# Prevalence of Cariogenic and Periodontopathic Bacteria in Japanese Children in the Primary and Mixed Dentitions

Junko Shimomura-Kuroki * / Kie Yamashita-Matsuda ** / Yukio Miyagawa *** / Shohachi Shimooka ****

***Aim:*** *The purpose of the present study was to evaluate the relationship between infection with cariogenic bacteria or periodontal pathogens and the oral condition of children in the primary and mixed dentition stages.* ***Method:*** *Children aged 3 to 11 years were selected. Detection of cariogenic and periodontopathic bacteria and single nucleotide polymorphism (SNP) analyses were performed, and the prevalence of infection with cariogenic bacteria or periodontal pathogens based on caries experience and dental stage was compared.* ***Results:*** *The prevalence of Streptococcus mutans in both stages was significantly higher in the caries group than in the caries-free group. The prevalence of Streptococcus sobrinus was significantly higher in the caries group only in the mixed dentition stage. The prevalence of periodontal pathogens was significantly higher in the mixed dentition stage than in the primary dentition stage, regardless of caries experience. However, there were no significant differences in the prevalence of the periodontal pathogens between the primary dentition and mixed dentition stages, based on caries experience.* ***Conclusion:*** *Our data suggested that cariogenic and periodontopathic bacteria have different infection patterns, and that the period of infection with these bacteria also differs.*

***Keywords:*** *Primary dentition, Mixed dentition, Children, Cariogenic bacteria, Periodontal pathogens.*
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## INTRODUCTION

Dental caries is one of the most common oral diseases in children,[1] so prevention of dental caries is essential. Periodontal disease affects a large portion of the general population, and its management constitutes a major part of general dental care. Although periodontal disease is not common in children, some researchers have reported that infection with periodontal pathogens occurs from early childhood.[2,3] Thus, prevention of periodontal disease is also necessary in children.

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It is well known that specific types of oral bacteria are related to these major oral diseases. Loesche[1] and Hamada *et al*[4] reported that *Streptococcus mutans* is associated with the initiation of dental caries, and Fujiwara *et al.*[5] and Hirose *et al*[6] noted that strains of *S. sobrinus* also had a high cariogenic potential. Ashimoto *et al*[7] reported that 5 periodontal pathogens (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, *Tannerella forsythia*) are associated with advanced periodontitis. In a previous study,[8] we revealed that one of the periodontal pathogens, *T. forsythia*, was associated with periodontal disease in Japanese adolescents. We further suggested that one of the human leukocyte antigen Class II alleles (HLA-DQB1) was related to localized aggressive periodontitis and had an association with *T. forsythia* infection. The atypical *Bam*HI site is located near the intron/exon border or the 5'-side of the third exon of HLA-DQB1 gene, which plays an important role in the recognition of foreign antigens in immune reactions.[9-11]

It is not easy to convince children of their susceptibility to dental caries and periodontal disease and the necessity of prevention when they have no symptoms. Caufield *et al*[12,13] reported that infants acquired *S. mutans* at a median age of 26 months and *S. sanguinis* at a median age of 9 months. However, detailed information regarding the period of infection with periodontal pathogens is not available. Pediatric dentists must be aware of the period of infectivity with cariogenic bacteria and periodontal pathogens, and they should

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explain the susceptibility and need for prevention to parents and guardians.

The purpose of the present study was to evaluate the relationship of infection with cariogenic bacteria and periodontal pathogens to the oral condition of children between the primary dentition and mixed dentition stages. The presence of 3 oral streptococci (*S. mutans*, *S. sobrinus*, and *S. oralis*), *Lactobacillus (L.B.*), and 5 periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola*, and *T. forsythia*) in dental plaque was examined using polymerase chain reaction (PCR) analysis, and the relationship of the bacterial infection to the oral environment of each child was investigated. In addition, we hypothesized that locating the atypical *Bam*HI in HLA-DQB1 gene might be useful to identify high susceptibility to periodontal disease in children. To test the hypothesis, HLA-DQB1 *Bam*HI site was selected to analyze the relationship with *T. forsythia* infection in the primary dentition and mixed dentition stages.

## MATERIALS AND METHOD

### *Subjects*

Subjects, aged 3 to 11 (mean, 7.04 years; SD = 2.05; 41 boys and 41 girls), who were in the primary dentition or mixed dentition stage, were chosen from the patients attending the Pediatric Dental Clinic at The Nippon Dental University Niigata Hospital. All subjects were Japanese, had no relevant medical history, and were not on antibiotic therapy. Their parents and guardians gave informed consent to the study protocol, which was approved by the ethics committee of The Nippon Dental University School of Life Dentistry at Niigata.

The dental caries experience in primary and permanent teeth was calculated using the decayed, missing, and filled teeth (DMFT) index without radiographs, according to the World Health Organization.[14] The patients were classified into a caries-free group and a caries group (≥ 1 dmft + DMFT) (Table 1).

**Table 1.** Subjects of this study

| | Caries group | | Caries-free group | | Total |
|---|---|---|---|---|---|
| | PD | MD | PD | MD | |
| Boys | 13 | 13 | 4 | 11 | 41 |
| Girls | 6 | 22 | 2 | 11 | 41 |
| Total | 19 | 35 | 6 | 22 | 82 |

PD, primary dentition stage; MD, mixed dentition stage.

### *DNA preparation for detection of cariogenic bacteria and periodontal pathogens*

Plaque samples were collected from the buccal surface of the maxillary left molars with a sterile cotton swab for 10 s before dental treatment and at least 1 h after tooth brushing. The swab was placed in 200 μL of sterile phosphate-buffered saline (PBS) in an Eppendorf tube, then the tube was vortexed for 1 min and centrifuged at 10,000 × g for 5 min at 4°C. The pellet was resuspended in 25 μL of sterile Tris-EDTA (TE) buffer, boiled at 100°C for 5 min, and kept frozen at −20°C until ready for PCR analysis.

### *Genomic DNA extraction for SNP analyses*

Genomic DNA was isolated from the buccal mucosal cells. The buccal mucosa was rubbed with a swab for 15 s, then the swab was stored in 200 μL of extraction buffer in an Eppendorf tube at room temperature. DNA was extracted using the ISOHAIR® kit (Nippon Gene Co, Toyama, Japan), according to the manufacturer's protocol.

### *Detection of cariogenic bacteria and periodontal pathogens*

PCR was performed to target regions of the glucosyltransferase (GTF) gene from 3 oral streptococci: *gtfb* and *gtfd* from *S. mutans*, *gtft* and *gtfi* from *S. sobrinus*, and *gtfr* from *S. oralis*.

For the detection of *L.B.* and the 5 periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola*, and *T. forsythia*), PCR targeting 16S rRNA was performed.

Specific primers used in this study have been reported in previous studies (Table 2).[8, 15-17]

**Table 2.** Species-specific primers for the detection of bacteria by PCR[8,15-17]

| Primer pairs (5'-3') | Base Position (bp) |
|---|---|
| *gtf b* | |
| ACT ACA CTT TCG GGT GGC TTG G | 793-1309 (517) |
| CAG TAT AAG CGC CAG TTT CAT C | |
| *gtf d* | |
| GGC ACC ACA ACA TTG GGA AGC TCA GTT | 325-755 (431) |
| GGA ATG GCC CCT AAG TCA ACA GGA T | |
| *gtf t* | |
| GAT GAT TTG GCT CAG GAT CAA TCC TC | 432-757 (326) |
| ACT GAG CCA GTA GTA GAC TTG GCA ACT | |
| *gtf i* | |
| GAT AAC TAC CTG ACA GCT GAC T | 871-1582 (712) |
| AAG CTG CCT TAA GGT AAT CAC T | |
| *gtf r* | |
| TCC CGG TCA GCA AAC TCC AGC C | 2962-3333 (372) |
| GCA ACC TTT GGA TTT GCA AC | |
| *L.B* | |
| TGG AAA CAG ATG CTA ATA CCG | 157-360 (204) |
| GTC CAT TGT GGA AGA TTC CC | |
| *A. actinomycetemcomitans* | |
| AAA CCC ATC TCT GAG TTC TTC TTC | 478-1,034 (557) |
| ATG CCA ACT TGA CGT TAA AT | |
| *P. gingivalis* | |
| AGG CAG CTT GCC ATA CTG CG | 729-1,132 (404) |
| ACT GTT AGC AAC TAC CGA TGT | |
| *P. intermedia* | |
| TTT GTT GGG GAG TAA AGC GGG | 458-1,032 (575) |
| TCA ACA TCT CTG TAT CCT GCG T | |
| *T. denticola* | |
| TAA TAC CGA ATG TGC TCA TTT ACA T | 193-508 (316) |
| TCA AAG AAG CAT TCC CTC TTC TTC TTA | |
| *T. forsythia* | |
| GCG TAT GTA ACC TGC CCG CA | 120-760 (641) |
| TGC TTC AGT GTC AGT TAT ACC T | |

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DNA samples were amplified by PCR in a final volume of 25 μL reaction mixture containing 22 μL of Platinum® PCR SuperMix (Invitrogen, Tokyo, Japan), 1 μL of 10 μM specific primers, and 1 μL of each DNA sample. Amplification of *S. mutans* and *S. oralis* was performed with an initial denaturation step at 95°C for 2 min followed by 45 cycles at 95°C for 30 s, 63°C for 1 min, 72°C for 1 min, and a final extension step of 72°C for 7 min. Amplification of *S. sobrinus* was performed with an initial denaturation step at 95°C for 2 min, followed by 45 cycles at 95°C for 30 s, 61°C for 1 min, 72°C for 1 min, and a final extension step of 72°C for 7 min. Amplification of the 5 periodontal pathogens and *L.B.* was performed with an initial denaturation step at 95°C for 2 min, followed by 45 cycles at 95°C for 30 s, 55°C for 1 min, 72°C for 1 min, and a final extension step of 72°C for 7 min.

***SNP analyses***

HLA-DQB1 *Bam*HI site polymorphism was genotyped as described previously.[8] Genomic DNA from each subject was amplified by PCR with specific primers, and the PCR reaction was performed in a final volume of 50 μL reaction mixture containing 45 μL of Platinum® PCR SuperMix (Invitrogen), 1 μL of 10 μM specific primers, 1 μL of genomic DNA sample and 2 μL of distilled water. PCR products were digested with *Bam*HI restriction endonuclease and separated by 2% agarose gel electrophoresis with ethidium bromide staining.

***Statistical Analyses***

Statistical analyses were performed using the computer software package SPSS Statistics 17.0 (SPSS, Tokyo, Japan). The relationship between two categorical variables was analyzed by 2 × 2 contingency tables. The significance of the difference between the two proportions was assessed with Pearson's chi-square test when all the expected frequencies in the table were more than 5 or with Fisher's exact test when any expected frequency was 5 or less.

## RESULTS

***Prevalence of cariogenic bacteria and periodontal pathogens based on caries experience***

The distribution and prevalence of each bacterium based on caries experience are shown in Tables 3 and 4. The prevalence of *S. mutans* (*gtfb*- or *gtfd*-positive) in the primary dentition stage was significantly higher in the caries group than in the caries-free group (Fisher; $P = 0.0055$); the prevalence of *S. mutans* in the mixed dentition stage was also significantly higher in the caries group than in the caries-free group ($\chi_o^2 = 28.24$; $P < 0.001$) (Table 3a). The prevalence of *S. sobrinus* (*gtft*- or *gtfi*-positive) in the mixed dentition stage was significantly higher in the caries group than in the caries-free group (Fisher; $P = 0.0043$), whereas in the primary dentition stage, there was no significant difference ($P > 0.05$) between the caries group and the caries-free group in the prevalence of *S. sobrinus* (Table 3b). There were no significant differences ($P > 0.05$) between the caries group and caries-free group in the prevalence of *S. oralis* and *L.B.* in the primary or mixed dentition stage (Table 4).

The distribution and prevalence of periodontal pathogens based on caries experience are shown in Table 4. There were no significant differences ($P > 0.05$) in the prevalence of any periodontal pathogens between the primary or mixed dentition stage.

**Table 3.** Prevalence of *S. mutans* (a) and *S. sobrinus* (b) based on caries experience

(a)

| | | *S. mutans*-positive | | *S. mutans*-negative | Total (%) |
|---|---|---|---|---|---|
| PD | Caries group | 16 (84.2) | ** | 3 (15.8) | 19 (100) |
| | Caries-free group | 1 (16.7) | | 5 (83.3) | 6 (100) |
| MD | Caries group | 31 (88.6) | *** | 4 (11.4) | 35 (100) |
| | Caries-free group | 4 (18.2) | | 18 (81.8) | 22 (100) |
| | Total | 52 | | 30 | 82 |

** p < 0.01; *** p < 0.001.

PD, primary dentition stage; MD, mixed dentition stage.

(b)

| | | *S. sobrinus*-positive | | *S. sobrinus*-negative | Total (%) |
|---|---|---|---|---|---|
| PD | Caries group | 6 (31.6) | | 13 (68.4) | 19 (100) |
| | Caries-free group | 0 (0) | | 6 (100) | 6 (100) |
| MD | Caries group | 10 (28.6) | ** | 25 (71.4) | 35 (100) |
| | Caries-free group | 0 (0) | | 22 (100) | 22 (100) |
| | Total | 16 | | 66 | 82 |

** p < 0.01.

PD, primary dentition stage; MD, mixed dentition stage.

**Table 4.** Distribution of each bacterium based on caries experience

| | | Positive bacteria | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *S. oralis* | *L.B.* | *P.i.* | *T.d.* | *T.f.* | *P.g.* | *A.a.* |
| PD | Caries group (n=19) | 19 | 19 | 0 | 2 | 11 | 1 | 1 |
| | Caries-free group (n=6) | 6 | 6 | 0 | 0 | 3 | 0 | 0 |
| MD | Caries group (n=35) | 35 | 35 | 0 | 7 | 32 | 3 | 3 |
| | Caries-free group (n=22) | 21 | 22 | 1 | 4 | 17 | 2 | 0 |
| | Total | 81 | 82 | 1 | 13 | 63 | 6 | 4 |

PD, primary dentition stage; MD, mixed dentition stage.

***Prevalence of S mutans and periodontal pathogens in different dental stages***

The distribution and prevalence of *S. mutans* and periodontal pathogens in the primary and mixed dentition stages are shown in Table 5. The prevalence of periodontal pathogens was significantly higher in the mixed dentition stage than in the primary dentition stage ($\chi_o^2 = 6.84$; $P < 0.01$) (Table 5a). On the other hand, there were no significant differences ($P > 0.05$) in the prevalence of *S. mutans* depending on dental stage.

***Relationship of infection by T forsythia to genetic factor in different dental stages***

The relationship of *T. forsythia* infection to the genetic carrier of HLA-DQB1-*Bam*HI site (*Bam*HI site +) is shown in Table 6. The *Bam*HI site + group constituted 25.6% (n = 21) of all subjects. Statistical analysis revealed a significant association of *T. forsythia* infection in both the primary and

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**Table 5.** Prevalence of *S. mutans* and periodontal pathogens in different dental stages

(a)

| | *S. mutans* | | Periodontal pathogens | |
|---|---|---|---|---|
| | Positive | Negative | Positive | Negative |
| PD | 17 (68.0%) | 8 (32.0%) | 15 (60.0%) ⎤ ** | 10 (40.0%) |
| MD | 35 (61.4%) | 22 (38.6%) | 49 (86.0%) ⎦ | 8 (14.0%) |
| Total | 52 | 30 | 64 | 18 |

** p < 0.01.

PD, primary dentition stage; MD, mixed dentition stage.

(b)

| | *S. mutans* - positive | | | Periodotal pathogens - positive | | |
|---|---|---|---|---|---|---|
| PD (n=25) | 17 (68.0%) | – | – | 15 (60.0%) | * | * |
| MD1 (n=32) | | 17 (53.1%) | – | | 27 (84.4%) | – |
| MD2 (n=25) | | | 18 (72.0%) | | | 22 (88.0%) |

* p < 0.05;   – p > 0.05

PD, primary dentition stage; MD1, under 8 years old in mixed dentition stage;   MD2, 8 years old and over in mixed dentition stage.

**Table 6.** Distribution of *T. forsythia* infection in the genetic carrier HLA-DQB1-*Bam*HI site group

| | *Bam* HI restriction site + | | Total (%) |
|---|---|---|---|
| | *T. forsythia*-positive | *T. forsythia*-negative | |
| PD | 2 (40.0) ⎤ * | 3 (60.0) | 5 (100) |
| MD | 15 (93.7) ⎦ | 1 (6.3) | 16 (100) |
| Total | 17 | 4 | 21 |

* p < 0.05.

PD, primary dentition stage; MD, mixed dentition stage.

mixed dentition stages (Fisher; $P = 0.028$) with *Bam*HI site + group. No statistically significant association ($P > 0.05$) was found between infection by cariogenic bacteria and other periodontal pathogens in the two different dental stages or the *Bam*HI site.

## DISCUSSION

Dental caries is the most common oral disease in children.[1] Periodontal disease also affects a large proportion of the general population. Some researchers have reported an association between the two diseases.[18-22] However, detailed information regarding the period of infection with periodontal pathogens, which is needed for pediatric dentists to motivate parents and guardians to prevent these two diseases, is not available. In the present study, we investigated the relationship of infection with cariogenic bacteria and periodontal pathogens to the oral condition of children in the primary and mixed dentition stages, and also investigated one of the polymorphisms, HLA-DQB1 *Bam*HI site, to analyze the relationship with bacterial infection for evaluation of the susceptibility to dental caries and periodontal disease in children.

Three oral streptococci (*S. mutans*, *S. sobrinus*, and *S. oralis*), and *Lactobacillus* associated with dental caries and 5 periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola*, and *T. forsythia*) associated with advanced periodontitis were selected for detection by PCR. Most oral streptococci produce GTF enzymes, which are necessary for biofilm formation on tooth surfaces and which play a key role in cariogenesis.[23,24] Some researchers have reported that the region of the *gtf* gene is a species-specific sequence for each of the oral streptococci.[15,16] Therefore we amplified the *gtf* gene to detect each oral streptococcus using PCR.

In the distribution and prevalence of each bacterium based on caries experience, the prevalence of *S. mutans* in the primary dentition stage was significantly higher in the caries group than in the caries-free group, and its prevalence in the mixed dentition stage was also significantly higher in the caries group than in the caries-free group (Table 3a). This result supports the fact that *S. mutans* has been implicated as the prime causative bacterium of human dental caries; children are infected by *S. mutans* through adults at an early age.[25-27] On the other hand, the prevalence of *S. sobrinus* in the mixed dentition stage was significantly higher in the caries group than in the caries-free group, whereas in the primary dentition, there was no significant difference between the caries group and caries-free group (Table 3b). It is known that *S. sobrinus* is frequently isolated from the oral cavity,[4] and in accordance with another study,[28] our results also suggest that infection with *S. sobrinus* developed at a later stage than that with *S. mutans*. In addition, there were no significant differences based on caries experience in the prevalence of all periodontal pathogens in the primary or mixed dentition stage (Table 4). This indicates that infection with these periodontal pathogens had no association with caries experience, and that cariogenic bacteria and periodontal pathogens have different infection patterns.

The prevalence of periodontal pathogens was significantly higher in the mixed dentition stage than in the primary dentition stage, and the prevalence of *S. mutans* did not significantly differ with dental stage (Table 5a). However, periodontal pathogen detection might increase with an increase in the number of permanent teeth. The mixed dentition stage (mean, 8.00 years, SD = 1.72) was thus divided into mixed dentition 1 (MD1; mean, 6.69 years, SD = 0.78) and mixed dentition 2 (MD2; mean, 9.61 years, SD = 1.04). MD1 included subjects in the mixed dentition stage under 8 years old and MD2 included subjects in the mixed dentition stage 8 years old and over. Children under 8 seldom have lateral segment teeth, so 8 years was considered the best age at which to divide the subjects. The prevalence of periodontal pathogens was significantly higher in both MD1 ($\chi_o^2 = 4.30$; $P < 0.05$) and MD2 ($\chi_o^2 = 5.09$; $P < 0.05$) than in the primary dentition stage, and there were no significant differences ($P > 0.05$) in terms of dental stage in the prevalence of *S. mutans* (Table 5b). These results coincided with the data shown in Table 5a. On the other hand, there were no significant differences ($P > 0.05$) between MD1 and MD2 in the prevalence of periodontal pathogens. These data suggest that infection with periodontal pathogens progresses with development of the dentition, especially during the transition from primary to mixed dentition. Wang *et al* [29] reported that genes affecting susceptibility to caries in the primary

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dentition may differ from those in permanent teeth. According to Carvalho *et al* [30] the formation, distribution, and maturation of plaque differ with dental stage. These results support the findings of the present study. In spite of the strong relationship with caries experience, infection with *S. mutans* had no association with development of the dentition. Thus, infection with *S. mutans* may occur in early childhood.

There was a significant association between *T. forsythia* infection and dental stage in the *Bam*HI site + group (Table 6). In a previous report,[8] we had suggested that HLA-DQB1 was associated with *T. forsythia* infection in Japanese adolescents. In the present study, we selected younger subjects (3 to 11 years) with unknown periodontal conditions. However, our present results also indicate an important association of *T. forsythia* infection and HLA-DQB1 with dentition development. Thus, we postulate that locating the atypical *Bam*HI in HLA-DQB1 gene might be useful to determine the tendency of high susceptibility to periodontal disease in children. Further studies with larger samples would be needed to confirm this hypothesis.

## CONCLUSIONS

Our data suggested that cariogenic bacteria and periodontal pathogens have different infection patterns and that the period of infection of these bacteria also differs. Thus, pediatric dentists must educate patients and their guardians regarding the susceptibility to dental caries and periodontal disease and the necessity of prevention from an early age.

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## REFERENCES

1. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev, 50: 353–380, 1986.
2. Okada M, Hayashi F, Nagasaka N. PCR detection of 5 putative periodontal pathogens in dental plaque samples from children 2 to 12 years of age. J Clin Periodontol, 28: 576–582, 2001.
3. Hayashi F, Okada M, Soda Y, Miura K, Kozai K. Subgingival distribution of *Campylobacter rectus* and *Tannerella forsythensis* on healthy children with primary dentition. Arch Oral Biol, 51: 10–14, 2006.
4. Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev, 44: 331–384, 1980.
5. Fujiwara T, Sasada E, Mima N, Ooshima T. Caries prevalence and salivary *mutans streptococci* in 0-2-year-old children of Japan. Community Dent Oral Epidemiol, 19: 151–154, 1991.
6. Hirose H, Hirose K, Isogai E, Miura H, Ueda I. Close association between *Streptococcus sobrinus* in the saliva of young children and smooth surface caries increment. Caries Res, 27: 292–297, 1993.
7. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol, 11: 266–73, 1996.
8 Shimomura-Kuroki J, Yamashita K, Shimooka S. *Tannerella forsythia* and the HLA-DQB1 allele are associated with susceptibility to periodontal disease in Japanese adolescents. Odontology, 97: 32–37, 2009.
9. Ohyama H, Takashiba S, Oyaizu K, Nagai A, Naruse T, Inoko H, Kurihara H, Murayama Y. HLA class II genotypes associated with early-onset periodontitis: DQB1 molecule primarily confers susceptibility to the disease. J Periodontol, 67: 888–894, 1996.
10. Takashiba S, Ohyama H, Oyaizu K, Kogoe-Kato N, Murayama Y. HLA genetics for diagnosis of susceptibility to early-onset periodontitis. J Periodont Res, 34: 374–378, 1999.
11. Takashiba S, Noji S, Nishimura F, Ohyama H, Kurihara H, Nomura Y, Taniguchi S, Murayama Y. Unique intronic variations of HLA-DQβ gene in early-onset periodontitis. J Periodontol, 65: 379–86 1994.
12. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: Evidence for a discrete window of infectivity. J Dent Res, 72: 37–45, 1993.
13. Caufield PW, Dasanayake AP, Li Y, Pan Y, Hsu J, Hardin M. Natural history of *Streptococcus sanguinis* in in the oral cavity of infacts: Evidence for a discrete window of infectivity. Infect Immun, 68: 4018–4023, 2000.
14. WHO. Dentition status and treatment needs. In: Oral health surveys: basic methods, 4th ed. Geneva; World Health Organization, 40–47, 1997.
15. Oho T, Yamashita Y, Shimazaki Y, Kushiyama M, Koga T. Simple and rapid detection of *Streptococcus mutans* and *Streptococcus sobrinus* in human saliva by polymerase chain reaction. Oral Microbiol Immunol, 15: 258–262, 2000.
16. Hoshino T, Kawaguchi M, Shimizu N, Hoshino N, Ooshima T, Fujiwara T. PCR detection and identification of oral streptococci in saliva samples using gtf genes. Diagn Microbiol Infect Dis, 48: 195–199, 2004.
17. Byun R, Nadkarni MA, Chhour KL, Martin FE, Jacques NA, Hunter N. Quantitative analysis of diverse *Lactobacillus* species present in advanced dental caries. J Clin Microbiol, 42: 3128–3136, 2004.
18. De Soete M, Dekeyser C, Pauwels M, Teughels W, Van Steenberghe D, Quirynen M. Increase in cariogenic bacteria after initial periodontal therapy. J Dent Res, 84: 48–53, 2005.
19. Quirynen M, Gizani S, Mongardini C, Declerck D, Vinckier F, Van Steenberghe D. The effect of periodontal therapy on the number of cariogenic bacteria in different intra-oral niches. J Clin Periodontol, 26: 322–327, 1999.
20. Tanner ACR, Milgrom PM, Kent R Jr, Mokeem SA, Page RC, Liao SIA, Riedy CA, Bruss JB. Similarity of the oral microbiota of preschool children with that of their caregivers in a population-based study. Oral Microbiol Immunol, 17: 379–387, 2003.
21. Bimstein E, Ram D, Irshied J, Naor R, Sela MN. Periodontal diseases, caries, and microbial composition of the subgingival plaque in children: A longitudinal study. J Dent Child, 69: 133–137, 2002.
22. Miyamoto E, Nakano K, Fujita K, Nomura R, Okawa R, Matsumoto M, Ooshima T. Bacterial profiles of oral streptococcal and periodontal bacterial species in saliva specimens from Japanese subjects. Arch Oral Biol, 54: 374–379, 2009.
23. Monchois V, Willemot RM, Monsan P. Glucansucrases: mechanism of action and structure-function relationships. FEMS Microbiol Rev, 23: 131–151, 1999.
24. Fujiwara T, Hoshino T, Ooshima T, Sobue S, Hamada S. Purification, characterization, and molecular analysis of the gene encoding glucosyltransferase from *Streptococcus oralis*. Infect Immun, 68: 2475–2483, 2000.
25. Berkowitz RJ, Jordan HV, White G. The early establishment of *Streptococcus mutans* in the mouths of infants. Arch Oral Biol, 20: 171–174, 1975.
26. Berkowitz RJ, Turner J, Green P. Primary oral infection of infants with *Streptococcus mutans*. Arch Oral Biol, 25: 221–224, 1980.
27. Berkowitz RJ, Jones P. Mouth-to mouth transmission of the bacterium *Streptococcus mutans* between mother and child. Arch Oral Biol, 30: 377–379, 1985.
28. Lindquist B, Emilson CG. Colonization of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes and caries development in children to mothers harboring both species. Caries Res, 38: 95–103, 2004.

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29. Wang X, Shaffer JR, Weyant RJ, Cuenco KT, DeSensi RS, Crout R, McNeil DW, Marazita ML. Genes and their effects on dental caries may differ between primary and permanent dentitions. Caries Res, 44: 277–284, 2010.
30. Carvalho JC, Figueredo CS, Mestrinho HD. Clinical report on plaque formation, distribution and maturation within the primary, mixed and permanent dentitions. Eur J Paediatr Dent, 10: 193–199, 2009.

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