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The Fidelity of Initial Acquisition of Mutans Streptococci by Infants from Their Mothers

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Abstract. Previous cross-sectional studies using bacteriocin profiles, serotyping, or genotyping suggest that mothers are the principle source of mutans streptococci (MS) to their infants. This study determined the commonality of MS genotypes between mothers and their infants at the time of initial acquisition. Oral bacteria of mothers and their infants were monitored from birth for approximately 3 years at three-month intervals. Genotypes of MS in infants appeared identical to those present in mothers in approximately 71% of 34 mother-infant pairs studied. Interestingly, female infants acquired MS genotypes identical to their mothers' with significantly greater fidelity than male infants (88% vs. 53%). Homology of genotypes between mothers and their infants at initial acquisition strongly suggests that MS strains were transmitted from mother to infant and that this transfer exhibited gender specificity. In no instance did we observe homology of genotypes between fathers and infants or fathers and mothers, further supporting the notion that acquisition of MS in humans follows maternal lines. Although the prevalence of dental caries was low in this young child population (11/34; 32%), we observed that male children who harbored the same genotype as their mothers had a 13 times greater likelihood of having caries than female children who acquired their mothers' strain; this difference was statistically significant ($p < 0.01$). Although we do not know the biological mechanisms governing fidelity of acquisition between a mother and her infant, our data suggest that caries outcome may be, in part, determined by both the source of MS and the presence of a specific genotype of MS. These findings point to continued studies which will further delineate the interrelation among gender, race, and caries outcome as a function of fidelity of transfer of MS from mothers to their infants.

Key words: mutans streptococci, acquisition, mother and infant, fidelity, DNA fingerprinting.

Introduction

The mutans streptococci (MS) are considered major etiologic agents in dental caries in humans. Because the presence of teeth or other non-desquamating surfaces is a prerequisite for stable colonization of MS, infants acquire MS after their teeth emerge (Berkowitz *et al.*, 1975; Stiles *et al.*, 1976; Caufield *et al.*, 1993). The question as to how and from whom infants acquire MS has not been definitively answered because most studies showing homologies among strains are cross-sectional and thus compare isolates after their initial acquisition. Moreover, the methods used for showing MS strain homology varied widely in their application and interpretation.

Previous research suggests that children acquire MS from their mothers after teeth emerge (Rogers, 1981; Caufield *et al.*, 1982, 1986, 1988, 1993; Davey and Rogers, 1984; Berkowitz and Jones, 1985; Masuda *et al.*, 1985; Caufield and Walker, 1989; Kulkarni *et al.*, 1989; Li and Caufield, 1993). This assumption seems reasonable, since mothers usually enjoy frequent and intimate contact with their infants in the first two years of life, when MS are initially transferred (Caufield *et al.*, 1993).

Pivotal to determining the source of MS in infants is the method for identifying individual strains of MS and showing that the strains found in the mother are the same as those found in her infant. Previous methods for examining strain homology among MS include phenotypic characterizations, such as bacteriocin typing and serotyping (Davey and Rogers, 1984; Berkowitz and Jones, 1985; Masuda *et al.*, 1985), and genotypic characterizations, including plasmid DNA profiles and chromosomal DNA fingerprinting (Caufield *et al.*, 1982, 1986, 1988, 1993; Caufield and Walker, 1989; Kulkarni *et al.*, 1989; Li and Caufield, 1993). Each method has limitations, but classification based upon genotypic rather than phenotypic traits appears more reliable (Caufield and Walker, 1989).

Here we present the results of a longitudinal study aimed at determining the natural history of the

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Table 1. Association between homology of MS strains within mother-infant pairs and various demographic attributes of the infant

Category	Genotypic Match ^a			Significance
	Yes	No	Total	
Mother-infant pair (%)	24 (70.6)	10 (29.4)	34	
<i>Gender</i>				
Female (%)	15 (88.2)	2 (11.8)	17	p = 0.02 ^b
Male (%)	9 (52.9)	8 (47.1)	17	
<i>Race</i>				
Black (%)	14 (87.5)	2 (12.5)	16	p = 0.06 ^c
White (%)	10 (55.6)	8 (44.4)	18	

^a DNA fingerprint patterns of the infant were compared with those of the mother.

^b Chi-square test.

^c Fisher's exact test.

transmission of MS from a mother to her infant. Using a DNA fingerprinting technique, we were able to make inferences as to the source of MS in infants. In addition, we found that the fidelity of MS acquisition correlated to the gender of the infant.

Materials and methods

Study population

The study population was derived from a larger group of mothers and their infants described in detail elsewhere (Dasanayake *et al.*, 1992; Wright *et al.*, 1992; Caufield *et al.*, 1993). Briefly, subjects were generally first-time mothers with an average age of 24 years. Mothers were selected if they harbored relatively high levels of MS in unstimulated saliva ($> 2.5 \times 10^4$ colony-forming units *per* milliliter; cfu/mL). In addition, mothers had, on average, a decayed, missing, and filled tooth surface (DMFS) score of 35. A total of 34 mother-infant pairs was included in the present study. Although not part of the original study design, MS samples were also obtained from seven fathers who shared the same households. Informed consent was obtained from each mother; all procedures received prior approval by the Institutional Review Board of the University of Alabama at Birmingham.

Isolation and cultivation of MS

Bacterial samples were obtained at three-month intervals from mothers and their infants over a period spanning infant birth to approximately 3 years of age (Caufield *et al.*, 1993). Isolates of MS were obtained from unstimulated saliva of mothers and from either plaque or saliva of infants as described elsewhere (Caufield *et al.*, 1993). Isolates were originally cultured from mitis salivarius-bacitracin agar medium (MSB) based upon colony morphology (Gold *et al.*, 1973), and confirmed by their fermentation characteristics (Shklair and Keene, 1974). Systematic efforts were made to select colonies at random, but colonies exhibiting different morphologies were deliberately included in each sample set. An average of 9 isolates of MS *per* individual was pure-cultured onto Todd-Hewitt agar and preserved at -70°C for later genetic characterization.

Chromosomal DNA isolation and DNA fingerprinting

For the initial determination of genotypes, a small-scale isolation procedure was used as described previously (Kulkarni *et al.*, 1989), except that Todd-Hewitt broth and agar were used as the growth media. A large-scale isolation of chromosomal DNA for repeated generation of DNA fingerprints has been described previously (Caufield and Walker, 1989). DNA was stored at 4°C until digested with restriction endonuclease. This previous study from our laboratory showed that digestion of chromosomal DNA with *Hae*III endonuclease provided good resolution of fragments in the 6- to 20-kilobase range. To determine the similarities between DNA fingerprints within each family unit, we visually compared a minimum of 10 *Hae*III restriction fragments. Two isolates were said to match if they had at least 9 of 10 bands in common. In only three of 34 mother-infant pairs, however, was polymorphism of a single band observed (Li *et al.*, 1992).

Statistical analyses

The Chi-square test and Fisher's exact test were used for comparison of the proportions among various category groups. The odds ratios were used to estimate the likelihood of genotypic matching in infants of different race, gender, or caries status, and 95% confidence intervals were calculated for each odds ratio. A p-value of less than or equal to 0.05 was considered statistically significant. The Student's *t* test or the analysis of variance (ANOVA) was used for comparison of mean values.

Results

Homology of strains in mother-infant pairs

DNA fingerprint patterns showed that 24 of 34 infants (70.6%) harbored genotypes of MS identical to those of their mothers (Table 1). MS genotypes from 10 infants (29.4%) did not match their mothers' genotypes. To illustrate the discerning power of DNA fingerprints, the Fig. shows genotypes of two families: one family in which mother and infant genotypes matched and another family in which no match was apparent. Among the seven families in which fathers' MS strains were available, none of the infants' fingerprint patterns matched their fathers' strains, nor did fathers' genotypes match those of their spouses.

Interestingly, mothers appeared to have transmitted strains of MS to their female offspring with greater fidelity (88%) than to their male offspring (53%), and the difference was statistically significant ($p = 0.02$; Table 1). In fact, genotypes of MS from female infants were greater than 6 times more likely to match their mothers' strains than were those of male infants (the odds ratio was 6.7 with a 95% CI of 1.2 to 38.6; $p = 0.02$). In addition, MS from black infants were more than 5 times more likely to match their mothers' strains than were those of white infants (odds ratio was 5.6 with a 95% CI of 0.97 to 32.2; $p = 0.06$), but this finding was not statistically significant.

The number of distinct genotypes of MS harbored by mothers was, on average, greater than the number of genotypes present in their infants (1.7 *vs.* 1.2); this difference was statistically significant ($p = 0.005$). We asked whether a difference existed between the numbers of genotypes present in matched and non-matched mother-infant pairs,

because the greater the number of genotypes in the mother, the less likely a similar genotype would be recovered in the infant. We also wanted to know if male or white infants acquired more MS genotypes than females or blacks; this may account for the observed non-matching with their mothers. For both queries, we found no significant differences between number of genotypes and gender or race (Table 2).

The relationship between first tooth emergence and genotypic matching in mother-infant pairs is shown in Table 2. The teeth of infants with strains matching those of their mothers emerged significantly earlier than those of the non-matching infants (6.6 vs. 7.9 mo; $p = 0.01$). When first tooth emergence was compared in reference to gender and race, however, there were no differences between male and female infants (7.2 vs. 6.8 mo, $p = 0.4$), or white vs. black infants (7.3 vs. 6.6; $p = 0.2$). When the times of initial MS acquisition were compared by gender and race, white children acquired MS earlier than blacks (21.3 mo vs. 29.3 mo; $p = 0.05$). We also noted that there was no significant difference between the time that infants whose genotypes matched their mothers' first became colonized with MS and those whose genotypes did not match (26.1 vs. 22.5 mo, respectively).

Dental development and caries activity

In spite of the relatively small sample population and a caries prevalence of 31% within the 34 children, several interesting and statistically significant findings arose. For example, eight of 11 children (73%) who experienced caries had MS that matched those of their mothers. This relationship was particularly pronounced in male children whose MS genotypes matched their mothers'; they were 13 times more likely to experience caries than female children (odds ratio = 13; 95% CI was 1.7 to 99; $p = 0.007$). Also, caries experience in terms of mean dmfs scores was more severe in males than in females (2.6 vs. 0.4). When race of the child was used as a variable, caries status was similar between black and white children, including those who matched and those who did not match their mothers' genotype. When genotypic matching, race, and gender were analyzed simultaneously by three-way ANOVA, no significant interactive effects with the children's caries outcome were found.

Discussion

Results from the present study strongly suggest that mothers are the major source of MS to their infants. We base this assertion on the observation that genotypes of MS isolated from infants at the time of initial acquisition were homologous to those isolated from the mother's saliva in 71% of the mother-infant pairs. The findings are further enhanced by the power of a prospective rather than a cross-sectional study, because isolates from mothers and infants were obtained at the time of initial acquisition of MS in the infant, which also increases the likelihood of DNA fingerprint matching, since we have observed a decreased concordance of genotypes between mother-child pairs as the child becomes older (Li and Caufield, unpublished observations). Others have reported commonality of oral

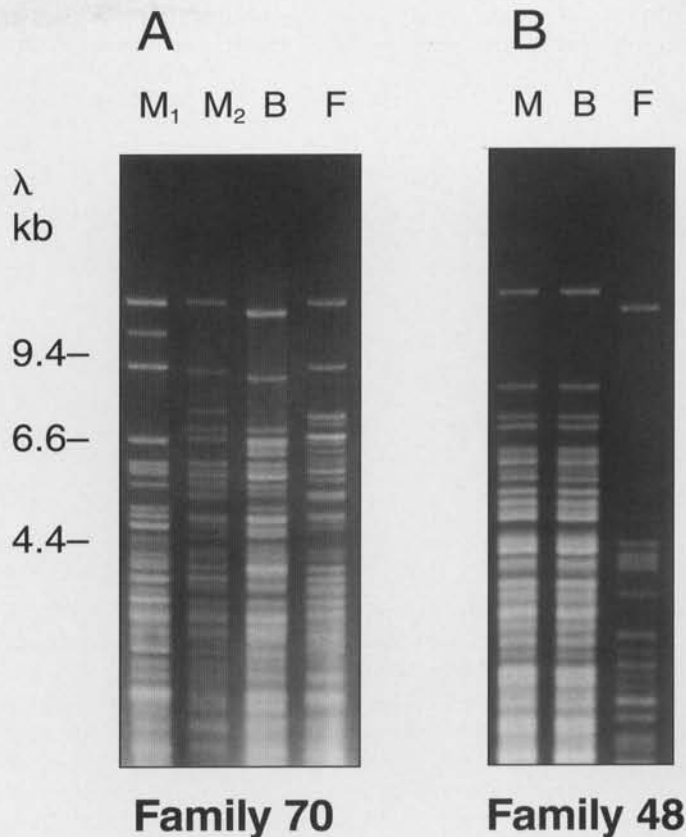


Figure. Chromosomal DNA fingerprints of MS from two families at time of initial MS acquisition in the infant. Panel A shows family 70, in which mother (M₁, M₂) and infant (B) strains of MS do not match. Panel B illustrates family 48, in which mother (M) and infant (B) harbor identical genotypes of MS. In neither family do strains of MS from the father (F) match those of the mother or infant. Chromosomal DNA patterns were generated by *Hae*III restriction endonuclease. Fragments were separated on a 0.55% gel as described in Caufield and Walker (1989).

streptococci besides MS among mother-infant pairs (Kelstrup *et al.*, 1970; Tagg *et al.*, 1983).

The high degree of fidelity between strains of MS in mothers and their female infants (88%), in contrast to those isolated from male infants (55%), indicates that the conservation of MS within mother-infant pairs is gender-specific, at least within the population studied here. Although the difference in fidelity between females and males was statistically significant, we do not know why MS was better conserved in female than in male offspring. We did observe, however, that infants whose teeth emerged early were significantly more likely to acquire MS from their mothers than infants whose teeth emerged later. That tooth emergence in females occurs sooner than in males is commonly accepted (Lunt and Law, 1974). We observed that tooth emergence in females was, on average, earlier (6.8 mo) than in males (7.2 mo), but the difference was not statistically significant. Earlier acquisition of MS might be correlated to early tooth emergence, but the data from a previous study (Caufield *et al.*, 1993) and the present study do not support this notion. In fact, the emergence of the first

Table 2. Comparison of the various dental and MS characteristics with demographic attributes of the infant

	n	Ave. No. Genotypes		1st Tooth Emerges (mo)		Init. MS Acquis. (mo)	
		Mean \pm SD	p ^a	Mean \pm SD	p	Mean \pm SD	p
<i>Genotypic match</i>							
Yes	24	1.2 \pm 0.4	0.6	6.6 \pm 1.3	0.01	26.1 \pm 13.2	0.4
No	10	1.3 \pm 0.7		7.9 \pm 1.4		22.5 \pm 8.2	
<i>Gender</i>							
Male	17	1.3 \pm 0.6	0.5	7.2 \pm 1.4	0.4	22.3 \pm 12.4	0.2
Fem.	17	1.2 \pm 0.4		6.8 \pm 1.5		27.8 \pm 11.2	
<i>Race</i>							
White	18	1.2 \pm 0.6	0.9	7.3 \pm 1.5	0.2	21.3 \pm 11.1	0.05
Black	16	1.3 \pm 0.5		6.6 \pm 1.4		29.3 \pm 11.7	
<i>Group</i>							
Mother	34	1.7 \pm 0.8	0.005	—	—	—	—
Infant	34	1.2 \pm 0.5					

^a P value, Student's *t* test.

teeth of infants whose MS matched their mothers' was similar to that in infants whose MS did not match, so earlier acquisition of MS did not foster parity of strains between mothers and infants. Perhaps children whose teeth emerge sooner are more advanced in other ways that might influence fidelity of acquisition, such as a more developed immune system. Moreover, there is evidence to suggest that females exhibit better immune responsiveness to antigens than do males; this may be related to female sex hormones (Ahmed *et al.*, 1985). The relationship between development of the immune system and fidelity of acquisition, however, has not been studied.

Our initial expectation was that the fidelity between genotypes of mothers and their infants would be better than the observed 71%, especially considering the fact that mothers were selected for high levels of MS and in view of previously published studies. Perhaps most of the infants acquired MS from their mothers, but we were unable to detect all genotypes present in mothers or in the infants. For example, we selected, on average, 9 isolates of MS from MSB plates of saliva samples. More extensive sampling was not practical because of time and cost limitations. Although we assume that saliva constitutes the vehicle of transfer from mother to child, we do not know whether MS found in saliva represent the total repertoire of MS on the tooth surfaces. Also, we do not have a good estimate as to the stability of the MS populations or of the MS genome. Previously, we reported the stability of DNA fingerprints over a three-year period in one mother-infant pair (Caufield *et al.*, 1989). As a side note, the chromosomal DNA fingerprint of MS strain Ingbritt, originally isolated 25 years ago by Krasse, showed a pattern identical to that of a recent isolate from this same subject (Li *et al.*, unpublished data), so in some individuals, MS appears to be stable. On the other hand, we have recently reported restriction fragment length polymorphisms within some genotypes of MS, suggesting that genetic rearrangements, deletions, or insertions occur at an as-yet-undetermined frequency (Li *et al.*, 1992). Hence, the lack of total homology with mothers' genotypes could be due, in part, to the instability of restriction patterns or a

form of genetic drift as a function of time.

The possible biological relevance between fidelity of acquisition and disease outcome may be illustrated by the fact that male children who acquired MS from their mothers were 13 times more likely than females to manifest caries at 3 years of age, suggesting that fidelity of MS acquisition may be an important predictor of which infants eventually manifest caries. Unfortunately, both the small sample size and the small number of children experiencing caries precluded our ability to explore in detail the relationship between fidelity of acquisition and caries outcome. Nonetheless, given the hypothesis that fidelity of acquisition is associated with caries outcome, we wonder why females who generally acquired mothers' genotypes did not manifest significantly more caries than males. Is it possible that mothers' strains of MS were more virulent in males compared with genotypes not from mothers? It should be noted that mothers included in this study were recruited because they harbored high numbers of MS in their saliva. These mothers also experienced, on average, a high caries rate (DMFS index = 35; Dasanayake *et al.*, 1992; Caufield *et al.*, 1993). At present, however, we have no way of assessing either the virulence or the transmissibility of individual genotypes. Evidence from an earlier study (Hagan *et al.*, 1989), now expanded to include ten families, shows that all ten of the MS-colonized children who were adopted prior to tooth emergence were caries-free. Since their natural mothers were not a likely source of MS, because MS does not colonize the oral cavity until after teeth emerge, it is tempting to speculate that a child who acquires MS from a source other than the mother may be less prone to caries. Further studies are needed, however, to confirm this association.

Among the seven families in whom MS was obtained from the father, the mother, and the infant, DNA fingerprints failed to show commonality of genotypes between fathers and infants or fathers and spouses. Similar findings were reported by others (Rogers, 1981; Davey and Rogers, 1984; Caufield *et al.*, 1988; Kulkarni *et al.*, 1989). The obvious explanation is that mothers enjoy the most contact

with their infants and, therefore, constitute the major source of MS. If, however, as we hypothesize, passively acquired immunoglobulins derived from the mother play a role in determining which strains of indigenous bacteria can colonize the infant, then fathers' strains may be excluded. The mechanism may be that intimacy between a mother and her spouse results in the appearance of an immunological recognition of the father's indigenous bacteria as non-self, and this immunological awareness is transferred to the infant through the placenta or breast milk. We also did not find commonality of genotypes among mother-father pairs. MS is apparently difficult to transmit outside the theoretical window period during childhood (Caufield *et al.*, 1993).

Conservation of specific strains of MS, not only among mother-child pairs but also within racial cohorts (Caufield *et al.*, 1988), underscores the notion that mother-to-child transfer of indigenous bacteria such as MS may be a multi-generational theme. We hypothesize that mothers transfer to their infants not only maternal immunoglobulins *via* the placenta and colostrum, but also a complementary set of indigenous bacteria capable of co-existing with these maternally-derived or -directed immunity factors. How passively acquired immunoglobulins from the mother influence the infant's "selection" of which bacteria are allowed to persist and those which are eliminated remains to be solved. Studies are under way to explore the role of the immune system, if any, on directing the acquisition of indigenous bacteria in infants.

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