Genetic polymorphism of the salivary mucin gene MUC7 in severe caries in Japanese pediatric patients

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Abstract  Caries is a major oral disease whose onset and advance in a broad age group is lifestyle-related, but the genetic background of individuals is also a very important factor. Nevertheless, the relationship between caries frequency and genetic background remains unclear, especially for genetic polymorphisms of salivary proteins. The salivary mucin MG2 plays a role in facilitating the clearance of bacteria, interacts with Streptococcus mutans, which may be a caries pathogen, and can function as an antimicrobial agent. Here, we evaluated the statistical relationship of genetic polymorphisms of tandem repeats in the gene encoding MG2 (MUC7) with the frequency of pediatric severe caries in the Japanese population. Genomic DNA from lingual mucosal cells of 70 healthy and 92 caries patients was purified, and the frequency of genetic polymorphisms in MUC7 was evaluated by PCR. The results indicated that alleles of 6 (the most common allele) and 5 tandem repeats were observed in control and caries groups. Differences in the frequencies of polymorphisms were analyzed by Fisher’s exact test. P-values for the frequencies of polymorphisms in MUC7 were as follows: 5-5 versus 5-6 repeats, 0.743; 5-5 versus both 5-6 and 6-6 repeats, 1.00; 5-6 versus 6-6 repeats, 0.254; 5-6 versus both 5-5 and 6-6 repeats, 0.206; 6-6 versus 5-5 repeats, 1.00; 6-6 versus both 5-5 and 5-6 repeats, 0.341. Our data suggest no significant association between genetic polymorphisms in the MUC7 gene and caries susceptibility in the Japanese pediatric population.

Key words  Caries, Genotype, MG2, Salivary mucin

Introduction  Dental caries, otherwise known as tooth decay, is a multifactorial disease in the oral cavity. Caries onset may be caused by many environmental factors, such as poor oral hygiene, oral microbial flora, and type of diet. Bacterial infection, in particular by Streptococcus mutans (S. mutans), is also thought to be a major risk factor for early childhood caries and future caries experience. Moreover, the physiological state of the host, for instance salivary flow and salivary buffering capacity, may affect caries onset. In addition, some salivary proteins (a genetic host factor) that have antibacterial properties and mineralization effects may affect the caries process. Thus, various factors are involved in caries onset and development.

Mucins (MG), which play multiple roles in the oral cavity, are salivary glycoproteins. Two distinct mucins, MG1 and MG2, are respectively synthesized from the MUC5B and MUC7 genes. These glycoproteins have been identified and characterized as preferentially expressed and secreted by submandibular, sublingual, and minor salivary glands. MG2, with a molecular weight of 150–180 kDa, is a...
A monomeric protein containing 30.4% protein, 68.0% carbohydrate, and 1.6% sulfate. The apomucin moiety of secreted MG2 is composed of 357 amino acid (aa) residues, and its molecular weight is 37 kDa. The structure of MG2 contains an N-terminal 144 aa serine, threonine, and proline rich domain; a 23 aa central domain consisting of 5, 6 or 8 nearly identical tandem repeats; and a C-terminal domain of 75 aa residues. In particular, the central domain of MG2 is thought to be very important for its modification and function. The tandem repeat region of MG2 contains 46 potential O-glycosylation sites. In general, glycosylated proteins are thought to be associated and agglutinated with various biological materials. MG2 interacts with oral bacteria, including S. mutans, which may be a caries pathogen. Moreover, MG2 agglutinates human immunodeficiency virus type 1 (HIV-1) and inhibits HIV infection by displacing the envelope glycoprotein gp120. Thus, MG2 is considered to be an important salivary factor of the non-immune defense system in the oral cavity.

To evaluate whether a relationship exists between diseases and the frequency of genetic polymorphisms, including single nucleotide polymorphisms (SNPs), many researchers use methods of SNP analysis. Recently, such studies have been conducted for oral diseases such as caries and periodontitis. For example, one study found no significant association between caries susceptibility and SNPs of the amelogenin (AMELX) or enamelin (ENAM) genes in subjects from various racial backgrounds. A recent study of ours also failed to find a correlation between the frequency of SNPs in AMELX and ENAM and pediatric severe caries among Japanese. However, analysis of genetic polymorphisms of potential target genes in relation to caries has only recently started.

Here, we report a lack of significant correlation between the frequency of a genetic polymorphism in the salivary mucin gene MUC7 and pediatric severe caries among Japanese. These findings help to elucidate the impact of host genetic background on the caries process.

### Materials and Methods

#### Subjects

All subjects were unrelated individuals of Japanese extraction. The 70 healthy controls (male: 33, female: 37) and 92 caries patients (male: 59, female: 33) ranged from 3 to 6 years of age (mean ± SD 4.8 ± 1.2 and 5.3 ± 0.96 years, respectively, Table 1). Table 1 also shows the numbers of total present and decayed teeth of subjects. All patients, who had no other diseases of the oral hard and soft tissues, have visited Matsumoto Dental University Hospital for their caries. None used orthodontic appliances and none needed pre-medication for dental treatment. No subject had a history of diabetes, hepatitis virus, or HIV infection. None were chronically taking anti-inflammatory drugs or were in a state of declining immunity. No subject had current acute necrotizing ulcerative gingivitis. Dmf (decayed, missing, and filled primary teeth) was adapted to the diagnostic classification of the control subjects (dmf = 0) and caries patients (dmf ≥ 10) (patients who had 10 or more decayed teeth were treated under general anesthesia, Table 1). The survey was conducted under the supervision of a pediatric dentist with 8 years of clinical experience.

#### Ethics

Subjects’ parents or legal guardians gave appropriate informed consent. The Ethics Committee of Matsumoto Dental University approved to the study protocol (No. 0061).

#### Genomic DNA extraction

Genomic DNA from lingual mucosal cells collected with a toothbrush was extracted using a previously described method. The concentration of purified DNA was measured by spectrophotometry and electrophoresis.
Genotype
The sequences of the primers used are as follows, hMG2Fw: 5′-GTAGCTACATTAGCACCAGTG-3′ and hMG2Rv: 5′-TTCAGAAGTGTCAGGTGCAAG-3′. PCR amplification of the target gene was carried out using a Gene Amp® PCR System 9700 (Applied Biosystems). The reactions were performed in a volume of 50 μl containing 0.1–0.5 μg genomic DNA, 1 × Taq buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 0.25 μM specific primers, and 1 U of Taq Polymerase (Biotech International). The PCR cycle conditions were an initial denaturation at 96°C for 2 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1.5 min, and extension at 72°C for 1 min, with a final extension at 74°C for 7 min. PCR products were electrophoresed on a 2% agarose gel and stained with ethidium bromide.

Statistical analysis
The differences in the frequency of genetic polymorphism for the MUC7 gene were analyzed by Fisher’s exact test.

Results
We have investigated the possibility of a relationship between pediatric severe caries and the incidence of genetic polymorphisms of the central domain, which may have important functions, in the mucin gene MUC7.

Genomic DNA was purified from Japanese pediatric subjects with or without (control) caries and then subjected to PCR with appropriate MUC7-specific primers. Following PCR, products were separated by 2% agarose gel electrophoresis, the gel was stained with ethidium bromide, and the pattern of genetic polymorphisms of tandem repeats in MUC7 were determined. Figure 1 shows an electrophoretic pattern of individuals from both the control and caries groups. In both groups, the sizes of PCR products for MUC7*5 and MUC7*6 alleles (5-5 and 6-6 genetic polymorphisms, respectively) were observed as 453 bp and 522 bp, respectively. The genetic polymorphism containing both 5 and 6 tandem repeats (5-6 genetic polymorphism) was also found in both groups. These results suggest
that the Japanese pediatric subjects in this study have genetic polymorphisms of either 5 or 6 tandem repeats, or both, in the central domain of \textit{MUC7}.

Next, we determined the statistical significance of these results for 70 control subjects and 92 caries patients. As can be seen in Table 2, the 6-6 genetic polymorphism (the most common) for the \textit{MUC7} gene was 54.3% in the control and 45.7% in the patient group, while the 5-5 polymorphism was 7.1% in the control and 6.5% in the patient group. The heterozygous 5-6 genetic polymorphism was 38.6% in the control and 47.8% in the patient group. The $P$-value of the 5-5 versus 5-6 genetic polymorphism frequency between control and caries patients was 0.743, and that of 5-5 versus both 5-6 and 6-6 polymorphisms was 1.00 (Table 3). The $P$-value of the 5-6 versus 6-6 genetic polymorphism frequency between the two groups was 0.254, and that of 5-6 versus both 5-5 and 6-6 polymorphisms was 0.266. The $P$-value of the 6-6 versus 5-5 genetic polymorphism frequency between the two groups was 1.00, and that of 6-6 versus both 5-5 and 5-6 polymorphisms was 0.341. These results suggest that there is no significant association between the genetic polymorphism of the tandem repeat domain in \textit{MUC7} and severe caries susceptibility in the Japanese pediatric population.

**Discussion**

Our previous study showed that the frequency of SNPs in the amelogenin gene at positions +287 and +522 and in the enamelin gene at position +2452 are not significantly associated with susceptibility to severe caries among the Japanese pediatric population\textsuperscript{18}. Although many researchers have studied the relationship between the frequency of caries and genetic background for particular genetic polymorphisms including SNPs, no consistent relationship has been found and further studies are necessary.

In this study, we investigated the relationship between the frequency of genetic polymorphisms in the \textit{MUC7} gene and Japanese pediatric severe caries patients. We found that both pediatric healthy subjects and patients with caries had \textit{MUC7*5} and \textit{MUC7*6} alleles, and that genetic polymorphisms in this gene consist of 5-5, 5-6, and 6-6 types. However, there was no significant difference between the frequency of these genetic polymorphisms and caries in the Japanese population.

\textit{MG2} is thought to play a role in facilitating the clearance of bacteria in the oral cavity. \textit{MG2} also interacts with \textit{S. mutans}, promoting their agglutination\textsuperscript{13,14}. Moreover, \textit{MG2} can function as an antimicrobial agent\textsuperscript{21}, suggesting that it is closely related to the innate immune system in the oral cavity. Such functions of \textit{MG2} may be affected by structural modifications of the protein. The central domain of \textit{MG2} contains tandem repeats, each composed of 23 amino acids\textsuperscript{6}. The most common allele contains 6 tandem repeats (\textit{MUC7*6}), while some alleles contain 5 (\textit{MUC7*5}) or 8 (\textit{MUC7*8}) tandem repeats depending on the ethnic background\textsuperscript{11}. This tandem repeat domain may be involved in a structural scaffold that ensures conservation of specific glycosylation motifs\textsuperscript{10}. The glycosylation content and formation of \textit{MG2} are also likely to be functionally significant. Salivary \textit{MG2a} and \textit{MG2b}, which are isoforms of \textit{MG2} with identical amino acid sequences, have been found to have distinct carbohydrate contents\textsuperscript{21,22}. This implies that the different modifications of \textit{MG2} might affect its functions (against caries), such as interacting with oral bacteria, and its antimicrobial activity. Because the tandem repeat region of \textit{MG2} contains 46 potential glycosylation sites, the number of tandem repeats (allele length) might be linked to quantitative and qualitative distinct modifications (e.g., \textit{MUC7*5} and \textit{MUC7*6} proteins), thereby influencing the function of \textit{MG2}\textsuperscript{21}. In fact, the \textit{MUC7*5} allele is less prevalent in patients with asthma, suggesting its protective role in respiratory function\textsuperscript{14}. Moreover, \textit{MUC1} (a polymorphic epithelial mucin) is one of the mucins secreted from various epithelial gland tissues, and includes an allele exhibiting a length polymorphism due to a variable number of tandem repeats; a large number of tandem repeats are associated with severe acne\textsuperscript{23}. Therefore, we investigated the relationship between
the frequency of genetic polymorphism of the tandem repeat region in the MUC7 gene and caries.

A previous report found a statistically significant difference in the allele frequency of MUC7*5 between asthmatics and non-asthmatics in UK residents of Northern European origin\(^{11}\). That report also showed an incidence of the genetic polymorphism in individuals of East Asian origin as follows: 5-5 polymorphism, 1 (2.5%); 5-6 polymorphism, 15 (38.5%); 6-6 polymorphism, 23 (59.0%). Our data showed an incidence of the polymorphism in Japanese subjects (total of control subjects and caries patients) as follows: 5-5 polymorphism, 11 (6.8%); 5-6 polymorphism, 71 (43.8%); 6-6 polymorphism, 80 (49.4%) (Table 2). Taken together, these results indicate a somewhat distinct distribution of polymorphisms in the MUC7 gene in Japanese compared with East Asian populations. As East Asian populations generally comprise several ethnic origins from various countries rather than a single ethnic origin from one country, frequencies of some genetic polymorphisms, including SNPs, are expected to be different among ethnicities in East Asia. It was previously shown that a SNP of the IL-1A gene at position –889 is present in 13.0% of Japanese, 15.4% of Chinese, and 7.0% of Korean populations\(^{24–26}\). Accordingly, the incidence of other SNPs should vary by ethnicity, even within East Asia.

Many researchers have attempted to find a relationship between the frequency of SNPs for some genes and caries. For MG2, although polymorphisms of its central domain and the single nucleotide polymorphism rs9982010 have been analyzed and evaluated in asthma patients, the relationship between the frequency of SNP in MUC7 and caries has not yet been evaluated\(^{27}\). Therefore, further studies are required to examine this issue.

In the present study, we found no relationship between the frequency of genetic polymorphisms in a salivary protein and severe caries among the Japanese pediatric population. These findings provide further evidence that the mechanisms of caries onset and development are influenced by the host genetic background. Consequently, we should investigate other potential target genes that are suspected to be involved in caries.

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**Abbreviations:** aa, amino acid(s); AMELX, the amelogenin gene; dmf, decayed, missing and filled primary teeth; ENAM, the enamelin gene; HIV-1, human immunodeficiency virus type 1; MG2, mucin glycoprotein 2; PCR, polymerase chain reaction; S. mutans, Streptococcus mutans; SNP(s), single nucleotide polymorphism(s)

**References**


