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Genotypic diversity of *Streptococcus mutans* and *Streptococcus sobrinus* in 3–4-year-old children with severe caries or without caries

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Background. The genotypic diversity of both *Streptococcus mutans* and *Streptococcus sobrinus* in children with different caries experience remains unclear.

Aim. To investigate the genotypic diversity of *S. mutans* and *S. sobrinus* in children with severe early childhood caries (SECC) and in caries-free (CF) children.

Methods. Stimulated saliva of 87 SECC and 91 CF children aged 3–4 years was collected and submitted to cultivation, and MS colonies were enumerated. The genomic fingerprint analysis of *S. mutans* and *S. sobrinus* was carried out using AP-PCR.

Results. One to five genotypes of *S. mutans* were colonized in an oral cavity of SECC and CF children; 85.5% SECC children and 57.9% CF children harboured more than one genotype of *S. mutans*. One to three genotypes of *S. sobrinus* were detected from each SECC child; 31.25% SECC children harboured more than one genotype of *S. sobrinus*. And one genotype was colonized in each CF child. *S. mutans* isolates from different individuals displayed distinctive DNA fingerprints.

Conclusions. DNA fingerprints of *S. mutans* and *S. sobrinus* isolates from 3- to 4-year-old children displayed genetic polymorphism, and *S. mutans* has greater genetic diversity than *S. sobrinus*. SECC children harboured more genotypes of *S. mutans* and *S. sobrinus* than CF children.

Introduction

The most common species of mutans streptococci in mankind are *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) with *S. mutans* dominating in prevalence[1]. Children harbouring both species show a higher number of total mutans streptococci and also a tendency to a higher caries experience than children carrying only *S. mutans*[2,3]. The methods of mutans streptococci identification from oral cavity include cultivation with selective medium such as trypticase yeast-extract cystine sucrose bacitracin (TYCSB) and mitis salivarius bacitracin (MSB)[4] and modern molecular biological techniques such as direct PCR detection of mutans streptococci in saliva or dental plaque[5]. PCR technique for certain bacterial detection is more convenient and has higher sensitivity than cultivation. Although cultivation with selective medium is more time-consuming and the isolation frequency of mutans streptococci to some extent depends on the selective medium used, it provides the information on the level of mutans streptococci and harvests clinical isolates of mutans streptococci for further studies such as genotypic diversity investigation.

AP-PCR technique has been widely used to discriminate genotypic diversity of mutans streptococci[6–8]. It has been observed that children harbour one to five distinct genotypes of mutans streptococci at different ages[9,10]. While few researches have investigated genotypic diversity of both *S. mutans* and *S. sobrinus* in the same sample. And it is unclear that whether there is any relationship between the level of mutans streptococci and the genetic diversity of *S. mutans* and *S. sobrinus*. Thus genomic diversity of mutans streptococci in different caries-experienced children, and if there were relationship among genomic diversity, level of mutans streptococci and caries scores remains unclear.

The aims of the study were to investigate the presence and genetic diversity of *S. mutans* and *S. sobrinus* in children suffering from severe early children caries and in children without caries, try to explore the relationship between

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genetic diversity of *S. mutans* and *S. sobrinus* and their relationship with the level of *S. mutans* and *S. sobrinus* and caries scores.

Materials and methods

Subjects

A total of cooperative 178 children aged 3–4 years were sampled from 14 urban kindergartens located within 10 km in Beijing, China. Eighty-seven children suffering from more than five decayed teeth (dt) were grouped into the experimental group (SECC group), and 91 caries-free (CF) children were grouped as control. Informed consent was obtained from their parents. Their general information of systemic medical histories was obtained from their parents and nurseries. All the children had no chronic diseases and did not receive any antibiotic therapy and fluoride therapy during the last 2 weeks before saliva collection. The ethical approval of this study was obtained from the Human Research Ethics Committee of Peking University Medical Science Centre (IRB00001052-5132).

Dental examination

Dental examination was carried out by two paediatric dentists in a knee-to-knee posture using a mouth mirror and a dental probe under natural light in the kindergartens. The examiners were calibrated for caries examination by examining twenty children younger than 4 years, with a Kappa value of 0.91. It was decided by both examiners if a child was excluded because of enamel hypoplasia. The teeth were cleaned using cotton pellet, and no radiograph was taken. Decayed, missing and filled primary teeth (dmft) and dt were assessed according to the dental caries diagnostic criteria of WHO[11]. Restored teeth with recurrent caries were considered as decayed.

Saliva sampling

Saliva sampling was carried out at 9–11 am in a different day with the dental examination. No food was permitted to consume within 1 h before saliva collection. Children were asked to chew 1 g paraffin (Orion Diagnostica, Espoo, Finland) and to spit saliva into a 15-mL sterilized centrifuge tube in 5 min. One-millilitre stimulated whole saliva was collected from each child. The saliva samples were stored in a 0°C ice bag and carried to the laboratory for inoculation within 2 h after collection.

Streptococcus mutans and Streptococcus sobrinus isolation and identification

Saliva in sterilized centrifuge tubes was agitated on a vortex test tube mixer for 15 s. Samples were diluted in sterilized PBS to 10^{-3}, and 100-μL aliquots were inoculated with glass rod on TYCSB (LAB) agar. After incubation in mixing air containing 5% CO_2 at 37°C for 72 h, colony characteristics of *S. mutans* and *S. sobrinus* were counted and the salivary levels of *S. mutans* and *S. sobrinus* were calculated. *S. mutans* and *S. sobrinus* colonies were identified with biochemistry test including mannitol, sorbitol, melitose, melibiose fermentation test and esculin, arginine hydrolysis test. Typical colonies of *S. mutans* and *S. sobrinus* appeared different (rough or relatively smooth appearance, adherent to or embedding into the agar and with glistening drops of liquid on or around the colony). One to two colonies per typical colony from each sample were preserved at −80°C in 20% glycerol until PCR analysis. Then, the isolates were subcultured on brain–heart infusion agar (Difco, Franklin Lakes, NJ, USA), and DNA was extracted with centrifugal columnar rapid bacterial genomic DNA extraction kit (Bioteke, Beijing, China). The content and purity of isolates DNA were measured by UV Spectrophotometer (Beckman Coulter, Brea, CA, USA). Template DNA was diluted into 25 ng/μL and stored at −20°C. The DNA extraction of isolates was reconfirmed as *S. mutans* or *S. sobrinus* by PCR with primers homologous to gtf B and gtf I described by Oho *et al.*[12] DNA of strain *S. mutans ATCC 25175* and *S. sobrinus ATCC 27607* was used as template DNA in positive control reactions. The resulting amplicons were submitted to electrophoresis in 1% agarose gel with TAE buffer (Tris–acetate 40 mM, EDTA 2 mM, pH 8.0), stained with goldview ethidium bromide

(Saibaisheng, Beijing, China), visualized under ultraviolet light, documented by the Photo system Biovision (1000/26M; Vilber Lourmat, Torcy, France). The 517 and 712-base-pair amplicons were identified as *S. mutans* and *S. sobrinus*, respectively.

Genotyping of Streptococcus mutans and Streptococcus sobrinus

AP-PCR fingerprinting for *S. mutans* was performed with the random primer OPA-02 (5′-TGC CGA GCT G-3′), and the reaction was processed by reference to Li[6]. The genotyping for *S. sobrinus* was performed with the random primer OPA-13 (5′-CAG CAC CCA C-3′), and the reaction was processed was processed by reference to Saarela *et al.*[13]

Amplification products were analysed electrophoretically in 1.5% agarose gel using TAE buffer and stained with goldview ethidium bromide, visualized with ultraviolet light. Different spectra of amplicons were indicative of genetic polymorphism. Images were captured with the Photo system (Biovision 1000/26M; Vilber Lourmat), and the amplicons were evaluated visually, according to the standard used by Grönroos and Alaluusua[7] To verify reproducibility, the reactions were performed twice in at least two independent amplifications.

Statistics

Software SPSS 13.0 was used to analyse the data in the present study. Mann–Whitney *U* test was used for the comparisons of amount of *S. mutans* and *S. sobrinus* in SECC and CF individuals. Associations between variables were tested by Spearman's rank correlation analysis. The Student's *t*-test or the analysis of variance (ANOVA) was used for the comparison of mean values. A *P*-value ≤0.05 was considered statistically significant.

Results

Detection and PCR identification of Streptococcus mutans and Streptococcus sobrinus

The average dt and dmft of 87 SECC children was 9.55 ± 2.66, 10.18 ± 3.01, respectively. *S. mutans* and *S. sobrinus* were isolated from 84 of 87 SECC children (96.55%) and 58 of 91 CF children (63.74%). The amount of *S. mutans* and *S. sobrinus* was calculated with $\log_{10}$ value, and the data were analysed with Mann–Whitney *U* test (Table 1). The data showed the amount of *S. mutans* and *S. sobrinus* detected in SECC children was significantly higher than that in CF children ($P < 0.001$). Spearman's rank correlation analysis suggested the amount of *S. mutans* and *S. sobrinus* in SECC children was not correlated with their caries index (dt, dmft) ($P > 0.05$).

Table 2 suggested detection frequencies of *S. mutans* and *S. sobrinus* in SECC children were significantly higher than those in CF children, respectively ($P < 0.05$). Fifteen SECC and two CF children harboured both *S. sobrinus* and *S. mutans* simultaneously. One child in each group harboured *S. sobrinus* only. The data showed that the average score of dmft (11.93 ± 2.49) in children harbouring both *S. mutans* and *S. sobrinus* was significantly higher than that (9.88 ± 3.05) in children harbouring *S. mutans* only ($P = 0.016$, $F = 3.153$). The salivary level of *S. mutans* and *S. sobrinus* was not increased with the presence of *S. sobrinus* ($P = 0.581$).

AP-PCR analysis

Seven hundred and eighty-three strains in all (i e. 730 *S. mutans* and 53 *S. sobrinus* strains) were obtained in this study. The details are illustrated in Fig. 1. Mann–Whitney *U* test showed that there was no significant difference in the colonies number of *S. mutans* between SECC and CF children ($Z = -1.178$, $P = 0.239$); 730 *S. mutans* isolates were classi-

Table 1. Amount of *Streptococcus mutans* and *Streptococcus sobrinus* in SECC and CF children.

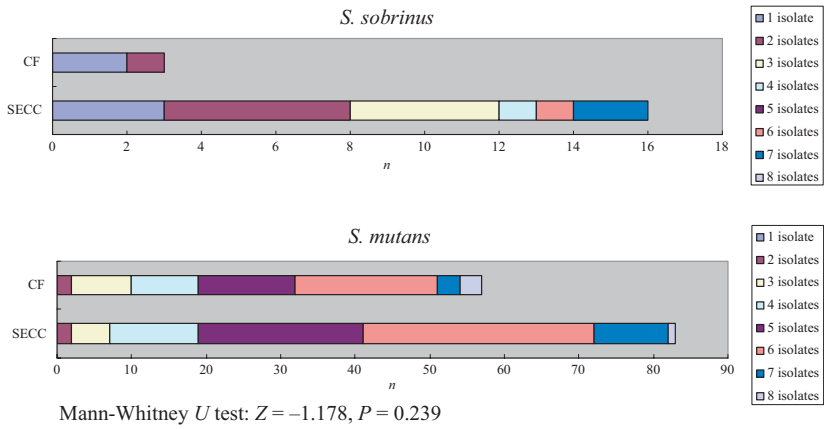
	SECC	CF	*Z*	*P*
log *S. mutans* and *S. sobrinus*	5.732 (0–7.301)	4.079 (0–7.079)	−8.085	<0.001**

CF, caries-free; SECC, severe early childhood caries.
P value was calculated using Mann–Whitney *U* test.
**$P = 0.000$.

Table 2. Average caries index scores (dt, dmft) of SECC children with *Streptococcus sobrinus* and *Streptococcus mutans* present or absent in saliva.

		CF	SECC		
		Number (%)	Number (%)	dt	dmft
S. mutans	*S. sobrinus*				
+	–	55 (60.44)	68 (78.16)	9.35 ± 2.76	9.88 ± 3.05*
+	+	2 (2.20)	15 (17.24)	10.73 ± 2.15	11.93 ± 2.49*
–	+	1 (1.10)	1 (1.15)	7	7
–	–	33 (36.26)	3 (3.45)	9.00 ± 1.00	9.33 ± 1.15
Total		91	87		

CF, caries-free; SECC, severe early childhood caries; dt, decayed teeth; dmft, decayed, missing and filled primary teeth.
Detection frequencies of *S. mutans* and *S. sobrinus* in SECC children were significantly higher than those in CF children, respectively (chi-square test: $P < 0.05$).
*One-way ANOVA analysis: $P = 0.016$, $F = 3.153$.



Mann-Whitney *U* test: $Z = -1.178$, $P = 0.239$

Fig. 1. The comparison of strain numbers of *Streptococcus mutans* and *Streptococcus sobrinus* obtained from severe early childhood caries and caries-free children.

fied into 337 distinct genotypes by fingerprint analysis, and 53 *S. sobrinus* isolates were classified into 23 distinct genotypes. One to five genotypes of *S. mutans* were colonized in one sample. As Table 3 showed, 85.5% (71/83) SECC children carrying *S. mutans* harboured more than one genotype, while 57.9% (33/57) CF children carrying *S. mutans* harboured more than one genotype. Fingerprinting images of representative genotypes of *S. mutans* were displayed in Figure 2. Mann–Whitney *U* test suggested the fingerprints of *S. mutans* detected from individuals in SECC group exhibited greater diversity than those from CF individuals ($P < 0.001$). Genotypes of *S. sobrinus* in SECC children ranged from 1 to 3. Each CF child harboured only one genotype of *S. sobrinus*.

Table 3. Comparison of genotypic diversity of *Streptococcus mutans* and *Streptococcus sobrinus* from SECC and CF children.

	Number (%)						
	1 genotype	2 genotypes	3 genotypes	4 genotypes	5 genotypes	*Z*	*P*
S. mutans							
SECC	12 (14.5)	28 (33.7)	21 (25.3)	17 (20.5)	5 (6.0)	–5.901	<0.001
CF	24 (42.1)	16 (28.1)	12 (21.0)	4 (7.0)	1 (1.8)		
S. sobrinus							
SECC	11 (68.75)	4 (25)	1 (6.25)	0	0		
CF	3 (100)	0	0	0	0		

CF, caries-free; SECC, severe early childhood caries.
P value was calculated using Mann–Whitney *U* test.

Streptococcus mutans from different individuals displayed distinctive DNA fingerprints, while identical genotype H of *S. sobrinus* was present in three SECC subjects –C15, C60 and C61 (Fig. 2).

Spearman's rank correlation analysis suggested that the number of *S. mutans* genotypes isolated from SECC children was not related to their caries index scores. In SECC children harbouring both *S. mutans* and *S. sobrinus*, the number of *S. mutans* genotypes was not related to caries index scores either. Whereas the number of *S. sobrinus* genotypes was negatively related to dmft scores (Spearman's rank correlation analysis, $r = -0.500$, $P = 0.048$) (Table 4). Among 15 SECC and two CF children harbouring both *S. mutans* and *S. sobrinus*, the number of *S. mutans* genotypes was correlated with the number of *S. sobrinus* genotypes harboured in the same subject (Spearman's rank correlation analysis, $r = 0.755$, $P < 0.001$) (Table 5). And the number of genotypes of *S. mutans* in SECC children harbouring both *S. mutans* and *S. sobrinus* had no significant difference as compared with those who harboured *S. mutans* only. Spearman's rank correlation analysis did not find any relationship between the amount and genotype numbers of *S. mutans* and *S. sobrinus*.

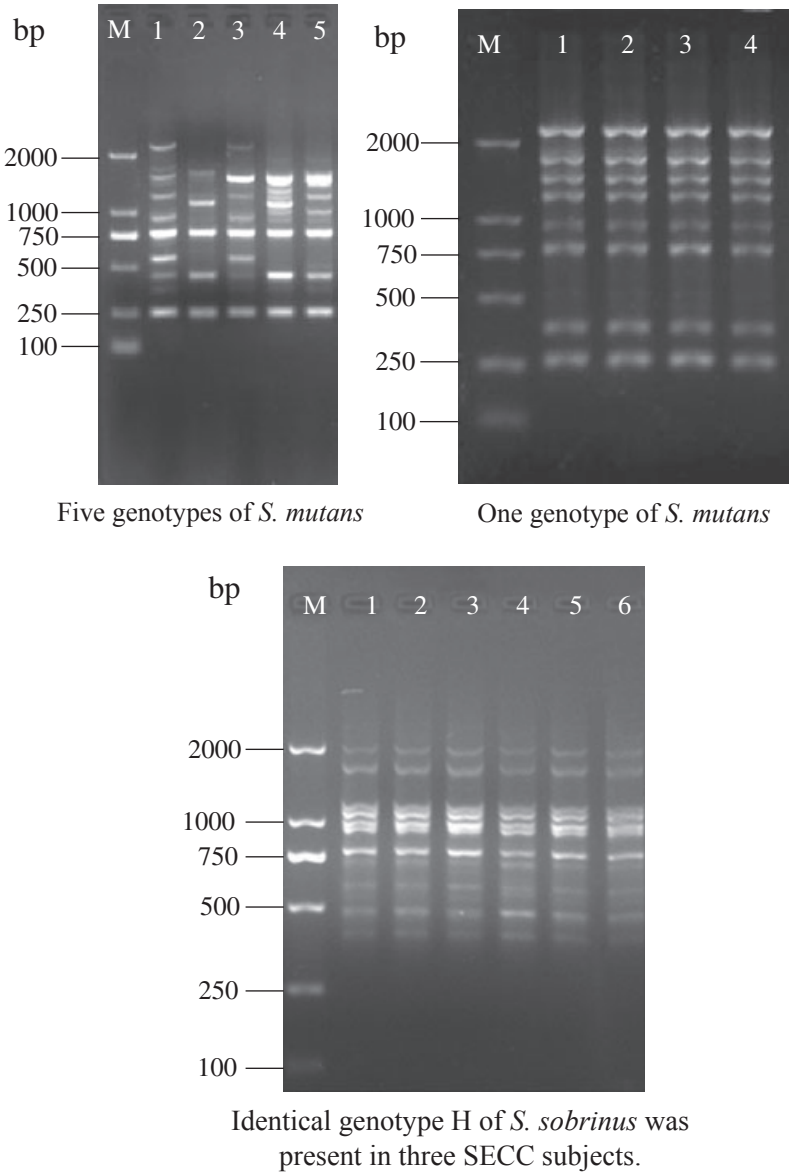


Fig. 2. Fingerprinting image of representative genotypes of *Streptococcus mutans* and *Streptococcus sobrinus.*

Table 4. The number of genotypes of both microbes and their corresponding caries index scores of SECC children with *Streptococcus sobrinus* detected.

SECC children	Number of genotypes		Caries index scores	
	Streptococcus mutans	*S. sobrinus**	dt	dmft
C2	5	2 (A,B)	10	11
C15	4	1 (**H**)	12	12
C16	2	1 (C)	7	7
C19	4	2 (D, E)	9	10
C20	1	1 (F)	8	8
C23	2	1 (G)	13	16
C30	2	1 (I)	8	11
C33	5	3 (J, K, L)	10	10
C36	4	2 (M,)	12	12
C39	0	2 (O, P)	7	7
C49	2	1 (Q)	10	13
C57	2	1 (R)	14	14
C60	2	1 (**H**)	13	13
C61	3	1 (**H**)	12	14
C62	1	1 (S)	13	15
C74	2	1 (T)	10	13
Total	41	20		

SECC, severe early childhood caries; dt, decayed teeth; dmft, Decayed, missing and filled primary teeth.
SECC children C15, C60 and C61 harboured identical genotype **H**.
*The number of *S. sobrinus* genotypes was related to their dmft score, Spearman's rank correlation analysis, $r = -0.500$, $P = 0.048$.

Table 5. Genotyping of *Streptococcus mutans* and *Streptococcus sobrinus* in children harbouring both microbes.

	Number of *S. mutans* genotypes (*n*)	Number of *S. sobrinus* genotypes (*n*)
SECC children		
C2	5	2
C15	4	1
C16	2	1
C19	4	2
C20	1	1
C23	2	1
C30	2	1
C33	5	3
C36	4	2
C49	2	1
C57	2	1
C60	2	1
C61	3	1
C62	1	1
C74	2	1
CF children		
F42	3	1
F45	2	1

CF, caries-free; SECC, severe early childhood caries.
Spearman's rank correlation analysis, $r = 0.755$, $P < 0.001$.

Discussion

Streptococcus mutans and *S. sobrinus* are generally considered to be the prime aetiological bacteria of human dental caries. It plays very important role in the formation of caries lesions. In the present study, the prevalence of *S. mutans* and *S. sobrinus* was 96.6% in SECC children and 63.7% in CF children respectively, which is in agreement with previous surveys of pre-school children[14,15]. MSB and TYCSB are most widely used media to cultivate *S. mutans* and *S. sobrinus*, and TYCSB is proved to be appropriate especially for the cultivation of *S. sobrinus*[4]. Studies using MSB or TYCSB as cultivation medium indicated the prevalence of *S. sobrinus* in individuals was different[15,16]. Several studies[2,3] suggested *S. sobrinus* starts to colonize human oral cavity later than *S. mutans* and the process continues as child develops, which means there is a tendency that the prevalence of *S. sobrinus* in children increases with their age. The prevalence of *S. sobrinus* in the present study was 18.39% in SECC children and 3.30% in CF children, respectively. And the children in this study were aged from 3 to 4 years namely in early stage of their lives. That might be a reason for the lower prevalence of *S. sobrinus* in the study. Previous study found that *S. sobrinus* commonly tend to be detected in combination with *S. mutans* in the same oral cavity[17]. In this study, however, one child in each group harboured only *S. sobrinus* without *S. mutans*. Similar cases were found in Köhler's research[18] which reported that *S. sobrinus* was usually found in combination with *S. mutans* in 4-year-old pre-school children, except for two subjects in whom *S. sobrinus* were the only species detected.

More recently, a hypothesis (the 'Ecological Plaque Hypothesis') has been proposed by PD Marsh[19,20] on the role of plaque bacteria in the aetiology of caries. He thought that the development of plaque-mediated disease at a site may be viewed as a breakdown of the homoeostatic mechanisms that normally maintain a beneficial relationship between

the resident oral microflora and the host. Potentially cariogenic bacteria may be found naturally in dental plaque, but these organisms are only weakly competitive at neutral pH and are present as a small proportion of the total plaque community. Besides mutans streptococci, some other oral bacteria like non-mutans streptococci, Actinomyces spp. and Bifidobacterium spp. are also acidogenic and aciduric. *Actinomyces gerensceriae* and Actinomyces spp. appeared to be associated with caries initiation. In addition to *A. gerensceriae* and *S. mutans, Bifidobacterium, Veillonella, Streptococcus salivarius, Streptococcus constellatus, Streptococcus parasanguis and Lactobacillus fermentum* were associated with caries as well[21,22]. In this study, three SECC children with very high dt scores as 8, 9 and 10, respectively, were not colonized with *S. mutans* and *S. sobrinus*. Some other oral microbes as described previously must exist contributing to caries initiation and procession, which was outside the subject of the present study.

Although the amount of *S. mutans* and *S. sobrinus* in SECC children was significantly higher than that in CF children as pointed out in other studies[2,3], it was not correlated with their caries scores in the present study. Maybe this is because all the SECC children involved in our study were in high level of carious activity, their carious scores were more than 5, and the dt was the major component of the dmft score of the SECC children which meant most of their carious teeth were untreated.

Previous studies[23,24] suggested that the cariogenicity of *S. sobrinus* was stronger than that of *S. mutans* for it is more acidogenic and aciduric. And clinical researches[17] found the amount of mutans streptococci was increased with the presence of *S. sobrinus*, which did not exist in this study. On the other hand, we found that the average dmft in SECC children harbouring both *S. mutans* and *S. sobrinus* was significantly higher than those harbouring *S. mutans* only. This could not be regarded simply, however, as a proof of contribution of *S. sobrinus* to early childhood caries.

In the present study, 730 *S. mutans* strains and 53 *S. sobrinus* strains were isolated for AP-PCR analysis. We selected one to two colonies for each colony with typical morphology. And the statistical analysis suggested that there was no significant difference in the colony numbers between SECC and CF children. Therefore, although it appeared to be a small number of strains obtained in our study, the strain isolates selected were very representative and the results should be reliable.

The results of the study showed that not only *S. mutans* but also *S. sobrinus* isolates displayed genetic diversity. One child was colonized with five genotypes of *S. mutans* and three genotypes of *S. sobrinus* at most. Previous study[25] approved that adults could be colonized with eight genotypes of *S. mutans* at most, and preschool children mostly were colonized with no more than four genotypes of *S. mutans*[10]. In the present study, *S. mutans* showed a more genetic diversity compared with *S. sobrinus*, which was in agreement with Klein's study[8]. Lindquist[26] found that nine adults carrying both *S. mutans* and *S. sobrinus* were colonized with 11 genotypes of *S. sobrinus* and 22 genotypes of *S. mutans*. Ota[27] compared 24 reference strains (serotype a–h) belonging to the mutans group of streptococci for DNA fragment patterns of rDNA after treatment with Hind III. It was shown that *Streptococcus cricetus* (serotype a), *Streptococcus rattus* (serotype b) and *Streptococcus downei* (serotype h) reveal comparatively homogeneous patterns while *S. mutans* (serotype c, e and f) exhibit differences between the different serotypes as well as within single serotypes, and *S. sobrinus* had an intermediary diversity. All these data suggested that genetic diversity of *S. mutans* was greater than that of *S. sobrinus*. It is necessary to point out that *S. mutans* isolates from different children in this study displayed different genetic fingerprints, which was also found by other investigators[28]. There were three SECC children (C15, C60 and C61) harboured an identical genotype of *S. sobrinus*. Their *S. mutans* strains, however, displayed distinct DNA fingerprints. We could not exclude the horizontal transmission between C60 and C61 because C60 and C61 were from the same kindergarten. C15 was from a different kindergarten and was not related to C60 and C61. Similar phenomenon was reported in

Kozai's[29] study, in which identical genotypes of *S. sobrinus* were harboured from several unrelated family members.

The data proved *S. mutans* and *S. sobrinus* isolates from SECC children displayed a more genotypic polymorphism than those from CF children in present study. Alaluusua[10] ribotyped the genetic diversity of mutans streptococci in six 1.5–3-year-old children with nursing bottle caries and in six CF, age-matched children. Among children with caries, four of them were colonized with more than one ribotype, whereas CF children had a low proportion of mutans streptococci in plaque and only one of them harboured more than one ribotype. Similarily, Xiaojing *et al.*[30] indicated that *S. mutans* from adults with caries had more genotypes than that from adults with no caries. Up to now, however, no researches on genotypic diversity of *S. sobrinus* in children with different caries experiences could be found.

And the question is: What correlation is there between the genotypes of *S. mutans* and *S. sobrinus* and dental caries? In this study, the number of *S. mutans* genotypes in SECC children was not correlated with their caries scores. The number of *S. sobrinus* genotypes was, however, positively correlated with dmft score. *S. sobrinus* is proved to have greater cariogenic potential than *S. mutans*[23,24]. And as known to all, cariogenicity is correlated with acidogenecity, aciduricity, polysaccharide synthesis ability from sucrose and adhesive ability. A positive relationship between caries activity and the genetic diversity of *S. mutans* and *S. sobrinus* is still controversial. Most of previous researches suggested that subjects with caries experience tend to carry more genotypes of *S. mutans* and *S. sobrinus*[8,10,25]. So we presume that among different genotypes of *S. mutans* and *S. sobrinus*, maybe, some genotypes are in dominance, have stronger cariogenic ability, and play very important role in caries process. On the other hand, Kreulen *et al.*[31] showed a negative correlation between caries activity and genotypic diversity of *S. mutans*. The microbiota resident in the oral biofilm are in competition with each other and subjected to many variable environmental stresses, including the availability or lack of nutrients, acidic pH and exposure to organic acids. Thus, strains with stronger cariogenicity may outcompete others and survive in oral cavity for they have better adaptability to environmental challenges. Thus, in this scenario, less diversity may mean more cariogenic.

So far, a few researches have targeted on the virulence traits of different genotype *S. mutans*. Alaluusua *et al.*[32]found polysaccharide synthesis ability of *S. mutans* isolates with different genotypes from caries-active children were different with those from CF children, although the difference was not significant. Some researches found that there were a larger number of genotypes of *S. mutans* with increased ability of synthesizing water-insoluble glucan in caries-active individuals[25,33]. While up to now, few studies on cariogenicity of different genotypes of *S. sobrinus* could be searched. It could only be presumed that different genotypes of *S. sobrinus* may play an important role in early childhood caries. Furthermore, in the present study, the genotype numbers of *S. mutans* were correlated with those of *S. sobrinus* in children harbouring both species which was not mentioned in other studies. Whether genetic diversity of *S. mutans* and *S. sobrinus* reflected microecological environment in oral cavity and whether different genotypes of *S. mutans* and *S. sobrinus* had effects on each other to make a more harmonious oral microecology are still unknown. The relationship between the genetic diversity of *S. mutans* and *S. sobrinus* and caries in children remains unclear and is needed to be further studied.

In conclusion, the prevalence and amount of *S. mutans* and *S. sobrinus* were significantly higher in SECC children than in CF children. The mean score of dmft in children harbouring both *S. mutans* and *S. sobrinus* was significantly higher than that in children harbouring *S. mutans* only. Isolates of *S. mutans* and *S. sobrinus* displayed genetic polymorphism, and *S. mutans* has greater genetic diversity than *S. sobrinus*. SECC children harboured more genotypes of *S. mutans* and *S. sobrinus* than CF children. In addition, identical genotype of *S. sobrinus* was present in unrelated subjects.

What the paper adds?

- Knowledge about the prevalence of *S. mutans* and *S. sobrinus* in CF children and in SECC children.
- This paper identifies DNA fingerprints of *S. mutans* and *S. sobrinus* isolates from 3- to 4-year-old children display genetic polymorphism and SECC children harbour more genotypes of *S. mutans* and *S. sobrinus* than CF children.
- It reports all CF and SECC children harbour distinct genotypes of *S. mutans,* while three unrelated individuals harbour identical genotype of *S. sobrinus*.

Why this paper is important to paediatric dentists?

- It provides the knowledge about the genotypic diversity of *S. mutans* and *S. sobrinus* in children with no caries and those with severe caries and suggests that genetic polymorphism of these two cariogenic microbes may correlate with the caries susceptivity of 3–4-year-old children.

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Author's contributions

Qiong Zhou, DDS, writer of the paper, participation in saliva sampling, bacteria cultivation, DNA extraction, AP-PCR of *Streptococcus mutans.* Xiurong Qin, MDS, participation in AP-PCR of *Streptococcus sobrinus.* Lihong Ge, PhD, doctoral student supervisor, modification and supervision of design and execution of this study. Man Qin, PhD, graduate student supervisor, design of experiment, modification of the paper.

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