD 1.9) years and 39.2 (SD 8.1) years for mothers (Table 1).

Most participants were Hispanic (71%), while 19% were Black and 10% reported another race. More than 70% of parents had at least a high school education. Girls received good dental care, as almost all children (96%) reported going to the dentist at least once a year, and 76% went twice a year for preventive visits (Tables 2 and 3).

All girls reported brushing at least once a day, and 76% reported more than once a day. However, only 52% reported flossing. The majority of the mothers brushed their teeth more than once a day (96%), reported flossing (61%) and having dental checkups at least once a year (70%). Few dental practices differed by age, race/ethnicity, BMI, or menarche for girls or mothers. Older girls flossed more often and had more frequent dental check-ups. Twelve girls had gingival disease by visual inspection whereas only two mothers reported having gum disease. The DMFT index (number of caries) was 1 or greater for 18/25 (72%) of the girls. Five dominant bacterial phyla were observed: *Actinobacteria* (6.8%), *Bacteroidetes* (27.4%), *Firmicutes* (29.8%) *Fusobacteria* (10.8%) and *Proteobacteria* (24.3%).

Characteristics of the microbiome of mothers and girls

The saliva oral microbiome was varied in the total sample of 46 mothers and daughters combined (Table 4). Richness of the saliva microbiome was similar among the girls and their mothers (mean Shannon index: 3.6 and 3.7, respectively; p=0.15) (Table 5). There was a wider range of the Shannon index in girls, (IQR=0.27) than mothers (IQR=0.13) (data not shown). Bacterial richness was lower among obese/overweight girls compared to normal weight girls, although only of borderline significance (p=0.08) (Table 5).

Richness was also reduced among mothers with gum bleeding, flossing and teeth brushing >1/day but not in girls. Diversity (beta-diversity, i.e., difference between individual microbiomes) was significantly greater for the 21 mothers than for the 23 daughters (p=0.021) (Figure 1).

We further assessed beta-diversity in children by several characteristics using NMDS plots. Overweight girls were found to have more diverse microbiomes than the normal-weight girls (p=.058) (Figure 2). When comparing normal-weight to overweight/obese subjects, we did not find significant differences in the *Firmicutes* to *Bacteroidetes* ratio in saliva samples for either girls or their mothers (p-value=0.47, 0.84, respectively by t test) (Data not shown).

Comparisons among mothers and daughters

Figure 3 shows the comparison of the number of microbiota from phylum to genus level between children and mothers. This Lefse analysis exhibited increased *Corynebacterium* in girls and in mothers, enrichment of *Porphyromonas, Tannerella, Atopobium, Fusobacterium, Campylobacter* and *Aggregatibacter.*

Comparisons of taxa by certain characteristics

Among girls, we observed differences in certain taxa features by body size (overweight vs normal weight), menarche and gingivitis (Figure 4). In post-menarcheal compared with pre-menarcheal girls, the relative abundance of *Flavobacteria* class, an unclassified genus in *Gammaproteobacteria* class and *Pseudomonadaceae,* were decreased while *Actinobacteria* phylum and *Mocrococcacea* family and its Rothia genus were increased. In obese/overweight children, relative abundance of *Megasphaera* and *Lactobacillales* order was increased and *CW040* order and *Lactobacillus* genus were decreased. In addition, girls with gingivitis showed increased relative abundance in the *Scardovia* genus and *Bifidobacteriales* order and decreased *Gemellaceae* family.

Discussion

The complex equilibrium between the diversity and relative proportion of species or taxa within the oral cavity maintains a healthy microbiome. Richness and diversity are desirable in the human microbiome, as they have been associated with better overall and dental health. The oral microbiome is only slightly less developed than the gut, which has been studied more extensively. Our analyses compared the diversity and richness by certain lifestyle, dental and biologic characteristics in girls and their biological mothers.

The oral microbiomes of girls and mothers in this study were diverse and exhibited a rich microbial environment, similar to previous reports of an ethnically diverse population of adults where the Shannon index ranged from 2 to 4 [29]. Microbiome richness was similar among mothers and their daughters; however, richness was lower among overweight than normal weight girls, while diversity was greater. Some studies have shown the oral microbiome of young children to be less complex than those of adults, with the number of different species detected increasing with child age [30–32], whereas others found the oral microbiome to vary most during childhood, when contact with external microbes is highest [33]. We observed differences in diversity between mothers and girls and in the Lefse analysis of variability, where mothers had more enriched taxa than girls (five *vs* two). It has been established that cohabitating individuals share bacterial biota; however, in many of these studies, the cohabitating individuals also may have had genetic relationships [34].

Obesity has been associated with periodontal disease and with altered oral microbiome. The inter-relationship between hormones, obesity and the oral microbiome is not well understood. Puberty is time when changes occur in the subgingival microbiome leading to a microbial profile that is similar to young adults. Studies report differences in bacteria of the oral microbiome associated with periodontal diseases in pre and post-pubertal children [14,35,36]. Girls in our study were on average about halfway through puberty, as the window spans about 9–15 years of age. The presence of gingivitis is a determinant of microbial changes so it may contribute to our findings since gingivitis had a prevalence of 50% in our population. Although richness and diversity were similar by menarche and by gingivitis in our study, differences were seen at the taxa level for several common bacteria. Overweight girls in our study had fewer taxa within *Lactobacillus* species but more in *Lactobacillales* order. Several studies [37–39] show that an increase of probiotics, specifically certain strains of gut *Lactobacillus* to be associated with weight loss in both human and animal studies. However, other studies reported the opposite. For instance, higher concentrations of *Lactobacillus* species have been observed in the gut microbiota of obese children [8] this report also found that obese children had an elevated *Firmicutes-to-Bacteroidetes* ratio compared to lean children. Evidence regarding a phylum level association between gut *Firmicutes*-to-*Bacteroidetes* ratios and BMI has been contradictory [40,41]. Several studies of the gut microbiome have found obese subjects have more *Firmicutes* and relatively less *Bacterioidetes* than normal weight adults [9,10,42] and children [8,43] whereas we and others [44,45] report no difference in the ratio of *Firmicute-to-Bacterioidetes* in either gut or oral microbiome.

There is increasing interest about the impact of human microbiota on human health, specifically, studying microbiome variability related to the multifactorial etiology of obesity in children [8,11]. A bi-directional pathway linking obesity and oral disease has been proposed. Central to this pathway is the role of altered inflammation which is a key feature of both obesity and dental microbial diseases such as periodontitis [6,7]. In an animal model, obesity has been reported to interfere with the ability of the immune system to appropriately respond to infection by the periodontal pathogen *Porphyromonas gingivalis* [46]. Fat is recognized as a reservoir for inflammatory cytokines, and it has been suggested that obesity likely affects periodontal disease through this pathway [47]. Lack of diversity in the gut microbiome has been associated with obesity [48]. Several mechanisms have been proposed including triggering inflammation, altering metabolic efficiency and promoting fat deposition [33,49]. Murphy reported increased dietary energy harvest, attributed to alterations in the gut microbiota (increased *Firmicutes* and decreased *Bacteroidetes),* suggesting that the obese microbiome possesses metabolic pathways that are highly efficient at extracting energy from food. Recent evidence suggests that gut microbiota is involved in energy regulation, and this should play a role in the pathophysiology of obesity [50,51]. The oral microbiota have been reported to differ between normal and overweight/obese children and adults [4,11] and in animal studies [52], although the direction is unclear. Differences in studies could be related to age population age differences and the fact that the oral microbiome is an open system and frequently exposed to exogenous bacteria in food, water and air. Data suggest that genetic factors could contribute to the difference in bacterial colonization in obese and normal weight adolescents and children [49,53–56].

Limitations

This pilot study with a small sample size limited our ability to see associations. The study was intended to test proof of concept to investigate oral health, microbiome, and health effects in a dental clinic. Our study population was recruited from an urban dental clinic with most girls being Hispanic or Black; therefore results are not generalizable to all girls. Another potential weakness is whether a single sample of the salivary microbiome provides a reasonable representation of a person's microbiome. Research is limited on temporal changes and factors that can influence one's oral microbiome. The relationship between the oral microbiome and its host is dynamic and in a healthy mouth, the composition of microbial communities appears to be relatively stable (after childhood) over time. Biological changes in a person's life such as pregnancy and puberty can affect the balance of the species, and lifestyle factors such as poor hygiene and smoking can also affect the balance.

Conclusion

Our study provides novel data on the association of health conditions with the oral microbiome in a multi-ethnic sample and how associations differ between mothers and daughters. We also observed new genus level taxonomic signatures of *Lactobacillales* obtained from the oral microbiota associated with obesity in children. Our data confirm the importance of genus level taxonomic classification in understanding patterns of microbiome alterations in disease and provides evidence that these alterations can be investigated using salivary samples from the human oral microbiome. Our study is consistent with Malamud

and colleagues who reported that saliva is capable of providing clinically informative markers of disease and is advantageous due to its non-invasive collection and storage.

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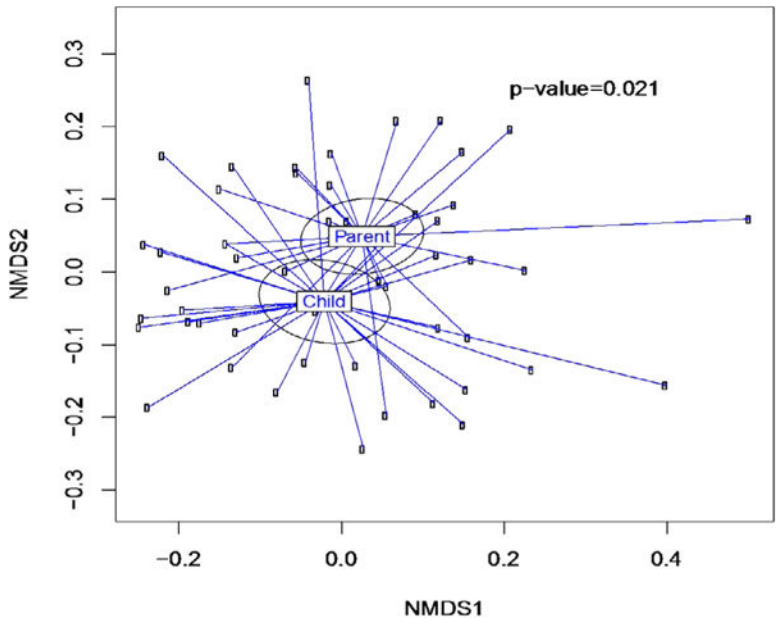

Figure 1.

The Beta-diversity of overall salivary microbiome of girls (n=21) and their mothers (n=21). Mothers had greater diversity than their daughters (p=.021). The weighted Unifrac distance matrices generated from taxa composition and relative abundance at genus level were visualized in nMDS plot. Ellipses were drawn to represent the standard error. The significance of the dissimilarity of overall microbiota between two groups was tested using PerMANOVA

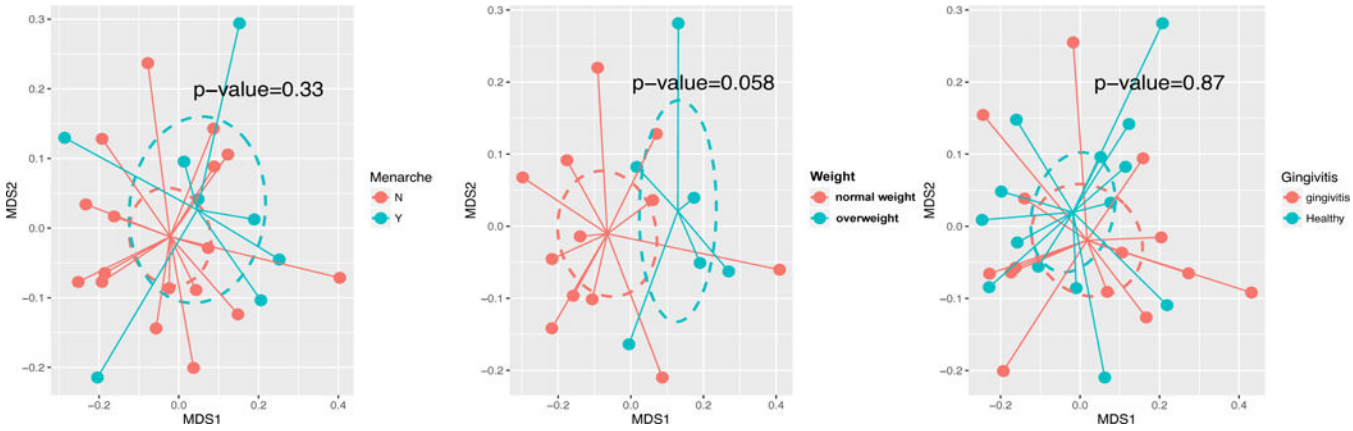

Figure 2.

Diversity of the salivary microbiome in girls by different health characteristics. Weighted Unifrac distance matrices of the girls' salivary microbiome data at genus level were visualized using nMDS plot. Diversity was greater among the overweight girls (n=21 p=. 058). Ellipses were drawn to represent the standard error. The significance of the dissimilarity of overall microbiota between those with and without health characteristics was tested using PerMANOVA.

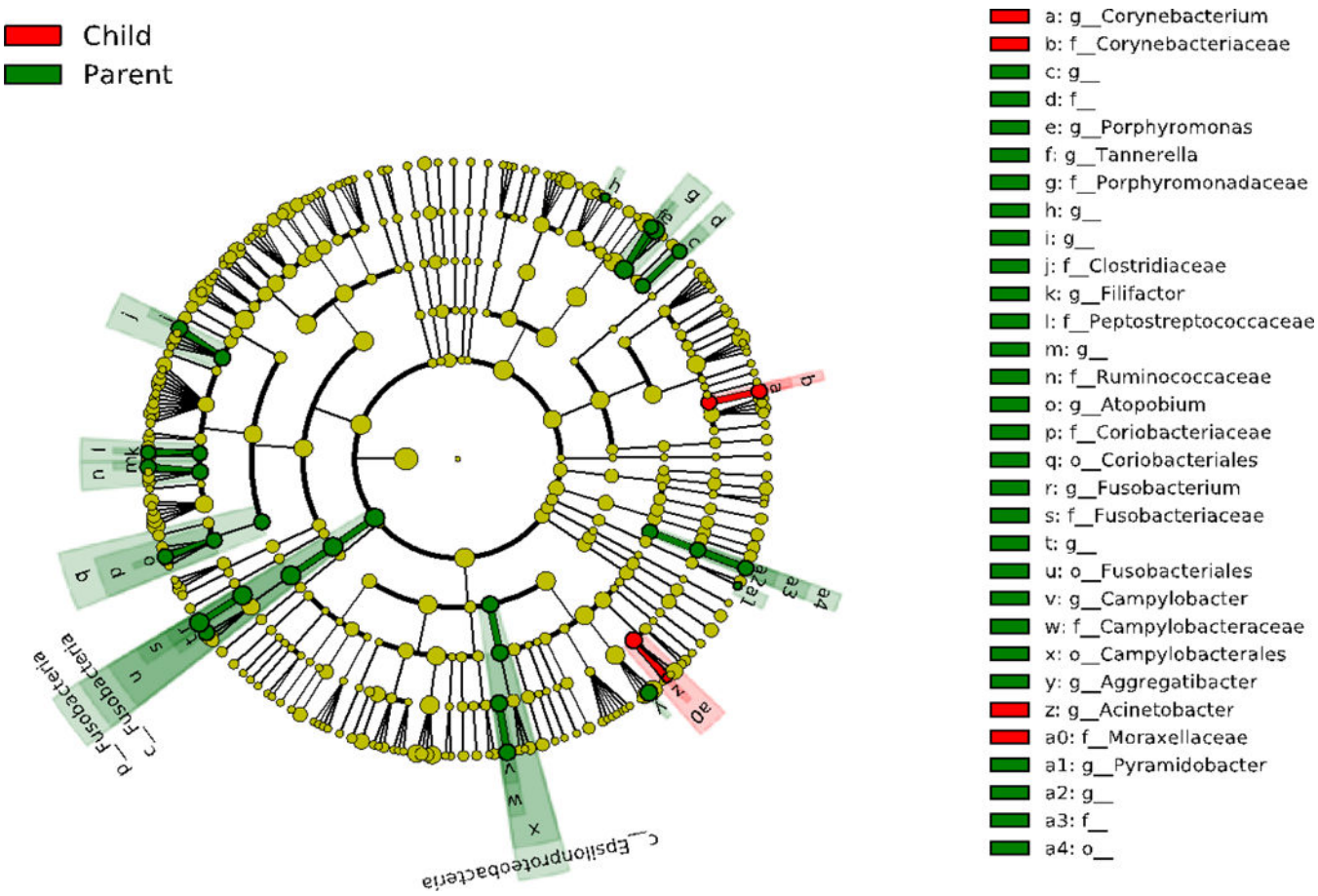

Figure 3.

Differential salivary microbiome composition of girls (n=25) and mothers (n=21). Cladogram plots compare the Lefse results from the salivary microbiome of children vs. mothers. Differences are represented in the color for the most abundant class (red indicating enrichment in girls, green indicating enrichment in parents). Each circle's diameter is proportional to the taxon's abundance. In girls, we observed enrichment of *Corynebacterium* and in mothers, enrichment of *Porphyromonas, Tannerella, Atopobium, Fusobacterium, Campylobacter* and *Aggregatibacter*

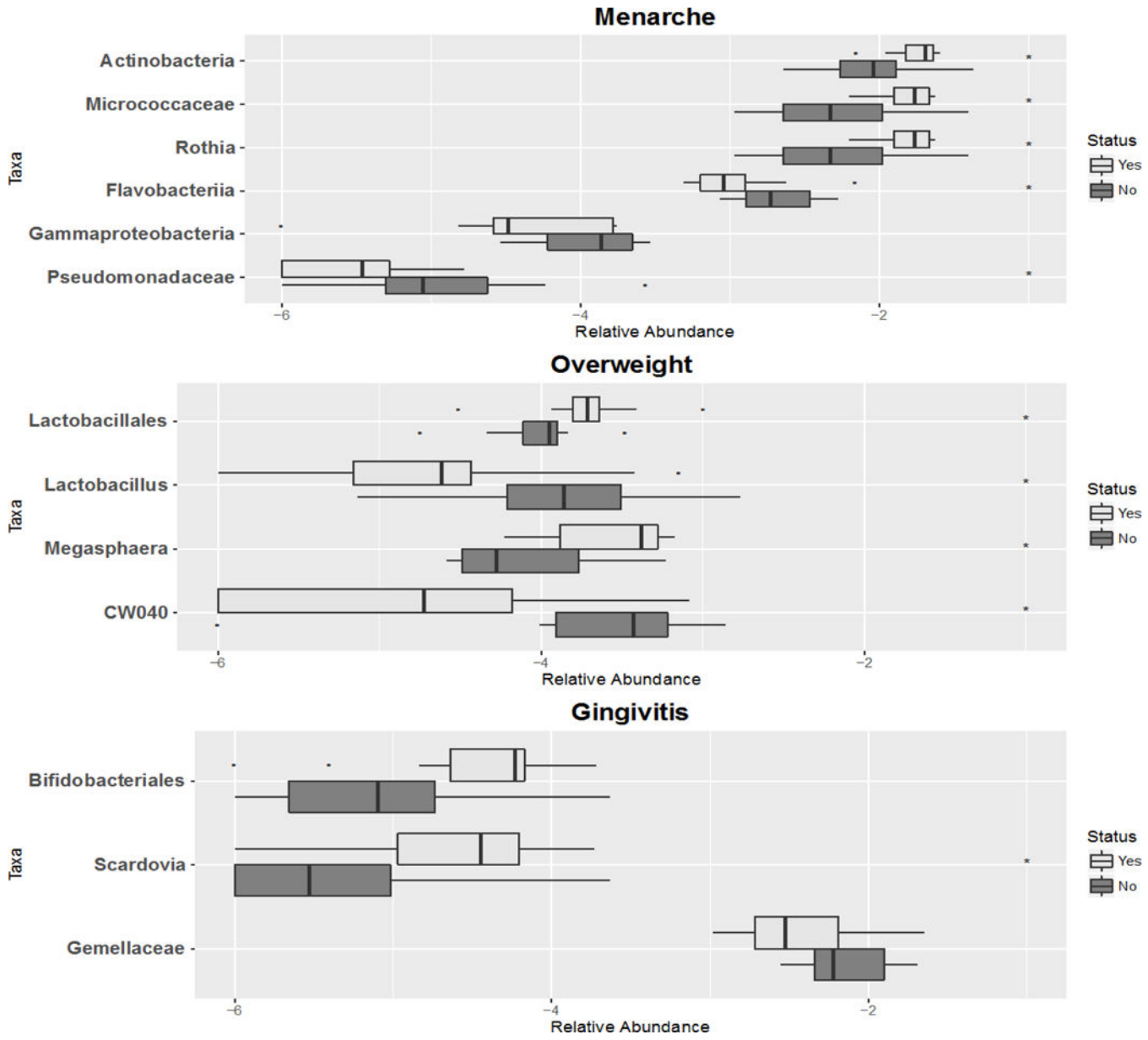

Figure 4.

Abundance of selected taxa features selected from phylum to genus level compared by Status of girls' Menarche, Overweight or Gingivitis (n=21). The boxplots show the median and the interquartile range of the relative abundance of selected taxa. Taxa were selected using random forest algorithm. Taxa that differed (p-value<0.05) are annotated with asterisk symbols.

Table 1

Demographics and anthropometric characteristics of participants in dental pilot MSSM 2013.

Characteristic	Girl (n=25)	Mother (n=23)*
	Mean ± sd or N (%)	Mean ± sd or N (%)
Age (yrs)	11.1 ± 1.9	39.2 ± 8.1
Range	7–15	28–63
Race		
Hispanic	17 (68%)	17 (74%)
Black	5 (20%)	4 (19%)
Other	3 (12%)	2 (10%)
Parental Education		
>High school	--	16 (70%)
Weight (kgs)	n=24	n=19
	50.0 ± 19.5	77.0 ± 16.0
Height (cm)	151.5 ±12.9	161.1 ± 8.8
[a]**Child BMI%**		
Normal (50–85%)	10 (53%)	--
Overweight (85–95%)	3 (16%)	--
Obese (>95%)	6 (32%)	--
[a]**Maternal BMI**		
Normal (18.5–24.9)	--	3 (17%)
Overweight (25–29.9)	--	7 (39%)
Obese (>30)	--	8 (44%)
Girl's Menarche	n=23	--
Yes	8 (35%)	--
No	15 (65%)	

[a]Note: BMI% is the standard measure used in children and is age-sex specific (CDC 2000); BMI is used in adults.

*25 guardians: Information on 23 mothers; there is no information on 2 fathers who accompanied daughters.

Table 2

Dental health indicators by demographic characteristics, MSSM dental study, 2013 (Daughters).

Daughters									
Dental Indicators		**N**	**Age (yrs)**	**BMI%-ile**	**RACE**			**Menarche**	
					Hispanic	**Black**	**Other**	**Yes**	**No**
			Mean (std)	**Median (IQR)**	**Proportion (N)**			**Proportion (N)**	
Brushing teeth	1/day	9	10.9 (1.5)	93.2 (31.2–97.8)	0.67 (6)	0.33 (3)	0.00 (0)	0.44 (4)	0.44 (4)
	>1/day	16	11.3 (2.1)	76.5 (73.6–88.7)	0.69 (11)	0.13 (2)	0.19 (3)	0.25 (4)	0.69 (11)
Flossing	Never	12	10.2 (1.5)*	76.4 (31.2–88.7)	0.67 (8)	0.17 (2)	0.17 (2)	0.17 (2)	0.67 (8)
	Ever	13	12.0 (1.8)	87.6 (75.9–97.6)	0.69 (9)	0.23 (3)	0.08 (1)	0.46 (6)	0.54 (7)
Dental Checkups	≤1/year	6	12.3 (1.9)**	96.9 (75.9–97.6)	0.50 (3)	0.33 (2)	0.17 (1)	0.67 (4)	0.33 (2)
	>1/year	19	10.7 (1.8)	76.5 (41.2–88.7)	0.70 (14)	0.16 (3)	0.11 (2)	0.21 (4)	0.68 (13)
DMFTa	None	7	11.7 (2.1)	91.3 (76.5–97.6)	1.00 (7)	0.00 (0)	0.00 (0)	0.43 (3)	0.43 (3)
	1+	18	10.9 (1.8)	76.4 (36.0 −88.8)	0.56 (10)	0.28 (5)	0.17 (3)**	0.28 (5)	0.67 (12)
Gums bleed	No	20	11.3 (2.1)	83.4 (35.9–96.3)	0.63 (12)	0.26 (5)	0.11 (2)	0.37 (7)	0.53 (10)
	Yes	5	11.0 (1.0)	87.0 (58.8–98.0)	0.80 (4)	0.00 (0)	0.20 (1)	0.20 (1)	0.80 (4)
Gingival Status	Healthy	12	11.1 (1.7)	76.5 (35.9–97.8)	0.67 (8)	0.17 (2)	0.17 (2)	0.33 (4)	0.50 (6)
	Gingivitis	12	11.2 (2.2)	84.9 (58–6-92.5)	0.67 (8)	0.25 (3)	0.08 (1)	0.33 (4)	0.67 (8)

Table 3

Dental health indicators by demographic characteristics, MSSM dental study, 2013 (Mothers).

Mothers							
				BMI	Race		
Dental Indicators		**N**	**Age (yrs) Mean (std)**	**Median (IQR)**	**Hispanic**	**Black**	**Other**
Mothers Over all		23	39.2 (8.1)	29.1 (27.4–31.4)	0.68 (17)	0.16 (4)	0.09 (2)
Brush Teeth	1/day	1	38	28.7	1.00 (1)	0.00 (0)	0.00 (0)
	>1/day	22	39.2 (8.3)	29.4 (27.4–31.4)	0.73 (16)	0.18 (4)	0.09 (2)
Flossing	Never	9	37.9 (8.8)	28.7 (23.4–31.4)	0.78 (7)	0.11 (1)	0.11 (1)
	Ever	14	40.0 (7.9)	29.4 (27.8–31.4)	0.71 (10)	0.21 (3)	0.07 (1)
Dental Checkups	Never	7	35.1 (4.5)	28.8 (28.0–29.4)	0.86 (6)	0.14(1)	0.00 (0)
	≤1/year	7	42.1 (6.6)	32.4 (28.2–35.2)	0.67 (6)	0.22 (2)	0.11 (1)
	>1/year	9	39.4 (11.5)	27.4 (24.6–30.9)	0.71 (5)	0.14 (1)	0.14(1)
Gums Bleed	No	19	39.2 (8.7)	28.8 (27.4–31.4)	0.68 (13)	0.21 (4)	0.11 (2)
	Yes	4	39.0 (4.9)	31.4 (24.6–33.3)	1.00 (4)	0.00 (0)	0.00 (0)
Dx Gum Disease	No	21	39.4 (8.4)	29.7 (27.6–32.4)	0.71 (15)	0.19 (0)	0.10(2)
	Yes	2	37.0 (5.7)	27.0 (24.7–29.4)	1.00 (2)	0.00 (0)	0.00 (0)

[a]Note: DMFT is the number of decayed, missing and filled permanent teeth in a child's mouth determined by oral exam by the dentist.

*Overall significant ($p<0.05$) by t-test, Wilcoxon or Kruskal-Wallis

**$p<0.10$

Table 4

Phylogenetic distribution of 282 taxa (bacterial strains) detected in 46 study participants in MSSM dental study, 2013.

Bacterium Phylum	Assigned Phyla (N (%))[a]	Published, Identified species[b]
Firmicutes	107 (37.9%)	38 (41.8%)
Bacteroidetes	39 (13.8%)	15 (16.5%)
Proteobacteria	72 (25.5%)	19 (20.9%)
Actinobacteria	33 (11.7%)	13 (14.3%)
Fusobacteria	6 (2.1%)	2 (2.3%)
Tenericutes	5 (1.8%)	0
Spirochaetes	4 (1.4%)	2 (2.3%)
TM7	4 (1.4%)	0
Cyanobacteria	3 (1.1%)	0
Synergistetes	2 (0.7%)	1 (1.1%)
Elusimicrobia	2 (0.7%)	0
Chlorobi	1 (0.4%)	0
Chloroflexi	1 (0.4%)	0
Deferribacteres	1 (0.4%)	1 (1.1%)
SR1	1 (0.4%)	0
GN02	1 (0.4%)	0
Total detected	282 (100%)	91 (32.3%)

[a]Note: Phyla refer to named species (both phyla and species) and to unidentified phylotypes at species level. Phylotypes are defined as clusters whose members have 97% full 16s rRNA sequence similarity. The data in this table are based on those in HOMD version 10.

[b]Named species are those with validly published names.

Table 5

Comparison of bacterial community richness and overall microbiome dissimilarity for selected dental and health characteristics in both children and mothers. MSSM Dental Study, 2013.

Children			
Characteristic	**Bacterial Community Richness (Shannon H index[a], mean+SD) by Characteristics**		
	Yes	**No**	**p-value[b]**
Health status			
Gingivitis	3.6 ± 0.3	3.7 ± 0.1	0.18
Gum bleed[c]	3.8 ± 0.2	3.6 ± 0.2	0.11
Overweight	3.5 ± 0.2	3.7 ± 0.2	0.08
Oral hygiene			
Flossing	3.6 ± 0.2	3.7 ± 0.2	0.34
Brush teeth[d]	3.6 ± 0.2	3.6 ± 0.2	0.67
Development			
Menarche	3.6 ± 0.2	3.6 ± 0.2	0.55
Mothers			
Characteristic	**Bacterial Community Richness (Shannon H index[a], mean+SD)**		
	Yes	**No**	**p-value[b]**
Health status			
Gum bleed[c]	3.8 ± 0.04	3.7 ± 0.2	0.07
Overweight	3.6 ± 0.2	3.8 ± 0.1	0.14
Oral hygiene			
Flossing	3.7 ± 0.2	3.8 ± 0.1	0.05
Brush teeth[d]	3.7 ± 0.002	3.8 ± 0.2	0.02

[a]Note: .H=The proportion of genus (i) relative to the total number of genus (pi) is calculated, and then multiplied by the natural logarithm of this proportion (lnpi). The resulting product is summed across genus, and multiplied by −1

[b].Shannon Index differences were tested using the Student t-test and the UNifrac distance by PerMANOVA.

[c].Bleeding while brushing, at least 3x per week.

[d].Brush teeth ≥ 1/ day