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Gene polymorphisms of matrix metalloproteinase-2, -9 and -12 in periodontal health and severe chronic periodontitis

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ABSTRACT

Aim: Matrix metalloproteinases (MMPs) are involved in periodontal tissue remodeling and degradation. MMP polymorphisms could alter transcription and function of these enzymes. The aim of this study was to investigate *MMP-2*, *MMP-9* and *MMP-12* gene polymorphisms in relation to susceptibility to severe chronic periodontitis (CP).

Methods: Genomic DNA was obtained from peripheral blood of 87 severe CP patients and 107 periodontally healthy subjects. *MMP-2 −735C/T*, *MMP-9 −1562C/T* and *MMP −12 357Asn/Ser* gene polymorphisms were genotyped by polymerase chain reaction and restriction fragment length polymorphism. Probing depth, clinical attachment loss, supragingival plaque accumulation and bleeding on probing were recorded. The data were analyzed by chi-square, logistic regression and Mann–Whitney-*U*-tests.

Results: The genotype distributions and allele frequencies of *MMP-2*, *MMP-9* and *MMP-12* genes were similar in CP and healthy subjects ($p > 0.05$). Differences between rare allele carriage rates of CP and healthy groups regarding *MMP-2*, *MMP-9* and *MMP-12* gene polymorphisms were not significant ($p > 0.05$). However, T allele carriers of *MMP-9 −1562* gene had less risk for CP (OR = 0.36; 95% CI = 0.16–0.81).

Conclusion: These data suggest that *MMP-2 −735C/T*, *MMP-9 −1562C/T* and *MMP-12 357Asn/Ser* polymorphisms are not associated with susceptibility to severe CP in Turkish population. However, T allele of *MMP-9 −1562* gene might be associated with decreased susceptibility to severe CP.

1. Introduction

Matrix metalloproteinases (MMPs) are a group of endogenous proteinases that contribute to degradation of extracellular matrix and basement membrane components.[1] Among these structurally related but genetically distinct enzymes, MMP-2 (gelatinase-A, 72 kDa gelatinase), MMP-9 (gelatinase-B, 96 kDa gelatinase) and MMP-12 (macrophage metalloelastase) are responsible for the breakdown of type IV collagen and non-collagenous components of the extracellular matrix.[2] In addition, MMP-2 but not MMP-9 is also able to cleave native type I collagen which

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is the abundant component of gingival connective tissue matrix.[3]

Elevated levels of MMP-2 and MMP-9 have been detected in gingival crevicular fluid[4–6], periimplant sulcular fluid[7] and gingival tissue[5,8–10] of periodontititis/periimplantitis patients. MMP-12 is a key enzyme for macrophage migration and macrophage-mediated matrix degradation.[11] It has been shown that osteoclast-derived MMP-12 cleaves bone matrix proteins pivotal for osteoclast-matrix interactions.[12] Furthermore, it has been demonstrated that MMP-2, -9, -13 and -14 which are potentially pivotal enzymes in periodontitis[13] are partly upregulated by a MMP-12-dependent mechanism.[14,15] Increased levels of MMP-12 are implicated in the pathogenesis of destructive diseases including rheumatoid arthritis[16,17], atherosclerosis[14] and aortic aneurisms.[18] Analogous activities and functions of MMP-2, -9 and -12 suggests that these enzymes synergistically orchestrate the destruction and remodeling of the periodontal tissues.

In recent years, many of genetic polymorphism studies have focused on molecular components of host response which are associated with periodontitis.[19–23] Polymorphisms in the MMP genes are potentially important because of their key role in periodontal tissue remodeling and destruction. Several polymorphisms have previously been identified in the promoter region of *MMP-2* gene that may effect expression and function.[24] Among these variants, the substitution of nucleic acid cytosine (C) to thymidine (T) at position 735 in the promoter region of *MMP-2* gene leads to altered transcription of the gene.[24] This single nucleotide polymorphism has been associated with heart failure and cancer.[25–27] 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.[28] The T allele results in a higher promoter activity than C allele due to higher affinity of a nuclear protein to −1562 T allele.[28] This functional polymorphism is characterized with increased mRNA, protein level and activity in −1562 T allele carriers of *MMP-9*[29] and has been associated with cardiovascular diseases.[28–33] Substitution of adenine to guanine at position 1082 in the coding region of *MMP-12* gene leads to a replacement in the amino acid sequence, asparagine (Asn) to serine (Ser), at residue 357. This gene polymorphism is proposed to potentially affect the catalytic activity of MMP-12.[34]

Considering the role of MMP-2, MMP-9 and MMP-12 in both remodeling and destruction of extracellular matrix, whether genetic variations influencing the transcription or function of these enzymes contribute to the susceptibility to chronic periodontitis (CP) was hypothesized. Currently there is only one report regarding association of *MMP-2* polymorphism and CP.[35] In addition, the results of previous studies on *MMP-9 −1562 C/T* polymorphism in CP are conflicting.[36–38] Also, association of *MMP-12 357Asn/Ser* gene polymorphism with periodontitis had only been studied in generalized aggressive periodontitis (G-AgP) patients.[39] Therefore, in the present study we aimed to evaluate the genotype distribution, rare allele carriage and allele frequencies of *MMP-2 −735C/T*, *MMP-9 −1562 C/T* and *MMP-12 357Asn/Ser* genes and their relation to susceptibility to severe CP in a Turkish population.

2. Materials and methods

2.1. Study population

A total of 194 unrelated Turkish subjects including 87 severe CP patients and 107 subjects with healthy periodontal conditions were recruited from the Department of Periodontology, School of Dentistry, Ege University over a period of 4 years between 2002 and 2006. All of the study subjects were residing in the Aegean region of Turkey and were in low to moderate socioeconomic level. All subjects underwent clinical and radiographic examination. Medical and dental histories were taken. Smokers in both CP and healthy groups were smoking more than 10 cigarettes per day for more than 5 years.[40] Subjects who had never smoked or quit smoking at least 5 years ago were considered as nonsmokers. None of the CP patients and healthy subjects had a history or current manifestation of systemic conditions which could modify the periodontal status including the diseases that has been associated with investigated MMP gene polymorphisms (cancer, cardiovascular diseases or respiratory diseases) or had transmissible infectious diseases (HIV, hepatitis). Pregnancy was also selected as exclusion criteria for the study. Participants eligible for the study were informed on the purpose of the study and gave written informed consent in accordance with Helsinki declaration. The study protocol was approved by the ethics committee of the Ege University, School of Medicine. CP patients were diagnosed in accordance with the clinical criteria for CP agreed by consensus at the World Workshop in Periodontics in 1999 as follows[41]:

2.1.1. CP group

The CP group included 32 females and 55 males ranged in age from 35 to 63 with a mean age of 48.3 ± 6.4. They had at least 20 teeth, had severe chronic periodontitis and exhibited at least four sites with clinical attachment loss (CAL) ≥ 5 mm in at least two quadrants. They also had bleeding on probing (BOP) at >80% of the proximal sites. The bone loss estimation was radiographically performed in each patient for assessment of the extent and severity of alveolar bone loss. Examinations were particularly focused on consistency of periodontal destruction with plaque accumulation in order to distinguish from aggressive forms of periodontitis.

2.1.2. Healthy control group

These individuals were periodontally healthy volunteers from the staff and other patients referring to the School of Dentistry. The healthy group consisted of 65 females and 42 males (mean age 43.6 ± 7.5 years; range 35–70 years). They had at least 20 teeth and at least 90% of measured tooth sites exhibited probing depth (PD) <3 mm and CAL ≤ 2 mm as well as BOP score <15% at examination and no alveolar bone loss present in radiography (i.e., distance between the cemento-enamel junction and bone crest <3 mm at >95% of the proximal tooth sites).

2.2. Determination of periodontal status

The clinical periodontal parameters were assessed at six sites around each tooth (mesio-buccal, mid-buccal, disto-buccal,