MMP-1 promoter polymorphism in Korean with generalized aggressive periodontitis

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ABSTRACT

Purpose: The aim of this study was to investigate matrix metalloproteinase 1 (MMP-1) gene polymorphism (1G/2G at -1607 and A/G at -519) in Korean subject and to assess the association between polymorphism and periodontal status.

Methods: Forty nine generalized aggressive periodontitis (GAP) patients and 57 periodontally healthy children were recruited and genomic DNA was extracted from buccal swab. The polymorphisms of MMP-1 promoter genes were determined by polymerase chain reaction and restriction fragment length product (PCR-RFLP) method. The distribution of genotype and allele frequency was compared between 2 groups by χ^2 test.

Results: There was a significant difference in the distribution of genotypes and frequency of alleles between the GAP and reference groups at the position - 519 of MMP-1 gene promoter ($P < 0.05$). Allele G carrier rate was significantly lower in GAP group than that of the reference group ($P < 0.001$). At the position -1607 of MMP-1 gene promoter, genotype distribution and allele frequency showed no statistically significant difference between the groups. However, in the female group, a significant difference was observed between the groups for the genotype distribution, allele frequency and allele 1G carrier rate ($P < 0.05$).

Conclusions: The DNA polymorphism at the MMP-1 gene promoter might be associated with GAP in Korean.

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KEY WORDS: aggressive periodontitis; genetic polymorphism; matrix metalloproteinase 1

INTRODUCTION

Inflammatory destruction of periodontal attachment apparatus is the hallmark of periodontal disease. The inflammatory reaction associated with periodontitis may damage the surrounding cells and connective tissue structures, including alveolar bone, causing tooth loss[1].

The current concepts of the etiology of periodontitis implicate bacterial infection by gram-negative organisms as the primary cause of the disease[2]. However, the simple presence, type or quantity of infection does not explain the different evolution and the poor prognosis in severe chronic periodontitis or aggressive periodontitis. Therefore, Michalowicz et al.[3] reported both genetic and environmental factors are involved in this inflammatory disease etiology of which is influenced by interaction of periodontal pathogens and host responses.

Matrix metalloproteinases (MMPs) are a family of metal-dependent proteolytic enzymes that mediate the degradation of extracellular matrix and basement membranes. Currently, at least 23 MMPs have been characterized[4]. A wide range of evidences has indicated that MMPs are found in the gingival crevicular fluid of patients with periodontitis in much larger amounts than in the control subjects[5]. There are also many evidences for the role of MMPs in the destructive processes of periodontal disease linked with the unbalanced production between MMPs and their endogenous tissue inhibitors[6]. Thus, these enzymes are thought to play a crucial role in the destruction of

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periodontal tissues.

MMP-1 is the most widely expressed MMP possessing proteolytic activities against fibrillar collagens (collagen types I, II and III), which are the most abundant protein components of periodontal extracellular matrix[6]. Normally, the expression levels of MMP-1 in most cells are low, but in pathological state, such as periodontitis, the gene transcript levels for MMP-1 are significantly upregulated by cytokines and growth factors[7]. Furthermore, MMP-1 levels were found to increase in periodontitis-affected gingival tissues[8], both the protein amounts and the activities of MMP-1 not only in gingival crevicular fluid but also in gingival tissue were reported to be higher in periodontitis affected sites than in healthy sites[9]. These findings suggest that MMP-1 is the key player acting in the periodontal extracellular matrix degradation and remodelling.

Kornman et al.[10] reported the association between interleukin (IL)-1 genotype and periodontitis severity, encouraging the search for some specific genetic risk factors for periodontitis. In Korean subjects, Jeong et al.[11] suggested that IL-1 gene polymorphism might have an influence on periodontal disease and Bang et al.[12] found a significant association between IL-6 gene polymorphisms and generalized aggressive periodontitis (GAP). However, Ryu et al.[13] failed to find anassociation between IL-10 gene polymorphisms and GAP.

DNA polymorphisms have been found in the promoter regions of several MMPs. Rutter et al.[14] reported an additional G at the nucleotide position -1607 in the promoter region of the MMP-1 gene creates a binding site for Ets family of transcription factors, 5'-GGA-3', as well as increased transcription of the MMP-1 gene and enzyme activity in normal fibroblasts and melanoma cells. Jurajda et al.[15] described the polymorphism at another position -519 of the MMP-1 gene promoter corresponding to an A/G change. They reported linkage between the -519 A/G and the -1607 1G/2G polymorphisms that the allele A at -519 is more often found with the allele 2G at -1607 and the allele G at -519 with the allele 1G at -1607, respectively.

Levels of MMP-1 expression can be influenced by these genetic variations and the cells expressing these polymorphism may provide a mechanism for more aggressive matrix degradation and hence this gene might be crucial in mediating connective tissue degradation in the pathogenesis of several diseases such as colorectal cancer[16], carotid artery disease[17], acute myocardial infarction[18] and ovarian cancer[19], including periodontitis.

A case-control study performed in Caucasian subjects showed a positive association between the allele 2G and the severity of chronic periodontal disease[20]. This same allele was also associated with early dental-implants failure[21]. Other studies performed in a Czech and Japanese population have found no association between this MMP-1 polymorphism and periodontitis[22,23].

All types of periodontitis, especially aggressive periodontitis, have at least a genetic basis for susceptibility to periodontal microbial infection and as single nucleotide polymorphism (SNP) displays ethnic variations and it is worthy to explore whether SNP of MMP-1 gene in Korean subjects is associated with GAP. Thus, this study was aimed to investigate MMP-1 gene polymorphism (1G/2G at -1607 and A/G at -5[19]) in Korean subject and to assess the association between polymorphism and periodontal status.

MATERIALS AND METHODS

1. Subjects

A total of 106 non-smoking Korean subject were recruited for this study. We included 49 GAP patients from the Department of Periodontics and 57 children from the Department of Pediatric Dentistry, Chonnam National University Hospital. A diagnosis of GAP was made on clinical and radiographic examination with the basis of medical and dental history questionnaires. Subjects in the GAP group were diagnosed by following

the criteria defined by the American Academy of Periodontology in 1999[24]. Each subject had no history of systemic diseases, and had not taken any medications. They are usually under 30 years of age, but may be older, and presented with more than 5 mm of clinical attachment loss, loss of generalized interproximal attachment and loss of alveolar bone at least three permanent teeth other than first molars and incisors.

This study protocol was approved by an Institutional Review Board (Chonnam National University IRB No.1-2007-09-070) and subjects signed a consent form after being advised of the nature of the study.

2. Clinical Measurement

Measurements of probing depth (PD) and clinical attachment level (CAL) were recorded at six points around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) except third molars using Williams probe (Hu-Friedy, Chicago, USA).

3. Buccal Swab and DNA Extraction

The sampling of epithelial buccal cells was performed as described by Trevilatto and Line[25]. Briefly, individuals undertook a mouthwash, buccal swab was taken with a sterile foam tipped applicator (Hardwood product company, Guilford, USA). The applicator was then frozen at −20 ℃ until used for DNA extraction. Genomic DNA was extracted with heating at 95 ℃ for 5 min in 200 ㎕ of 50 mM NaOH and neutralization with 20 ㎕ Tris buffer (pH 8.0).

4. Analysis of SNPs

The polymorphisms of promoter were determined by PCR-RFLP method as previously described[20]. The sequences of the PCR primers (Bioneer, Daejeon, Korea) are shown in Table 1. For the MMP-1-1607, the reverse primer was specially designed to introduce a recognition site of restriction enzyme *XmnI* (New England Biolabs, Ipswich, USA). The allele 1G has this recognition site, whereas the allele 2G destroy the recognition site by inserting a G[20]. Primer sequence for the MMP-1-519 introduce a recognition site of restriction enzyme *KpnI* (BEAMS biotechnology, Sungnam, Korea). Allele A/G substitution at position −519 removes the recognition site of this enzyme[15].

PCR was carried out in a total volume of 25 ㎕, containing 4 ㎕ of genomic DNA, 10 pmol of each primer and 12.5 ㎕ of GoTaq® Green Master Mix (premixed solutions containing GoTaq® DNA Polymerase, dNTPs, $MgCl_2$ and reaction buffers), (Promega, Madison, USA) using thermal cycler (Geneamp PCR system 2700®), (Biosystems, Middletown, USA). The PCR cycling conditions of MMP-1-1607 were 1 min at 95 ℃ followed by 35 cycles of 30 s at 95 ℃, 30 s at 55 ℃ and 30 s at 72 ℃, with a final extension of 72 ℃ for 5 min. The PCR cycling of MMP-1-519 was constructed of initial denaturation at 95 ℃ for 2 min, followed by 30 cycles of denaturation at 93 ℃ for 30 s, annealing at 49 ℃ for 30 s, elongation at 72 ℃ for 20 s and final elongation at 72 ℃ for 4 min. The PCR products were electrophoresed in 3% agarose gel and stained with 0.1% ethidium bromide.

Table 1. The Sequences of the PCR Primers, Restriction Enzymes and Fragment Length

	Primer (5' - 3')	Restriction enzyme	Fragment (bp)
MMP-1-1607	F: 5'-TCG TGA GAA TGT CTT CCC ATT-3' R: 5'-TCT TGG ATT GAT TTG AGA TAA GTG AAA TC-3'	*XmnI* (37℃)	118 (29+89)
MMP-1-519	F: 5'-CAT GGT GCT ATC GCA ATA GGG T-3' R: 5'-TGC TAC AGG TTT CTC CAC ACA C-3'	*KpnI* (37℃)	200 (176+24)

MMP-1 : matrix metalloproteinase-1.

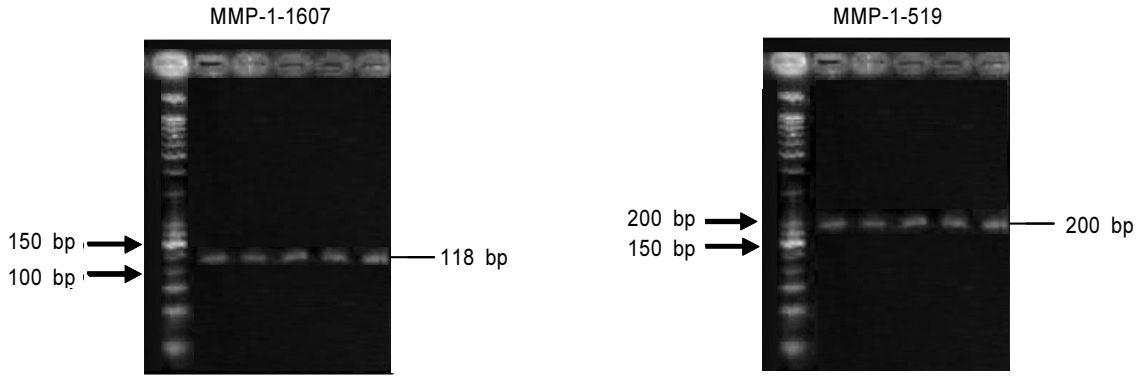

Figure 1. *The PCR products of MMP-1-1607 and MMP-1-519 were electrophoresed in 3% agarose gel and stained with 0.1% ethidium bromide. The PCR products were 118 bp and 200 bp for MMP-1-1607 and* MMP-1-519, *respectively.*

A 15 μl of resulting product was digested with restriction enzyme; 20 U/ml of *XmnI* for MMP-1-1607, 3 U/ml of *KpnI* for MMP-1-519 at 37℃ overnight. The restriction fragments were determined on 3% agarose gel electrophoresis and stained with 0.1% ethidium bromide. The sequences of the PCR primers, restriction enzymes and fragment length are shown in Table 1.

5. Statistical Analysis

Data analysis was performed with the SPSS 14.0K software program. The distribution of genetic polymorphism in the subjects was calculated using descriptive statistical analysis. The x^2 test was used to analyze the data obtained to determine the association between GAP and MMP-1-1607 and MMP-1-519 genotypes. The P value of 0.05 determined the statistical significance of the results.

RESULTS

1. Clinical Assessments

A total of 106 subjects were enrolled in this study. Forty nine non-smoking patients (26 males and 23 females) met the criteria for the GAP group with mean PD of 4.7 mm, mean CAL of 4.8 mm and mean 43.1 years of age when diagnosis was made. Fifty seven children (23 male and 34 female) who were periodontally healthy were included in the reference group.

2. Analysis of MMP-1 Gene Polymorphism

1) Gel Electrophoresis Results

The PCR products were 118 bp and 200 bp for MMP-1-1607 and MMP-1-519, respectively (Fig. 1).

Since *XmnI* digested the allele 1G of MMP-1-1607, allele 1G gave product of 89 bp and allele 2G gave product of 118 bp. For the MMP-1-519, allele A and allele G gave products of 176 bp and 200 bp respectively (Fig. 2).

2) Distribution of Genotypes and Allele Frequency

Genotype and allele distribution of MMP-1-1607 and MMP-1-519 are shown in Table 2. For MMP-1-1607, the allele 2G was observed at a frequency of 68.4% and 79.8% in GAP and reference group respectively. The 1G/1G, 1G/2G and 2G/2G genotype was found at a frequency of 6.1%, 51.0% and 42.9% for GAP group, 3.5%, 33.3% and 63.2% for reference

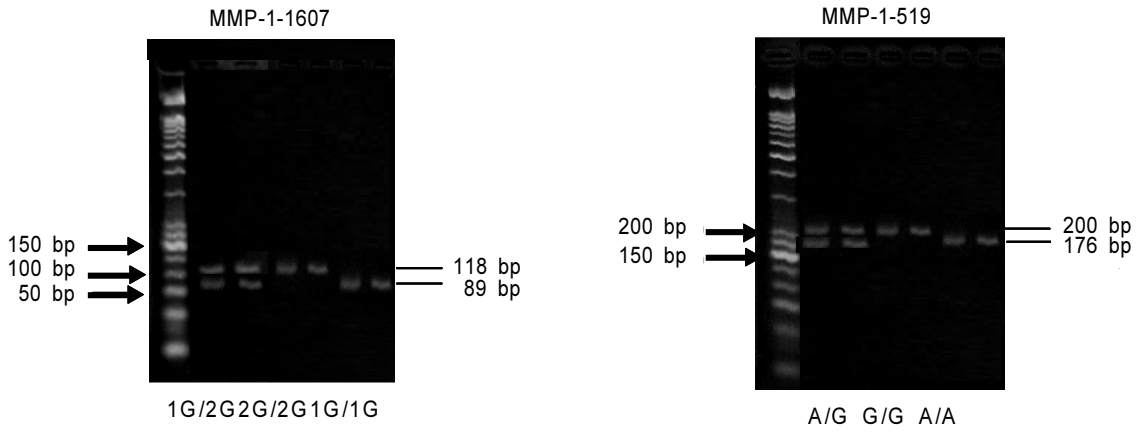

Figure 2. *SNPs at MMP-1-1607 and MMP-1-519 after restriction enzyme digestion were electrophoresed in 3% agarose gel and stained with 0.1% ethidium bromide. The allele 1G of MMP-1-1607 gave product of 89 bp and allele 2G gave product of 118 bp. For the MMP-1-519, allele A and allele G gave products of 176 bp and 200 bp respectively.*

Table 2. Distribution of Genotypes and Alleles of MMP-1-1607 and -519

	GAP (n=49)	Reference (n=57)	*P*-value
MMP-1-1607			
1G / 1G	6.1	3.5	0.060
1G / 2G	51.0	33.3	
2G / 2G	42.9	63.2	
Allele 1G frequency	31.6	20.2	0.060
Allele 2G frequency	68.4	79.8	
MMP-1-519			
A/A	69.4	31.6	0.000*
A/G	28.6	64.9	
G/G	2.0	3.5	
Allele A frequency	83.7	64.0	0.020*
Allele G frequency	16.3	36.0	

The data were presented in percentage (%).
MMP-1 : matrix metalloproteinase-1
Allele frequency : proportion of specific allele to the total number of alleles.
* Statistically significant at $P<0.05$ by χ^2 test compared to the reference group.

group, no significant difference was found between the two groups.

For MMP-1-519, allele A was shown in 83.7% and 64.0% for each group, the A/A, A/G and G/G genotype was found at a frequency of 69.4%, 28.6% and 2.0% for GAP group, and 31.6%, 64.9% and 3.5% for the reference group, which was significantly different between two groups ($P<0.05$) (Table 2).

The distribution of 1G/1G, 1G/2G and 2G/2G genotype at -1607 position of MMP-1 promoter was 0.0%, 57.7% and 42.3% for GAP group, 8.7%, 43.5% and 47.8% for reference group in male, which was not significantly different. However, it was 13.0%, 43.5% and 43.5% for GAP group, 0.0%, 26.5% and 73.5% for reference group in female subject which was significantly different ($P<0.05$). Allele 1G frequency of

MMP-1-1607 was shown in 28.8% and 30.4% in male, 34.8% and 13.2% in female that a significant difference was observed in female subject between the GAP and the reference groups ($P<0.05$).

At the position of MMP-1-519, A/A, A/G and G/G genotype distribution was 73.1%, 26.9% and 0.0% for male and 65.2%, 30.4% and 4.3% for female in GAP group, 34.8%, 60.9% and 4.3% for male 29.4%, 67.6% and 2.9% for female in the reference group. Both male and female showed a significant difference between the GAP and reference group ($P<0.05$). Allele A frequency of MMP-1-519 was 86.5%, 80.4% in GAP group, and 65.2%, 63.2% in the reference group for male and female respectively. Statistically significant difference between 2 groups was observed in male subject only (Table 3).

3) Distribution of Allele Carrier

At the position of MMP-1-1607, allele 1G carrier rate was 57.1% and 36.8% for GAP and reference group respectively which is not significantly different between two groups. For the MMP-1-519, allele G carrier rate was significantly higher in the reference group than that in the GAP group (68.4% versus 30.6%) (Table 4).

The allele 1G carrier rate of MMP-1-1607 was 57.7% for the GAP group, 52.2% for the reference group in male, 56.5% for the GAP group and 26.5% for the reference group in female. A statistic significant difference was in female subject ($P<0.05$). The allele G carrier rate at the position of -519 for the GAP group and reference group was 26.9%, 65.2% in male, 34.8%, 70.6% in female, and both gender subject showed a significant difference between groups (Table 5).

DISCUSSION

MMPs are a group of endogenous proteinases that contribute to degradation of extracellular matrix and basement membrane components. Among these structurally related but genetically distinct enzymes, MMP-1 is the key proteolytic enzymes capable of degrading fibrillar collagen, especially type I and III, which are the predominant types of interstitial collagen in gingiva[26]. Evidences indicated that levels and the activities of MMP-1 are found to be increased higher in periodontitis affected sites than in healthy sites[9,27]. Thus, MMP-1 might be a key player acting in the periodontal extracellular matrix degradation and remodelling.

The MMP and/or their inhibitors are regulated at various levels under tight control by factors such as regulation of gene expression, activation of zymogens and inhibition of activated enzymes by specific inhibitors[4]. Previous reports indicated that sequence variations in the promoters of MMPs may influence critical steps in binding to transcription factors or overall transcriptional efficiency, which results in differential expression of MMPs. These promoter polymorphisms are reported to be associated with susceptibility to diseases such as coronary artery disease, cancers and periodontitis[28].

A common single nucleotide G insertion/deletion polymorphism in the MMP-1 promoter at the position -1607 was reported to create a binding site for ets family transcription factor which have been shown to increase transcription of MMP-1[14] and the linkage between MMP-1-1607 polymorphism and A/G substitution polymorphism at the MMP-1-519 was reported[15]. The association of this gene polymorphism and periodontitis has been reported, but no studies have been reported in Korean subjects. Therefore, the purpose of this study was to investigate the association of the MMP-1-1607 (1G/2G) and MMP-1-519 (A/G) polymorphisms with the susceptibility to GAP in Korean subjects.

De Souza et al.[20] showed an association between MMP-1-1607 polymorphism and severe chronic periodontitis in 87 Brazilian subject. However, their recent

Table 3. Distribution of Genotypes and Allele Frequencies of MMP-1-1607 and -519 According to Genders

	Male			Female		
	GAP (n=26)	Reference (n=23)	*P*-value	GAP (n=23)	Reference (n=34)	*P*-value
MMP-1-1607						
1G/1G	0.0	8.7	0.303	13.0	0.0	0.020*
1G/2G	57.7	43.5		43.5	26.5	
2G/2G	42.3	47.8		43.5	73.5	
Allele 1G frequency	28.8	30.4	1.000	34.8	13.2	0.010*
Allele 2G frequency	71.2	69.6		65.2	86.8	
MMP-1-519						
A/A	73.1	34.8	0.013*	65.2	29.4	0.015*
A/G	26.9	60.9		30.4	67.6	
G/G	0.0	4.3		4.3	2.9	
Allele A frequency	86.5	65.2	0.017*	80.4	63.2	0.061
Allele G frequency	13.5	34.8		19.6	36.8	

The data were presented in percentage (%).
MMP-1 : matrix metalloproteinase-1
Allele frequency : proportion of specific allele to the total number of alleles.
*Statistically significant at $p<0.05$ by χ^2 test compared to the reference group.

Table 4. Distribution of Allele 1G Carrier of MMP-1-1607 and Allele G Carrier of MMP-1-519 in Each Group

	GAP (n=49)	Reference (n=57)	*P*-value
MMP-1-1607			
Allele 1G noncarrier	42.9	63.2	0.051
Allele 1G carrier	57.1	36.8	
MMP-1-519			
Allele G noncarrier	69.4	31.6	0.000*
Allele G carrier	30.6	68.4	

The data were presented in percentage (%).
MMP-1 : matrix metalloproteinase-1
*Statistically significant at $p<0.05$ by χ^2 test compared to the reference group.

Table 5. Distribution of Allele 1G Carrier of MMP-1-1607 and Allele G Carrier of MMP-1-519 in Each Group According to Genders

	Male			Female		
	GAP (n=26)	Reference (n=23)	*P*-value	GAP (n=23)	Reference (n=34)	*P*-value
MMP-1-1607						
Allele 1G noncarrier	42.3	47.8	0.778	43.5	73.5	0.029*
Allele 1G carrier	57.7	52.2		56.5	26.5	
MMP-1-519						
Allele G noncarrier	73.1	34.8	0.010*	65.2	29.4	0.014*
Allele G carrier	26.9	65.2		34.8	70.6	

The data were presented in percentage (%).
MMP-1 : matrix metalloproteinase-1
*Statistically significant at $P<0.05$ by χ^2 test compared to the reference group.

larger population study (n = 223) showed no significant association[29]. The discrepancy might occur due to methodological differences, in that the periodontitis group had not been classified by disease severity. In Japanese, Itagaki et al.[23] did not find any differences in MMP-1 allele and genotype distributions between healthy and chronic periodontitis groups. However, Cao et al.[30] reported that a single nucleotide polymorphism in the MMP-1 promoter region of -1607 may be associated with GAP in Chinese population. In this study, at the position -1607 of MMP-1 gene, distribution of genotypes failed to show any significant difference between the GAP and the reference group except in the female group. However, For the MMP-1-519, allele A was observed at the frequency of 83.7% that was significantly larger than that of reference group. The genotype of A/A, A/G and G/G of MMP-1-519 was 31.6%, 64.9% and 3.5% respectively and also showed a statistically significant difference between 2 groups. These results show that MMP-1 polymorphism can be associated with prevalence of GAP in Korean subject.

Ju et al.[31] conducted SNP analysis in Korean subject using the TaqMan assay method, which constitutes PCR and probing. Similar genotype distribution and allele frequency of the MMP-1-1607 gene in control group with my study was observed. The allele frequency of 2G in the cervical cancer group was 66.1%, and 66.9% in the control group, showing Korean with specific polymorphism in MMP-1 are neither more susceptible to develop cervical cancer nor more vulnerable for cancer progression. Nho et al.[32] however, reported MMP-1-1607 and MMP-1-519 polymorphisms can be associated with Body Mass Index with age ≥50 years in Korean by using the pyrosequencing SNP Reagent kits. They suggest that allele 1G in MMP-1-1607 and allele A in MMP-1-519 polymorphisms may play a protective factor against the increase in BMI in Korean population. But their control group showed a large different distribution of genotypes and alleles from other studies (Table 6).

These contradictory results on genes polymorphisms are might occur due to several reasons. Difference races and/or populations may have different risk alleles for the same disease. There are many regulatory mechanisms that may control the action of MMPs on the components of the extracellular matrix[33]. There are also various regulatory steps involved in destruc-

Table 6. Genotype Distribution and Allele Frequency of the Control Group Versus Previous Reports

	Czech[22]	Brazil[29]	Turkey[35]	Korea(this study)	Korea[32]	Korea[31]	Japan[23]	China[36]
MMP-1-1607								
1G/1G	29.8	22	60.8	3.5	32.6	9.9	14.8	26
1G/2G	46.1	47.7	27.8	33.3	43.3	46.4	43.7	50
2G/2G	24.1	30.3	11.4	63.2	24.1	43.7	41.5	24
Allele 2G frequency	47.2	54.1	25.3	79.8	45.8	66.9	63.4	49
Allele 2G carrier	70.2	78	39.2	96.5	67.4	90.1	85.2	74
MMP-1-519								
A/A	31.9	18	51.5	31.6				
A/G	45.4	64	41.3	64.9				
G/G	22.7	18	7.2	3.5				
Allele A frequency	54.6	50	72.2	64				
Allele G carrier	68.1	82	48.5	68.4				

The data were presented in percentage (%).

tion of periodontal connective tissue and/or bone; from bacterial infection into a host cell, host immune responses including the cytokine networks, MMPs and tissue inhibitors of MMPs regulation. Therefore, an increase in mRNA transcription only which is the result of MMP-1 polymorphism may not necessarily lead to the increased enzyme activity on the extracellular matrix. Furthermore, a study proved that some MMP members share common extracellular matrix substrates and compensate these functions for each other[34]. These shared functions indicate that a single gene polymorphism of an MMP may not have enough effect on disease susceptibility or progression.

In the present study, DNA samples from the subjects were limited in number, and the associations among SNP, clinical stage and prognosis in a larger number of patients were not investigated. In addition, this study may be limited by biases resulting from observational investigation and the ethnic composition since only Koreans were included. However, on the basis of these results, there may be some association between MMP-1 polymorphisms and susceptibility of GAP and it may contribute to the periodontal tissue destruction in Korean subjects. Further series studies are needed to search for other risk gene polymorphism that may synergistically elevate a susceptibility to the periodontal disease and haplotype analysis of these genes could be more important. In the future, the assessments of diagnostic periodontal risk gene may give an assurance in the detection of periodontitis susceptible patients.

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