Association Between Glucan Synthesis by *Streptococcus mutans* and Caries Incidence in Schoolchildren Receiving a Fluoride Mouth Rinse

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**Purpose:** To determine whether variation in glucan synthesis by *Streptococcus mutans* isolates is associated with caries development in children receiving a fluoride mouth rinse (FMR).

**Materials and Methods:** Of 122 children (aged 9 to 10 years), 64 had received FMR (FMR(+) group) and the remaining 58 children had not (FMR(–) group). The number of decayed and filled teeth (DFT) and increases in the number of DFT in 1 year (dDFT) were recorded. Saliva samples were collected to isolate the clinical *S. mutans* strains. The isolates were incubated in heart infusion broth supplemented with 1% sucrose, then the amount of water-insoluble glucan (WIG) formed on a glass tube surface was evaluated.

**Results:** In the FMR(–) group, children carrying *S. mutans* had a higher DFT (\(P = 0.039\)) and tended to have a higher dDFT (\(P = 0.080\)) than the others. In the FMR(+) group, although the differences between children with and without *S. mutans* were not significant, children carrying *S. mutans* that produced a high amount of WIG had higher dDFT than the other *S. mutans*-positive children (\(P = 0.034\)).

**Conclusions:** This study revealed that the variation in glucan synthesis by *S. mutans* is associated with caries development in children receiving a FMR.

**Keywords:** child, dental caries, glucans, *Streptococcus mutans*

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*S. mutans* is considered a primary pathogen for dental caries (Loesche, 1986; Kohler and Bjarnason, 1987; O’Sulivan and Thibodeau, 1996). Recently however, a reduction in the caries predictive power of the test involving counting *S. mutans* in saliva has been observed in fluoride-exposed populations with a low incidence of caries. Twetman and Petersson (1996) reported that the caries predictive ability of estimations of the number of *S. mutans* in saliva and general incidence of caries decreased with increasing fluoride exposure in preschool children. Petti and Hausen (2000) reported that the predictive power of multiple salivary *S. mutans* counts was adequate for children with low fluoride exposure and a high plaque index, while it was low for other groups. In populations with a low incidence of caries, this test cannot be considered sufficiently predictive to justify its use in identifying high-risk individuals. These
trends emphasise the need to develop additional caries activity tests for screening high-risk children from populations with a low incidence of caries.

In bacteriological caries assessments, the specific salivary level or general presence of cariogenic bacteria such as S. mutans, S. sobrinus, and lactobacilli have been taken into account. Such tests assume that the cariogenicity of a certain group of bacteria is generally uniform. However, different strains belonging to a single bacterial species sometimes show divergent characteristics, especially in the case of cariogenic bacteria. It was reported that the rate of acid production by oral streptococci including S. mutans, S. sobrinus, S. mitis, S. oralis and other species varies widely, and that the differences were not only between different species but also within a single species (de Soet et al., 2000). Alaluusua et al (1997) reported that the S. mutans glucosyltransferase (GTF) production of clinical isolates exhibiting different ribotypes showed variability. GTFs of S. mutans are considered a cariogenic virulence factor owing to their ability to synthesise glucan, which facilitates sucrose-dependent adherence and cell-to-cell accumulation of bacteria (Koga et al, 1986).

A previous study revealed that irrespective of the use of a fluoride mouth rinse for a long period, most children carried S. mutans in their saliva; however, only a few children who used a fluoride mouth rinse for a long period developed caries (Kaneko et al., 2006). In order to identify high-risk factors in children exposed to fluoride, it may be necessary to take not only the specific salivary level or general presence of S. mutans into account but also differences in the cariogenicity of each isolate of S. mutans.

Although many studies have demonstrated the importance of glucan synthesis by S. mutans in the pathogenesis of dental caries (Johnson et al, 1977; Hamada and Slade, 1980; Yamashita et al, 1993), only a few studies have investigated whether variation in glucan synthetic activity between isolated S. mutans strains has a significant effect on dental caries (Alaluusua et al, 1997; Mattos-Graner et al, 2000). Furthermore, no study has been conducted on fluoride-exposed children with a low incidence of caries. This study aimed to determine whether variation in glucan synthesis among S. mutans isolates is associated with caries development in children using a fluoride mouth rinse for a long period.

**MATERIALS AND METHODS**

**Subjects**

One hundred and twenty-two children (aged 9 to 10 years) attending two neighbouring schools participated in this study. The schoolchildren lived in similar social and economic environments in one area. Furthermore, the range of occupations (agriculture/fishing, factory, service) of the parents was identical between the schools. All children were Japanese.

Among the subjects, 64 children (FMR(+) group) had used a daily fluoride mouth rinse with 500 ppm sodium fluoride solution for 2 years (from the age of 5 or 6) at nursery school, followed by a weekly fluoride mouth rinse with 2,000 ppm at elementary school. The fluoride mouth rinse was used after lunch at nursery school and at about 10 a.m. at elementary school under the supervision of teachers. In Japan, preschool children are considered able to perform fluoride mouth rinsing safely and effectively (Sakuma et al, 2004). As a control group, 58 children (FMR(−) group) at another elementary school did not use fluoride mouth rinse.

In both groups, in addition to regular dental health education once a year by dental hygienists, the children received routine dental health education from the school nurse, teachers and occasionally the school dentist, including toothbrushing instructions and advice on limiting the consumption of sweets. The children in both groups resided in fluoride-deficient communities (<0.2 ppm fluoride in tap water).

**Dental caries examination**

Clinical examinations using dental mirrors and probes under artificial white light, without bite-wing radiographs, were performed at baseline and after 1 year according to WHO criteria (World Health Organization, 1986) by trained and calibrated examiners. The total numbers of decayed and filled permanent teeth (DFT) were recorded. The intra- and interexaminer reliabilities using Cohen’s Kappa were 0.95–0.97 and 0.88–0.93, respectively (Maeda et al, 2000).
Clinical isolates

At the baseline, samples of 3-min paraffin-stimulated saliva were collected at least 2 h after breakfast or oral hygiene procedures in the morning. The saliva samples were then stored on ice and transported to the laboratory. Saliva samples were serially diluted with phosphate-buffered saline (PBS) and plated using the Eddy Jet spiral plating system (IUL Instruments; Barcelona, Spain) onto mitis salivarius bacitracin agar (Gold et al, 1973). The samples were then incubated in anaerobic jars for 48 h at 37°C with Anaero Pack (Mitsubishi Gas Chemical; Tokyo, Japan). The colonies growing on each mitis salivarius bacitracin agar were categorised morphologically, and the colony-forming units (CFUs) of each morphological group were counted separately. One colony from each morphological group in each saliva sample was placed in heart infusion broth (Nippon Becton Dickinson; Tokyo, Japan) and incubated overnight for the following identification tests.

Phenol red broths (Nippon Becton Dickinson) with mannitol, sorbitol, melibiose, raffinose, N-acetylglucosamine or glucose added at 1% were prepared before culture. Each isolate in 20 μl of heart infusion broth was inoculated into 800 μl of these phenol red broths with various sugars. After 24 h incubation, changes in broth colours from red to yellow were observed, and isolates were identified as *Streptococcus mutans* or other species (Beighton et al, 1991; Bergey’s Manual, 1994).

Evaluation of adherent WIG synthesis

Isolates of *S. mutans* from frozen stocks were passage cultured in brain-heart infusion broth (Nippon Becton Dickinson). After growth at 37°C for 16 h, 20 μl of culture was transferred into 2 ml of heart infusion broth supplemented with 1% sucrose, then incubated at 37°C for 16 h in a test-tube rack tilted at a 45-degree angle (Kaneko et al, 2006). After incubation, the glass tubes were vortexed for 10 s to desorb loosely adherent WIG and cells from the glass wall, and then the medium was discarded. The tubes were gently washed with 2 ml of PBS (phosphate buffer solution), and 2 ml of 0.5N NaOH were added to dissolve the remaining WIG on the glass wall. This firmly adherent WIG solution was centrifuged to remove the remaining cells, and then the concentration of WIG was determined by the phenol sulfuric acid method. The amount of firmly adherent WIG derived from 1 ml of heart infusion broth supplemented with 1% sucrose was calculated as the adherent WIG synthesis by the isolate of *S. mutans*.

Statistical analysis

The increase in DFT between baseline and 1 year (dDFT) was calculated. To compare the differences in quantitative data such as DFT and dDFT, the Mann-Whitney U test was used. Differences in proportional data such as gender and infection rate were evaluated by Fisher’s exact test. P values of 0.05 or less were considered significant.

In the analysis of an association between WIG synthesis by *S. mutans* isolates and caries, children who did not carry *S. mutans* were excluded. When a number of morphologically distinguishable *S. mutans* strains were isolated from one child, adherent WIG synthesis by each strain was determined separately as above, and a weighted average using the CFU of each isolate in saliva as the weight was considered as the adherent WIG synthesis by *S. mutans* derived from the child.

SPSS software (version 17.0J, SPSS Japan; Tokyo, Japan) was used for all analyses.

RESULTS

Table 1 shows characteristics of the groups with and without FMR. The DFT score (*P* < 0.001) and the dDFT score (*P* < 0.001) of the FMR(+) group were significantly lower than those of the FMR(–) group. The FMR(+) group had a lower prevalence of *S. mutans* than the FMR(–) group (81.3% vs 89.7%; *P* = 0.212), although the difference was not statistically significant. *S. mutans* carried by children of the FMR(+) group tended to exhibit a higher level of WIG synthesis than those of the FMR(–) group (87.5 ± 52.4 μg/ml broth vs 68.8 ± 36.4 μg/ml broth; *P* = 0.059).

Presence of *S. mutans* and caries incidence

The caries incidences at baseline and the increase over 1 year of children carrying or not carrying *S. mutans* are shown in Table 2. In the FMR(–) group, children carrying *S. mutans* had a significantly higher DFT scores (1.92 ± 1.68 vs 0.50 ± 1.22; *P* = 0.039) and tended to have a higher dDFT score (0.75 ± 1.28 vs 0.00 ± 0.00; *P* = 0.080) than the others. In the FMR(+) group, children carrying *S.
had higher DFT (0.12 ± 0.32 vs 0.08 ± 0.29; \( P = 0.750 \)) and dDFT scores (0.13 ± 0.60 vs 0.00 ± 0.00; \( P = 0.325 \)) than the others; however, these differences were not statistically significant.

**Glucan synthesis by S. mutans isolates and caries incidence**

The distributions of WIG synthesis by S. mutans carried by children with and without caries incidence after 1 year are shown in Fig 1. In the FMR(+) group, WIG synthesis by S. mutans carried by children who had caries incidence after 1 year was significantly higher than WIG synthesis by S. mutans carried by the other children (\( P = 0.022 \)).

Comparisons of DFT and dDFT scores between children carrying S. mutans isolates that produce adherent WIG at a level at or above the 75th percentile and below the 75th percentile of all isolates are shown in Table 3. In the FMR(−) group, children carrying S. mutans that produce WIG at a level at or above the 75th percentile had significantly higher dDFT (\( P = 0.034 \)).
DISCUSSION

The results of this study revealed that in the FMR(+) group, children whose S. mutans strains were in the highest quarter in terms of the distribution of adherent WIG synthesis had higher caries incidence over the 1 year of the study than children whose S. mutans strains were not in the highest quarter in terms of adherent WIG synthesis. This finding suggests that the variation in glucan synthesis activity of S. mutans strains is associated with caries development and that a caries activity test which takes the variation in glucan synthetic activity of S. mutans strains into account may improve the power of predicting caries incidences.

The differences in the levels of WIG synthesis between strains could be associated with different levels of virulence. Previous studies that investigated the variation in glucan synthetic activity of clinically isolated S. mutans strains reported that S. mutans isolates from young adults with caries synthesised significantly more WIG than those from caries-free young adults (Shklair and Gaugler, 1981), and that WIG synthesis by S. mutans isolates from children with caries and those who are caries-free showed a positive correlation with caries incidence (Mattos-Graner et al., 2000). We also observed that in the FMR(+) group in particular, the mean amount of adherent WIG synthesised by the isolates from children who had caries at 1 year was significantly higher than that from children without caries.

It has been demonstrated that WIG produced from sucrose makes dental biofilm more cariogenic (Paes Leme et al., 2006). Previous studies demonstrated that glucan synthesised in a salivary pelli-

cle promotes selective adherence (Schilling and Bowen, 1992) and accumulation of cariogenic streptococci on human teeth (Rolla, 1989). Furthermore, glucan increases the volume and porosity of biofilm, which enables more substrates to diffuse to enamel surfaces (Dibdin and Shellis, 1988). As a result, acidogenic bacteria in deeper layers of dental biofilm produce more acid from substrates, and hydrogen ions diffuse through the porous biofilm more rapidly (Hata and Mayanagi, 2003), which enhances the development of dental caries (Cury et al., 1997).

In the FMR(–) group, however, differences in both DFT and dDFT scores were not significant between children whose S. mutans strains were in the highest quarter in terms of adherent WIG production and children whose S. mutans strains were not in the highest quarter. In this group, not only children carrying S. mutans strains that produced a high level of WIG but also children carrying S. mutans strains that produced a low level of WIG developed caries at 1 year (Fig 1). As regards the association between carrying S. mutans and dental caries, children who carried S. mutans had a significantly higher DFT score ($P = 0.039$) and tended to have a higher dDFT score ($P = 0.080$) than the others. In the children with low fluoride exposure, the presence or absence of S. mutans may be more important than the variation in glucan synthetic activity by S. mutans strains. The children who participated in this study were allowed to use fluoridated toothpaste. A preliminary study conducted at the same two schools showed that the proportions of children using fluoridated toothpaste were 86.3% in the school of the FMR(+) group and 84.2% in the school of the FMR(–) group, although the data are...
not shown. There was no significant difference between the two schools in this regard ($P = 0.588$). It was assumed that the children in all the groups in our study had similar trends of fluoridated toothpaste use.

CONCLUSIONS

In the group using a fluoride mouth rinse over a long period, children carrying $S. \text{mutans}$ isolates that produced a high level of adherent WIG showed a significantly higher level of caries development than those that carried isolates that produced a low level of adherent WIG. Taking the variation in glucan synthesis by $S. \text{mutans}$ isolates into account may improve the power of predicting caries incidence in fluoride-exposed populations with a low incidence of caries.

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REFERENCES