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Genetic polymorphisms of the sortase A gene and early childhood caries in two-year-old children

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ARTICLE INFO

Article history:
Accepted 1 February 2012

Keywords:
Streptococcus mutans
Sortase
Caries

ABSTRACT

Objective: To explore and compare the genetic polymorphisms of the sortase A (srtA) gene found in *Streptococcus mutans* (*S. mutans*) infecting two-year-old children suffering early childhood caries to those found in caries-free children through molecular identification methods.

Methods: Clinical *S. mutans* strains were isolated from the dental plaques of two-year-old children. Fifteen strains of *S. mutans* from the caries-active group and 15 strains of *S. mutans* from the caries-free group were collected. Genomic DNA was extracted from the *S. mutans* isolates. DNA fragments, including the srtA gene, were amplified by PCR. The PCR products were purified, sequenced and analyzed. A chi-square test and BioEdit software were used to analyze the sequencing results.

Results: All 30 clinically isolated *S. mutans* strains had a 741 base pair (bp) srtA gene. There were no nucleotide sequence insertions or deletions observed in the srtA genes. Twenty mutations were identified in the srtA genes that taken from the 30 clinical strains. There were 10 silent point mutations at the 78, 99, 150, 165, 186, 222, 249, 261, 312, and 636 bp positions. The other 10 mutations were point mutations resulting in a missense mutation at the 23, 34, 36, 47, 112, 114, 168, 176, 470, and 671 bp positions. None of the positions were enzyme-activity sites of srt A. The missense mutation rates of the two groups did not exhibit statistically significant differences.

Conclusion: There were no genetic polymorphisms of the sortase A gene associated with early childhood caries in two-year-old children.

1. Introduction

Streptococcus mutans (*S. mutans*) is considered a principal etiological agent of human dental caries.[1] The main virulence factors associated with cariogenicity include adhesion, acidogenicity, and acid tolerance.[2] The adherence of *S. mutans* to dental surfaces is the first step in the formation of a plaque biofilm and is mediated by both sucrose-dependent and sucrose-independent mechanisms.[3,4] Incipient adherence of *S. mutans* is a sucrose-independent mechanism. In the absence of sucrose, *S. mutans* expresses several surface proteins that can bind to salivary components to form the required pellicle on the teeth. This sucrose-independent adhesion is implicated in initial *S. mutans* colonization of the teeth in vivo.

Recently, it was demonstrated that the sortase A (SrtA) enzyme is responsible for sorting and anchoring surface proteins to the cell wall of *S. mutans*.[5] SrtA is a transpeptidase essential for anchoring the majority of the LPXTG motif-containing proteins of gram-positive bacteria,[6] and inactiva-

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0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2012.02.002

tion of the srtA gene in several gram-positive bacteria has been reported to cause multiple pathogenesis defects.[7,8] *S. mutans* strains with a mutation in srtA that disables the enzyme are almost incapable of colonizing the teeth in the absence of sucrose,[9] and srtA is responsible for the cariogenicity of *S. mutans*.[5]

Early childhood caries (ECC) provides one of the major disease impacts on children's health. Early Childhood Caries is defined as "the presence of 1 or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces" in any primary tooth in a 71-month or younger child.[10] ECC develops early and is distributed in a polarized pattern with most dental decay occurring in a small number of children. There are many reports that *S. mutans* has a strong relationship with ECC.[11,12] It has been proposed that the strains of *S. mutans* associated with ECC are genetically distinct from those found in caries-free (CF) children.[13] As srtA is important in the control of surface proteins during the initial *S. mutans* colonization of the teeth, we hypothesized that the srtA gene might possess genetic polymorphisms in different strains of *S. mutans*.

The purpose of this study was to explore and compare the genetic polymorphisms of the srtA gene found in *S. mutans* associated with ECC to those found in *S. mutans* in CF children through molecular identification methods in clinical isolates obtained from two-year-old children.

2. Materials and methods

2.1. Bacterial strains

All clinical isolates were preserved from our previous study.[14] Fifteen strains of *S. mutans* were isolated separately from caries-active (dmft $\geq$ 5) children and caries-free (dmft = 0) children. The dmft index is the number of decayed, missing and filled deciduous teeth. The children were two-year-old who participated in an epidemiological survey.

This survey included 394 children, with 109 having ECC and 285 being CF.[15] Clinical examinations for ECC were conducted with the aid of CPI probes, disposable mouth mirrors, and an intra-oral LED light source using the criteria recommended by World Health Organization.[16] We selected 32 caries-active (dmft $\geq$ 5) children from 109 children with ECC and randomly selected 32 CF (dmft = 0) children from 285 CF children matched with sex. Dental plaque samples from all 64 children were cultured and *S. mutans* colonies were isolated only from 24 children in the caries-active group and 12 children in the CF group. Then, we randomly picked 2–4 colonies according to morphology from each child for gram staining and biochemical tests and got 75 isolated *S. mutans* strains from caries-active group and 34 strains from the CF group. 15 strains from each of the two groups were randomly selected as research objects. The *S. mutans* strains were grown in brain heart infusion broth under anaerobic conditions (10%H_2, 10% CO_2, 80% N_2) in anaerobic jars. *S. mutans* UA159 was used as a reference strain.

2.2. DNA extraction

The clinical isolates were transferred to 2 ml of brain heart infusion broth and incubated overnight at 37 °C until reaching stationary phase. The cells were harvested by centrifugation at 12,000 rpm for 5 min and washed twice with phosphate buffered saline. The bacterial cells were suspended in 5% Chelex 100, a chelating exchange resin,[17] treated with 5 units (20 mg/ml) proteinase K at 37 °C for 1 min, then digested at 56 °C for 4 h followed by boiling for 10 min and immediate cooling on ice for 3 min. After centrifugation at 12,000 rpm for 3 min, the supernatant was collected as a PCR template. The DNA concentration and purity were determined spectrophotometrically by measuring the A260 and A280 (Varian, USA). The genomic DNA samples were stored at −80 °C before use. Genomic DNA from *S. mutans* UA159 was used as the reference.

2.3. Amplification of srtA gene

The PCR primers were designed according to the UA159 srtA sequence. The srtA gene was localized in the 1053014–1053754 bp position within the UA159 whole genome, and the total length of srtA was 741 bp. Primer Express 2.0 software was used for designing the primers. The designed primer was used to amplify a 941 bp DNA fragment carrying the srtA gene. The primers were synthesized using an ABI 3900 DNA synthesizer.

Forward primer: 5′-TTGTTATTACGTTTGCAATGCTCA-3′
Reverse primer: 5′-CAAAGTGATGCGCCTAGATGAA-3′

The PCR amplification was performed in a 50 μl total reaction volume. Using a hot start protocol, the samples were preheated at 93 °C for 3 min followed by amplification under the following conditions: denaturation at 93 °C for 45 s, annealing at 55 °C for 45 s, and elongation at 72 °C for 45 s. A total of 40 cycles were performed and followed by a final elongation step at 72 °C for 45 s. Eight microliters of each amplified product was electrophoresed in 2% (wt/vol) agarose gel along with a molecular size marker (Takara, Japan) in parallel. The Tris–borate–EDTA buffer electrophoresis was performed at 110 V for 0.5 h. The gel was stained with ethidium bromide and visualized under short-wavelength UV light. The results were captured with an Alpha IS-1000 digital imaging system (Alpha Innotech Corp., San Leandro, CA).

2.4. Sequencing of srtA gene

The PCR products were collected and purified using a Qiaquick Gel Extraction Kit (QIAgen, Hilden, Germany). Each amplified product was sequenced at both directions by the Shanghai Shenggong Bioengineering Company (Shenggong, China). The sequences of the PCR products were compared with known srtA gene sequences in GenBank from the UA159 strain using BioEdit software (GenBank accession number NC_004350).

2.5. Statistical analysis

SPSS17.0 software was used for statistical analysis. A chi-square test was used to compare different srtA sequences between the caries-active group and the caries-free group. A P-value below 0.05 was used to indicate a statistically significant