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MMP-8 -799 C>T genetic polymorphism is associated with the susceptibility to chronic and aggressive periodontitis in Taiwanese

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Abstract

Aim: Matrix metalloproteinase (MMP)-8 is a protease that degrades numerous extracellular molecules and has been implicated in the pathogenesis of periodontitis. Polymorphism in the MMP-8 could affect the susceptibility to disease. Our aim was to evaluate the association between periodontitis and MMP-8 -799 C>T polymorphism.

Material and methods: Genomic DNA was obtained from 361 chronic periodontitis patients (CP), 96 aggressive periodontitis patients (AgP), and 106 periodontally healthy controls (HC). MMP-8 -799 C>T polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The frequencies of genotypes in diseased groups were similar but were significantly different from those in the HC. Multivariate logistic regression analysis with adjustment for age, gender and smoking indicated that increased risks of AgP and CP were associated with the -799 T allele (in AgP, adjusted OR = 1.99, $p = 0.04$; in CP, adjusted OR = 1.87, $p = 0.007$). To avoid the confounded effect of smoking on MMP-8 polymorphism to periodontitis, the analysis was conducted on non-smokers and the associations were significant.

Conclusions: These results suggested that non-smoking Taiwanese with the MMP-8 -799 T allele were associated with the risks of both CP and AgP. Further studies in other ethnic populations are necessary.

Key words: gene polymorphism; MMP-8; periodontitis

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Conflict of interest and source of funding statement

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Periodontitis is a multifactorial disease which 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 (Landi et al. 1997, Chen et al. 2007). The degradation of periodontium is essentially controlled by matrix metalloproteinases (MMPs), a family of zinc and calcium-dependent proteolytic enzymes that regulate the aggravation of extracellular matrix and basement membranes (de

Souza et al. 2003). MMPs play an important role in physiological and pathological events, including the repair and breakdown of connective tissue due to inflammatory response (Birkedal-Hansen 1993, Ingman et al. 1996). Excessive production of MMPs due to genetic polymorphisms may influence the manifestation and development of periodontal diseases (Lee et al. 1995).

Matrix metalloproteinase-8, or neutrophil collagenase, is one of the pivotal biomarkers in the connective tissue breakdown in periodontitis and is a potential candidate for use as a diagnostic aid (Mantyla et al. 2003, 2006). Significantly higher MMP-8 levels in oral fluids, gingival crevicular fluid (GCF) and saliva occur in patients with periodontitis as compared to healthy controls (HC). Numerous studies have demonstrated that MMP-8 associates with the initiation and progression of periodontitis and reflects its severity (Sorsa et al. 2004, 2006).

Besides the destructive role, MMP-8 has defensive and anti-inflammatory effects. Owen et al. (2004) indicated that MMP-8 had an unexpected, anti-inflammatory role in the lung during lipopolysaccharide (LPS)-mediated acute injury in mice by down-regulating the alveolar polymorphonuclear neutrophils burden. To clarify the role of MMP-8 in periodontitis and systemic inflammatory responses, Kuula et al. (2009) investigated the *Porphyromonas gingivalis*-induced periodontitis in MMP 8-deficient ($MMP8^{-/-}$) mice. Since the $MMP8^{-/-}$ mice had significantly more severe site-specific alveolar bone loss, the authors concluded that the presence of MMP-8 caused at least a partially defensive local inflammatory response against the *P. gingivalis*-induced alveolar bone destruction. Most recently, Hernández et al. conducted the lipopolysaccharide-induced CXC chemokine in *P. gingivalis*-induced experimental periodontitis in MMP-8 null mice. CXC chemokine is a potent chemoattractant for neutrophils and involves in regulating neutrophil influx to periodontium to form the first line of defence against periodontal pathogens. They reported that the CXC chemokine level was reduced in MMP-8 null mice compared to the wild-type mice (Hernandez et al. 2011).

Matrix metalloproteinase-8 gene is located on *11q 22.3*. Its genetic polymorphism in -799 C>T (rs11225395) at the promoter region of the MMP genes can lead to change in MMP-8 expression. *In vitro* experiments showed that the T allele had higher promoter activity than the C allele in chorion-like cytotrophoblast cells (Wang et al. 2004) and in breast cancer cells (Decock et al. 2007). Therefore, the T allele had a greater strength in driving MMP-8 gene expression. It is reasonable to hypothesize that genetic variation affecting the expression or activity of MMP-8 may influence the susceptibility and severity of periodontitis. To the best of our knowledge, no study has reported an association between MMP-8 -799 C>T polymorphism and periodontitis. Therefore, we undertook the present study to determine whether or not the MMP-8 -799 C>T polymorphism is associated with susceptibility to periodontitis in Taiwanese.

Materials and Methods

Determination of the sample size

Genetic Power Calculator (Purcell et al. 2003) was utilized to estimate power calculation. This sample size including 96 aggressive periodontitis (AgP), 361 chronic periodontitis (CP) and 106 HC had 80% power to detect susceptibility with a genotypic relative risk $\geq$ 1.5 at a significance level of 0.05 for a SNP with a risk allele frequency $\geq$ 0.42 (Altshuler et al. 2010).

Study population

A case–control study encompassed the recruitment of study subjects visiting the Division of Periodontics at Kaohsiung Medical University Hospital between January 2004 and May 2010. Personal information on demographic data, medical history, tobacco use and family history of periodontitis was collected via a structured questionnaire and trained interviewer. All subjects had at least 18 teeth when they were enrolled in the study (Hu et al. 2009, Ho et al. 2010). Exclusion criteria included systemic diseases associated with destructive periodontal disease, such as diabetes mellitus, immunosuppression, human immunodeficiency virus infection and polymorphonuclear or monocyte defects determined via the questionnaire and medical history review. Subjects who had taken antibiotics in the previous 3 months, were pregnant, were lactating, or were in need of antibiotic prophylaxis before periodontal treatment were also excluded from this study. The study protocol was approved by the Research Ethics Committee of Kaohsiung Medical University Hospital, and written informed consent was obtained from each subject.

Determination of periodontal status

Based on clinical examinations (probing depth and attachment loss) and radiographic patterns of alveolar bone destruction, each subject in this study was diagnosed as having AgP, CP, or as being a HC. All dentists involved in this study received calibration training on the diagnosis of periodontitis. Periodontal diagnostic criteria for AgP and CP were assigned in accordance with the classification agreed to the World Workshop for Periodontics and the American Academy of Periodontology (Armitage 1999). Specifically, subjects more than 35 years of age, with an attachment loss $\geq$5 mm at more than one tooth, with more than three sites of probing depth >6 mm, and lesions distributed at more than two teeth in each quadrant were diagnosed with CP. Subjects who had more than eight teeth with an attachment loss >5 mm and a probing depth >6 mm, at least three affected teeth other than first molars and incisors, and the level of attachment loss that was not consistent with the plaque level or local contributing factors were diagnosed with AgP. Subjects having no evidence of attachment loss at more than one site or a probing depth less than 3 mm were diagnosed as periodontally HC. The HC recruited were older than 35 years, otherwise, they may have been too young to have periodontitis and may have been misclassified. The smoking status of participants was categorized as non-smoker or smoker. Current and former smokers were included in the smoker group.

Sample collection

A 10-ml sample of peripheral blood was collected into EDTA coated vacutainer tubes by a standard venipuncture method from each subject. Genomic DNA was extracted from peripheral leucocytes using standard phenol/chloroform extraction and precipitation with ethanol (Blin et al. 1976). The DNA concentration was determined by ultraviolet light spectrophotometry at 260 nm.

Analysis of MMP-8 genotypes

The single nucleotide polymorphism for the MMP-8 genotype at position -799 from the transcription start site was detected by PCR amplification followed by restriction enzyme digestion (Qiu et al. 2008). For analysis of the MMP-8 -799 C/T polymorphism, an amplification of a 255-bp fragment using the forward primer 5′-GCCAGAGACTCAAGTGGGAGACTACCATGCAGATC-3′ and reverse primer 5′-TTATGATTGCCCAGACATTTG-3′ was generated in a thermocycler (PE Applied Biosystems, Foster City, CA, USA). The10 μl PCR product was digested with 2U *Bgl*II (NewEngland Biolabs, Ipswich, MA, USA) and separated on 3% agarose gel (Cambrex, Rockland, ME, USA). Three restriction fragments were present at 255, 224, and 31 bp representing CT heterozygous subjects. The homozygous TT genotype yielded the two bands at 224 and 31 bp and the CC homozygote remained as the only uncut 255 bp band. The accuracy of genotyping data obtained from PCR-RFLP analyses was validated by direct sequencing of 15% of a randomly selected sample of cases and controls. No discrepancy between the two results occurred.

Statistical analysis

Chi-square (χ^2) analysis was utilized to test for deviation of genotype frequencies from Hardy–Weinberg equilibrium. Comparisons of descriptive statistics in the three groups are shown as mean (±SD) and within-group proportions. The χ^2-test and ANOVA test were used to compare means and proportions and to evaluate the statistical significance of differences among the three groups. The risk associated with genotypes and periodontitis was calculated by 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, gender and smoking status which were used as independent variables. A p-value <0.05 was considered statistically significant. All data analysis was performed using the statistical package JMP 9.0 (SAS Institutes Inc., Cary, NC, USA).

Results

The genotype frequencies of MMP-8 in our subjects population agreed with the Hardy–Weinberg equilibrium ($\chi^2 = 2.03$, $p = 0.15$). Table 1 presents the demographic characteristics of the participants. The age (mean ± SD) of AgP (38.2 ± 7.4) was significantly younger than that of CP (53.2 ± 9.4) and HC (48.8 ± 11.8). Although the difference was not significant among groups, more smokers were in the CP and AgP groups than in the HC group. The probing depth and clinical attachment level in AgP and CP groups were significantly higher than those in HC ($p < 0.01$).

Table 2 presents the distribution of the MMP-8 genotypes in the different groups. The distribution of the MMP-8 genotypes between CP patients and HC was significantly different ($\chi^2 = 9.62$, $p = 0.008$), whereas the difference between AgP patients and HC was not significant ($p = 0.09$).

Table 1. Demographic characteristics of study subjects classified as patients with aggressive periodontitis (AgP), chronic periodontitis (CP) and healthy controls (HC) (comparisons performed by χ^2-test or ANOVA)

	AgP	CP	HC	*p*-value
	n = 96 (%)	*n* = 361 (%)	*n* = 106 (%)	
Gender, *n* (%)				
Male	59 (61.5)	199 (55.1)	55 (51.9)	0.38
Female	37 (38.5)	162 (44.9)	51 (48.1)	
Age (mean ± SD)*	38.19 ± 7.43	53.15 ± 9.43	48.83 ± 11.79	<0.0001
Smoking status, *n* (%)†				
Smoker	24 (25.0)	92 (25.5)	16 (15.1)	0.08
Non-smoker	72 (75.0)	269 (74.5)	90 (84.9)	
Probing depth (mm)	3.34 ± 0.66	3.51 ± 0.46	2.45 ± 0.27	<0.01
Clinical attachment level (mm)	4.26 ± 0.29	4.27 ± 0.73	2.56 ± 0.23	<0.01

*Age: $p < 0.0001$ for AgP *versus* HC; $p < 0.0001$ for AgP *versus* CP; $p = 0.0001$ for CP *versus* HC.
†Smoking status: $p = 0.08$ for AgP *versus* HC; $p = 0.92$ for AgP *versus* CP; $p = 0.03$ for CP *versus* HC.

Table 2. Genotypes of matrix metalloproteinase (MMP)-8 -799 C>T polymorphism in patients with aggressive periodontitis (AgP), chronic periodontitis (CP) and healthy controls (HC)

Genotype	AgP	CP	HC	*p*-value	AgP *versus* HC	CP *versus* HC
	n = 96 (%)	*n* = 361 (%)	*n* = 106 (%)		*p*-value	*p*-value
CC	34 (35.4)	122 (33.8)	53 (50.0)	0.04*	0.09	0.008*
CT	50 (52.1)	191 (52.9)	40 (37.7)			
TT	12 (12.5)	48 (13.3)	13 (12.3)			
CC	34 (35.4)	122 (33.8)	53 (50.0)	0.009*	0.04*	0.002*
CT + TT	62 (64.6)	239 (66.2)	53 (50.0)			

The total number of subjects and percentages of genotypes are: 37.1% CC (209/563), 49.9% CT (281/563), 13% TT (73/563), 62.9% (CT + TT).
*Comparisons performed by chi-square test.

The frequency of the C/C genotype was higher in the HC group (50.0%) than in the AgP and CP groups (35.4% and 33.8%, respectively). The C/T genotype was more prevalent in the AgP (52.1%) and CP (52.9%) groups than that in the HC group (37.7%) ($\chi^2 = 9.86$, $p = 0.04$). Owing to the fewer number of patients with the T/T genotype, C/T and T/T were assembled as the T allele carrier group to increase statistical power. The distributions of the T allele genotypes (C/T + T/T) and non-T carrier (C/C) were significantly different among groups ($\chi^2 = 9.36$, $p = 0.009$).

The associations of the MMP-8 genotype with disease types are compiled in Table 3. The analysis of genotypes in AgP and HC groups found that the C/T genotype and the T allele carriers were associated with increased risk of AgP (crude OR = 1.95, 95% CI = 1.08–3.57, $p = 0.03$ and crude OR = 1.82, 95% CI = 1.04–3.23, $p = 0.04$, respectively). After adjustment for age, gender and smoking status, the associations were still significant (C/T: adjusted OR = 2.30, 95% CI = 1.15–4.69, $p = 0.02$; T allele carriers: adjusted OR = 1.99, 95% CI = 1.04–3.87, $p = 0.04$). Based on the C/C genotype, the crude OR was 2.07 for C/T (95% CI = 1.30–3.33, $p = 0.002$) and 1.96 for C/T + T/T (95% CI = 1.26–3.04, $p = 0.003$) in comparison with CP and HC. After adjustment for age, gender and smoking status, the C/T genotype and C/T + T/T genotypes were associated with increased risk of CP (adjusted OR = 1.93, 95% CI = 1.20–3.13, $p = 0.007$; adjusted OR = 1.87, 95% CI = 1.19–2.93, $p = 0.007$, respectively).

Smoking is a strong confounding factor for exploring the polymorphism in a population with periodontitis. Because the smokers in our study population were few, we could not stratify the genotype distribution according to the smoking status. To avoid the confounding effect of smoking on MMP-8 polymorphism to periodontitis, we conducted the analysis in non-smokers (Table 4). A significant difference in the distribution of the MMP-8 genotype in both AgP *versus* HC group and CP *versus* HC group was detected. Based on the C/C genotype, the crude OR was both 2.52 for C/T (95% CI = 1.29–5.04, $p < 0.05$) and C/T + T/T (95% CI = 1.33–4.87, $p < 0.05$) in comparison with AgP and HC. Similarly, the crude OR was 1.97 for C/T (95% CI = 1.18–3.32, $p < 0.05$) and 1.91 for C/T + T/T (95% CI = 1.18–3.09, $p < 0.05$) for CP patients. Even after adjustment for age and gender, the genotypes associated with periodontitis still remained significant in the AgP group (the adjusted OR was 2.54 for C/T, 95% CI = 1.18–5.62, $p < 0.05$; the adjusted OR was 2.33 for T allele carriers, 95% CI = 1.14–4.90, $p < 0.05$, respectively) and the CP group (the adjusted OR was 1.86 for C/T, 95% CI = 1.10–3.16, $p < 0.05$; the adjusted OR was 1.84 for T allele carriers, 95% CI = 1.12–3.02, $p < 0.05$, respectively).

Discussion

Matrix metalloproteinase-8, a member of the MMP family of enzymes, plays an important role in extracellular matrix degradation, cell proliferation, differentiation, apoptosis and angiogenesis. It is present at acute inflammation sites and potently degrades type I collagen, thus, contributing to processes of extracellular

Table 3. Analysis of matrix metalloproteinase (MMP)-8 polymorphisms in aggressive periodontitis (AgP), chronic periodontitis (CP) patients and healthy controls (HC)

Genotype	AgP *versus* HC				CP *versus* HC			
	Crude OR (95% CI)	*p*-value	Adjusted OR* (95% CI)	*p*-value	Crude OR (95% CI)	*p*-value	Adjusted OR* (95% CI)	*p*-value
CC	1		1		1		1	
CT	1.95 (1.08–3.57)	0.03	2.30 (1.15–4.69)	0.02	2.07 (1.30–3.33)	0.002	1.93 (1.20–3.13)	0.007
TT	1.44 (0.58–3.54)	0.43	1.25 (0.44–3.46)	0.67	1.60 (0.82–3.31)	0.171	1.66 (0.84–3.49)	0.150
CC	1		1		1		1	
CT + TT	1.82 (1.04–3.23)	0.04	1.99 (1.04–3.87)	0.04	1.96 (1.26–3.04)	0.003	1.87 (1.19–2.93)	0.007

CI, confidence interval; OR, odds ratio.
*Adjusted by age, gender and smoking status in logistic regression analysis.

Table 4. Comparison of matrix metalloproteinase (MMP)-8 genotypes in non-smokers

Genotype	AgP	CP	HC	AgP *versus* HC		CP *versus* HC	
	n = 72 (%)	*n* = 269 (%)	*n* = 90 (%)	Crude OR (95% CI)	Adjusted OR† (95% CI)	Crude OR (95% CI)	Adjusted OR† (95% CI)
CC	21 (29.2)	95 (35.3)	46 (51.1)	1	1	1	1
CT	39 (54.2)	139 (51.7)	34 (37.8)	2.52*(1.29–5.04)	2.54*(1.18–5.62)	1.97*(1.18–3.32)	1.86*(1.10–3.16)
TT	12 (16.6)	35 (13.0)	10 (11.1)	2.51 (0.94–6.83)	1.79 (0.60–5.37)	1.68 (0.79–3.84)	1.78 (0.82–4.15)
CC	21 (29.2)	95 (35.3)	46 (51.1)	1	1	1	1
CT + TT	51 (70.8)	174 (64.7)	44 (48.9)	2.52*(1.33–4.87)	2.33*(1.14–4.90)	1.91*(1.18–3.09)	1.84*(1.12–3.02)

*$p < 0.05$.
†Adjusted by age and gender in logistic regression analysis.

matrix degradation and tissue remodelling (Galis et al. 1994). We hypothesized that a genetic polymorphism in the MMP-8 gene might alter the expression of this gene, modulate the inflammatory response and consequently affect the risk of periodontitis. The results in this study indicated that the MMP-8 -799 T allele was associated with increased risk of AgP and CP in Taiwanese. After adjustment for gender, age and smoking status, the association was still significant. This implied that subjects with the MMP-8 -799 T allele were susceptible to periodontitis.

Because the sample size is critical in any polymorphism association with certain diseases, the larger the number of subjects in a case-control study, the more reliable the conclusion is. In our study, the number of subjects was 563, which exceeded that of many studies investigating SNPs and periodontitis. Therefore, it provided sufficient power (Purcell et al. 2003) to confirm the hypothesis.

Matrix metalloproteinase-8 is mainly secreted by polymorphonuclear leucocytes, but increasing evidence has shown that MMP-8 can be expressed from other cells, such as plasma cells, fibroblasts (Sorsa et al. 1992, Van Lint & Libert 2006), human chondrocytes (Cole et al. 1996), rheumatoid synovial and gingival fibroblasts (Hanemaaijer et al. 1997), endothelial cells (Hanemaaijer et al. 1997), epithelial cells (Tervahartiala et al. 2000), odontoblasts (Palosaari et al. 2000) and oral cancer cells (Moilanen et al. 2002). Elevated MMP-8 level was found in saliva from advanced periodontitis subjects (Ramseier et al. 2009) and was substantiated in periodontal pockets or sites with poor response to treatment (Mantyla et al. 2006). The severity of periodontitis is positively correlated with the MMP-8 levels (Kinane et al. 2003). However, in the *P. gingivalis*-infected MMP-8 deficient mice, the bone loss was more extensive related to the *P. gingivalis*- infected wide mice (Kuula et al. 2009). The results suggested that the physiological levels of MMP-8 exert defensive and anti-inflammatory functions during periodontal infection, whereas the pathologically elevated MMP-8 levels cause destructive response against periodontal pathogens.

The functional analyses of MMP-8 promoter showed the minor allele (T allele) of -799 site had increased promoter activity than the major allele (C allele) (Decock et al. 2007), that is, the T allele could enhance the MMP-8 gene expression and increase the MMP-8 production. We can speculate that the MMP-8 -799 T allele carriers have more MMP-8 production in the periodontal environment with bacterial challenge compared with the non-T allele carriers. The Elevated levels of MMP-8 have pathological effects to induce destruction of periodontium. This is the possible explanation of our findings that the T allele carriers had increased risk of AgP and CP. The exact mechanism with which -799 C>T polymorphism of the MMP-8 gene affects the susceptibility to periodontitis is still not clear. The electrophoretic mobility shift assays showed that the binding ability of the allelic variant of -799 C>T polymorphism to the nuclear extract proteins prepared from different cells was variable (Wang et al. 2004, Decock et al. 2007). The impact of MMP-8 gene promoter activity at the -799 SNP site had a cell-specific response and influenced the function and effect of MMP-8 in the evolution and manifestation of different diseases. So far, the basis for the cell-specific promoter activity is not clear. The MMP-8 gene promoter activity depends on the affinity, which DNA sequence at the SNP site of the T or C allele interacted with the nuclear protein from the inspected cell. This implied that the role of destruction or protection at MMP-8 -799 C>T polymorphism was not yet decided in various diseases. Nevertheless, the outcome of the *in vitro* experiments did not represent the biological effect of MMP-8 SNP in the periodontal environment and should be cautiously evaluated in the mechanism of periodontitis.

Significant differences in C-reactive protein (CRP) and MMP-8 levels in whole saliva between periodontitis patients and normal subjects were reported (Christodoulides et al. 2007). The MMP-8 level was positively correlated with CRP level in serum in rheumatoid arthritis (Rajasekhar et al. 2004). Most recently, significantly higher serum CRP, MMP-8, MMP-9, TNF-α levels have been reported in dementia and periodontitis patients in comparison with HC (Rai et al. 2010). Increased amounts of MMP-8 (as well as MMP-1, -3 and myeloperoxidase) in saliva and the presence of putative periodontal pathogens in HIV-patients' periodontal pockets have been reported (Mellanen et al. 1996). Periodontal treatment eliminated the Chlamydia pneumonia in dental plaque and reduced GCF MMP-8 level in periodontitis patients with Chlamydia pneumonia (Mantyla et al. 2004). The combinations of these salivary biomarkers with red-complex anaerobic periodontal pathogens (*P. gingivalis* and *Treponema denticola*) provided highly accurate prediction of periodontal disease activity. In *P. gingivalis*-induced-periodontitis ($MMP8^{-/-}$) mice, the serum total LPS activity and the immunoglobulin G-class antibody levels were significantly elevated (Kuula et al. 2009).

Smoking is a known risk factor for periodontitis. Neutrophils are a major cellular source of MMP-8; tobacco-induced degranulation events in neutrophils and increases in pro-inflammatory mediator burden could influence MMP-8 expression levels in smokers' periodontal environment (Liu et al. 2006). In the investigation of inspecting the association of periodontitis and genetic polymorphism, the smoking status should be considered as a confounding factor. The number of smokers in our study population was small, so we selected non-smokers for the advanced exploration. An association with increased risk and genotypes in both groups was found.

Studies had been performed to evaluate the association between MMP-8 -799 C>T polymorphism and various diseases. In African Americans, no significant association between the individual MMP-8 polymorphism and preterm premature rupture of membrane (PPROM) was found, but the MMP-8 promoter constructs containing the three minor allele haplotypes (-799 C>T, -381 A>G, +17 C>G) had a threefold greater activity in chorion-like trophoblast cells than those containing the major alleles and an increased odds ratio of PPROM (Wang et al. 2004). In Chinese women with breast cancer, the

MMP-8 -799 T allele was associated with reduced cancer relapse and greater survival than those with the C allele (Decock et al. 2007). An increased risk of hepatocellular carcinoma was found to be associated with -799 C/T genotype in non-hepatitis B virus carriers (Qiu et al. 2008). But after correction for multiple comparisons, the association was not again significant.

As in other case-control studies, limitations in our study should be ameliorated. We only evaluated one polymorphism of the MMP-8 gene, which is part of a cluster of MMP genes which localize to chromosome *11q 22.3*. Other variants of this gene and the putative effect of haplotypes were not validated in this study. We cannot rule out that the studied polymorphism is linked to certain functional SNPs through linkage disequilibrium. As mentioned above, the periodontal inflammation is associated with biomarkers levels, such as MMP-8, CRP in tissue fluids. We did not detect the biomarkers levels such as MMP-8 and CRP in saliva, serum or GCF, the putative periodontal pathogens and the serum antibody. Therefore, the interrelation between MMP-8 -799 polymorphism and inflammatory activity was not clarified. Further investigations combined with genetic polymorphisms, function and level of MMP-8, and levels of inflammatory biomarkers are necessary to establish a definite association of MMP-8 polymorphism with periodontitis.

In conclusion, this is the first study to show that the MMP-8 -799 C>T polymorphism is significantly associated with a risk for both AgP and CP in the non-smoking Taiwanese. The presence of the T allele in MMP-8 -799 polymorphism may increase the risk of periodontitis. Further studies involving other ethnic populations and the functional mechanisms of SNP are needed to confirm the findings in this article.

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Clinical Relevance

Scientific rationale for the study: Periodontitis is a multi-factorial disease regulated by bacterial, host genetic and environmental aspects. MMP-8 is an important enzyme involved in the pathogenesis of periodontitis. Genetic polymorphism at the promoter region of MMP-8 gene may influence the expression of gene and modulate the inflammatory response. This is the first study examining whether or not MMP-8 SNP affects the susceptibility to chronic and aggressive periodontitis in a Taiwanese population.
Principal findings: The -799 T allele of MMP-8 gene was significantly associated with the risks of chronic and aggressive periodontitis in non-smokers.
Practical implications: Non-smoking Taiwanese who are MMP-8 -799 T allele carriers (C/T and T/T genotypes) may be more susceptible to periodontitis.