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Investigation of matrix metalloproteinase-1 − 1607 1G/2G polymorphism in a Turkish population with periodontitis

Kemal Ustun[1], Nilgün Özlem Alptekin[2], Sema S. Hakki[2] and Erdogan E. Hakki[3]

[1]Oral and Dental Health Center, Selçuklu, Konya, Turkey; [2]Department of Periodontology, Faculty of Dentistry, Selcuk University, Konya, Turkey; [3]Department of Field Crops, Faculty of Agriculture, Selcuk University, Konya, Turkey

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Abstract

Aim: Matrix metalloproteinase-1 (MMP-1) is a proteolytic enzyme that degrades extracellular matrix and plays a fundamental role during destruction of periodontal tissues. The aim of this study was to examine the association between MMP-1 − 1607 1G/2G polymorphism and chronic periodontitis susceptibility in a Turkish population.

Material and Methods: A total of 180 subjects were enrolled in this study. All the subjects received a periodontal examination including full-mouth clinical attachment loss measurements, probing depths, plaque index scores, gingival index scores and radiographic bone loss ratios. Three groups formed according to periodontal conditions were healthy, moderate periodontitis and severe periodontitis groups. MMP-1 − 1607 1G/2G gene promoter polymorphism was genotyped using a polymerase chain reaction-restriction fragment length polymorphism method.

Results: Analysis of the polymorphism showed no differences in distribution of the MMP-1 − 1607 1G/2G polymorphism among healthy, moderate periodontitis and severe periodontitis groups ($p > 0.05$). When the groups were further stratified by smoking status, we found no significant differences in genotype distributions, allele frequencies and carriage rates among any groups either ($p > 0.05$).

Conclusions: On the basis of the results, no significant association is found for the MMP-1 − 1607 1G/2G polymorphism with susceptibility to periodontitis. Moreover, smoking status did not seem to affect this result.

Key words: genetics; MMP-1; periodontitis susceptibility; polymorphism

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Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, bone resorption and characterized by pocket formation and/or gingival recession (Armitage 1999). Current understanding of the aetiology of periodontitis implicates that bacterial infection is the primary cause of the disease (Flemmig 1999), while the pathology is the result of the interactions between pathogens and host. Host response involves immune mechanisms that are under the influence of genetic and environmental factors known as risk determinants (Kornman & Newman 2000). Role of these risk factors is not clearly understood, while data have been accumulating on the involvement of genetic determinants in the initiation and progression of periodontitis (Kornman et al. 1997, Kornman & Newman 2000, Michalowicz et al. 2000). Multiple aspects of periodontitis could be affected by genetic mechanisms such as the predisposal of the individual to develop the disease or experience its more severe forms (Shapiro et al. 1997). A genetic predisposal to periodontitis may exhibit population-based variations (Kinane & Hart 2003), while some human groups are significantly more prone to various forms suggesting a chromosomal specificity (Fujita et al. 2005). In addition to the chromosome-based linkage studies, single nucleotide polymorphisms (SNPs) have also been studied in detail and shown to be linked to certain types of periodontitis (Kornman & Newman 2000). Disease forms linked

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to SNPs have shown that these types of genetic analyses could potentially explain the differences between individuals as well as populations in their response to the pathogens and the patterns of tissue destruction.

As a result of pathological events in periodontium, the most important component of the periodontium lost is collagen type I, which is found in the extracellular matrices of the periodontal ligament and alveolar bone (Birkedal 1993, Seguier et al. 2001). There are several pathways of collagen degradation and these pathways can be initiated in disease phenomena. Matrix metalloproteinases (MMPs) are the most important enzymes in the connective tissue destruction degrading the periodontal ligament attachment and bone matrix proteins MMPs are a family of proteolytic enzymes that mediate the degradation of extracellular matrix macromolecules, including the interstitial and basement membrane collagens, fibronectin, laminin and proteoglycan core protein (Uitto et al. 2003). On the basis of the published sequence of the human genome, the MMP gene family encodes a total of 24 homologous proteinases, stromelysins, membrane-type MMPs and other MMPs depending on their substrate specificity and molecular structure (Bartold & Narayanan 2006). MMPs are involved in physiological processes such as tissue development, re-modelling and wound healing, and play important roles in the regulation of cellular communication, molecular shedding and immune functions by processing bioactive molecules including cell surface receptors, cytokines, hormones, adhesion molecules and growth factors (Sorsa et al. 1995, Uitto et al. 2003). Many types of MMPs have been identified in inflamed periodontal tissues and gingival crevicular fluid. These enzymes are thought to play an important role in tissue destruction in periodontal diseases. Fibroblast-type collagenase (MMP-1) is the major type of proteolytic enzyme that can cleave native interstitial collagens type I and type III, which are the most abundant protein components of periodontal extracellular matrix (Birkedal 1993).

Many reviews have been published in recent years supporting the evidence that genes influence an individual's predisposition for the initiation and progression of periodontal disease (Takashiba & Naruishi 2006). Investigators predict that information concerning polymorphism will be useful in the prevention and therapy of periodontitis, as well as in the recognition of patients in need for more comprehensive therapy. Cytokines, HLA alleles, immunoreceptors, proteases and structural molecules such as vitamin D, Catepsin C are being largely investigated for polymorphisms that may be related with periodontal disease (Takashiba & Naruishi 2006).

A polymorphism in the promoter region of human MMP-1 gene has been described (Rutter et al. 1998). The two alleles (1G and 2G) are formed by an insertion/deletion of a guanine at position -1607. This results in creation of a binding site for the Ets family of transcription factors as well as the increased transcription of the MMP-1 gene and increased enzyme activity (Rutter et al. 1998). These polymorphisms have been investigated in different races but contradictory results have been obtained. Therefore, the present study was designed to investigate the polymorphism of MMP-1 at -1607 position among individuals with different levels of chronic periodontal disease in a Turkish population.

Material and Methods

Subjects

All the subjects were Caucasians of exclusively Turkish nationality. The study population consisted of 180 unrelated subjects (129 males and 51 females) who refereed to the Department of Periodontology, Faculty of Dentistry, Selcuk University. All the subjects were over 35 years of age and had at least 20 teeth present in the mouth. The study was approved by the Local Ethic Committee at Selcuk University (2004–132), and written informed consent was obtained from all the participants before inclusion in the study in accordance with the Helsinki declaration. Subjects with potential confounding factors such as diseases of oral hard or soft tissues except caries and periodontal disease, a history of diabetes, hepatitis, or HIV infection, in need for pre-medication for dental treatment, chronic usage of anti-inflammatory drugs, immunosuppressive chemotherapy, history of any disease known to severely compromised immune function, current acute necrotizing ulcerative gingivitis or current pregnancy or lactation, or use of orthodontic appliances were excluded from the study (De Souza et al. 2003). After intra-oral and radiographic examinations, patients diagnosed as aggressive periodontitis were not included in this study. The subjects phenotype and genotype status were assigned after inclusion of the study by a single investigator (K. U.). The study included both smokers and non-smokers where the smoking status was classified based on cigarette consumption calculated in pack years (Bernaards et al. 2001).

Periodontal examination

Diagnosis and classification of disease severity were based on clinical parameters and consisted of physical and radiographic examination, medical and dental history, probing depth (PD), assessment of clinical attachment loss (CAL), plaque index (PI) (Silness & Löe 1964), gingival index (Löe & Silness 1963) and tooth mobility. Measurements of PD and CAL were recorded at six points around each tooth. Radiographic bone loss (RBL) was assessed as a percentage of the expected bone height, calculated to the nearest 10% using a Schei ruler (Schei et al. 1959).

The examinations were performed by a single investigator (K. U.).

The subjects were divided into three groups. These groups and their categorization criteria were the same as described by de Souza et al. (2003).

Healthy: subjects found to exhibit no signs of periodontal disease as determined by the absence of CAL and no sites with PD above 3 mm.

Mild periodontitis: patients with teeth exhibiting $\geqslant 3$ mm and < 7 mm CAL.

Moderate to severe periodontitis: patients with teeth exhibiting $\geqslant 7$ mm CAL.

The demographic informations as well as the clinical profile of the patients are shown in Table 1. Box–whisker plots for CAL, RBL and PD are given below (Figs 1–3). The characteristics of the groups are as follows:

Healthy group: Healthy group consisted of 54 (19 females and 35 males) individuals. The mean age and standard deviation (SD) of the group was 40.3 ± 5.5 and ranged between 35 and 56 years in this study. Nineteen of the participants were smokers and the mean smoking status and SD was 5.13 ± 9.5 pack years and ranged between 2 and 40 pack years.

Mild periodontitis group: Mild periodontitis group consisted of 42 (eight females and 34 males) patients. The

Table 1. Clinical parameters of the study population

	Healthy	Mild periodontitis	Severe periodontitis	Kruskal–Wallis test score	*p*
Age (years)	40.3 ± 5.5	45.3 ± 8.2	44.9 ± 7.5	16.828	0.000
PPD (mm)	2.4 ± 0.2	2.9 ± 0.3	3.8 ± 0.9	115.755	0.000
RBL (%)	0.0 ± 0	4.3 ± 3.4	20.0. ± 12.1	148.774	0.000
PI	1.4 ± 0.6	1.7 ± 0.6	2.2 ± 0.6	46.630	0.000
GI	1.2 ± 0.4	1.6 ± 0.5	2.1 ± 0.5	63.107	0.000

Parameters are mean ± SD.

p-values are according to Kruskal–Wallis test score.

PPD, probing pocket depth; RBL, radiographic bone loss; PI, plaque index; GI, gingival index.

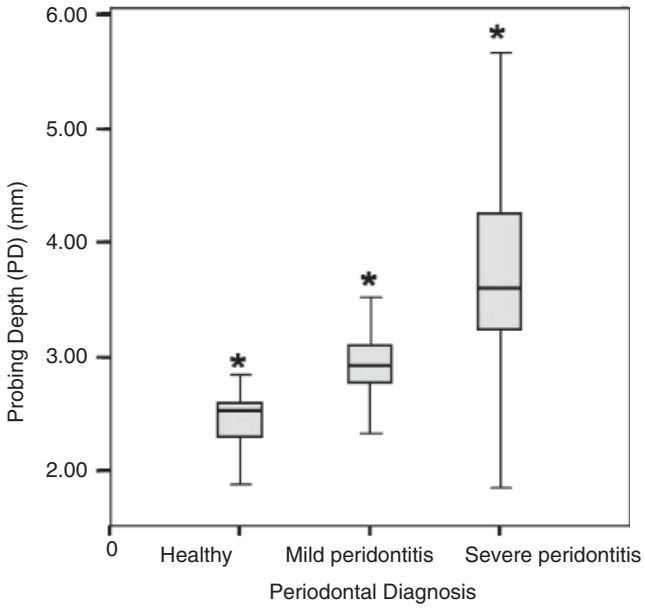

Fig. 1. Box–Whisker plot graphic for PD and periodontal diagnosis. *$p < 0.05$ according to Mann–Whitney *U* test.

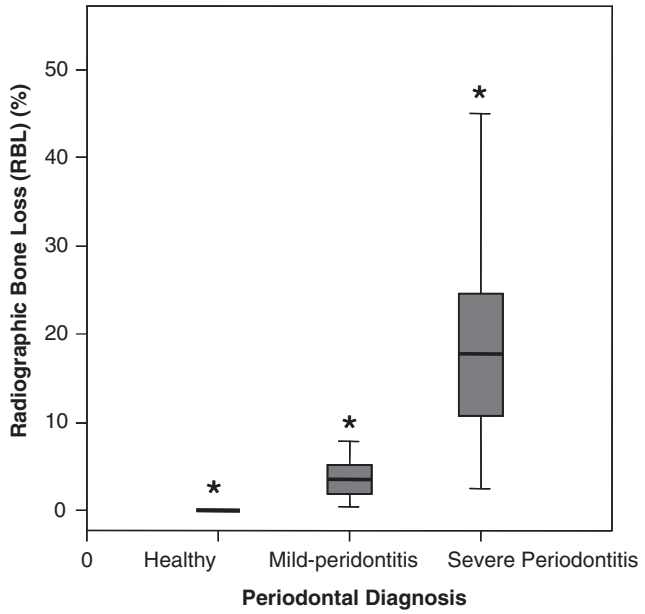

Fig. 2. Box–Whisker plot graphic for RBL and periodontal diagnosis. *$p < 0.05$ according to Mann–Whitney *U* test.

mean age and SD of the group was 45.3 ± 8.2 years and ranged between 35 and 68 years. Eighteen of the patients were smokers. The mean smoking status and SD was 6.5 ± 10.4 pack years and ranged between 1 and 45 pack years.

Moderate to severe periodontitis group: Moderate to severe periodontitis group consisted of 84 (23 females and 61 males) patients. The mean age and SD of the group was 44.9 ± 7.5 years and ranged between 35 and 64 years. Forty-five of the patients were smokers. The mean smoking status and SD was 9.9 ± 13.7 pack years and ranged between 1 and 58 pack years.

Genotype identification and polymerase chain reaction (PCR)

The genomic DNA was isolated from peripheral blood leucocytes by a standard method using the proteinase K digestion of cells. The MMP-1 −1607 1G/2G polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as previously described (De Souza et al. 2003). The sequences of PCR primers were 5′-TCGTGAGAA TGTCTTCCCATT-3′ forward primer and 5′-TCTTGGATTGATTTGAGATA AGTGAAATC-3′ reverse primer. PCR was carried out in a total volume of 50 μl, containing 100 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1 μM of each primer, 100 mM each dATP, dCTP, dGTP, dTTP and 4 U Taq DNA polymerase (Bioron GmbH, Ludwigshafen, Germany.) The solution was incubated for 5 min. at 95°C, followed by 36 cycles of 30 s at 95°C, 30 s at 55°C, 30 s at 72°C, with a final extension of 72°C for 7 min.

A 20 μl aliquot of PCR products was mixed with 3 μl Tango Buffer 10 × (Fermentas International Inc., Burlington, Canada), 1 μl Xmnl (10 U/ml) (Fermentas International Inc.) and 6 μl ddH_2O. The solution was incubated at 37°C for 16 h. The aliquot of the digest was mixed with 5 μl loading buffer (6 ×) and 20 μl of the mixture was electrophoresed on a 3% microphore agarose gel at 80 V for 45 min.

A representative gel image is shown in Fig. 4.

Data analysis

Statistical analysis of data was performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

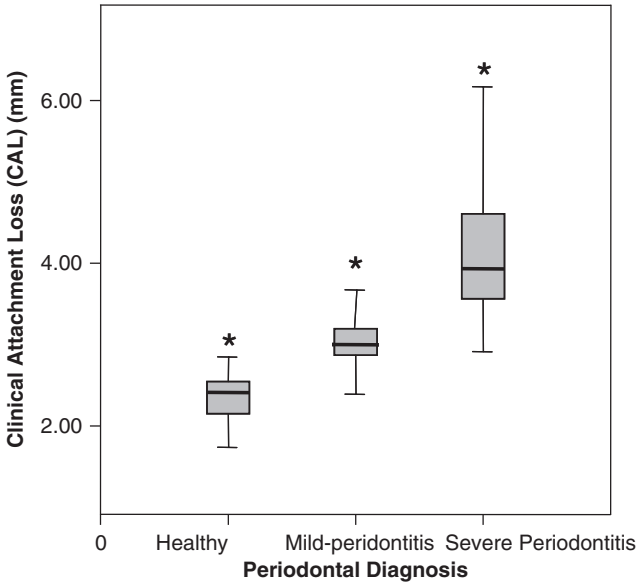

Fig. 3. Box–Whisker plot graphic for CAL and periodontal diagnosis. $*p < 0.05$ according to Mann–Whitney *U* test.

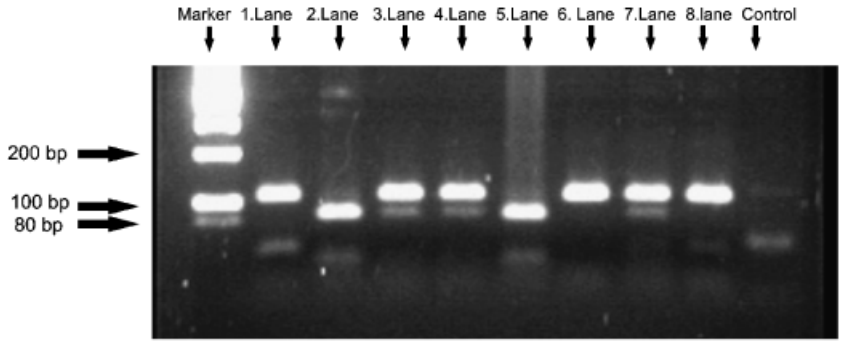

Fig. 4. A representative image of the gel used in genotyping.
Lane 1: DNA Ladder (Fermentas MassRuler #SM0383)
Lane 2,7,9: 2G/2G genotype
Lane 3,6: 1G/2G genotype
Lane 4,5,8: 1G/2G genotype
Lane 10: Control.

The differences between disease categories and age, PI, CAL, RBL were determined using Kruskal–Wallis test. Significant results were further analysed by Mann–Whitney *U* test with Bonferroni correction.

Differences in genotype distribution from those expected by Hardy–Weinberg equilibrium and the significance of the differences in observed frequencies of the polymorphism in healthy, moderate periodontitis and severe periodontitis groups were assessed by the χ^2 test. A *p*-value $\leqslant 0.05$ was considered statistically significant.

A multivariate logistic regression was used to determine the role of MMP-1 – 1607 2G/2G polymorphism in periodontitis while adjusting for potential confounder smoking. Odds ratios were calculated with 95% confidence interval.

Results

The study population consisted of 180 subjects of three groups (healthy, mild periodontitis and moderate to severe periodontitis). Eighty-two of the subjects were smokers (19 healthy, 18 mild periodontitis and 35 moderate to severe periodontitis) and 98 of them were non-smokers (35 healthy, 24 mild periodontitis and 39 moderate to severe periodontitis). Table 2 shows the MMP-1 – 1607 1G/2G genotype distributions. Allele frequencies and carriage rates for healthy, mild periodontitis and moderate to severe periodontitis subjects are presented. Genotype distributions were not significantly different from Hardy–Weinberg equilibrium ($p > 0.05$). No statistically significant differences ($p > 0.05$) in allele, genotype frequencies or carriage rates of the MMP-1 – 1607 gene were found among groups. Age and gender distribution was not found to significantly impact the polymorphism distribution in the study groups.

As smoking is a known risk factor for periodontitis, a logistic regression analysis was performed. A significant ($p < 0.05$) association is detected between periodontitis and smoking 10 or more cigarettes per day. Results of the test are given in Table 3.

Discussion

Chronic periodontitis is regarded as an ''eco-genetic'' disease, the onset and severity of which are influenced by bacteria on the tissues, gene polymorphisms, host immune and inflammatory responses, local and systemic environmental factors, and various medications (Bartold & Narayanan 2006). A study using 117 pairs of adult twins estimated that about half of the variance found in the expression of chronic periodontitis can be attributed to genetic factors (Michalowicz et al. 2000). Kornman et al. (1997) were the first to show that a specific genetic variation of the IL-1 gene cluster was associated with chronic periodontitis. Since then, many genetic polymorphisms that may have a potential impact on periodontal disease are being investigated.

This study represents an investigation of the role of the MMP-1 – 1607 polymorphism in chronic periodontitis in a Turkish population. Polymorphism in the MMP-1 – 1607 region was investigated in Japanese (Itagaki et al. 2004), Czech (Holla et al. 2004), Brazilian (De Souza et al. 2003, Astolfi et al. 2006) and Chinese (Cao et al. 2005a, b, 2006) populations. Contradictory results have been obtained. Some of the investigations found an association between the 2G allele and periodontal disease (De Souza et al. 2003, Cao et al. 2005a, 2006), some found an association with the 1G allele (Holla et al. 2004) and the others did not find any association (Itagaki et al. 2004, Astolfi et al. 2006). Because previous studies have suggested that a polymorphism on this

Table 2. Allele and genotype distributions of the MMP-1

	Healthy	Mild	Moderate to severe	Total	*p*
			Total		
MMP-1 −1607	*n* = 54	*n* = 42	*n* = 84	*n* = 180	
1G/1G	11 (20.4%)	9 (21.4%)	15 (17.9%)	35 (19.4%)	0.961
1G/2G	28 (51.9%)	23 (54.8%)	44 (52.4%)	95 (52.8%)	
2G/2G	15 (27.8%)	10 (23.8%)	25 (29.8%)	50 (27.8%)	
1G	50 (46.2%)	41 (48.8%)	74 (43%)	165 (45.8%)	0.769
2G	58 (53.8%)	43 (51.2%)	94 (57%)	195 (54.2%)	
2G+	11 (20.3%)	9 (21.4%)	15 (17.8%)	35 (19.4%)	0.874
2G−	43 (79.7%)	33 (78.6%)	69 (82.2%)	145 (80.6%)	
			Smoking		
MMP-1 −1607	*n* = 19	*n* = 18	*n* = 45	*n* = 82	
1G/1G	4 (21%)	3 (16.6%)	7 (15.5%)	14 (17%)	0.623
1G/2G	12 (63.1%)	8 (44.4%)	25 (55.5%)	45 (54.8%)	
2G/2G	3 (15.9%)	7 (39%)	13 (33%)	23 (28.2%)	
1G	20 (52.6%)	14 (38.8%)	39 (43.3%)	73 (44.5%)	0.446
2G	18 (47.4%)	22 (61.2%)	51 (56.7%)	91 (55.5%)	
2G+	4 (21%)	3 (16.6%)	7 (15.5%)	14 (17%)	0.886
2G−	15 (79%)	15 (83.4%)	38 (84.5%)	68 (83%)	
			Non-smoking		
MMP-1 −1607	*n* = 35	*n* = 24	*n* = 39	*n* = 98	
1G/1G	7 (20%)	6 (25%)	8 (20.5%)	21 (21.4%)	0.442
1G/2G	16 (45.7%)	15 (62.5%)	19 (48.7%)	50 (51%)	
2G/2G	12 (34.3%)	3 (12.5%)	12 (30.8%)	27 (27.6%)	
1G	30 (42.8%)	27 (56.25%)	35 (44.8%)	92 (46.9%)	0.321
2G	40 (57.2%)	21 (43.75%)	43 (55.2%)	104(53.1%)	
2G+	7 (20%)	6 (25%)	8 (20.5%)	21 (21.4%)	0.885
2G−	28 (80%)	18 (75%)	31 (79.5%)	77 (78.6%)	

p-values are according to χ^2 test.
MMP-1, matrix metalloproteinase-1.

Table 3. Logistic regression analysis for susceptibility to periodontitis

	β	SE	OR	95% CI of OR	*p*
MMP-1 2G/2G	−0.038	0.370	0.963	0.467–1.987	0.919
Smoking					
0–9 pack years to non-smoking	0.235	0.501	1.265	0.474–3.379	0.639
10⩽pack years to non-smoking	0.779	0.387	2.180	1.022–4.650	0.044*
Constant	0.598	0.234	1.819		

*Statistically significant ($p < 0.05$).
Periodontitis was analysed as a dependent variable, covariates were smoking status and MMP-1 −1607 2G/2G polymorphism.
OR, odds ratio; CI, confidence interval; MMP-1, matrix metalloproteinase-1.

position may have a functional consequence, as it affects the functions of the MMP-1 enzyme (Rutter et al. 1998), we have hypothesized that such an association could be found between the presence and severity of periodontitis where MMP-1 is responsible for collagen degradation (De Souza et al. 2003). There are three polymorphisms identified in the promoter region of MMP-1 gene. The one largely studied is the −1607 1G/2G polymorphism and the others are −519A/G and −422A/T polymorphisms. In the literature, −519A/G (Jurajda et al. 2002) and −422A/T (Thiry-Blaise et al. 1995) polymorphisms have not been associated with periodontal disease. So, we studied the −1607 1G/2G polymorphisms susceptibility with periodontal disease.

In our study, we did not see any significant association between the MMP-1 −1607 polymorphism and susceptibility to periodontitis. This finding, although not excluding the potential role of such a polymorphism in larger and other populations, suggests that a variation at MMP-1 at −1607 position does not play direct role in development or severity of chronic periodontitis in adults studied. Further studies may be designed to investigate other positions; however, −1607 position is the only scientifically justified polymorphism that has been linked to MMP-1 function (Tower et al. 2003). Therefore, the biological significance of other positions should be documented before such analyses. Still, the current data is useful when future studies would be designed to analyse composite polymorphisms in subjects with periodontitis or when various groups of humans with different ethnic and genetic backgrounds are studied.

On the other hand, at least three important regulatory mechanisms may control the action of MMPs on the components of the extracellular matrix: regulation of transcription levels, activation of the pro-enzyme into the extracellular matrix and the inhibition of MMPs by their tissue inhibitors of MMPs. Control of transcription has been considered the key step in the regulation of MMPs activity. However, the control of protein activity is of key importance. MMPs activity is controlled by endogenous activators, inhibitors and factors that influence MMP secretion, cell surface localization and MMP degradation and clearance (Sternlicht & Werb 2001). They explained that some MMP members share common extracellular matrix substrates and compensate these functions for each other. These shared functions indicate that a single gene polymorphism of an MMP may not have enough effect on disease susceptibility or progression (Itagaki et al. 2004).

When we look at polymorphism studies, there is no absolute distinction between common and single-gene diseases, because polymorphism may not cause disease until the carrier of the gene is exposed to a specific environmental agent. For example, an individual with the *β*-globulin gene polymorphism that leads to sickle cell anaemia will have different clinical disease experiences at the sea level and at high altitudes, where oxygen is more limited (Kornman & Newman 2000). As Armitage et al. (2000) mentioned, the role of genetics in multifactorial diseases such adult periodontitis is quiet complex. For example, one patient may have increased susceptibility due to heavy smoking, another due to poorly controlled diabetes, another due to a genetic polymorphism and fourth due to a chronically heavy bacterial challenge. This is similar to the risk factors for coronary artery disease. It is reasonable to assume that in certain population groups, an individual risk factor may explain a different proportion of severe periodontitis cases than in another population group. For example, some popu-

lation groups may have a higher prevalence of diabetes than another group, and therefore diabetes would be expected to explain a greater part of the disease in that population than in another population (Armitage et al. 2000). Individuals with different genetic backgrounds have different susceptibilities; therefore, aetiology of periodontitis is genetically heterogenous, that is, different genetic mechanisms lead to the same clinical end point. Another reason for the contradictory results on gene polymorphism disease associations is that distinct races and/or populations may have different risk alleles for a same disease (Itagaki et al. 2004). Even in the same race, discrepancies may occur due to methodological differences as sample size and disease classification methods (Astolfi et al. 2006). In this study, 180 individuals with different periodontal conditions except aggressive forms of periodontitis were included. One of the limitations of this study is that although the sample size is not very low compared with the studies in the literature, still it is unable to show the risk factors that may have a slight contribution to the pathogenesis of periodontal disease.

Smoking is known to be a major risk factor for periodontal disease. Some polymorphisms show strong associations only in smoking patients with periodontitis (Kornman & Newman 2000). Genetic susceptibility to periodontitis can easily evincible in non-smokers because degradation of connective tissue in smokers may occur as a consequence of smoking (Holla et al. 2004) and thus may mask the influence of individual variants of the MMP-1 polymorphism. Holla et al. (2004) detected a borderline association of inverse 1G allele with chronic periodontitis. The association between 1G/2G polymorphism was most strongly observed in non-smoking subjects. In our study, we detected an important association with heavy smoking and periodontitis susceptibility. But there was no association with MMP-1 2G/2G polymorphism.

As a result, most of the multifactorial diseases are influenced, at least in part, by genetic factors. It is believed that the combination of several significant risk gene polymorphisms in certain individuals synergistically elevates a susceptibility to the disease (Michalowicz et al. 2000, Kornman 2003). Further studies with larger sample sizes are needed to search for other genetic markers. Investigation of more polymorphisms that may contribute to pathogenesis of periodontitis at the same study population would be more beneficial as some gene interactions could be monitored. It could advance our understanding of the pathogenesis of this common eco-genetic disease, facilitate the early identification of individuals at high risk and may provide new targets for the design of novel preventive and therapeutic measures.

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Address:
Kemal Ustun
Selçuklu Ağız ve Diş Sağlığı Merkezi
Konya
Turkey
E-mail: k_ustun@yahoo.com

Clinical Relevance

Scientific rationale for the study: Periodontitis is recently described as an eco-genetic disease which environmental and genetic factors act together to form the disease. MMP-1 −1607 polymorphism has been associated with periodontal disease in some populations. Genetic susceptibilities may vary in different races. Identification of genetic polymorphisms associated with periodontal disease in different races should permit a better understanding of disease aetiology, allowing improved classification, diagnosis and treatment of periodontal diseases. This is the first study reporting MMP-1 −1607 polymorphisms and susceptibility to periodontal disease in a Turkish population.

Principal findings: No significant association is found for the MMP-1 −1607 polymorphism with susceptibility to periodontitis in a Turkish population.