The Role of Genetic Factors in the Outbreak Mechanism of Dental Caries

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Objective: *The aim of the present study was to investigate the relationships between cariogenic bacterial infection and single nucleotide polymorphisms (SNPs) in candidate genes associated with dental caries, and to explore the factors related to caries in children.*

Study design: *Children aged 3 to 11 years were selected. Detection of cariogenic bacteria (Streptococcus mutans, Streptococcus oralis, Streptococcus sobrinus and Lactobacillus) from the plaque of each patient, and SNP analyses of five candidate genes (MBL2, TAS2R38, GLUT2, MMP13 and CA6) were performed using DNA isolated from buccal mucosal cells. The dental caries experience in primary and permanent teeth was determined using the decayed, missing and filled teeth (DMFT) index, and the effects of the observed factors on the DMFT value were analyzed by multiple regression analysis.* ***Results:*** *The results of the multiple regression analysis showed that the DMFT value significantly increased in the presence of S. mutans or S. sobrinus ($p < 0.001$), while the dmft/DMFT value decreased in the presence of nucleobase C in MBL2 ($p < 0.05$).* ***Conclusion:*** *These results suggest that the MBL2 gene is related to the pathogenesis of dental caries.*

Key words: *Caries risk, children, cariogenic bacteria, MBL2*

INTRODUCTION

Dental caries is one of the most common chronic oral diseases in children[1], and the prevention of dental caries is essential for maintaining oral health. Cariogenic bacteria in plaque are one of the direct causes of dental caries. In addition, genetic, environmental and behavioral factors have also been reported to be associated with susceptibility to dental caries[2].

To date, a number of genetic studies on dental caries have been performed, leading to the identification of several susceptibility genes for the disease[3-6]. Furthermore, there have been many studies on the genetic factors associated with dental caries, and many of these studies investigated single nucleotide polymorphisms (SNPs) in candidate genes for positive markers of dental caries[7, 8]. In particular, focus has been placed on the mannose-binding lectin 2 (*MBL2*), taste receptor 2 member 38 (*TAS2R38*), glucose transporter 2 (*GLUT2*), matrix metalloproteinase 13 (*MMP13*) and carbonic anhydrase 6 (*CA6*) genes as candidate genes.

Mannose-binding lectin (MBL) is a liver-derived serum protein that plays an important role in innate immunity[6, 9]. The human MBL gene is named *MBL2*. Six polymorphisms in the *MBL2* gene were reported to be associated with variations in the quantity and function of MBL in serum[10], and the C(-290)G polymorphism was shown by Olszowski *et al* to be associated with carious intensity in children with primary dentition[11].

Some reports have indicated that taste pathway genes, such as those involved in taste preference, might influence caries risk. Wendell *et al.* performed multiple SNP assays for each gene and their results suggested that *TAS2R38* was a candidate gene associated with caries in primary dentition[12].

Eny *et al* reported that the presence of a SNP in the gene for GLUT2, a member of the glucose transport protein family, was associated with a higher habitual consumption of sugar and suggested that GLUT2 played a role in glucose sensing that affects food intake in humans[13]. Kulkarni *et al* reported that participants who were carriers of the Ile variant of *GLUT2* had a significantly higher DMFT caries score and suggested that the *GLUT2* genotype was associated with caries risk[14].

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Matrix metalloproteinases (MMPs) are major regulators of extracellular matrix turnover and they constitute a family of zinc-dependent endopeptidases. It is well known that MMPs play an important role in the organization of enamel and dentin organic matrix, suggesting their involvement in the control and progression of caries[15]. MMP13 is a collagenase 3 expressed in pulp dental tissue[14] that can degrade extracellular matrix components, such as collagens, gelatin, perlecan and fibronectin[16], and is involved in bone development and repair[17]. Tannure *et al.* reported that genetic variations in MMP13 caused by gene-environment interactions may contribute to caries susceptibility[18].

Carbonic anhydrase VI is a secreted enzyme that catalyzes the hydration of carbon hydroxide in saliva and other body fluids; this protein is encoded by the *CA6* gene. Some reports suggest that a polymorphism in the *CA6* gene is associated with the salivary buffer capacity[19, 20].

Despite these findings, there is still no positive marker that can estimate the risk of contracting dental caries; thus, additional studies are essential for obtaining more useful information in the clinical setting. The aim of the present study was to investigate the relationships between cariogenic bacterial infection and SNPs in candidate genes associated with dental caries, and to explore the factors related to caries in children.

MATERIALS AND METHOD

Subjects, aged 3 to 11 years (mean, 7.04 years; SD, 2.05 years; 40 boys and 41 girls), who were in the primary dentition or mixed dentition stage, were selected from the patients of the Pediatric Dental Clinic at The Nippon Dental University Niigata Hospital[21]. All subjects were Japanese and living in Niigata prefecture, had no relevant medical history and were not on antibiotic therapy. Their parents or guardians provided informed consent for inclusion in the study, and the study protocol was approved by the ethics committee of The Nippon Dental University School of Life Dentistry at Niigata (ECNG-H-151).

The dental caries experience in primary and permanent teeth was calculated using the decayed, missing and filled teeth index (dmft and DMFT for primary and permanent teeth, respectively) without radiographs, according to the World Health Organization[22]. The patients were classified into a caries-free group (n = 28; 15 boys and 13 girls) and a caries group (≥1 dmft + DMFT; n = 53; 25 boys and 28 girls).

Measurements of the pH of saliva and buffering capacity

For the measurement of the pH and buffering capacity of stimulated saliva, each subject was asked to chew on an unflavored paraffin pellet for 5 min and to spit approximately 5 ml of saliva into a test tube. The pH of saliva and buffering capacity of the stimulated saliva were measured using a hand-held Checkbuf™ pH meter (Horiba Ltd., Tokyo, Japan) according to the manufacturer's instructions.

DNA preparation and detection of cariogenic bacteria and periodontal pathogens

DNA preparation from plaque samples was performed as described previously[23]. Plaque samples were collected from the buccal surface of the maxillary left molars by swabbing 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 in an Eppendorf tube, which was then 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, heated at 100°C for 5 min, and then kept frozen at -20°C until used for polymerase chain reaction (PCR) analysis. PCR for the detection of cariogenic bacteria was performed targeting regions of the glucosyltransferase (GTF) gene of the three oral streptococci: *gtfb* and *gtfd* in *Streptococcus mutans*, *gtft* and *gtfi* in *Streptococcus sobrinus* and *gtfr* in *Streptococcus oralis.* In addition, PCR targeting 16S rRNA for the detection of *Lactobacillus* (*LB*) and five periodontal pathogens (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Tannerella forsythia*) was also performed as described previously[18].

Genomic DNA extraction and SNP analyses

Genomic DNA was isolated from the buccal mucosa. The buccal mucosa was rubbed with a swab for 15 s and the swab was inserted into 200 µl of extraction buffer in a microtube. The extraction of DNA was performed with the ISOHAIR® kit (Nippon Gene Co., Ltd., Toyama, Japan) according to the manufacturer's protocol. SNPs in five candidate genes, *MBL2, TAS2R38, GLUT2, MMP13, CA6* (one SNP for each), were analyzed by PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis. Genomic DNA samples from each subject were amplified by PCR with specific primers in a final volume of 50 µl of reaction mixture containing 45 µl of Platinum® PCR SuperMix (Invitrogen, Tokyo, Japan), 1 µl of 10 µM specific primers, 1 µl of each genomic DNA sample and 2 µl of distilled water. Each PCR product was digested with the appropriate restriction endonuclease. The products were run on 8% polyacrylamide gels, which were stained with ethidium bromide. The primer sets and restriction enzymes used are summarized in Table 1. *BsaJ*I digestion yields fragments of 162 bp (homozygote GG), and 128 bp and 34 bp (homozygote CC). *Cac8*I digestion yields fragments of 168 bp (homozygote CC), and 135 bp and 33 bp (homozygote GG). *Pst*I digestion yields fragments of 138 bp (homozygote TT), and 112 bp and 26 bp (homozygote CC). *Bsr*I digestion yields fragments of 158 bp (homozygote AA), and 117 bp and 41 bp (homozygote GG). *BtsC*I digestion yields fragments of 458 bp (homozygote CC), and 271 bp and 187 bp (homozygote TT).

Table 1. Species-specific primers and enzymes used for the detection of SNPs

Candidate gene	Primer pair (5′- 3′)	Enzyme
MBL2	CTTGTGACACTGCGTGACTAG GCAATGCACGGTCCCATTTG	*BsaJ*I
TAS2R38	TCTAACTCGCATCCGCACTG CACAGCAGCACACAATCACTG	*Cac8*I
GLUT2	GAACCCAAAACCAACCCCTTG TCCCCAAGCCACCCACCAAAGAATGCTGC	*Pst*I
MMP13	CCCTGCTGAAACAAGAGATGC CCTGCAATGGTGAGTCATCAC	*Bsr*I
CA6	AGTCCTTGGAGTTGACCTCG CTCTGTGCAAGCCATTCCTG	*BtsC*I

Statistical analysis

The effects of the observed factors (gender, pH of saliva, buffering capacity, prevalence of cariogenic bacteria, prevalence of periodontal pathogens and allele frequencies of each SNP) on the dmft/DMFT value were analyzed by multiple regression analysis using IBM SPSS Statistics ver. 22.0 (IBM Corp., Armonk, NY, USA). The categorical data (gender, prevalence of cariogenic bacteria, prevalence of periodontal pathogens and allele frequencies of each SNP) were coded for the multiple regression analysis. A stepwise method was used to obtain the multiple regression equation.

RESULTS

The relationship between caries experience and each factor

The distribution and prevalence of each of the cariogenic bacteria and periodontal pathogens based on caries experience are shown in Table 2. The dmft/DMFT value was over 15 in the presence of *S. mutans* or *S. sobrinus*. In addition, the dmft/DMFT value was over 15 in the presence of at least two types of periodontal pathogens. The relationships between caries experience and pH or buffering capacity of the stimulated saliva are also shown in Table 2. The pH value remained almost the same regardless of caries prevalence, but the buffering capacity varied with caries prevalence.

The allele frequencies of SNPs in the five candidate genes and the caries experience are shown in Table 3. The frequencies of the GG allele in the *MBL2* gene, the CC allele in the *GLUT2* gene and the TT allele in the *CA6* gene were all 0% in the caries-free group of primary dentition.

Effects on the dmft/DMFT value based on multiple regression analysis

Table 4 shows the effects of the variables that significantly affected the dmft/DMFT value. The analysis of variance (ANOVA) results indicated that the dmft/DMFT value could be predicted by the obtained regression model significantly ($p < 0.001$), although the coefficient of determination was not so high (0.464). Unstandardized coefficients indicated how much the dependent variable varied with an independent variable when all of the other independent variables were held constant. The unstandardized coefficient for the buffering capacity of the stimulated saliva was -1.414. This meant that for one unit increase in buffering capacity, there was a decrease in the dmft/DMFT value of 1.414. Based on the standardized coefficients, it was shown that the prevalence of cariogenic bacteria and periodontal pathogens increased the dmft/DMFT value significantly.

In contrast, the increase in buffering capacity and the CC allele in the *MBL2* gene decreased the dmft/DMFT value significantly. The scores of the *MBL2* genotypes in the multiple regression analysis were 1 for the GG allele, 2 for the CG allele and 3 for the CC allele. The other independent variables (gender, pH of saliva, *TAS2R38*, *GLUT2*, *MMP13* and *CA6*) did not affect the dmft/DMFT value significantly ($p > 0.05$). The tolerance and variance inflation factor (VIF) values in Table 4 show that the multicollinearity in the obtained multiple regression equation can be ignored because the tolerance values were much greater than 0.1 and the VIF values were much lower than 10.

Table 2. The distribution of cariogenic bacteria and periodontal pathogens, and the pH and buffering capacity of stimulated saliva based on caries experience

dmft+DMFT	Number of subjects	Number of subjects infected with cariogenic bacteria*				Number of subjects infected with periodontal pathogens**				Mean value of saliva pH	Mean value of buffering capacity
		1	2	3	4	1	2	3	4		
0	28	1	22	5	0	8	13	7	0	7.4	6.0
1	6	0	1	5	0	1	5	0	0	7.4	5.5
2	3	0	0	2	1	1	2	0	0	7.3	6.5
3	2	0	0	2	0	0	1	1	0	7.2	6.5
4	3	0	0	3	0	2	1	0	0	7.3	6.3
5	5	0	4	1	0	1	3	1	0	7.5	6.1
6	6	0	0	2	4	2	3	1	0	7.3	5.6
7	3	0	0	2	1	0	2	1	0	7.2	5.3
8	7	0	0	4	3	1	3	3	0	7.3	6.2
9	2	0	0	1	1	0	2	0	0	7.1	5.7
10	5	0	0	3	2	0	4	1	0	7.3	5.3
11	3	0	1	2	0	1	1	1	0	7.5	5.9
12	1	0	0	1	0	0	1	0	0	7.1	5.7
13	2	0	1	0	1	1	1	0	0	7.2	5.2
14	0										
15	1	0	0	0	1	0	0	1	0	7.2	3.9
16	1	0	0	0	1	0	0	1	0	7.0	5.8
17	1	0	0	1	0	0	0	1	0	7.4	6.8
18	0										
19	1	0	0	1	0	0	0	0	1	7.3	5.9
20	1	0	0	0	1	0	0	1	0	6.9	3.9

*Coded numbers for ANOVA: 1. *LB*; 2. *LB*, *S. oralis*; 3. *LB*, *S. oralis*, *S. mutans*; 4. *LB*, *S. oralis*, *S. mutans*, *S. sobrinus*

**Coded numbers for ANOVA: 1. No infection; 2. Infected with one type of periodontal pathogen; 3. Infected with two types of periodontal pathogens; 4. Infected with four types of periodontal pathogens

Table 3. Allele frequencies of SNPs in five candidate genes based on caries experience

Gene polymorphism (rs number)	Genotype	Caries-free group, n (%)	Caries group, n (%)
MBL2 C/G	CC	24 (85.7)	42 (79.2)
(rs7096206)	CG	4 (14.3)	9 (17.0)
	GG	0 (0.0)	2 (3.8)
TAS2R38 G/C	GG	8 (28.6)	10 (18.9)
(rs713598)	GC	16 (57.1)	32 (60.4)
	CC	4 (14.3)	11 (20.7)
GLUT2 C/T	CC	0 (0.0)	1 (1.9)
(rs5400)	CT	4 (14.3)	7 (13.2)
	TT	24 (85.7)	45 (84.9)
MMP13 A/G	AA	7 (25.0)	9 (17.0)
(rs2252070)	AG	11 (39.3)	23 (43.4)
	GG	10 (35.7)	21 (39.6)
CA6 C/T	CC	15 (53.6)	34 (64.2)
(rs2274327)	CT	13 (46.4)	15 (28.3)
	TT	0 (0.0)	4 (7.5)

DISCUSSION

Several reports have indicated that infection with cariogenic bacteria is a cause of dental caries[24-26]. Similarly, in this study, the presence of cariogenic bacteria increased the dmft/DMFT value significantly, suggesting that infection with cariogenic bacteria is a major factor of dental caries.

The prevalence of periodontal pathogens was also significantly higher in the subjects with a higher caries score. Some reports have suggested that cariogenic bacteria and pathogenic periodontal disease bacteria are antagonistic to each other[27, 28]. In this study, the results suggested the presence of relationships between cariogenic bacteria and periodontal pathogens in the development of oral diseases (Table 4).

The measurement of buffering capacity is an effective method to check for caries susceptibility[29, 30]. Our results also suggested that buffering capacity acts as an effective marker of caries activity.

Five SNPs in candidate genes previously reported as factors related to dental caries were analyzed in this study. Of these, only one SNP in the *MBL2* gene showed a significant effect in this study. *MBL2* is known to play an important role in the innate immune system[6, 9, 10] and a polymorphism in this gene is responsible for increasing the susceptibility to some infectious diseases. In this study, the presence of nucleobase C in *MBL2* decreased the dmft/DMFT value, suggesting that *MBL2* may be involved in the pathogenesis of dental caries. However, further studies with larger samples will be needed to confirm this hypothesis.

Table 4. The effects of the variables that significantly affected the dmft/DMFT value

Variables	Unstandarized coefficients	Standardized coefficients	*p* value	Multicollinearity Tolerance	VIF
(constant)	5.488		0.183		
Cariogenic bacteria	3.343	0.480	<0.001	0.939	1.065
Buffering capacity	-1.414	-0.282	0.001	0.949	1.053
Periodontal pathogens	1.822	0.249	0.005	0.938	1.066
MBL2	-1.960	-0.173	0.042	0.994	1.006

The results of the stepwise multiple regression analysis

CONCLUSION

Our data suggested that the *MBL2* gene is related to the pathogenesis of dental caries. Thus, it may be effective that pediatric dentists do not only evaluate the presence of cariogenic bacteria or measure the buffering capacity, but also analyze the SNP in the *MBL2* gene to know the susceptibility to dental caries of children.

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