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# Matrix metalloproteinase-2, -9, and tissue inhibitor of MMP-2 gene polymorphisms in Taiwanese periodontitis patients

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**Abstract** *Background/purpose:* Matrix metalloproteinases (MMPs) and tissue inhibitor of MMPs (TIMPs) have been shown to play an important role in the pathogenesis of tissue destruction in periodontitis. The associations between single nucleotide polymorphisms (SNPs) in the promoter regions of MMP-2, MMP-9, and TIMP-2 genes and the risk of aggressive periodontitis (AgP) and chronic periodontitis (CP) were investigated in a Taiwanese population.
*Materials and methods:* MMP-2 C-1306T, C-735T, T-790G, and MMP-9 C-1562T and TIMP-2 G-418C SNPs were genotyped by polymerase chain reaction-restriction fragment length polymorphism analysis in 69 patients and 129 patients with AgP and CP, respectively, and 117 periodontal healthy individuals who served as healthy controls (HC). Chi-square test and logistic regression analysis were used to investigate the possible association of genotypes with periodontitis.
*Results:* No significant differences in the distributions of the C-1306T and C-735T variants between periodontitis and HC were detected. Patients with genotype of MMP-2 -790 TT or T allele of MMP-2-790T/G as compared to genotypes of GT + GG genotypes or G allele, were less susceptible to CP [odds ratio (OR) = 0.50, 95% confidence interval (CI) = 0.25–1.00 and OR = 0.52, 95% CI = 0.28–0.96, respectively]. The frequencies of TIMP-2 G-418C gene polymorphisms in nonsmokers were statistically significantly different among AgP, CP, and HC groups (P = 0.024). The nonalcohol drinking participants with C allele of MMP-9 C-1562T as compared to T allele, were less susceptible to AgP (adjusted OR = 0.4; 95% CI, 0.18–0.90).

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*Conclusion*: It is suggested that MMP-2 T-790G, MMP-9 C-1562T, and TIMP-2 G-418C gene polymorphisms might be associated with periodontitis in the Taiwanese Han population.
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## Introduction

Periodontitis is a multifactorial disease that involves microbial challenge and host responses. Although bacteria are the initial factors for human periodontitis, their impact may be modified by an individual's predisposition, which can determine the manifestation and progression of the disease.[1] Matrix metalloproteinases (MMPs) play an important role in physiological and pathological events, including the repair and breakdown of connective tissue because of the inflammatory response.[2] Excessive production of MMPs because of genetic polymorphisms may influence the manifestation and development of periodontal diseases.[3]

MMP-2 can broadly break down both type IV collagen and noncollagenous components of extracellular matrix, and also native type collagen type I collagen in the gingival connective tissue matrix.[4,5] MMP-2 has effects on stimulating cell proliferation and inhibiting apoptosis. MMP-2 also affects fibroblast growth factor-1, leading to the release of the active soluble ectodomain of the receptor[6]; therefore, it has the potential to modulate the mitogenic and angiogenic activities of the fibroblast growth factor. These findings suggest that MMP-2 can contribute not only to tissue destruction but also to tissue repair. Elevated levels of MMP-2 have been reported in gingival crevicular fluid and gingival tissue of periodontitis.[7,8]

The constitutive and induced expression of MMP-2 is likely to be subjected to regulation by transcription factors.[9,10] Several single nucleotide polymorphisms (SNPs) in the MMP-2 promoter region have been identified including MMP-2 -1306 C>T (rs243865), MMP-2 -735C>T (rs2285053), and MMP-2 -790T>G (rs243864).[11–14] A C to T transition located at nucleotides −1306 and a C to T allelic variation located at −735 might disrupt a SP1-binding site and consequently diminish promoter activity.[11,14] The MMP-2 -790GG genotype was observed to be marginally less frequent in patients with chronic periodontitis (CP).[15]

A large number of molecular epidemiological studies have been performed to evaluate the role of these MMP-2 gene polymorphisms in various tumors.[14,16–19] Considering the role of MMP-2 in both remodeling and destruction of extracellular matrix, we hypothesize that MMP-2 genetic variations influencing the transcription or function of this enzyme might contribute to the susceptibility to periodontitis.

MMP-9 (gelatinase-B, 96 kDa gelatinase) is one of the MMPs responsible for the breakdown of type IV collagen and noncollagenous components of the extracellular matrix.[4] In addition, MMP-2—but not MMP-9—is also able to cleave native type I collagen, which is the abundant component of gingival connective tissue matrix.[5] Elevated levels of MMP-9 have been detected in gingival crevicular fluid and gingival tissue of periodontitis patients.[2,8] A single-base substitution variant in the MMP-9 gene is located at the promoter site at position −1562 relative to the transcription start site where a transition between C and T occurs. The functional polymorphism is characterized with increased mRNA, protein level, and activity in MMP-9 -1562 T allele carriers of MMP-9.[15]

Tissue inhibitor of metalloproteinase-2 (TIMP-2) is particularly important because of its dual functions in terms of regulating MMP-2 activity.[20–22] It is reasonable to postulate that the polymorphism may downregulate TIMP-2 expression and consequently cause an imbalance between the activities of TIMP-2 and MMP-2, which are believed to have a significant impact on periodontitis development and progression.[23,24]

Although some reports revealed that the polymorphisms of MMP-2 (-1306 C/T, -735 C/T, and -790 T/G) and TIMP-2 -418 G/C were related to the risk of some diseases, so far reports on the correlation between these polymorphisms and the risk of periodontitis development/susceptibility were rare and showed different results.[1,15,25–27]

Considering the role of MMP-2, MMP-9, and TIMP-2 in both remodeling and destruction of the extracellular matrix, whether genetic variations influencing the transcription or function of these enzymes contribute to the susceptibility to CP and aggressive periodontitis (AgP), we aimed to evaluate the genotype distributions and allele frequencies of *MMP-2 -1306C/T*, *MMP-2 -735C/T*, *MMP-2 -790T/G*, *MMP-9 -1562C/T*, and *TIMP-2 -418G/C* genes and their relation to the susceptibility to severe periodontitis in a Taiwanese Han population.

## Materials and methods

### Study population

A total of unrelated Taiwanese individuals including 129 CP and 69 AgP patients and 117 participants with healthy periodontal condition were recruited from the Department of Periodontology, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan and Chung Shan Medical University Hospital, Taichung City, Taiwan, between May 2006 and July 2012. All participants underwent clinical and radiographic examination. Medical and dental histories were taken. Current smokers or former smokers who were smoking > 10 cigarettes per day for > 5 years were tagged as the smoking group. Participants who had never smoked were classified as the nonsmoking group. None of the CP and AgP patients and healthy participants had a history or current manifestation of systemic conditions, which could modify the periodontal status including the diseases that have been associated with investigated MMP gene polymorphisms (cancer, cardiovascular diseases, or respiratory diseases) or had transmissible infectious diseases (human immunodeficiency virus, hepatitis). Pregnancy was also

selected as one of the exclusion criteria for the study. Participants eligible for the study signed the informed consent. The Research Ethics Committee of Kaohsiung Medical University Hospital (KMUH-IRB970384) and Chung Shan Medical University Hospital (CSMUH No. CS09107) approved the study protocol. Periodeontitis patients were diagnosed in accordance with the clinical criteria for CP or AgP as established by consensus at the World Workshop in Periodontics in 1999.[28]

## Genomic DNA preparation and quantitation

A 20-mL sample of whole blood was collected into EDTA-coated vacutainer tubes using the standard venipuncture method, then centrifuged at 4°C (1600 *g*) for 10 minutes. Genomic DNA was extracted from peripheral leukocytes using standard phenol/chloroform extraction techniques, and precipitated by ethanol using the DNA isolation kit (Puregene, Gentra System, Minneapolis, MN, USA) according to the procedure described by Blin and Stafford.[29] DNA concentration was determined by spectrophotometry at 280 nm (UV) wavelength and diluted as 10 ng/$\mu$L.

## Genotyping of MMP-2, MMP-9, and TIMP-2

The SNPs of the genotypes at positions of MMP-2 -1306, MMP-2 -790, MMP-2 -735, MMP-9 -1562, and TIMP-2 -418 from the transcription start site were detected by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion. The synthetic oligonucleotide primer sequences, PCR conditions for MMP-2, MMP-9, and TIMP-2 restriction fragment length polymorphisms and PCR products are outlined in Table 1.

## Statistical analysis

PowerMarker version 3.25 (GlaxoSmithKline, NC, USA) was used to test for deviation of genotype frequencies from Hardy—Weinberg equilibrium. Comparisons of descriptive statistics in the three groups are shown as mean (± standard deviation) and within-group proportions. The $\chi^2$ test and analysis of variance test were used to compare the proportions and means, respectively, and to evaluate the statistical significance of differences among the three groups. The risk associated with genotype and periodontitis was calculated using simple logistic regression and displayed as the odds ratio (OR) with 95% confidence interval (CI). A multivariate logistic regression was used to assess the relationship of the genotype to disease status while adjusting for the potential confounding effects of age, sex, and smoking status, which were used as independent variables. A P value $< 0.05$ was considered statistically significant. All data analyses were performed using the statistical package SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

# Results

Overall, 87 CP patients, 69 AgP patients, and 117 healthy controls (HCs) had fulfilled the minimal required sample size for genotyping test. The demographic parameters of the study groups are summarized in Table 2. Significant differences in age, habits of betel quid chewing, smoking, and alcohol drinking were noted among these three groups. No significant differences in the genotype distributions of the MMP-2 C-1306T and C-735T, and MMP-9 C-1562T among periodontitis and control groups were detected in our study (Table 3). There was only a trend of difference in the allele distributions of the MMP-2 T-790G and genotype distributions of TIMP-2 G-418C (Table 3, P = 0.08). The MMP-2 T-790G and TIMP-2 G-418C genotypes in the CP and healthy groups were found in accordance with those expected by the Hardy—Weinberg equilibrium.

Next, the study participants were subcategorized according to their smoking, betel quid chewing, and alcohol drinking habits. Multiple logistic regression analysis was used to evaluate the association of the rare allele-carrying genotypes with periodontal disease susceptibility, while adjusting for modifying factors such as age, sex, and smoking. The logistic regression indicated that TIMP-2 G-

**Table 1** PCR conditions for MMP-2, MMP-9, and TIMP-2 restriction fragment length polymorphisms.

| Gene | Primes | Restriction enzyme | |
|---|---|---|---|
| *MMP-2 -1306C/T* | 5′-CTTCCTAGGCTGGTCCTTACTGA-3′ (forward) | *Xsp*I | 188 + 162 + 26 bp (CT); 188 bp (CC) |
| | 5′-CTGAGACCTGAAGAGCTAAAGACCT-3′ (reverse) | | 162 + 26 bp (TT) |
| (95°C, 10 min, 35 cycles then 95°C, 45 s then 55°C, 45 s; 72°C, 45 s) | | | |
| *MMP-2 -790T/G* | 5′-GGGTCTTTTGTGACCTCGATC-3′ (forward) | *Pvu*I | 118 + 99 + 19 bp (TG); 118 bp (TT) |
| | 5′-GGTAAAATGAGGCTGAGACCTG-3′ (reverse) | | 99 + 19 bp (GG) |
| (95°C, 10 min, 40 cycles then 95°C, 45 s then 55°C 45 s, 72°C, 45 s) | | | |
| *MMP-735C/T* | 5′-ATAGGGTAAACCTCCCCACATT-3′ (forward) | *Hin*fI | 300 + 254 + 46 bp (CT); 300 bp (CC) |
| | 5′-GGTAAAATGAGGCTGAGACCTG-3′ (reverse) | | 254 + 46 bp (TT) |
| (95°C, 10 min, 40 cycles then 95°C, 45 s then 55°C 45 s, 72°C, 45 s) | | | |
| *TIMP-2 -418G/C* | 5′-CGTCTCTTGTTGGCTGGTCA-3′ (forward) | *Bso*BI | 230 + 51 +23 bp (GG); 230 + 51 bp (CC) |
| | 5′-CCTTCAGCTCGACTCTGGAG-3′ (reversed) | | 253 + 230 + 51 + 23 bp (CC) |
| (94°C, 4 min, 35 cycles then 94°C, 1 min, then 56°C 45 s, 72°C, 45 s) | | | |
| *MMP-9 -1562C/T* | 5′- GGGATAATTTGGCTCTGGTCTTCAA-3′ (forward) | *Sph*I | 436 bp (CC); |
| | 5′-CCATGGGAACCATAGAAAAGA-3′ (reversed) | | 436 + 194 + 242 bp (CT) |
| 94°C, 10 min, 35 cycles then 95°C, 30 s, 59°C, 45 s, 72°C, 45 s | | | |

MMP = matrix metalloproteinase; PCR = polymerase chain reaction; TIMP, tissue inhibitor of MMPs.

**Table 2** Demographic data of participants in healthy control (HC), aggressive periodontitis (AgP), and chronic periodontitis (CP) groups.

| | HC $n = 117$ (%) | AgP $n = 69$ (%) | CP $n = 129$ (%) | $\chi^2$ | P |
|---|---|---|---|---|---|
| Age (mean ± SD) | 45.5 ± 11.3 | 39.0 ± 10.1 | 50.8 ± 8.6 | 31.48 | 0.000 |
| Sex | | | | | |
| Male | 57 (48.7) | 41 (59.4) | 74 (57.4) | 2.68 | 0.260 |
| Female | 60 (51.3) | 28 (40.6) | 55 (42.6) | | |
| Habit | | | | | |
| BQ chewing | | | | | |
| Yes | 8 (6.8) | 10 (14.5) | 28 (21.7) | 10.88 | 0.004 |
| No | 109 (93.2) | 59 (85.5) | 101 (78.3) | | |
| Smoking | | | | | |
| Yes | 20 (17.1) | 26 (37.7) | 51 (39.5) | 16.47 | 0.000 |
| No | 97 (82.9) | 43 (62.3) | 78 (60.5) | | |
| Al drinking | | | | | |
| Yes | 30 (25.6) | 29 (42.0) | 65 (50.4) | 16.00 | 0.000 |
| No | 87 (74.4) | 40 (58.0) | 64 (49.6) | | |

Al drinking = alcohol drinking; BQ chewing = betel quid chewing; SD = standard deviation.

**Table 3** Association of MMP-2-1306, -790, and -735, MMP-9-1562 and TIMP2 418 genotypes, and allele with healthy control (HC), aggressive periodontitis (AgP), and chronic periodontitis (CP) groups.

| Genotype | HC $n = 117$ (%) | AgP $n = 69$ (%) | CP $n = 129$ (%) | $\chi^2$ | P |
|---|---|---|---|---|---|
| MMP-2-1306 | | | | | |
| CC | 105 (89.7) | 62 (89.9) | 114 (88.4) | 1.49 | 0.83 |
| CT | 12 (10.3) | 7 (10.1) | 14 (10.9) | | |
| TT | 0 (0.0) | 0 (0.0) | 1 (0.8) | | |
| C allele | 222 (94.9) | 131 (94.9) | 242 (93.8) | 0.35 | 0.84 |
| T allele | 12 (5.1) | 7 (5.1) | 16 (6.2) | | |
| MMP-2-735 | | | | | |
| CC | 57 (48.7) | 39 (56.5) | 75 (58.1) | 2.98 | 0.56 |
| CT | 48 (41.0) | 26 (37.7) | 44 (34.1) | | |
| TT | 12 (10.3) | 4 (5.8%) | 10 (7.8) | | |
| C allele | 162 (69.2) | 104 (75.4) | 194 (75.2) | 2.71 | 0.26 |
| T allele | 72 (30.8) | 34 (24.6) | 64 (24.8) | | |
| MMP-2-790 | | | | | |
| TT | 98 (83.8) | 52 (75.4) | 95 (73.6) | 5.80 | 0.21 |
| GT | 18 (15.4) | 17 (24.6) | 31 (24.0) | | |
| GG | 1 (0.9) | 0 (0.0) | 3 (2.3) | | |
| T allele | 214 (91.5) | 121 (87.7) | 219 (84.9) | 5.00 | 0.08 |
| G allele | 20 (8.5) | 17 (12.3) | 39 (15.1) | | |
| TIMP-2-418 | | | | | |
| GG | 73 (62.4) | 48 (69.6) | 94 (72.9) | 8.51 | 0.08 |
| GC | 44 (37.6) | 19 (27.5) | 31 (24.0) | | |
| CC | 0 (0.0) | 2 (2.9) | 4 (3.1) | | |
| G allele | 188 (81.7) | 114 (83.8) | 219 (84.9) | 0.89 | 0.64 |
| C allele | 42 (18.3) | 22 (16.2) | 39 (15.1) | | |
| MMP-9-1562 | | | | | |
| CC | 90 (76.9) | 48 (69.6) | 96 (74.4) | 1.48 | 0.83 |
| CT | 28 (21.4) | 19 (27.5) | 31 (24.0) | | |
| TT | 2 (1.7) | 2 (2.9) | 2 (1.6) | | |
| C allele | 205 (87.6) | 115 (83.3) | 223 (86.4) | 1.35 | 0.51 |
| T allele | 29 (12.4) | 23 (16.7) | 35 (13.6) | | |

MMP = matrix metalloproteinase; TIMP, tissue inhibitor of MMPs.

**Table 4** Genotypes and allele distributions in nonsmokers of healthy control (HC), aggressive periodontitis (AgP), and chronic periodontitis (CP) groups.

| Genotype | HC $n = 97$ (%) | AgP $n = 43$ (%) | CP $n = 78$ (%) | $\chi^2$ | P |
|---|---|---|---|---|---|
| MMP-2-1306 | | | | | |
| CC | 88 (90.7) | 40 (93.0) | 68 (87.2) | 2.535 | 0.64 |
| CT | 9 (9.3) | 3 (7.0) | 9 (11.5) | | |
| TT | 0 (0.0) | 0 (0.0) | 1 (1.3) | | |
| C allele | 185 (95.4) | 83 (96.5) | 145 (92.9) | 1.691 | 0.43 |
| T allele | 9 (4.6) | 3 (3.5) | 11 (7.1) | | |
| MMP-2-735 | | | | | |
| CC | 45 (46.4) | 24 (55.8) | 40 (51.3) | 2.117 | 0.71 |
| CT | 41 (42.3) | 17 (39.5) | 31 (39.7) | | |
| TT | 11 (11.3) | 2 (4.7) | 7 (9.0) | | |
| C allele | 131 (67.5) | 65 (75.6) | 111 (71.2) | 1.920 | 0.38 |
| T allele | 63 (32.5) | 21 (24.4) | 45 (28.8) | | |
| MMP-2-790 | | | | | |
| TT | 81 (83.5) | 33 (76.7) | 58 (74.4) | 5.258 | 0.26 |
| GT | 16 (16.5) | 10 (23.3) | 18 (23.1) | | |
| GG | 0 (0.0) | 0 (0.0) | 2 (2.5) | | |
| T allele | 178 (91.8) | 76 (88.4) | 134 (85.9) | 3.068 | 0.22 |
| G allele | 16 (8.2) | 10 (11.6) | 22 (14.1) | | |
| TIMP-2-418 | | | | | |
| GG | 60 (61.9) | 29 (67.4) | 61 (78.2) | 11.270 | 0.024 |
| GC | 37 (38.1) | 13 (30.2) | 14 (17.9) | | |
| CC | 0 (0.0) | 1 (2.4) | 3 (3.9) | | |
| G allele | 156 (81.3) | 70 (83.3) | 136 (87.2) | 2.245 | 0.33 |
| C allele | 36 (18.7) | 14 (16.7) | 20 (12.8) | | |
| MMP-9-1562 | | | | | |
| CC | 77 (79.4) | 32 (74.4) | 57 (73.1) | 4.967 | 0.29 |
| CT | 18 (18.6) | 9 (20.9) | 21 (26.9) | | |
| TT | 2 (2.0) | 2 (4.7) | 0 (0.0) | | |
| C allele | 172 (88.7) | 73 (84.9) | 135 (86.5) | 0.842 | 0.66 |
| T allele | 22 (11.3) | 13 (15.1) | 21 (13.5) | | |

MMP = matrix metalloproteinase; TIMP, tissue inhibitor of MMPs.

418C gene polymorphisms in nonsmokers were statistically significantly different among AgP, CP, and HC groups ($P = 0.024$, Table 4); the participants with MMP-2 -970 TT genotypes and T allele as compared to GT + GG genotypes and G allele, were less susceptible to CP (adj. OR = 0.50, 95% CI = 0.25–1.00 and adj. OR = 0.52, 95% CI = 0.23–0.96, respectively; Table 5), and the nonalcohol drinking participants with MMP-9-1562 C allele as compared to those with T allele, were less susceptible to AgP but not to CP (adj. OR = 0.4, 95% CI = 0.18–0.90; Table 6).

## Discussion

The human *MMP-2* gene exhibits sequence variations, and several functional SNPs in the MMP-2 promoter have been described.[30,31] A C→T polymorphism located at nucleotide −1306 site disrupts an Sp1 regulatory element, and the T allele thus has a strikingly lower promoter activity compared with the C allele.[20] Recently, a previous study showed that another C→T polymorphism located at nucleotide −735 also destroys an Sp1 binding element, with the T allele being associated with significantly diminished promoter activity.[31] The −1306C→T and −735C→T polymorphisms are in a linkage disequilibrium and the $T^{-1306}$–$T^{-735}$ haplotype displays an even lower promoter activity and mRNA expression compared with the haplotype consisting of only one T allele at the −1306 or −735 site, indicating an interactive effect of these two SNPs on MMP-2 transcriptional function.[31] The study observed an increased risk of the cancer associated not only with the −1306C allele but also with the −735C allele; furthermore, an even greater association was observed between elevated risk of developing esophageal squamous cell carcinoma (SCC) and $C^{-1306}$ and $C^{-735}$-allele containing haplotypes.[32] A contribution of MMP-2 $C^{-1306}$ and $C^{-735}$-allele containing haplotypes, alone or in combination with smoking, to the risk of developing lung cancer in a case–control study has been reported.[33] A joint effect between the MMP-2 promoter polymorphisms and tobacco smoking was evident. The −1306*CC* and −735*CC* genotypes or the $C^{-1306}$–$C^{-732}$ haplotype were significantly associated with lung cancer risk in nonsmokers; however, the risk was markedly elevated in smokers, particularly heavy smokers, suggesting an additive gene–smoking interaction. Tobacco smoking is an established etiological factor for lung cancer; therefore,

**Table 5** Logistic regression analysis of the associations of the genotypes and allele frequencies of MMP-2 -1306, -790, and -735, MMP-9 -1562, and TIMP-2 -418 genes, and chronic periodontitis (CP) risks.

| Genotype | HC<br>*n* = 117 (%) | CP<br>*n* = 129 (%) | Crude OR<br>(95% CI) | Adjusted OR[a]<br>(95% CI) |
|---|---|---|---|---|
| MMP-2-1306 | | | | |
| CT/TT | 12 (10.3) | 15 (11.6) | 1 | 1 |
| CC | 105 (89.7) | 114 (88.4) | 0.87 (0.39–1.94) | 0.94 (0.39–2.26) |
| T allele | 12 (5.1) | 16 (6.2) | 1 | 1 |
| C allele | 222 (94.9) | 242 (93.8) | 0.82 (0.38–1.77) | 0.87 (0.38–2.00) |
| MMP-2-735 | | | | |
| TT | 12 (10.3) | 10 (7.8) | 1 | 1 |
| CT | 48 (41.0) | 44 (34.1) | 1.1 (0.43–2.80) | 1.18 (0.42–0.34) |
| CC | 57 (48.7) | 75 (58.1) | 1.58 (0.64–3.91) | 1.78 (0.64–4.93) |
| T allele | 72 (30.8) | 64 (24.8) | 1 | 1 |
| C allele | 162 (69.2) | 194 (75.2) | 1.35 (0.91–2.00) | 1.41 (0.91–2.18) |
| MMP-2-790 | | | | |
| GT/GG | 19 (16.2) | 34 (26.4) | 1 | 1 |
| TT | 98 (83.8) | 95 (73.6) | 0.54 (0.29–1.02) | 0.50* (0.25–1.00) |
| G allele | 20 (8.5) | 39 (15.1) | 1 | 1 |
| T allele | 214 (91.5) | 219 (84.9) | 0.53* (0.30–0.93) | 0.52* (0.28–0.96) |
| TIMP-2-418 | | | | |
| GC/CC | 44 (37.6) | 35 (27.1) | 1 | 1 |
| GG | 73 (62.4) | 94 (72.9) | 1.62 (0.94–2.78) | 1.46 (0.81–2.63) |
| G allele | 188 (81.7) | 219 (84.9) | 1 | 1 |
| C allele | 42 (18.3) | 39 (15.1) | 0.8 (0.50–1.29) | 0.9 (0.53–1.51) |
| MMP-9-1562 | | | | |
| TT | 2 (1.7) | 2 (2.9) | 1 | 1 |
| CT | 28 (21.4) | 19 (27.5) | 1.24 (0.16–9.44) | 0.94 (0.11–7.91) |
| CC | 90 (76.9) | 48 (69.6) | 1.07 (0.15–7.73) | 0.99 (0.13–7.83) |
| T allele | 29 (12.4) | 35 (13.6) | 1 | 1 |
| C allele | 205 (87.6) | 223 (86.4) | 0.9 (0.53–1.53) | 1.04 (0.58–1.83) |

* $P < 0.05$.
HC = healthy control; MMP = matrix metalloproteinase; OR = odds ratio; TIMP, tissue inhibitor of MMPs.
[a] Adjustment for age, sex, and habits of betel quid chewing, alcohol drinking, and smoking.

this gene–smoking interaction is biologically plausible. A higher risk of lung cancer among smokers who carried the susceptible genotypes or haplotypes may be attributed to many transformed or preinvasive lung cells caused by tobacco carcinogens, which in turn increases the possibility that one of these cells will become an invasive tumor under the condition of higher lifetime expression of MMP-2. In addition, MMP expression can be induced by smoking.[32,34] Given these conditions, it would be expected that individuals who smoked and carried the −1306CC and −735CC genotypes or the $C^{-1306}$–$C^{-732}$ haplotype were more susceptible to developing lung cancer. A trend to decreased frequency of the MMP-2-790 GG homozygotes was observed in patients with CP compared to HC ($P = 0.036$, corrected $P > 0.05$).[29]

TIMP-2 expression was identified in fibroblasts, macrophages, and epithelial cells and was significantly increased in periodontitis-affected gingival tissues when compared to healthy gingival tissues.[35] Gene polymorphisms of MMP-2 did not show any association with the generalized AgP (GAgP), but the frequencies of TIMP-2-418 G to C gene polymorphism revealed significant differences between patients and controls (a significant increasing trend of TIMP-2 -418C carrier in GAgP patients occurred).[1] Considering the roles of MMP-2 in cell proliferation, apoptosis, and angiogenesis,[30] it is no surprise that participants with functional genotypes in the MMP-2 promoter are genetically more susceptible to carcinogenesis. Within this context, our results suggested that TIMP-2 G-418C may be associated with the risk of different subtypes of periodontitis (AgP vs. CP).

To date, only a few studies have evaluated the association between MMP-9 -1562 C/T polymorphism and periodontitis.[36–38] No association was revealed between MMP-9 -1562 T/T genotype and T allele distribution of this gene and CP in a Brazilian population. The similar frequencies of MMP-9 -1562T/T genotype and allele frequencies of this polymorphism were also found in CP patients and healthy individuals in a Czech population.[36] The T allele of the *MMP-9 -1562* gene was significantly higher in severe CP when compared to the moderate form of CP.[36] By contrast, a significantly different genotype distribution has been found between CP patients and healthy controls regarding MMP-9 -1562 C/T polymorphism in a Turkish population.[37] T allele carriage of MMP-9 -1562 gene polymorphisms in Turkish GAgP patients was found to be lower than that of healthy individuals and was found to be associated with decreased susceptibility to GAgP (adjusted OR = 0.52).[26] Although not significant ($P = 0.06$), higher T allele carriage in the healthy group than in the CP group was

**Table 6** Associations of genotypes and allele frequencies of MMP-2 -1306, -790, and -735, MMP-9 -1562 and TIMP-2 -418 genes, and AgP risks in nonalcohol-drinking participants using logistic regression analysis.

| Genotype | HC<br>*n* = 87 (%) | AgP<br>*n* = 40 (%) | Crude OR<br>(95% CI) | Adjusted OR[a]<br>(95% CI) |
|---|---|---|---|---|
| MMP-2-1306 | | | | |
| CT/TT | 7 (8.0) | 3 (7.5) | 1.00 | 1.00 |
| CC | 80 (92.0) | 37 (92.5) | 1.08 (0.26—4.41) | 0.88 (0.19—3.98) |
| T allele | 7 (4.1) | 3 (3.7) | 1.00 | 1.00 |
| C allele | 165 (95.9) | 77 (96.3) | 1.08 (0.27—4.27) | 0.86 (0.20—3.72) |
| MMP-2-735 | | | | |
| TT | 9 (10.4) | 2 (5.0) | 1.00 | 1.00 |
| CT | 31 (35.6) | 15 (37.5) | 2.18 (0.42—11.36) | 1.96 (0.34—11.30) |
| CC | 47 (54.0) | 23 (57.5) | 2.20 (0.44—11.03) | 2.06 (0.38—11.26) |
| T allele | 49 (28.5) | 19 (23.7) | 1.00 | 1.00 |
| C allele | 123 (71.5) | 61 (76.3) | 1.26 (0.68—2.32) | 1.26 (0.65—2.43) |
| MMP-2-790 | | | | |
| GT/GG | 12 (13.8) | 9 (22.5) | 1.00 | 1.00 |
| TT | 75 (86.2) | 31 (77.5) | 0.55 (0.21—1.44) | 0.63 (0.23—1.75) |
| G allele | 13 (7.6) | 9 (11.2) | 1.00 | 1.00 |
| T allele | 159 (92.4) | 71 (88.8) | 0.64 (0.26—1.56) | 0.69 (0.27—1.79) |
| TIMP-2-418 | | | | |
| GC/CC | 32 (36.8) | 11 (27.5) | 1.00 | 1.00 |
| GG | 55 (63.2) | 29 (72.5) | 1.53 (0.68—3.48) | 1.73 (0.71—4.22) |
| G allele | 140 (82.4) | 68 (85.0) | 1.00 | 1.00 |
| C allele | 30 (17.6) | 12 (15.0) | 0.80 (0.39—1.66) | 0.72 (0.33—1.56) |
| MMP-9-1562 | | | | |
| CT/TT | 18 (20.7) | 14 (35.0) | 1.00 | 1.00 |
| CC | 69 (79.3) | 26 (65.0) | 0.48 (0.21—1.11) | 0.40 (0.16—1.02) |
| T allele | 19 (11.0) | 16 (20.0) | 1.00 | 1.00 |
| C allele | 153 (89.0) | 64 (80.0) | 0.49 (0.24—1.01) | 0.40* (0.18—0.90) |

* $P < 0.05$.
AgP = aggressive periodontitis group; CI = confidence interval; HC = healthy control group; MMP = matrix metalloproteinase; OR = odds ratio; TIMP, tissue inhibitor of MMPs.
[a] Adjustment for age, sex, and habits of betel-quid chewing and smoking.

in agreement with these previous Turkish population studies.[26,37] Moreover, logistic regression analysis revealed an adjusted OR of 0.36, indicating a decreased susceptibility to CP in T allele carriers. Genotype distribution and rare allele carriage of *MMP-9 -1562 C/T* gene polymorphism in a Turkish population seem to be in contrast to the probable linkage between MMP-9 -1562 T allele carriage and increased MMP-9 function and consequently to the periodontal destruction. These results are also in contradiction to the results of previous studies in other populations.[36] The higher frequency and carriage of T allele in the healthy group might be of importance in supporting the recent findings indicating defensive anti-inflammatory properties of MMP-9.[36—40] In addition, it should be kept in mind that MMP-9 activity is also modulated by activation of the proenzyme and by regulation of MMP inhibitors.

In conclusion, a significant difference (P = 0.024) in the frequencies of TIMP-2 G-418C polymorphisms among nonsmokers in the AgP, CP, and HC groups were observed. However, the logistic regression analysis revealed that MMP-2 -790TT genotype and T allele might be a protective factor for CP. The MMP-9-1562 C allele was found to be a significant protective factor for AgP after adjustment for age, sex, betel quid chewing, and smoking in nonalcohol drinking individuals. Additional multicenter studies are necessary to clarify whether genetic variations within the MMP genes contribute to interindividual differences in susceptibility to severe CP and AgP.

## Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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