Distribution of mutans streptococci in families: a longitudinal study

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The purpose of this study was 1) to investigate whether a group of children not colonized by mutans streptococci (MS) at the age of 3 years were colonized several years later, and 2) to study whether MS that appeared in the children were identical to those found in the parents. In a previous study no MS were found in 13 3-year-old first-born children. In 10 of these children pooled plaque samples were again collected after 5 years, and in the other 3 children after 2 years. Additionally, separate plaque samples were obtained from the children's first permanent molars when the teeth were present. Pooled samples were also obtained from all the parental pairs at follow-up and from three pairs at baseline. MS were isolated, and genotyping was done through DNA fingerprinting by restriction endonuclease analysis. In 10 children MS were still not found. Among the three children with detectable MS the DNA fingerprints of the bacteria were the same in one girl and her mother and in one boy and his father; in the other boy no similar pattern was found in either mother or father. None of the individuals in the 13 parental pairs showed identical genotypes of MS. The results indicate an opportunity to remain MS-negative if not colonized at the age of 3 years. The difficulty of being colonized with MS from the spouse is highlighted. □ *Colonization; genotypes; preschool*

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Mutans streptococci (MS), once established, seem to persist in the oral cavity. The colonization-level does not fluctuate much over time (1, 2). The same genotype of the bacteria has been found again after several months and years in children and adults (3–6) and even after treatment with chlorhexidine varnish (7). Mothers have been pointed out as important sources of transmission of MS to their children (8–10). There are also examples of fathers who may have been the source and of the possibility of extra-familial transmission of the bacteria (4, 11–13).

Initial acquisition of MS has been suggested to occur during a discrete period between the age of 19 and 31 months of age, designated the 'window of infectivity' (14). A 'second window' would possibly open when the first permanent teeth emerge and new ecological niches will be available, facilitating the colonization. If the colonization of the bacteria is delayed, the caries experience later in childhood will be less than early colonization (15–17).

The aim of this study was to investigate whether children who were not colonized by MS at 3 years of age were colonized 2 or 5 years later. If colonized, the MS were DNA-fingerprinted, and the genotypes found were compared with those in their parents for possible identity and source. The results indicate that it is possible to remain free of mutans streptococci for years if not colonized by the bacteria at the age of 3 years.

Materials and methods

Subjects

Thirteen first-born children, 6 girls and 7 boys, who at baseline were 3 years old and MS-free (11), were re-examined 2 years later (Families 22, 23, and 24) or 5 years later (the other 10 children). The children's ages at follow-up ranged from 5 years and 1 month to 8 years and 7 months (mean, 7.3 years). Genotyping of MS had been performed at baseline for only 3 parental pairs. However, in this second examination, all parents were included.

Bacterial sampling and examination

Pooled plaque samples from all the subjects were obtained in the same manner as in the baseline study. In brief, the tip of a wooden toothpick was passed along the gingival margin of the upper and lower jaws, and another toothpick along the fissures of the teeth. The tips of the toothpicks were cut off in VMGIII medium (18). For the children this method was used for the primary dentition. For their first permanent molars a site-specific method was used as follows: Quick sticks, plastic sticks with a small cotton pellet on the top (DAB, Stockholm, Sweden), were used to obtain plaque separately from the buccal and the occlusal tooth surfaces. The procedure was repeated for all erupted molars. The plaque was transferred from each Quick stick to a pad on a specially designed plastic strip, site-specific Strip mutans (Orion Diagnostica, Helsinki, Finland), by moving the stick with gentle pressure (19). The strips were incubated in selective sucrose-bacitracin broth for 48 h at 37°C.

The Strip mutans test was used for evaluating the colonization level of MS (20). Four colony density classes are used in the test, corresponding to the following number of colony-forming units (CFU)/ml saliva by conventional methods: 0 and 1 = low levels of mutans streptococci in saliva; less than 10^5 CFU/ml; 2 = high levels of mutans

Table 1. Strip mutans classes for the subjects at baseline/follow-up and presence of mutans streptococci (MS) and caries prevalence for the children at follow-up

	Strip mutans classes			MS in child's		deft/DMFT
Family no.	Mother	Father	Child	Pooled sample	Site sample	Child
1	1/2	2/1	0/0	−	−	0/0
2	2/2	3/3	0/0	−	−	3/0
3	2/2	1/2	0/0	−	−	0/0
4	2/0	1/2	0/0	−	−	0/0
7	3/2	*/3	0/1	+	+	1/0
10	2/1	*/2	0/0	−	−	0/0
11	0/1	*/2	0/0	−	−	1/0
15	1/0	*/2	0/1	−	+	6/4
16	0/1	*/2	0/0	−	−	0/‡
19	2/2	*/1	0/0	+	−	0/3
22†	1/1	2/2	0/0	−	−	0/‡
23†	3/1	2/1	0/0	−	−	1/‡
24†	3/3	3/2	0/0	−	−	4/‡

− = no MS detected; + = MS detected.
* Did not participate.
† 2nd examination at 5 years of age.
‡ No first permanent molar erupted.

streptococci in saliva; between 10^5 and 10^6 CFU/ml; and 3 = very high levels of mutans streptococci in saliva, about 10^6 CFU/ml or more.

Questions on the subject's general health and medicinal use, including antibiotic therapy during the last 2 months, were established. For each year it was recorded who had been the child's principal caregiver since the baseline examination. At baseline and follow-up, caries prevalence in the children was recorded in accordance with the WHO criteria (21).

Bacterial analysis

Within 24 h after collection each toothpick tip was transferred from the VMGIII medium to 2 ml phosphate-buffered saline, pH 7.2, and agitated on a Vortex test-tube mixer for 15 sec. One portion of the plaque/phosphate-buffered saline suspension and one from the VMGIII medium were cultivated on MSB agar (22). Additionally, for the children a portion of the colonies from each pad on the site-specific Strip mutans was dissolved in phosphate-buffered saline and spread and grown separately on MSB-agar.

Serotyping and DNA fingerprinting of the isolates were done in accordance with the procedures described by Redmo Emanuelsson et al. (11). In brief, colonies (the aim was 10/subjects from the pooled plaque sample and 10/first permanent molar when the tooth was present) representing all morphological types were transferred to separate tubes of dialyzed tryptose yeast extract medium (23). From this medium one portion was taken for Gram's stain control, and a second portion was used for serotyping with an immunofluorescent technique (24). If these two analyses indicated a non-contaminated isolate, a third portion was cultivated on MSB agar. After one more morphology check, isolates were transferred to blood agar and further to skim milk (Difco Laboratories, Detroit, Mich., USA) and stored in a refrigerator. For the DNA analysis the isolates were cultivated on Todd–Hewitt plates (Difco Laboratories), followed by cultivation in Todd–Hewitt broth. Glycine was added and after 45 min at 37°C centrifugation and rinsing with Tris-hydroxymethyl aminomethane, pH 8 (Tris; IBI, Cambridge, UK). This was followed by another centrifugation, re-suspension of the pellet in lysis buffer, and sonication. Lysis buffer containing lysozyme (chicken egg white, lyophilized powder; USB, Cleveland, Ohio, USA) was added, followed by NaCl and sodium dodecyl sulfate (SDS) (IBI). The extraction of DNA was done with phenol-chloroform (IBI) followed by rinsing with 95% and 70% cold ethanol. Finally, the DNA-pellet was dissolved in 50 µL double-distilled water. For the digestion with restriction endonuclease *Hae*III (BRL, Gaithersburg, Md., USA), 25 µL of the suspension was used. The DNA-fragments were separated by means of 0.55% (w/v) agarose gel electrophoresis and stained with ethidium bromide, and the gel was photographed under ultraviolet light. The DNA-fragment patterns on the gels were visually compared for identity in the presence or absence of bands. Samples representing each subject, and for the children also digests from the site-specific colonies, were re-run together on a single gel to ensure good conditions for comparison and to test the reproducibility.

Results

All subjects were healthy without chronic diseases and medication except the father in Family 1, who had taken systemic antibiotics approximately 4 weeks before sampling. In nine children all first permanent molars had

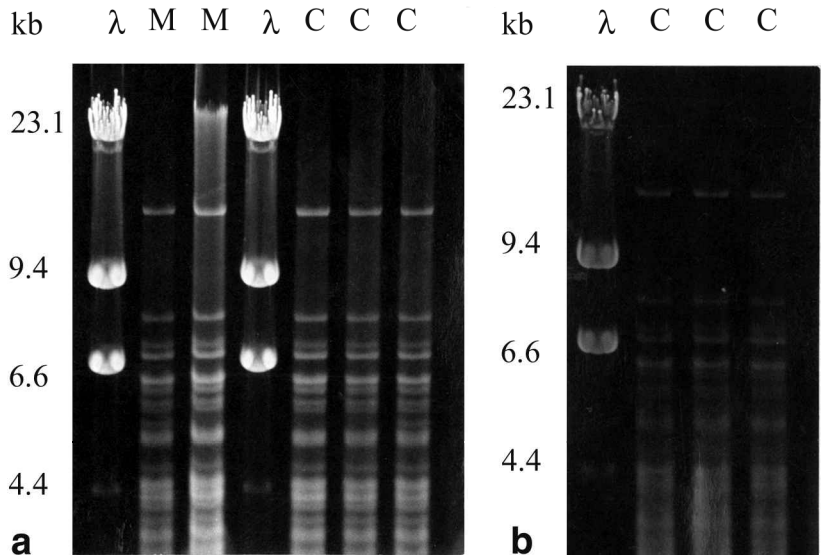

Fig. 1a. DNA fingerprints of mutans streptococci (MS) representing genotypes found in the pooled plaque samples obtained from the mother (M) and the child (C) in Family 7. First lane, lambda *Hind*III digest as molecular-size marker. 1b. DNA fingerprints of MS representing genotypes found in the site-specific plaque samples obtained from the first permanent molars of the Child 7.

erupted; in four of them all these molars were sealed or filled (Families 2, 7, 15, and 19). One child (Family 4) had two first permanent molars sealed, and in four children all first permanent molars were sound (Families 1, 3, 10, and 11). Four children had no erupted first permanent molar. The caries prevalence ranged from 0 to 6 deft in the primary dentition and from 0 to 4 in the permanent dentition. Thirty-one of 33 subjects examined with the Strip mutans test at both examinations had the same or one class difference between the two sampling occasions. Two mothers (Families 4 and 23) showed two classes less than at baseline (Table 1).

Prevalence of genotypes

In 10 children no MS were found, either in the pooled or in the site-specific plaque samples. In three children MS were found: one showed MS in the pooled sample and in the site specific samples from the occlusal surfaces of all four permanent molars (Child 7). A second showed MS in the site-specific samples from both buccal and occlusal surfaces of the permanent molars (Child 15), and the third only in the pooled sample (Child 19). MS were found in the plaque samples from all the parents. In total, 289 isolates were analyzed: 230 from the pooled plaque samples (1 to 10 isolates per adult (mean, 8.3), and for the children 9 (Child 7) and 3 (Child 19) isolates, respectively) and 59 from the site-specific samples.

Of the three children colonized at detectable levels, one had a genotype identical with a strain found in the mother (Child 7, pooled and site-specific samples). Another child harbored the same genotype as found in the father (Child 15, site-specific samples). The third child showed his 'own' genotype, not found in any of the parents (Child 19, pooled sample). None of the individuals in the parental pairs showed identical MS genotypes. In Table 2 the genotypes are given for each subject. Fig. 1 illustrates the DNA fingerprints obtained from mother and child in Family 7.

In the three parental pairs (Families 22, 23, and 24) that were re-sampled, 12 different DNA fingerprints were present at baseline; 10 of these were still detected in the same subjects at the follow-up examination. In addition, one person (the father in Family 22) had gained a further two genotypes.

One of the parents, the father in Family 22 harbored strains belonging to both serotype d/g (*Streptococcus sobrinus*) and serotype c/e/f (*S. mutans*) at baseline and follow-up. All the remaining subjects harbored strains representing serotype c/e/f only.

Principal caregiver

Fig. 2 illustrates who was the principal caregiver of the child from birth to 5 or 6 years of age. The periods with different caregivers in relation to the children's ages altered between the families. The children started school when they were 6 years old. In Family 3 the mother was the principal caregiver to the daughter during the daytime from birth until she started school. The most frequent combination was the mother half of the time and the day caregiver/kindergarten the other half. The three children harboring MS were within this group (Children 7, 15, and 19).

Table 2. The age of the subjects and the different mutans streptococci (MS) genotypes presented by the subjects in the plaque samples

		Follow-up		Baseline
Family no.	Subject	Age*	Genotype†, pooled and site-specific	Genotype, pooled
1	Mother	37y	A	
	Father	38y	B	
	Boy	8y, 1m	—	
2	Mother	41y	A	
	Father	36y	BC	
	Girl	8y, 5m	—	
3	Mother	37y	A	
	Father	45y	B	
	Girl	8y, 1m	—	
4	Mother	32y	AB	
	Father	36y	CD	
	Boy	8y, 7m	—	
7	Mother	38y	***A***‡	
	Father	37y	B	
	Girl	7y, 8m	***A***	
10	Mother	32y	A	
	Father	37y	BC	
	Boy	8y, 6m	—	
11	Mother	36y	A	
	Father	37y	BC	
	Girl	7y, 3m	—	
15	Mother	36y	A	
	Father	41y	B***C***DE	
	Boy	7y, 5m	***C***	
16	Mother	47y	AB	
	Father	55y	C	
	Boy	7y, 5m	—	
19	Mother	35y	AB	
	Father	36y	CDEF	
	Boy	7y, 11m	G	
22	Mother	31y	A	A
	Father	34y	BCDE	BC
	Girl	5y, 1m	—	—
23	Mother	30y	A	A
	Father	36y	C	BC
	Girl	5y, 2m	—	—
24	Mother	35y	ABC	ABCD
	Father	37y	EF	EF
	Boy	5y, 1m	—	—

* For age, y = years, m = months.
† The designation of genotypes by capital letters is only valid within each family.
‡ Bold, italics indicate identical genotype between individuals within a family. − = no MS detected.

Discussion

Twenty-five years ago Carlsson et al. (25) found that *S. mutans* was only established in half of 25 studied infants when they had reached 5 years. In this follow-up study our aim was to shed some further light on the establishment of the bacteria in the oral cavity and on the risk of being colonized by MS when the first permanent teeth emerge. Children not colonized by MS at the age of 3 years (baseline) participated 2 or 5 years later. During the period studied the first permanent molars had erupted in nine children, but just three of them showed MS in their plaque samples. In two of these the Strip mutans score had also increased from 0 to 1. The four children without erupting molars were still MS-negative. The possibility of acquiring MS after the so-called 'window of infectivity' was suggested recently when 22 of 30 children (73%) were positive for MS at the age of 11 but not at the age of 5 years (16). The present findings may give some further support for a second window infectivity. Although we compared the proportion of children becoming MS-positive after the window of infectivity, it is obvious that it is smaller in the present study (23%). This could be because few permanent teeth had erupted in these children while they were younger. Other factors involved in the colonization of the bacteria may further differ between the groups. However, the present results are also indicative of the possibility to remain uncolonized, having emerging teeth or not.

In previous studies identical genotypes in father/child pairs have been observed, but homology of genotypes between mother and child is more common (3, 4, 6, 9–13).

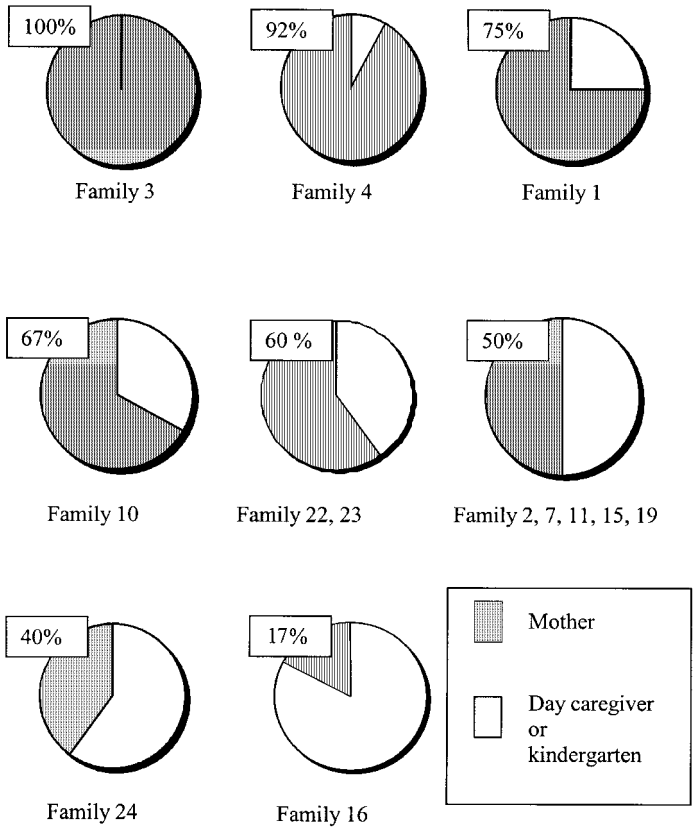

Fig. 2. Graphic illustration of who was the caregiver of the child in each family, on the basis of information for each half-year.

When comparing the DNA fingerprints found in these children and parents, we observed three different patterns: identical genotype between mother/child and father/child and not identical between parent/child. These results support the hypothesis that the father also may be of importance as source of transmission of MS, as may individuals outside the family.

All six individuals in the three parental-pairs who were re-sampled showed at least one identical genotype compared with the genotypes found at baseline. This finding, with a predominant, stable genotype, is in accordance with earlier studies (3–7). Different observations with regard to spouses sharing MS genotypes have been presented (4, 6, 9, 11–13, 26). Diverse cultural backgrounds including, for example, diet and habits, use of antimicrobial agents, receiving dentures, and heredity may be factors that can explain why acquiring the bacteria from a husband/wife seems to be more or less easy. Another explanation is that the bacteria from the spouse are present at less than detectable levels. In the present study a low risk of acquiring the bacteria from one's husband/wife is further highlighted, indicated by the unique pattern of DNA fragments found in the individuals.

In the present group of children the caries prevalence was low. It is worth pointing out that only the two children (Children 15 and 19) who had the first permanent molars filled (DMFT 3 and 4, respectively) showed MS in their plaque samples. In four children (Children 2, 11, 23, and 24) deft was between 1 and 4, but no MS could be found either in the plaque samples or in the Strip mutans test. This finding can reflect the fact that the numbers of MS were below the detection level of MSB. Other explanations can be that our sample did not properly reflect the situation when these lesions developed or that other bacteria were responsible for caries in these primary teeth.

In previous studies the mother as the principal caregiver during the child's first years of life has been in correlation with the MS colonization of the child and homology of genotypes between mother and child (11, 14). In this study 11 children (Children 1–4, 7, 10, 11, 15, 19, 22, and 23) had the mother as the principal caregiver 50% of the time or more during the period between 0 and 5 or 6 years (Fig. 2). Nevertheless, eight of them were still MS-free even though six mothers (Mothers 1, 2, 3, 4, 10, and 23) had Strip mutans 2 or 3 at any of the sampling occasions. It seems as if several concurrent factors are necessary for the transmission of MS to end up in colonization of the bacteria, as already suggested by van Houte & Green (27). Most likely, the pre-school period is important for the early establishment of the bacteria, since, for example, almost

the same frequency of mutans streptococci has been shown in 7- and 12-year-old children (28). Today's research has a challenge in ascertaining which factors are important or perhaps essential to remain MS-negative or keep a low colonization level.

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