The relationship between Matrix Metalloproteinase-1 gene promoter (-1607) polymorphism and aggressive periodontitis

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Abstract
Aim: The purpose of this study was to investigate the role of Matrix metalloproteinase-1 (MMP-1) -1607 1G/2G promoter polymorphism in aggressive periodontitis (AgP).

Material and Methods: One hundred sixty-five systemically healthy volunteers comprising of 65 AgP and 100 periodontally healthy subjects were enrolled in this study. Full mouth radiological and clinical assessments of the periodontal tissues were performed. Genomic DNA was isolated from peripheral blood samples and amplified by PCR and analyzed by a restriction endonuclease.

Results: There was statistically no significant difference in genotype (p=0.79) and allele frequencies (p=0.99) of MMP-1 -1607 polymorphism between AgP patients and periodontally healthy control group. Additionally, multiple logistic regression analysis was performed to adjust for possible confounding factors, such as age, gender and smoking. Yet, the association still remained non-significant (p=0.57). The odds ratio (OR) 1G/1G vs. 1G/2G+2G/2G was 0.75(0.28-2.0; p=0.57).

Discussion: Early identification of individuals at high-risk of developing periodontal diseases might be helpful for prevention and may contribute to the treatment of disease. MMP-1 is biologically a promising candidate gene that is involved in pathogenesis of AgP. However, our results showed that the MMP-1 (-1607) promoter polymorphism did not have a role in the susceptibility to AgP disease risk in Turkish population.

Keywords
Genetic predisposition to disease; Periodontitis; Genetic association studies; Matrix Metalloproteinase-1
Introduction

Periodontal diseases are multifactorial diseases with the possible involvement of multiple genes and interaction of genes with environmental factors [1]. Epidemiologic studies demonstrated that periodontal disease risk is not the same for every individual [2]. Although pathogenic bacteria in microbial dental plaque are essential for the development of periodontitis, this alone is not enough to cause disease. Inflammatory and immune response mounted by the host against the bacteria and their byproducts, has a crucial role in the development and progression of periodontal diseases. This host response is mostly under genetic control. The genetic differences in the host response may cause variation in the release of the cytokines and inflammatory mediators in response to microbial deposits on the tooth surface that can potentially exacerbate or alleviate the damage.

Aggressive periodontitis (AgP), is a rare, severe form of periodontal disease characterized by rapid attachment and bone loss of the tooth-supporting structures [3]. Although the primary etiological factor of the disease is microbial dental plaque and pathogenic microorganisms in it, the ultimate clinical outcome is determined by the interaction of environment and genetic factors [4].

Several lines of evidence support the genetic component of AgP. This disease seems to affect members of the same family, which suggests the presence of a genetic component [5]. Additionally, AgP is a clinical manifestation of some monogenic syndromes such as Papillon-Lefèvre syndrome, Chédiak-Higashi syndrome, leukocyte adhesion deficiency, cyclic neutropenia. The presence of severe form of AgP in these syndromes demonstrates that a genetic mutation at a single locus can lead to periodontal breakdown and susceptibility to disease. Furthermore, segregation analyses, however, showed that different modes of inheritance models in diverse populations suggest that genetic factors play a role in AgP [1, 4, 6].

Matrix metalloproteinases (MMP) are a large family of proteolytic enzymes responsible for the breakdown and remodeling of extracellular matrix components. MMPs are involved in physiological events such as tissue regeneration, but also pathological conditions such as periodontal disease [7, 8]. Extracellular matrix destruction seen in periodontal disease forms the basis of tissue damage in the disease process [8]. Initial studies failed to fully determine the cellular resource of MMPs. Bacteria and their byproducts held responsible for the tissue breakdown seen in periodontitis. It was known that periodontal pathogens such as Aggregatibacter (Actinobacillus) Actinomycetemcomitans and Porphyromonas gingivalis can produce bacterial collagenase to invade tissue. However, these bacterial proteinases do not have an important role in periodontal tissue destruction [9]. In periodontitis, mainly host- and partly bacteria-derived proteolytic enzymes are responsible for the periodontal breakdown [10].

MMP-1, which plays a vital role in the destruction of matrix components, is a key player in the process of tissue destruction in periodontal diseases. Several studies have reported that MMP-1 increases the immunoreactivity in periodontal tissues of AgP and chronic periodontitis (CP) compared to those of healthy periodontal individuals [11, 12]. In the study in which crevicular fluid levels (GCF) of MMP-1 in healthy, AgP and CP individuals’ gingival were evaluated with the fluorometric substrate method, MMP levels in the diseased sites of AgP patients were higher than in the other groups [13]. This finding is supported by another study showing that the MMP-1 levels of GCF in the CP group were higher compared to those of healthy individuals. Furthermore, the MMP-1 levels decreased after non-surgical periodontal treatment compared to pretreatment MMP-1 levels [14]. All these studies underline the role of MMP-1 in AgP and periodontal diseases.

Several studies evaluating the relationship between MMP-1 gene -1607 promoter polymorphism and periodontal disease in various populations have been conducted [15, 16]. However, the vast majority of these studies have investigated the relationship of MMP-1 gene -1607 polymorphism in CP [15, 16]. Only two studies assessed the relationship of MMP-1 -1607 promoter polymorphism with AgP, and both were in the Asian population. One of these studies was carried out in the Japanese [17], and the other one in the Chinese [18]. To the best of our knowledge, no research has yet been conducted to examine the relationship between MMP-1 gene and AgP in Turkish population. Therefore, to identify useful markers that could predict susceptibility AgP, we investigated the single-nucleotide polymorphisms in the promoter of the MMP1 gene in Turkish population.

Material and Methods

Study Subjects

This case-control study was conducted from 2007 to 2009 among 165 participants who sought treatment at the Department of Periodontology, Ondokuz Mayis University. This study was approved by the Ondokuz Mayis University local Ethics committee (approval date: 25.12.2006, and no: 138) and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants.

Systemically healthy unrelated individuals were included. Volunteers, who received periodontal treatment within six months, using chronic anti-inflammatory drugs, pregnant or in lactation period, were excluded.

All participants were evaluated clinically and radiographically. Clinical case definition was based on the definition of the Division of Oral Health at the Centers for Disease Control and Prevention for use in population-based surveillance of periodontitis [19]. Clinical examination included clinical attachment level (CAL), pocket depth, plaque index and gingival index. All measurements were performed by a single investigator with a Williams probe in the six sites of each tooth, excluding 3rd molars.

Healthy subjects: Subjects who showed neither attachment loss nor pocket depths of >3 mm and no radiographic evidence of bone loss were classified as healthy controls.

Aggressive Periodontitis: Systemically healthy patients with an interproximal attachment loss on at least two permanent teeth, of which at least one was 1st molar or an incisor, and involving no more than two teeth other than first molars and incisors, and the number of microbial deposits were inconsistent with the severity of the periodontal disease was classified as localized AgP. AgP patients were categorized as generalized if interproximal attachment loss affected more than two teeth.
other than the first molars and incisors.  

**DNA extraction and genotyping:**

DNA isolation was performed using a genetic extracting kit high Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). MMP-1 -1607 promoter region was amplified using the following primers set listed below with ‘Auto-Q Server Gradient Termal Siklus (Quanta Biotech, UK).

Forward: 5’ TCGTGAGAATGTCTTCCCATT 3’  
Reverse: 5’ TCTTGGATTGATTTGAGATAAGTGAAATC 3’

Genotyping analysis of MMP-1607 gene polymorphisms was performed by restriction fragment length polymorphism (RFLP) method. PCR amplification was done using Auto-Q Server Gradient Termal Siklus (Quanta Biotech, UK) and started with an initial denaturation at 95 °C for 5 min then the reaction mixture was subjected to 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 56 °C, and 30 s extension at 72 °C, followed by a final 10 min extension step at 72 °C. PCR products, digested with XmlI and were electrophoresed in 3% agarose gels at 90 volts for 60 min.

**Statistical Analysis:**

Statistical analysis in this study was done using R2.9.1 program (The R Project for Statistical Computing, available at: https://www.r-project.org/). Statistical significance limit was set as p <0.05. The categorical data were expressed as percentages and compared with the χ² test. Continuous variables were expressed as the mean and standard deviation (SD) and compared using the Student t-test. The Hardy-Weinberg equilibrium was tested using the Chi-square (χ²) goodness-of-fit test. Allele frequencies were assessed with the allele-counting method. Comparison of allele frequencies in control and AgP groups was done with the Z test. Differences in genotype frequencies between AgP patients and controls were tested with the Chi-square test. Multiple logistic regression models were used to estimate odds ratios for the association of genetic marker with AgP. The estimated ORs were adjusted for the effects of the established risk factors such as age, gender, and smoking.

### Results

**Study population characteristics:**

Our study included a total of 165 individuals (115 women, 50 men), 65 of whom were AgP patients, and 100 were periodontally healthy controls. There were 47 women (72.3%) and 18 men (27.7%) in the AgP group, 68 women (68.00%) and 32 men (32.00%) in the healthy control group. No statistically significant difference was found in terms of gender distributions between groups (p=0.67; Table 1). Eleven of the participants in the AgP group were smokers, whereas there were no smokers in the control group. The mean age of AgP group was 24.87 ± 4.50 years and ranged between 18-41 years. In the healthy group, the mean age was 24.87 ± 4.50 and ranged between 18-45 years. Statistically, a significant difference was observed in the mean age between the groups (p= 0.001; Table 1). When the periodontal variables between AgP and healthy control groups were compared, as expected, the difference between all clinical variables was statistically significant (Table 1).

The distributions of the MMP-1 genotypes in both AgP and healthy controls were in Hardy-Weinberg equilibrium (p> 0.05; 2 <3.84). The distribution of the genotypes and the allele frequencies of the MMP-1 gene are shown in Table 1. There was no statistically significant difference in MMP-1 gene -1607 promoter region 1G/1G, 1G/2G, and 2G/2G genotype distributions between AgP and healthy control groups (p = 0.79; Table 2). MMP-1 gene 1G allele frequency was 0.369 % in AgP group and 0.370 % in the healthy control group, the difference was not statistically significant (p=0.99; Table 2).

### Table 1. Study population characteristics

| Clinical Variables | AgP patient n=65 | Control n=100 | p-value |
|--------------------|------------------|---------------|---------|
| Probing depth (mm) | 3.72±0.81        | 1.70±0.36     | >0.001  |
| Clinical attachment level | 4.34±1.25 | 1.70±0.36 | >0.001 |
| Bleeding on probing (%) | 48 % | 16 % | >0.001 |
| Plaque index (%) | 52.80±38.46 | 24.83±16.29 | >0.001 |
| Mean Age (range) | 28.64±8.41(18-41) | 24.87±4.50(18-45) | >0.001 |
| Gender (women) n(%) | 47 (%72.3) | 68(68.0) | 0.67 |

### Table 2. Distribution of the MMP1 -1607 1G> 2G Genotype and Allele Frequencies

| Genotype | Cases | Controls |
|----------|-------|----------|
|          | n     | %        | n     | %        |
| 1G/1G    | 7     | 13.21    | 18    | 18.00    |
| 1G/2G    | 23    | 43.40    | 38    | 38.00    |
| 2G/2G    | 25    | 43.40    | 44    | 44.00    |
| Total    | 53    | 100      |       |          |
| p=0.69 (unadjusted) |     |         |       |          |

**Allele frequency**  
C | 0.349 | 0.370 |    |    |
T | 0.651 | 0.630 |    |    |

**Age, sex adjusted OR**  
1G/1G vs. 1G/2G+2G/2G | 0.75(0.28-2.01 p=0.57) |

### Table 3. Distribution of the MMP1 -1607 1G> 2G Genotype and Allele Frequencies among non-smokers

| Genotype | Cases | Controls |
|----------|-------|----------|
|          | n     | %        | n     | %        |
| 1G/1G    | 7     | 13.21    | 18    | 18.00    |
| 1G/2G    | 23    | 43.40    | 38    | 38.00    |
| 2G/2G    | 23    | 43.40    | 44    | 44.00    |
| Total    | 53    | 100      |       |          |
| p=0.69 (unadjusted) |     |         |       |          |

**Allele frequency**  
C | 0.349 | 0.370 |    |    |
T | 0.651 | 0.630 |    |    |

**Age, sex adjusted OR**  
1G/1G vs. 1G/2G+2G/2G | 0.75(0.28-2.01 p=0.57) |
Moreover, multivariate logistic regression analysis was used for the association of genetic marker with AgP in the presence of confounding factors. Adjustment for the established risk factors age, gender and smoking status did not alter this association. There was no statistically significant difference in terms of genotype distributions when age and gender correction was made in the logistic regression analysis (p = 0.74). The age-, sex-, and smoking status-adjusted OR between 1G/1G and 1G/2G, 2G/2G genotype was 0.75(0.28-2.0; p=0.57).

Additionally, we also examined the role of the MMP-1 promoter polymorphism with quantitative traits of AgP, including probing depth (p=0.48), attachment level (p=0.52), plaque index (p=0.11) and bleeding on probing (p=0.11). However, the MMP-1 promoter polymorphism revealed no significant association with none of the tested variables.

Finally, considering the adverse effects of smoking on periodontal tissues, we also evaluated non-smokers separately. Similar to overall results, no difference was found between the genotype distributions and allele frequencies of non-smoker patients and the control group (Table 3).

**Discussion**

The goal of this study was to investigate the relationship of -1607 1G/2G promoter polymorphism in MMP-1 gene with susceptibility to AgP and quantitative traits (e.g. probing depth) related to AgP in Turkish population. The selection of these SNPs was based on their prior reported associations with AgP risk in at least one case-control sample. The results of this study showed that the -1607 1G/2G polymorphism in MMP gene was not associated with the susceptibility to AgP and that the frequency of 2G alleles was very close to each other in both the individuals with AgP and the healthy control group. To the best of our knowledge, this is the first study investigating the association of MMP-1 gene -1607 1G/2G polymorphism with AgP in Turkish population.

Rutter et al. (1998) [20] first revealed the presence of a polymorphism in the MMP-1 gene promoter region in humans. Two different (1G and 2G) alleles are formed by adding/deleting G/GA in the -1607 promoter region. This newly formed 2G allele creates a binding site (5'-GGG-3') for Ets, which is one of the transcription factors. This binding site in turn increases MMP-1 transcription. The carriage of a 2G allele of MMP-1 gene may increase MMP-1 gene expression, in turn, excessive MMP-1 protein production occurs and enzyme activity increases. In such a case, the excessive collagenolytic activity may affect the risk, severity of periodontal diseases, or response to periodontal therapy [8]. Despite the biological relevance of MMP-1 with AgP, our case-control study did not provide evidence of the association of MMP-1 with AgP.

Although many studies examined the relationship between MMP-1 -1607 1G/2G polymorphism and susceptibility to periodontal disease, most of the studies have been conducted on patients with CP. Only two studies assessed the relationship between MMP-1 -1607 promoter polymorphism and AgP risk and were in the Asian population [17, 18], one of which was in Japanese [17] and the other one was in Chinese [18]. The results of these studies on MMP-1 -1607 polymorphism and both risks of AgP and CP gave conflicting results.

The first study examining the relationship between MMP-1-1607 polymorphism and AgP was conducted by Itagaki et al. (2004) [17] in Japanese. This study compromised of 37 GAP patients and 142 periodontally healthy controls. No significant difference in the genotype distribution, the allele frequency of MMP-1 -1607 polymorphism between GAP and controls in Japanese were reported. Contrary to these results, in Cao et al's (2005) [18] study consisting of 40 GAPs and 52 controls reported that MMP-1 promoter -1607 region polymorphism might be a risk factor for GAP in Chinese. Our results were in parallel with Itagaki et al.'s (2004) study which did not show a relationship between -1607 1G / 2G polymorphism and AgP. Interestingly, a more careful inspection of these studies showed that while the 1G allele frequency in the Turkish population (37%) was similar to those of Japanese (36.6%), the Chinese had relatively higher 1G allele carriage (51%) compared to these two populations. This result suggests that inter-ethnic differences might be the underlying reason of the conflicting results seen in these populations [21].

Periodontal diseases such as AgP are multifactorial diseases, that is, both genetic and environmental factors play a role in the pathogenesis of the disease [1, 4]. The fact that the disease is genetically complex and that more than one gene plays a role in its pathogenesis makes it difficult to replicate the genetic association studies.

Additionally, the conflicting results in different studies may partly be explained by the fact that MMP-1 gene transcription is regulated by the combined effect of a large number of local factors, such as cytokines, hormones, growth factors, host defense system elements, and bacterial products [22]. Some MMPs, such as MMP-1, have common extracellular matrix substrates [23]. In such cases, MMPs can compensate for each other's function.

Smoking is one of the most important environmental risk factors for AgP [24]. Smoking has been associated with more severe periodontal breakdown, as shown by increased bone loss, pocket depth, clinical attachment, gingival recession, and a higher rate of tooth loss. Duration of smoking (year) and a dose of smoking (more than 10 / day) also increase these risks [24]. A gene-environmental interaction has been shown between IL-1 polymorphisms and smoking. Korman et al. (1997) [25] demonstrated that IL-1α and IL-1β polymorphisms were associated with severe adult periodontitis in non-smoking individuals. In contrast, no such relationship between IL-1 gene polymorphism and CP in smokers was reported. This result revealed that smoking is an important risk factor that needs to be considered in genetic association studies. Therefore, we tried not to include non-smokers in our study when forming our study groups. However, since the AgP is a rare disease, our sample size was small; therefore, we did not exclude 11 smokers from AgP group. Considering the effects of smoking on periodontitis, we also performed analyzes in the non-smoking group only. Similar to the overall analysis, statistically no significant difference was found in the distribution of patients and control groups among nonsmokers (p = 0.57) and non-smoking LAP (p = 0.74) individuals. Moreover, in the overall analysis, in addition to smoking, other environmental risk factors such as age, gender were evaluated as corrective factors in the logistic regression.
analysis, and no changes were detected in the results. Another limitation of our study is that the control group was younger than the AgP (24.87±4.50) group (28.64±5.84). However, the disease has its onset at puberty [1], considering that our control group age ranged between 18-45 years, the individuals in the control group should have had this disease by that time if they were a risk group for AgP. Additionally, in order to address this issue, we adjusted our analysis for age.

In conclusion, we evaluated the relationship between MMP-1 -1607 1G/2G polymorphism and AgP in Turkish population. Our research revealed that there is no difference in the allelic or genotypic frequencies of MMP-1 gene -1607 polymorphism between AgP and healthy control group. Although MMP-1 is a good candidate for AgP, our study did not provide any evidence of association of MMP-1 with AgP. However, we cannot exclude the possibility that other polymorphisms in the MMP-1 gene may be associated with the risk of AgP. Therefore further studies are required to examine other polymorphisms in the MMP-1 gene.

Scientific Responsibility Statement
The authors declare that they are responsible for the article’s scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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References
1. Albandar JM. Global risk factors and risk indicators for periodontal diseases. Periodontal. 2000. 2000;2:299-302. DOI: 10.1016/S2000-2910.9.x. 2. Griffiths G, Wilton J, Curtis M, Maiden M, Gillett L, Wilson D, et al. Detection of high-risk groups and individuals for periodontal diseases: clinical assessment of the periodontium. J Clin Periodontal. 1988;15:403-9.
3. Albandar JM, Brown L, Tjäderhane L, Ho. Clinical features of early-onset periodontitis. J Am Dent Assoc. 1997;128:1933-9.
4. Vieira AR, Albandar JM. Role of genetic factors in the pathogenesis of aggressive periodontitis. Periodontal. 2000. 2002;29:177-206. DOI: 10.1038/j.1600-0757.2002.290109.x.
5. Novak M, Novak K. Early-onset periodontitis. J Clin Periodontol. 1997;24:71-72.
6. Brahman-R K. Syndromes Associated With Aggressive Periodontitis. Clin Dent. 2013;974-3979:7.
7. Bryand JM. Gelob LM. Modulation of matrix metalloproteinase activities in periodontitis as a treatment strategy. Periodontal. 2000;299-302. DOI: 10.1016/S2000-2910.9.x.
8. Sorsa T, Tjäderhane J, Konttinen YT, Lauho A, Salo T, Lee HM, et al. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Ann Med. 2000;383:518-21.
9. Sadek J, Perler C. Matrix metalloproteinase in periodontal tissue remodelling. Matrix. 1992; (Suppl. 1):352-62.
10. Knutper V, Will H, López-Otin C, Smith B, Atkinson SJ. Stanton H, et al. Cellular mechanisms for human procollagenase-3 (MMP-13) activation Evidence that M71-MMP (MMP-14) and gelatinase a (MMP-2) are able to generate active enzyme. J Biol Chem. 1996;271:17124-31.
11. Dong W, Xiang J, Li C, Yao Z, Huang Z. Increased expression of extracellular matrix metalloproteinase inducer is associated with matrix metalloproteinase-1 and -2 in gingival tissues from patients with periodontitis. J Periodontal. 2009;4:125-32.
12. Ingman T, Tervahartiala T, Ting Y, Scheske H, Haaeian A, Kinane DF, et al. Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. J Clin Periodontal. 1996;23:17124-31.
13. Afshar B, Shaddox LM, Toddler J, Magnusson I, Aukhil I, Walker C. Matrix metalloproteinase levels in children with aggressive periodontitis. J Periodontal. 2008;79:819-26.