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Changes in Dental Plaque Microbial Richness and Oral Behavioral Habits during Caries Development in Young Chinese Children

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Key Words
Children · Denaturing gradient gel electrophoresis · Dental plaque · Longitudinal study · Microbial richness

Abstract
Objective: To detect changes in the microbial richness of dental plaque and oral behaviors during caries development in young Chinese children. ***Methods:*** Supragingival plaque samples and a survey of oral behaviors of 130 children aged 3 at baseline were analyzed at 6 months and 12 months. Total DNA was isolated from all samples and PCR-denaturing gradient gel electrophoresis analysis was conducted. ***Results:*** In the follow-up, 44 children had caries or cavity fillings at 6 months, a further 28 children had caries or cavity fillings at 12 months. The other 58 children remained caries-free at 12 months. According to the changes in caries status at the 12-month follow-up, all participants were divided into three groups: caries-free, caries at 6 months and caries at 12 months. The changes in oral behaviors during the 12-month follow-up were not significantly different in the three groups. The frequency of eating sweets and eating sweets before sleeping was significantly different among the three groups at baseline. At baseline, the average detectable bands of caries in the 12-month caries group were similar to those of the caries-free group; both of them were higher than that of the 6-month caries group. At 6 months, the average detectable bands of the 12-month caries group were significantly lower than that of the caries-free group although the children of the 12-month caries group were caries-free at that time. ***Conclusions:*** For young Chinese children, the high frequency of eating sweets and eating sweets before sleeping are risk factors of caries onset, and the decrease in microbial richness could occur 6 months before the onset of caries.

As a multifactorial disease, physical, biological, environmental, behavioral and lifestyle-related factors may contribute to caries initiation and development [Selwitz et al., 2007; Zhou et al., 2011]. Among these, cariogenic dental plaque is the etiologic agent of caries [Filoche et al., 2010; Kanasi et al., 2010a]. Mutans streptococci (mainly *Streptococcus mutans* and *Streptococcus sobrinus*) are the most frequently isolated microbial group from human cariogenic dental plaque, which is involved in caries onset and development [van Houte et al., 1994]. However, studies indicate that dental caries is caused by potentially pathogenic microbial communities rather than a single pathogen [Jenkinson and Lamont, 2005; Kuramitsu et al., 2007].

The formation of dental plaque in a healthy individual as a result of an ordered pattern of colonization by a range of bacteria forms the basis of the ecological plaque hypothesis

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[Marsh, 2003]. The composition of the resident microflora remains relatively stable over time following the establishment of a dynamic balance [Marsh, 2003]. However, loss of this balance can result in the outgrowth of pathogenic bacteria. For example, an increased prevalence of acidogenic and aciduric bacteria, which rapidly metabolize dietary sugars to acid, leads to a reduction in pH and demineralization of the enamel [Kuramitsu et al., 2007]. Therefore, a study of the pathogenic theory from a microbial community perspective is required to more fully elucidate the role of bacteria in the initiation and development of caries.

Microbial factors are essential but not adequate for developing clinical disease, which is aggravated by dietary factors [Kanasi et al., 2010b]. Diet also contributes to caries development [Palmer et al., 2010]. Dietary factors including a high frequency of consuming sugary snacks or beverages [Thitasomakul et al., 2009; Warren et al., 2008], poor dietary habits [Palmer et al., 2010], irregular feeding practices [Feldens et al., 2010b; Arora et al., 2011], nutritional problems [Feldens et al., 2010a], and undesirable oral hygiene [Hashim et al., 2013] were found to be relevant to caries in children. The above-mentioned studies have identified a number of dietary risk factors associated with caries. Whether caries-free children with such dietary risk factors would develop caries in the future is not known yet.

Cultivation-independent molecular methods, based mainly on 16S rDNA sequences, are now available for the detection of uncultivable bacteria in the oral cavity [Ledder et al., 2007; Jiang et al., 2011; Jiang et al., 2013]. Among them, polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) has been used to identify bacterial richness profiles associated with oral disease status [Rasiah et al., 2005; Siqueira et al., 2008; Jiang et al., 2011]. This technique allows the simultaneous analysis of multiple samples and provides genomic profiles of microbial communities as well as the possibility of identifying community members on the basis of sequencing of excised bands [Li et al., 2007]. Using PCR-based 16S rRNA gene DGGE, Li et al. [2005] demonstrated reduced dental plaque bacterial richness and complexity in children suffering from severe caries compared with their caries-free counterparts. However, most current investigations are cross-sectional, with longitudinal studies of the dynamic microbial changes before and after the onset of caries being rarely reported.

The purpose of this study was to detect changes in dental plaque microbial community profiles and oral behavioral habits during the transition from a caries-free to a caries state using PCR-DGGE in 3-year-old caries-free children followed up for 12 months.

Subjects and Methods

Subjects

A total of 230 children aged 3 years from six urban kindergartens located within a 10-km radius in the Haidian district of Beijing, China, underwent routine oral examination in March 2011. Any subject who met the following criteria was included in the study: caries-free; intact primary dentition; teeth without enamel hypoplasia or dentin hypoplasia or white spots; without systemic disease; no use of antibiotics or topical fluoride application in the previous 3 months. One hundred and forty-four of the 230 children were recruited for this study, all of whom were followed up for 12 months. Written informed consent was obtained from the parents or guardians of all the participants prior to enrollment. The study design, protocol, and informed consent details were approved by the Ethics Committee of Peking University Health Science Center (IRB00001052-5132).

Experimental Design

Full-mouth supragingival plaque samples and surveys of children's oral behavioral habits were collected from all participants and their parents or guardians, respectively, after caries examinations in the same location at baseline, 6 and 12 months. The questionnaire was verified for the clarity of the questions in the previous study [Qin et al., 2008]. If children were found to be suffering from caries during the follow-up, their parents were recommended to take them to a dental clinic. According to the potential changes of caries status at the 12-month follow-up, all the participants were divided into three groups: caries-free group (caries-free children at the 12-month follow-up), 6-month caries group (children who were found with caries or cavity fillings at the 6-month review) and 12-month caries group (children who were caries-free at 6 months but found with caries or cavity fillings at 12 months).

Clinical Examination and Sampling

Both baseline and follow-up dental examinations were conducted by two pediatric dentists (in the knee-to-knee position with respect to the patient) in a medical examination room of the kindergarten. Caries status and the decayed (cavitated), missing, or filled teeth (dmft) index was scored according to the modified World Health Organization [1997] caries diagnostic criteria. At baseline, the children with white spots on their teeth were excluded from the study while white spots were coded as caries during follow-up appointments. No radiographs were taken. Consistency in examination and caries diagnosis was ensured by the provision of training for dental examiners prior to the initiation of the study. The κ value for intraexaminer agreement in the diagnosis of caries was 0.834.

All participants were instructed to refrain from cleaning their teeth for 12 h and to avoid food and drink for 2 h before sample collection. The plaque samples were collected between 9 and 11 a.m. The teeth were gently air-dried, and a pooled plaque sample was collected from all smooth surfaces using a sterile dental excavator. The plaque sample was pooled in a sterile 1.5-ml centrifuge tube containing 50 μl TE (50 mM Tris-HCl, 1 mM EDTA; pH 8). Samples were immediately shipped on dry ice to the microbiology laboratory at Peking University School of Stomatology within 60 min. On receipt, all samples were washed in 1 ml TE buffer twice, centrifuged, and the precipitates were stored at –80°C prior to further processing.

DNA Extraction

Bacterial samples were thawed at 4°C and total genomic DNA was isolated using the Wizard Genome DNA Purification Kit (Promega, Madison, Wisc., USA). DNA products were evaluated by electrophoresis in 0.5% agarose gels run at 100 V for 30 min, and the DNA sizes (23 kb) were confirmed according to a molecular size standard. DNA quality and quantity were measured with an ultraviolet spectrophotometer at 260 and 280 nm (DU® 640, Beckman Instruments, Inc., Fullerton, Calif., USA). All DNA was stored at –20°C before further analysis.

PCR Amplification

The concentration of each DNA sample was adjusted to 10 ng/μl for PCR amplification. PCR was performed using the Gene Amp PCR System 9700 (PE Applied Biosystems, Foster, Calif., USA). The V2–V3 region of the 16S ribosomal DNA was amplified using universal bacterial primers HDA1-GC (5′-GC CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG T-3′) and HDA2 (5′-GTA TTA CCG CGG CTG CTG GCA C-3′) as employed by Rasiah et al. [2005]. It was used to generate an approximately 240-bp amplicon. PCRs were carried out in a total volume of 25 μl containing 2.5 μl template, 12.5 μl Gotap™ Colourless Master Mix (Promega), 1 μl of each primer (10 μmol/l), and 8 μl of nuclease-free water (Promega). The PCR conditions were as follows: initial denaturation at 95°C for 5 min and 28 cycles consisting of 30 s at 94°C, 30 s at 57°C, and 30 s at 72°C, plus an additional cycle of 10 min at 72°C for chain elongation. The PCR products were evaluated by agarose gel (1%) electrophoresis run at 100 V for 30 min. By applying a molecular size standard, the sizes of all amplicons were confirmed.

DGGE Analysis

Samples of PCR-amplified products (10 μl) were loaded onto the DGGE gel and separated using the Bio-Rad Dcode™ System (Hercules, Calif., USA). A 30–60% linear DNA denaturing gradient (100% denaturant is equivalent to 7 mol/l of urea and 40% demonized formamide) was formed in 8% (w/v) polyacrylamide gels. The PCR products and one DGGE standard marker (Wako, Japan) were electrophoresed (60 V/60°C/16 h) in 1× Tris-acetate-EDTA buffer (pH = 8). Subsequently, the gels were rinsed with deionized water, fixed for 15 min in deionized water containing 10% ethanol and 0.5% glacial acetic acid. Then the gels were rinsed with deionized water twice and stained for 15 min with deionized water containing 0.5% silver nitrate and 200 μl formaldehyde. After the gels were rinsed with deionized water twice, they were kept in deionized water containing 1.5% sodium hydroxide and 1.25 ml formaldehyde for 5–8 min till the bands appeared. DGGE images were then digitally captured with an imaging system (Vilber Fusion Fx5, France).

DGGE images were normalized according to the DGGE reference markers and analyzed using Quantity One 4.62 (Bio-Rad). The gel background was subtracted by use of mathematical algorithms according to the spectral analysis of overall densitometry curves. A minimal profiling setting (1.0%) was employed for the band search for all DGGE gels. The number of DGGE bands detected per lane was determined. Cross-comparisons of multiple DGGE profiles were made by transferring the results to a microbial database. The number of bands in each DGGE profile was considered to be an indicator of the richness of the dental plaque microbial community.

Table 1. Caries status of the 130 children at 6 and 12 months

Outcome variable (n = 130)	6 months	12 months
Children with caries and fillings, n (%)	44 (33.8)	72 (55.4)
dft (range)	3.4±0.5 (1–5)	4.1±1.6 (2–7)
dt (range)	2.5±0.2 (1–3)	3.2±0.9 (2–5)
dfs (range)	5.4±2.1 (3–8)	7.6±1.5 (5–10)
ds (range)	3.6±1.1 (2–5)	5.3±0.8 (4–7)

dft = Decayed and filled teeth; dt = decayed teeth (including filled teeth with secondary caries); dfs = decayed and filled tooth surfaces; ds = decayed tooth surfaces (including filled tooth surface with secondary caries).

Statistical Analyses

All statistical analyses were carried out with SPSS 13.0 software (SPSS Inc., Chicago, Ill., USA). Fisher's exact test was employed to analyze the distribution of the socioeconomic and developmental characteristics and oral behavioral habits at baseline among the caries-free group, the 6-month caries group and the 12-month caries group. The changes in oral behavioral habits at baseline, 6 months and 12 months in each group were also compared by Fisher's exact test. Numbers of DGGE bands at baseline, 6 months and 12 months in each of the three groups were analyzed using repeated-measures ANOVA and post hoc Student-Newman-Keuls-q (S-N-K-q) testing so that multiple comparison could be conducted. In order to determine if sphericity was violated, a Mauchly's test was carried out. If sphericity was violated, a Geisser-Greenhouse correction was employed. The difference in the numbers of DGGE bands among the three groups at each time point was also compared using ANOVA analysis and post hoc S-N-K-q testing; p values lower than 0.05 were considered to indicate statistical significance.

Results

General Characteristics of the Subjects

One hundred and thirty of 144 children who completed the 12-month review were included in our study; 44 children were found to have caries or cavity fillings at 6 months, a further 28 children were found to have caries or cavity fillings at 12 months. The occlusal surface of posterior teeth was found to be the most caries-susceptible site. The other 58 children remained caries-free at the 12-month follow-up (table 1). There was no tooth exaction induced by caries. Five teeth in 3 children were missing owing to dental trauma.

Questionnaire Survey

The changes in oral behavioral habits in the caries-free group, the 6-month caries group and the 12-month caries group at the 12-month follow-up were not significantly

Table 2. Comparison of oral behavior among the caries-free group, the 6-month caries group and the 12-month caries group at baseline

Variable[a]	Caries-free group	6-month caries group	12-month caries group
Frequency of eating sweets**			
≥2 times/day	18 (31.0)	26 (59.1)	15 (53.6)
<2 times/day	40 (69.0)	18 (40.9)	13 (46.4)
Frequency of eating sweets before sleeping**			
≥3 times/week	19 (32.8)	25 (56.8)	16 (57.1)
<3 times/week	39 (67.2)	19 (43.2)	12 (42.9)
Frequency of toothbrushing*			
≥1 time/day	17 (29.3)	27 (61.4)	14 (50.0)
<1 time/day	41 (70.7)	17 (38.6)	14 (50.0)
Sleep with a pacifier			
Yes	32 (55.1)	25 (56.8)	16 (57.1)
No	26 (44.8)	19 (43.2)	12 (42.9)
Drinking habits			
Water only	16 (27.6)	10 (22.7)	6 (21.4)
Mainly water	14 (24.1)	11 (25)	8 (28.6)
Mainly sugar-containing beverage	14 (24.1)	12 (27.2)	8 (28.6)
Sugar-containing beverage only	14 (24.1)	11 (25)	6 (21.4)
How to clean child's mouth*			
Parent(s) brush for the child	39 (67.2)	11 (25)	12 (42.8)
Brushing by the child	19 (32.8)	33 (75)	16 (57.1)
Rinse with only water	0	0	0
None	0	0	0
Use of fluoride toothpaste*			
Yes	40 (69.0)	15 (34.1)	11 (39.3)
No	15 (25.9)	26 (59.1)	13 (46.4)
Not known	3 (5.2)	3 (6.8)	4 (14.3)

Number and percent of children. * $p < 0.05$; ** $p < 0.01$. [a] By Fisher's exact test for each variable.

different (online suppl. table, www.karger.com/doi/10.1159/000366505). The distribution of oral behavioral habits among the caries-free group, the 6-month caries group and the 12-month caries group at baseline was then compared. The frequency of eating sweets and eating sweets before sleeping was significantly different among the three groups ($p < 0.001$). The children in the caries-free group brushed their teeth more frequently than the children in the other two groups ($p < 0.05$). No significant difference was observed in the drinking habits and sleeping with a pacifier ($p > 0.05$). Compared with 69.0% (40 of 58) of children in the caries-free group who use fluoride toothpaste, 34.1% (15 of 44) of children in the 6-month caries group and 39.3% (11 of 28) of children in the 12-month caries group did not use fluoride toothpaste ($p < 0.05$). Children who had their teeth brushed by their parents accounted for a higher proportion in the caries-free group than in the 6-month and 12-month caries groups ($p < 0.05$; table 2). Besides, the distribution of gender, socioeconomic background and developmental characteristics among the three groups at baseline was not significantly different (table 3).

DGGE Band Pattern

DGGE profiles were obtained from supragingival plaque samples at baseline, at 6-month and 12-month reviews. Representative PCR-DGGE analysis images of the caries-free group, the 6-month caries group and the 12-month caries group are shown in figure 1. The mean numbers of bands in the profiles, which represent microbial richness of the three groups at baseline, 6 months and 12 months, are shown in figure 2. The effect of time and caries status on the mean numbers of DGGE bands was evaluated by repeated-measures ANOVA analysis. The results showed a significant effect of time ($F = 26.315$, $p < 0.01$), a nonsignificant effect of the grouping factor ($F =$

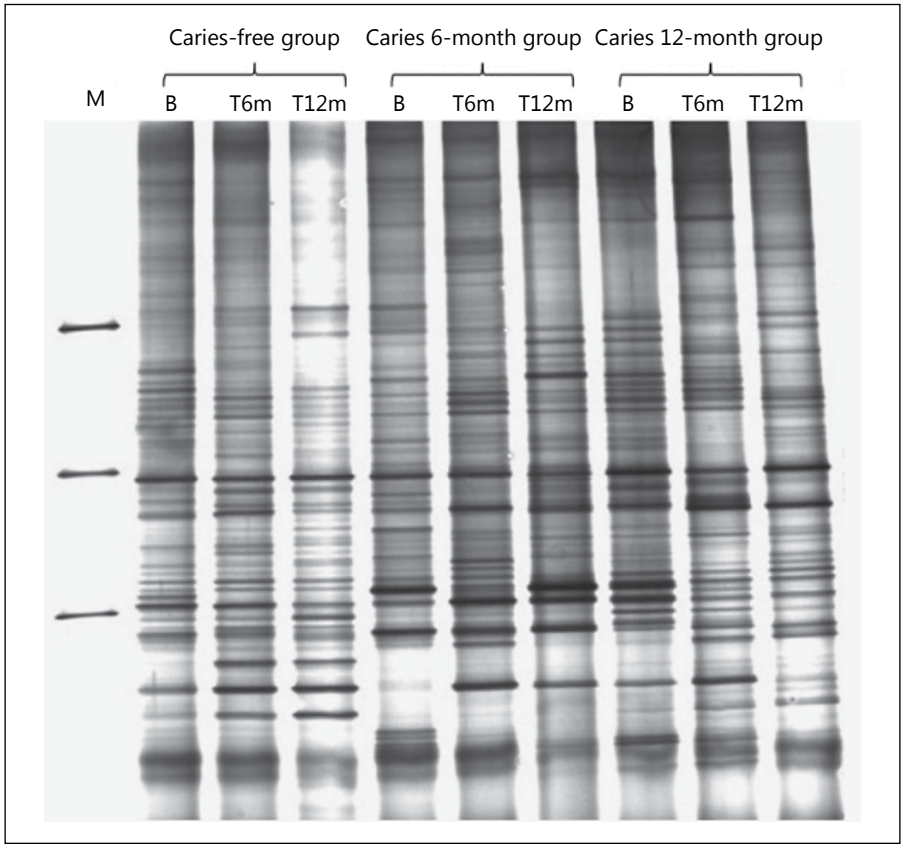

Fig. 1. Representative PCR-DGGE analysis images of the caries-free group, the 6-month caries group and the 12-month caries group from baseline (B) to 6-month review (T6m) and 12-month review (T12m). A total of 130 sets (130 participants) of DGGE gel images were produced; one set of each group is included in the figure for illustration.

Table 3. Comparison of the socioeconomic background and developmental characteristics of the caries-free group, the 6-month caries group and the 12-month caries group at baseline

Variable[a]	Caries-free group	6-month caries group	12-month caries group
Gender			
Male	29 (50.0)	21 (47.7)	13 (46.4)
Female	29 (50.0)	23 (52.3)	15 (53.6)
Mother's schooling at child's birth			
≥12 years	41 (70.7)	31 (70.5)	20 (71.4)
<12 years	17 (29.3)	14 (29.5)	8 (28.6)
Mother's occupation at child's birth			
Employer/professional	6 (10.3)	4 (9.1)	3 (10.7)
Employee/nonprofessional	52 (89.7)	40 (90.9)	25 (89.2)
Unemployed	0	0	0
Gestational age			
≥37 weeks	56 (96.6)	42 (95.5)	27 (96.4)
<37 weeks	2 (3.4)	2 (4.6)	1 (3.6)
Mode of delivery			
Vaginal	15 (25.9)	11 (25.0)	7 (25.0)
Cesarean section	43 (74.1)	33 (75.0)	21 (75.0)
Weight at birth			
≥2,500 g	58 (100)	44 (100)	28 (100)
<2,500 g	0	0	0

Number and percent of children. [a] By Fisher's exact test for each variable; all comparisons not significant ($p > 0.05$).

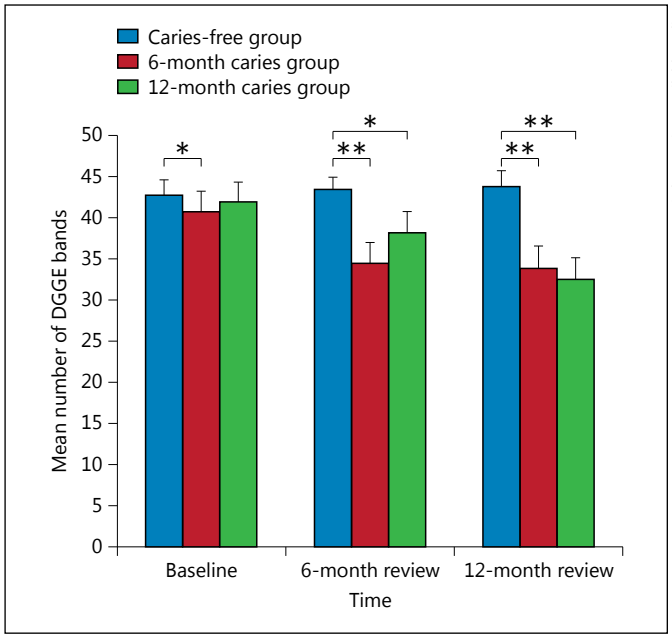

Fig. 2. Column view showing the mean number of detectable bands from the caries-free group, the 6-month caries group and the 12-month caries group at baseline, 6-month review and 12-month review (* $p < 0.05$; ** $p < 0.01$).

0.843, $p = 0.432$) and the interaction of time and grouping ($F = 4.246$, $p = 0.021$). Further, we compared the mean numbers of detectable bands among the caries-free group, the 6-month caries group and the 12-month caries group from baseline, 6 months and 12 months (post hoc S-N-K-q testing). At baseline, the mean numbers of detectable bands of caries in the 12-month caries group (41.9 ± 3.7 bands) were similar to those in the caries-free group (42.9 ± 1.7 bands, $p > 0.05$). Both of them were higher than that in the 6-month caries group (40.8 ± 2.5 bands, $p < 0.05$). At 6 months, the mean numbers of detectable bands of the 12-month caries group (38.3 ± 3.4 bands) were significantly lower than that of the caries-free group (43.5 ± 1.4 bands), although the children of the 12-month caries group were caries-free at that time ($p < 0.05$).

Then we compared the mean numbers of detectable bands at baseline, 6 months and 12 months in the caries-free group, the 6-month caries group and the 12-month caries group (post hoc S-N-K-q testing). In the caries-free group, there was no significant difference in the mean numbers of detectable bands at baseline (42.9 ± 1.7 bands), 6 months (43.5 ± 1.4 bands) and 12 months (43.8 ± 1.9 bands, $p > 0.05$). In the 6-month caries group, the mean numbers of detectable bands at the 6-month review (34.5 ± 3.4 bands) were lower than that at baseline (40.8 ± 2.5 bands). In the 12-month caries group, the mean numbers of detectable bands at the 6-month review (38.3 ± 3.4 bands) were significantly lower than that at baseline (41.9 ± 3.7 bands, $p < 0.05$), although this group was caries-free at 6 months.

Color version available online

Discussion

Longitudinal studies of the composition of the oral microflora during caries development can provide valuable information about the etiology of caries. To gain a thorough understanding of caries initiation and development, the selection of an appropriate study population is critical. Caries progression is often slow in adults, but rapid in children. In a survey of US preschool children, caries prevalence increased with age: 19.6% of 3-year-old children were found to have tooth decay (dmft = 3.9) compared with 29.3% of 5-year-old children (dmft = 5.7) [Phipps et al., 2012]. An epidemiological survey report covering multiple regions in China indicated that the incidence of caries was 25% or less in 3-year-old urban children, but that the level rose to 66% among 5-year-old children [Wang et al., 2002]. These data indicated that the incidence of caries suddenly reached a peak in children aged 3–5 years; therefore, we selected 3-year-old caries-free children as our target population in this study. The epidemiological results of our study were similar to those for caries prevalence reported in children of the same age-group in Beijing [Wang et al., 2002].

Tao et al. [2013] monitored the longitudinal changes in oral microbial richness of 12 children with severe early childhood caries compared with caries-free controls at 8, 14, 20, 26 and 32 months of age and found that caries was accompanied by a decrease in microbial richness, but the decrease did not quite reach statistical significance. In our study, intragroup comparison of the mean numbers of bands at baseline, 6 months and 12 months revealed that the microbial richness noticeably decreased in the 6-month caries group at baseline and in the 12-month caries group at the 6-month review (fig. 2). The results imply that microbial richness may undergo change at least 6 months before the appearance of caries.

Current understanding of caries development is encapsulated in the ecological plaque hypothesis [Marsh, 2003], which proposes that a shift in the balance of the resident plaque bacteria underlies the initiation and development of caries. When low populations of acidogenic and aciduric bacterial species, previously in balance with the oral environment and other plaque species, increase following high-frequency carbohydrate exposure, then caries oc-

curs. As caries progresses, a direct result of that may be that a shrinking number of species cannot survive the harsh conditions, which leads to a reduction in bacterial community richness. This notion was supported by several studies. Gross et al. [2010] demonstrated an overall loss of community richness with caries progression in young permanent teeth with the species that were significantly decreased including the *Streptococcus mitis*, *pneumoniae* and *infantis* groups, *Corynebacterium matruchotii*, *Streptococcus gordonii*, *Streptococcus cristatus*, *Capnocytophaga gingivalis*, Eubacterium IR009, *Campylobacter rectus*, and *Lachnospiraceae* sp. C1. The lower pH and greater demineralization associated with caries may be the result of the loss of these species. The current study provides additional support for the ecological plaque hypothesis about caries, confirming a decline in the richness of dental plaque before caries onset. Our next step will focus on discovering the species which decrease or increase during the transition from a caries-free state to caries in young children.

Qin et al. [2008] demonstrated that night feeding and excessive sugar intake were important contributors to the development of severe early childhood caries. Other studies suggested that sweets or beverage frequencies were independently associated with children with severe early childhood caries [Arcella et al., 2002; Marshall et al., 2005]. In Swedish 2- and 3-year-old children, eating at bedtime and during the night, in particular, was strongly correlated with severe early childhood caries. Compared to 56% of the children with severe early childhood caries, most caries-free children reported no nighttime snacks or beverages [Bankel et al., 2006]. In our study, for the caries-free group, the 6-month caries group and the 12-month caries group, respectively, there was no significant difference found in the oral behaviors from baseline to the 12-month review, which suggested that the eating habits and oral care behavior of these children in each group were relatively stable over time. However, the oral behaviors such as frequency of eating sweets, eating sweets before sleeping, toothbrushing, use of fluoride toothpaste and the method of mouth cleaning among the three groups at baseline were significantly different (table 3). It is implied that children with improper eating and oral care habits were more likely to have caries at 6 or 12 months.

DGGE band analysis provides a method for the determination of microbial richness in a microbial community. Furthermore, DGGE analysis over time allows the investigation of shifts in the microbial composition at a population level. The present study proves that PCR-DGGE is a valuable tool for monitoring the dynamics of an oral microbial community with caries causation and development. However, this technique is limited for the analysis of complex communities [Ercolini, 2004] by the presence of minor bacterial populations in samples at a level below the limit of detection. More recently, high-throughput bar-coded parallel 454 pyrosequencing analysis of bacterial richness in severe early childhood caries showed greater complexity in the bacterial phenotypes than was previously reported [Jiang et al., 2013]. Because of the large sample size in our study, broad-range primers were utilized for PCR-DGGE fingerprinting analysis of the changes in bacterial richness during the process of caries development; in this way, we were able to select representative or particular samples for future research.

In summary, a significant variation in DGGE profiles was observed during the progression from the caries-free to the caries state. Most significantly, a decrease in bacterial richness was detected at least 6 months before caries onset. Besides, children with improper eating and oral care habits were more likely to have caries at 6 months or 12 months. This study provides a preliminarily confirmation that reduced microbial richness is associated with caries onset. These data provide novel insights into the role of potentially pathogenic communities in the development of caries and they represent the basis for further investigations into the design of more effective interventions for this disease. Further research should take advantage of PCR-DGGE combined with other techniques and it should pay attention to a specific group of bacteria of dental plaque during the caries process, which can put the etiology of caries into a broader context.

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Disclosure Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service or company that could be construed as influencing the position presented in the present article.

References

Arcella D, Ottolenghi L, Polimeni A, Leclercq C: The relationship between frequency of carbohydrates intake and dental caries: a cross-sectional study in Italian teenagers. Public Health Nutr 2002;5:553–560.

Arora A, Scott JA, Bhole S, Do L, Schwarz E, Blinkhorn AS: Early childhood feeding practices and dental caries in preschool children: a multi-centre birth cohort study. BMC Public Health 2011;11:28.

Bankel M, Eriksson UC, Robertson A, Kohler B: Caries and associated factors in a group of Swedish children 2–3 years of age. Swed Dent J 2006;30:137–146.

Ercolini D: PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. J Microbiol Methods 2004;56:297–314.

Feldens CA, Giugliani ER, Duncan BB, Drachler M de L, Vitolo MR: Long-term effectiveness of a nutritional program in reducing early childhood caries: a randomized trial. Community Dent Oral Epidemiol 2010a;38:324–332.

Feldens CA, Giugliani ER, Vigo A, Vitolo MR: Early feeding practices and severe early childhood caries in four-year-old children from southern Brazil: a birth cohort study. Caries Res 2010b;44:445–452.

Filoche S, Wong L, Sissons CH: Oral biofilms: emerging concepts in microbial ecology. J Dent Res 2010;89:8–18.

Gross EL, Leys EJ, Gasparovich SR, Firestone ND, Schwartzbaum JA, Janies DA, Asnani K, Griffen AL: Bacterial 16S sequence analysis of severe caries in young permanent teeth. J Clin Microbiol 2010;48:4121–4128.

Hashim R, Williams S, Thomson WM: Oral hygiene and dental caries in 5- to 6-year-old children in Ajman, United Arab Emirates. Int J Dent Hyg 2013;11:208–215.

Jenkinson HF, Lamont RJ: Oral microbial communities in sickness and in health. Trends Microbiol 2005;13:589–595.

Jiang W, Jiang Y, Li C, Liang J: Investigation of supragingival plaque microbiota in different caries status of Chinese preschool children by denaturing gradient gel electrophoresis. Microb Ecol 2011;61:342–352.

Jiang W, Zhang J, Chen H: Pyrosequencing analysis of oral microbiota in children with severe early childhood dental caries. Curr Microbiol 2013;67:537–542.

Kanasi E, Dewhirst FE, Chalmers NI, Kent R, Moore A, Hughes CV, Pradhan N, Loo CY, Tanner AC: Clonal analysis of the microbiota of severe early childhood caries. Caries Res 2010a;44:485–497.

Kanasi E, Johansson I, Lu SC, Kressin NR, Nunn ME, Kent R, Tanner AC: Microbial risk markers for childhood caries in pediatricians' offices. J Dent Res 2010b;89:378–383.

Kuramitsu HK, He X, Lux R, Anderson MH, Shi W: Interspecies interactions within oral microbial communities. Microbiol Mol Biol Rev 2007;71:653–670.

Ledder RG, Gilbert P, Huws SA, Aarons L, Ashley MP, Hull PS, McBain AJ: Molecular analysis of the subgingival microbiota in health and disease. Appl Environ Microbiol 2007;73:516–523.

Li Y, Ku CY, Saxena D, Caufield PW: Genetic profiling of the oral microbiota associated with severe early-childhood caries. J Clin Microbiol 2007;45:81–87.

Li Y, Ku CY, Xu J, Saxena D, Caufield PW: Survey of oral microbial richness using PCR-based denaturing gradient gel electrophoresis. J Dent Res 2005;84:559–564.

Marsh PD: Are dental diseases examples of ecological catastrophes? Microbiology 2003;149:279–294.

Marshall TA, Eichenberger Gilmore JM, Broffitt B, Stumbo PJ, Levy SM: Diet quality in young children is influenced by beverage consumption. J Am Coll Nutr 2005;24:65–75.

Palmer CA, Kent R, Loo CY, Hughes CV, Stutius E, Pradhan N, Dahlan M, Kanasi E, Arevalo Vasquez SS, Tanner AC: Diet and caries-associated bacteria in severe early childhood caries. J Dent Res 2010;89:1224–1229.

Phipps KR, Ricks TL, Manz MC, Blahut P: Prevalence and severity of dental caries among American Indian and Alaska Native preschool children. J Public Health Dent 2012;72:208–215.

Qin M, Li J, Zhang S, Ma W: Risk factors for severe early childhood caries in children younger than 4 years old in Beijing, China. Pediatr Dent 2008;30:122–128.

Rasiah IA, Wong L, Anderson SA, Sissons CH: Variation in bacterial DGGE patterns from human saliva: over time, between individuals and in corresponding dental plaque microcosm. Arch Oral Biol 2005;50:779–787.

Selwitz RH, Ismail AI, Pitts NB: Dental caries. Lancet 2007;369:51–59.

Siqueira JF, Rocas IN, Debelian GJ, Carmo FL, Paiva SS, Alves FR, Rosado AS: Profiling of root canal bacterial communities associated with chronic apical periodontitis from Brazilian and Norwegian subjects. J Endod 2008;34:1457–1461.

Tao Y, Zhou Y, Ouyang Y, Lin H: Dynamics of oral microbial community profiling during severe early childhood caries development monitored by PCR-DGGE. Arch Oral Biol 2013;58:1129–1138.

Thitasomakul S, Piwat S, Thearmontree A, Chankanka O, Pithpornchaiyakul W, Madyusoh S: Risks for early childhood caries analyzed by negative binomial models. J Dent Res 2009;88:137–141.

van Houte J: Role of micro-organisms in caries etiology. J Dent Res 1994;73:672–681.

Wang HY, Petersen PE, Bian JY, Zhang BX: The second national survey of oral health status of children and adults in China. Int Dent J 2002;52:283–290.

Warren JJ, Weber-Gasparoni K, Marshall TA, Drake DR, Dehkordi-Vakil F, Kolker JL, Dawson DV: Factors associated with dental caries experience in 1-year-old children. J Public Health Dent 2008;68:70–75.

World Health Organization: Oral Health Surveys: Basic Methods, ed 4. Geneva, World Health Organization, 1997.

Zhou Y, Lin HC, Lo EC, Wong MC: Risk indicators for early childhood caries in 2-year-old children in southern China. Aust Dent J 2011;56:33–39.