Oral Microbiology Immunology 2004: 19: 408–410
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Oral Microbiology and Immunology

Short communication

Transmission of *Streptococcus mutans* in a group of Turkish families

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Ersin NK, Kocabas EH, Alpoz AR, Uzel A. Transmission of Streptococcus mutans *in a group of Turkish families.*
Oral Microbiol Immunol 2004: 19: 408–410. © Blackwell Munksgaard, 2004.

Background/aims: To investigate the transmission of *Streptococcus mutans* in a group of Turkish families using AP-polymerase chain reaction (PCR) detection.
Methods: Eight mothers who had high *S. mutans* levels in unstimulated saliva and 8 children aged between 2 and 3 years participated in the study. Plaque samples from each child were collected with the tips of sterile toothpicks for *S. mutans* counts. Although not part of the original study design, *S. mutans* samples were also obtained from the unstimulated saliva of the three fathers who shared the same households. Three typical isolates of *S. mutans* were isolated from TYCSB agar of each subject and identified by sugar fermentation tests. *S. mutans* ATCC 10449 was used as the reference strain. AP-PCR was conducted with OPA-05 primer.
Results: All of the mothers and fathers shared the similar genotypes within their children. The fathers also harbored similar genotypes to their spouses.
Conclusion: The mothers or the fathers could be the source for the transmission of *S. mutans* to their children.

Key words: AP-polymerase chain reaction; *Streptococcus mutans*; transmission

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Accepted for publication May 14, 2004

Mutans streptococci play a major role in the development of caries in humans (1, 4). Children are more likely to acquire mutans streptococci in their oral flora during the early years of their lives (2). Berkowitz et al. (3) found that the acquisition of mutans streptococci occurs between 19 and 31 months of age, although other studies reported that younger children may also be colonized (4, 10, 17). Many studies suggested the mothers as the principal source of mutans streptococci to their infants (2, 3, 5, 22). However, other possible transmission routes, such as extrafamilial acquisition of mutans streptococci in children, intrafamilial transmission between mother, father and child, and transmission between spouses, have been reported (6, 8, 12, 13, 15, 16, 18, 19, 24).

The purpose of the study was to investigate the transmission of *Streptococcus mutans* between mother, father and child in a group of Turkish families, using AP-polymerase chain reaction (PCR).

Eight mother–infant pairs participated in the study. Mothers who harbored high levels of *S. mutans* in their unstimulated saliva ($\geq 10^6$ colony-forming units (CFU)/ml) were selected. The eight infants were 2–3 years old. Although not part of the original study design, *S. mutans* samples from unstimulated saliva were also obtained from three fathers who shared the same households. Isolates of *S. mutans* were obtained from the plaque of infants with sterile toothpicks between 9 and 11 a.m. Samples were dispersed in a Vortex mixer for 30 s to dislodge the plaque and to obtain a homogeneous suspension. The plaque samples and unstimulated saliva from fathers were cultivated on *S. mutans* selective TYC agar supplemented with 0.2 U/ml bacitracin (Sigma Chemical Co., St. Louis, MO) and sucrose 15% (25). The plates were incubated anaerobically in an atmosphere containing 10% CO_2 at 37°C for 72 h. The isolates were examined under a dissecting microscope and identified by their distinctive colony and confirmed by sugar fermentation tests. Three isolates of *S. mutans* were picked. *S. mutans* ATCC 10449 was used as a reference strain. The isolates were pure cultured into 50% glycerol and stored at − 70°C for later DNA extraction. DNA isolations were done as described before (19). Briefly, *S. mutans* isolates were first grown in 5 ml of Todd–Hewitt broth culture and incubated at 37°C for 2 days. After the incubation, bacterial cells were harvested

by centrifugation (4000 rev/min (1430 ***g***) for 10 min) and cells washed with 1 ml of TE buffer. Cells were resuspended in 100 µl TE, 50 µl of 10% sodium dodecyl sulfate was added, and cells were incubated for 30 min at 65°C. The Eppendorf tubes containing the cells were placed in a microwave oven and heated for 2 min 30 s at 490 W. The pellets were dissolved in 250 µl TE and the Eppendorf tubes were frozen at − 20°C. Before AP-PCR detection, the suspension was melted, centrifuged and the supernatant was used in AP-PCR. PCR's were carried out in a 50 µl reaction mixture containing the DNA template (2 µl of 1 : 100 diluted DNA), 0.2 mM of each deoxynucleoside triphosphate/liter, 0.4 µmol primer/liter (OPA-05; 5′-AGGGGTCTTG-3′) (Thermo Bio Science GmbH, Ulm, Germany), 3.0 mM $MgCl_2$ and 2.5 U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania) in the manufacturers' recommended buffer. DNA amplification was performed in a programmable thermal cycler (Crocodile III, Appligene, Oncor, Illkirch, France). The cycling parameters used were initial denaturation at 94°C for 5 min, followed by 35 cycles each of 1 min at 94°C, 2 min at 36°C, and 2 min for 72°C. After the last cycle, the PCR tubes were incubated at 72°C for 5 min and held at 4°C. A reagent blank, which contained all the components of the reaction mixture with the exception of template DNA (which was substituted with sterile distilled water), was included in every PCR assay. PCR products were analyzed electrophoretically in 1% agarose gel containing 0.5 µg/ml ethidium bromide and visualized with ultraviolet light. Gene Ruler DNA ladder mix (MBI, Fermentas) was run as a molecular-size marker in the gel. The AP-PCR fingerprints were analyzed by side-by-side visual comparison. Fingerprints were considered similar when all major bands were identical. Any repeatable difference regarding the strong bands was considered discriminatory. In addition, the size of the base pairs was measured by BioDoc Analyze (Biometra Ti5, Goethingen, Germany) and compared within the subjects.

In the present study, transmission of genotypes of *S. mutans* occurred in all mother, father and child pairs. The AP-PCR patterns are shown in Fig. 1. The reproducibility of AP-PCR was good; all isolates that were repeatedly typed with AP-PCR gave identical profiles in each case.

The present study demonstrated a high degree of homology between *S. mutans* strains among members of the same family. Tedjosasongko & Kozai (21) found 33.3% homologies of strain types between child and mother and 8.3% homologies between child and father. Grönroos et al. (11) have found maternal transmission of MS in 64% of her culture-positive children and Li & Caufield (15) have also found identical genotypes of MS in approximately 71% of 34 mother-infant pairs. In previous studies, mothers but not fathers have been frequently observed to harbor strains in common with their children, suggesting that intrafamilial transmission originates mainly from the mother. However, in recent studies, it is reported that mutans streptococci transmission relates not only to the mother but also to the father or others both outside and within the family, which supports our results (8, 12). Van Houte & Green (23) pointed out that successful transmission of bacteria between human hosts is complex and depends on a variety of interrelated factors, including bacterial affinity to potential colonization sites and number of infecting bacterial cells available for attachment. The vehicle could be a domestic object contaminated with saliva, such as spoons or a toothbrush (9, 20). A greater percentage of matching mutans streptococci genotypes were reported in an American population (15) than in Swedish families (6) (71% and 24%, respectively). In Japan, 31.4% of genotypes harbored by 0–11-year-old children matched genotypes detected in their fathers; 30.5% of the children carried genotypes not found in their parents (12).

In the present study, when the fathers were examined, both father and mother shared the same *S. mutans* strains. This finding suggests a possible horizontal transmission between spouses. This is in accordance with other studies (12, 19) but contrasts with others (6, 7). Van Loveren et al. (24) showed that there might be a similarity between mutans streptococci in mother, father and child, indicating that transmission between the family members occurs.

Further prospective studies involving a larger study group with a large number *S. mutans* isolates are necessary to explore the frequency of transmission in Turkish families.

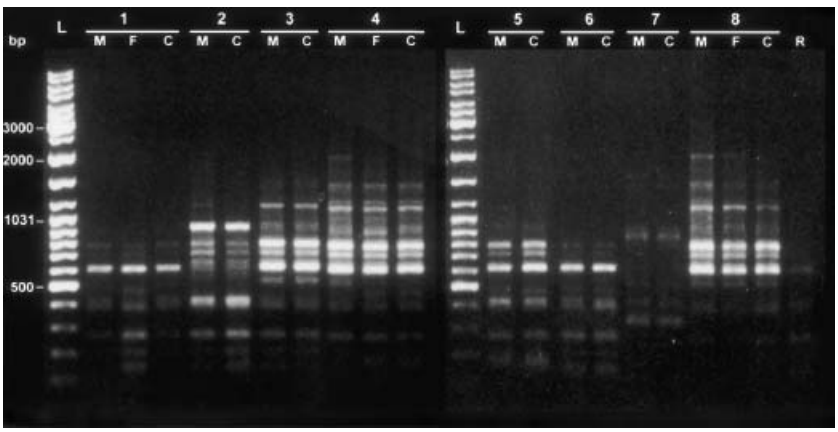

Fig. 1. AP-PCR patterns of *S. mutans* strains isolated from eight families including mother (M), father (F), and child (C), obtained with primer OPA-05. L: molecular-size marker. R: reference strain of *S. mutans*.

References

1. Alaluusua S. Transmission of mutans streptococci. Proc Finn Dent Soc 1991: **87**: 443–447.
2. Alaluusua S, Renkonen O-V. *Streptococcus mutans* establishment and dental caries experience in children 2–4 years old. Scand J Dent Res 1983: **91**: 453–457.
3. Berkowitz RJ, Jones P. Mouth-to-mouth transmission of the bacterium *Streptococcus mutans* between mother and child. Arch Oral Biol 1985: **30**: 377–379.
4. Berkowitz RJ, Turner J, Green P. Primary oral infection of infants with *Streptococcus mutans*. Arch Oral Biol 1980: **25**: 221–224.
5. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptoccocci by infants: evidence for a discrete window of infectivity. J Dent Res 1993: **72**: 37–45.
6. Emanuelsson R, Li Y, Bratthall D. Genotyping shows different strains of mutans streptococci between father and child and within parental pairs in Swedish families. Oral Microbiol Immunol 1998: **13**: 271–277.
7. Emanuelsson R, Thornqvist E. Distribution of mutans streptococci in families: a longitudinal study. Acta Odontol Scand 2001: **59**: 93–98.
8. Emanuelsson R, Wang W. Demonstration of identical strains of mutans streptococci within Chinese families by genotyping. Eur J Oral Sci 1998: **106**: 788–794.

9. Emilson CG, Ciardi JE, Olsson J, Bowen WH. The influence of saliva on infection of the human mouth by mutans streptococci. Arch Oral Biol 1989: **34**: 335–340.
10. Grindefjord M, Dahllof G, Wikner S, Hojer B. Mode'er T. Prevalence of mutans streptococci one-year-old children. Oral Microbiol Immunol 1991: **6**: 280–283.
11. Grönroos L, Saarela M, Mattö J, Tanner-Salo U, Vuorela A, Alaluusua S. Mutacin production by *Streptococcus mutans* may promote transmission of bacteria from mother to child. Infect Immun 1998: **66**: 2595–2600.
12. Kozai K, Nakayama R, Tedjosasongko U, Kuwahara S, Suzuki J, Okada M, Nagasaka N. Intrafamilial distribution of mutans streptococci in Japanese families and possibility of father-to-child transmission. Microbiol Immunol 1999: **43**: 99–106.
13. Köhler B, Andreen I, Jonsson B. The earlier the colonization by mutans streptococci, the higher the caries prevalence at 4 years of age. Oral Microbiol Immunol 1998: **3**: 14–17.
14. Köhler B, Lundberg A-B, Birkhed D, Papapanou PN. Longitudinal study of intrafamilial mutans streptococci ribotypes. Eur J Oral Sci 2003: **111**: 383–389.
15. Li Y, Caufield Pw. The fidelity of initial acquisition of mutans streptococci by infants from their mothers. J Dent Res 1995: **74**: 681–685.
16. Li Y, Wang W, Caufield PW. The fidelity of mutans streptococci transmission and caries status correlate with breast-feeding experience among Chinese families. Caries Res 2000: **34**: 123–132.
17. Matsuda N, Tsutsumi N, Sobue S, Hamaada S. Longitudinal survey of the distribution of various serotypes of *Streptococcus mutans* in infants. J Clin Microbiol 1979: **10**: 497–502.
18. Mattos-Graner RO, Li Y, Caufield PW, Duncan M, Smith DJ. Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. J Clin Microbiol 2001: **39**: 2313–2316.
19. Saarela M, von Troil-Linden B, Torkko H, Stucki AM, Alaluusua S, Jousimies-Somer H, Asikainen S. Transmission of oral bacterial species between spouses. Oral Microbiol Immunol 1993: **8**: 349–354.
20. Svanberg M. Contamination of toothpaste and toothbrush by *Streptococcus mutans*. Scand J Dent Res 1978: **86**: 412–414.
21. Tedjosasongko U, Kozai K. Initial acquisition and transmission of mutans streptococci in children at day nursery. ASDC J Dent Child 2002: **69**: 284–288.
22. Thorild I, Lindau-Jonson B, Twetman S. Prevalence of salivary *Streptococcus mutans* in mothers and their preschool children. Int J Paediatr Dent 2002: **12**: 2–7.
23. Van Houte J, Green DB. Relationship between the concentration of bacteria in saliva and the colonization of teeth in humans. Infect Immun 1974: **9**: 624–630.
24. Van Loveren C, Buijs JF, ten Cate JM. Similarity of bacteriocin activity profiles of mutans streptococci within the family when the children acquire the strains after the age of 5. Caries Res 2000: **34**: 481–485.
25. Van Palenstein Helderman WH, Ijsseldijk M, Huis in't Veld JHJ. A selective medium for the two major subgroups of the bacterium *Streptococcus mutans* isolated from human dental plaque and saliva. Arch Oral Biol 1983: **7**: 599–603.