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MMP-13 promoter polymorphisms in patients with chronic periodontitis: effects on GCF MMP-13 levels and outcome of periodontal therapy

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Abstract

Aim: The aims of this study were to investigate (a) the matrix metalloproteinase-13 (MMP-13) promoter polymorphisms in severe, generalized chronic periodontitis (CP), (b) the relationship of periodontal therapy outcome with these genotypes and (c) gingival crevicular fluid (GCF) MMP-13 level–MMP-13 genotype correlation.

Materials and Methods: Genomic DNA was obtained from peripheral blood of 102 patients with severe, generalized CP, and 98 periodontally healthy subjects. MMP-13 −77A/G and 11A/12A polymorphisms were determined by the polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing methods, respectively. Fifty-eight CP patients received non-surgical periodontal therapy and were followed for 6 months. Clinical periodontal parameters and GCF samples were collected at baseline and at 6 months. GCF MMP-13 levels were analysed by an enzyme-linked immunosorbent assay.

Results: The distribution of MMP-13 −77AG genotypes and allele frequencies did not differ significantly between study groups ($p > 0.05$). Study subjects, except 3, had the 11A/11A genotype. MMP-13 −77G allele carriers had similar GCF MMP-13 levels and clinical periodontal parameters compared with AA genotypes after non-surgical periodontal therapy ($p < 0.05$).

Conclusions: These data suggest that the −77A/G and 11A/12A polymorphisms of MMP-13 gene are not associated with susceptibility to severe, generalized CP in a Turkish population. It seems that −77G allele carriage may not influence the outcome of periodontal therapy.

Key words: GCF; gene polymorphism; MMP-13; periodontal therapy; periodontitis

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Conflict of interest and sources of funding statement

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Extracellular matrix degradation in periodontitis is mediated by complex cascades of events involving both host- and microbial-derived proteinases (Uitto et al. 1989, Sorsa et al. 1992, Reynolds 1996). Matrix metalloproteinases (MMPs) are one of the most important groups of these proteinases (Birkedal-Hansen 1993, Sorsa et al. 2006). Among the MMPs, MMP-13 (collagenase-3) expression was first discovered in breast cancer (Freije et al. 1994). MMP-13 is expressed by osteoblastic cells adjacent to the osteoclasts at sites of active bone resorption, gingival sulcular epithelium, gingival fibroblasts, macrophage-like cells and plasma cells (Fuller & Chambers 1995, Uitto et al. 1998, Tervahartiala et al. 2000, Wahlgren et al. 2001, Kiili et al. 2002). MMP-13 is considered

as an enzyme responsible for bone resorption and cartilage destruction in rheumatoid arthritis and osteoarthritis (Martel-Pelletier & Pelletier 1996). Elevated gingival crevicular fluid (GCF) MMP-13 levels in patients with chronic periodontitits (CP) might suggest that MMP-13 could also contribute to alveolar bone destruction in periodontitis (Golub et al. 1997, 1998, Tervahartiala et al. 2000, Ilgenli et al. 2006, Hernández et al. 2007).

The MMP-13 gene is defined on chromosome 11q22.3 (Ye 2000). Genetic polymorphisms located in the promoter region of the MMP genes could lead to changes in protein expression, structure and function and could be associated with predisposition to various diseases (Takashiba & Naruishi 2006). Previously, several studies investigating MMP gene polymorphisms in CP patients of distinct populations yielded conflicting findings (Hollá et al. 2004, 2005, Itagaki et al. 2004, de Souza et al. 2005, Astolfi et al. 2006, Cao et al. 2006, Keles et al. 2006, Gürkan et al. 2008, Pirhan et al. 2008). On the other hand, to date, there are no data on MMP-13 gene polymorphisms in the periodontitis patients. However, MMP-13 gene polymorphisms have been reported to be associated with several systemic diseases including cardiovascular diseases and rheumatoid arthritis that are related to periodontitis (Yoon et al. 2002, Ye et al. 2007). Therefore, the aim of the present study was threefold: first, to investigate MMP-13 gene polymorphisms (-77A/G and 11A/12A) in Turkish subjects with severe, generalized CP; second, to assess the association between polymorphisms of the MMP-13 gene (A/G and 11A/12A) and the GCF MMP-13 level as well as periodontal status, and finally to determine the effects of MMP-13 genotypes on the outcomes of non-surgical periodontal therapy.

Materials and Methods

Study population

A total of 200 unrelated Turkish subjects from the Aegean region including 102 severe, generalized CP patients and 98 periodontally healthy subjects were recruited from the patient pool of the Department of Periodontology at the Ege University over a period of 4 years between 2002 and 2006. The control group included periodontally healthy volunteers from the staff and other subjects referring to the School of Dentistry. All subjects had to be in good general health and had a low-to-moderate socioeconomic level. Subjects who had never smoked (non-smokers) or who smoked <10 cigarettes/day for <5 years (light smokers) were included in the present study. Exclusion criteria were: oral diseases other than caries and periodontal disease, ongoing orthodontic therapy, a history of systemic or local disease with an influence on the immune system (cancer, cardiovascular and respiratory diseases), diabetes mellitus, hepatitis or HIV infection, immunosuppressive chemotherapy or current pregnancy or lactation. After completion of personal medical and dental questionnaires, written informed consent was obtained. The study protocol was approved by the Ethics Committee of the Medical Faculty of the Ege University according to the declarations of Helsinki.

The diagnosis of subjects was established on the basis of clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions (Armitage 1999).

Severe, generalized CP group

The CP group included 39 females and 63 males ranging in age from 35 to 65, with a mean age 47.9 ± 7.6 years. CP patients had $\geqslant 15$ teeth exhibiting $>30\%$ of measured sites with $\geqslant 5$ mm clinical attachment loss (CAL). They also had bleeding on probing (BOP) at $>50\%$ of the proximal sites and exhibited alveolar bone loss of $\geqslant 50\%$ in at least two different quadrants. 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.

Healthy control group

Fifty females and 48 males between the ages of 35 and 70 (mean age 43.7 ± 7 years) were included in the healthy group. They had no history of periodontal disease and had at least 20 teeth. Subjects had no sites with >2 mm CAL and no sites with >3 mm probing depth (PD). Furthermore, they had a BOP score $<20\%$ at examination and no alveolar bone loss present in radiography (i.e. distance between the cemento-enamel junction and bone crest $\leqslant 3$ mm at $>95\%$ of the proximal tooth sites).

Determination of periodontal status

All subjects were evaluated clinically and radiographically at the first visit, and the following clinical parameters were assessed by a single investigator (D. P.): PD, CAL, supragingival plaque accumulation (Ainamo & Bay 1975) and BOP (Mühlemann & Son 1971). The clinical periodontal parameters were assessed at six sites around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations) for the whole mouth excluding third molars. The cemento-enamel junction was accepted as a reference point in measurements of CAL. PD and CAL measurements were performed using a manual Williams probe. BOP (deemed positive if it occurred within 15 s after periodontal probing) and supragingival plaque accumulation were recorded dichotomously.

DNA separation and MMP-13 genotyping

Two millilitres of peripheral venous blood sample was collected into EDTA-anticoagulant tubes by the standard venipuncture method. Genomic DNA was prepared from whole blood using the genomic DNA isolation kit (NucleoSpin Blood L, Macherey-Nagel, GmbH & Co. KG, Düren, Germany) according to the manufacturer's protocol. The DNA concentration was determined by spectrophotometry at 280 nm and diluted as 50 ng/μl in 200 μl volume. To quantitate DNA, electrophoresis was performed with ethidium bromide-stained 1% agarose gel, using a volume of 2 μl (100 ng). Agarose gel was visualized with respect to standard control DNA markers with known base pairs (bp) on a digital gel imaging system. All genotyping processes were performed by verified and quantified genomic DNA.

The MMP-13 -77A/G polymorphisms were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) methods as described previously (Yoon et al. 2002). The 11A/12A polymorphism (insertion/deletion at nt -291) was determined by the direct DNA sequencing method. All genotyping was performed

blindly with respect to clinical diagnosis by a single investigator (A. B.).

Collection of GCF samples and enzyme-linked immunosorbent assay (ELISA) for MMP-13

In the CP group, GCF samples were collected from two approximal sites of the non-adjacent teeth with $\geqslant 7$ mm PD. In the healthy group, GCF samples were collected from two approximal sites of the non-adjacent teeth with $\leqslant 2$ mm PD. Before GCF sampling, the supragingival plaque was removed from the interproximal surfaces with a sterile curette; these surfaces were dried gently by an air syringe and were isolated by cotton rolls. GCF was sampled with a filter paper (Periopaper, ProFlow Inc., Amityville, NY, USA). Paper strips were carefully inserted into the crevice until 1 mm and left there for 30 s (Lamster et al. 1985). Care was taken to avoid mechanical injury. Strips contaminated with blood were discarded (Cimasoni 1983). The absorbed GCF volume of each strip was determined by an electronic device (Periotron 8000, ProFlow Inc., Amityville) and the strips were placed into a sterile polypropylene tube and kept at -40°C until analysis. The readings from the electronic device were converted to an actual volume (μl) by reference to the standard curve.

The absorbed fluid was eluted from each strip into 75 μl 50 mM Tris-HCl, pH 7.8, containing 0.2 M NaCl and 1 mM $CaCl_2$ for 2 h at 22°C on the shaker. The eluted GCF samples were frozen until the ELISA.

GCF MMP-13 levels were determined using a commercial ELISA kit as recommended by the manufacturers (GE Healthcare, Amersham, Little Chalfont, UK). The assay was based on a two-site ELISA ''sandwich'' format, where standards and samples were incubated in microtitre wells pre-coated with anti-MMP-13 antibody, respectively. Any MMP-13 present would be bound to the wells: other components of the sample were removed by washing and aspiration. The second antibody to MMP-13 was horseradish peroxidase conjugated. The amount of peroxidase bound to each well was determined by the addition of a tetramethylbenzidine (TMB) substrate. The reaction was stopped by adding an acid solution, and the resultant colour was read at 450 nm in a microplate spectrophotometer (Labsystems Multiskan RC, VWR™ International, Espoo, Finland). The concentrations of MMP-13 in samples were determined by interpolation from standard curves. All determinations were carried out as duplicates. The incubation times between each steps were performed at room temperature on the shaker for 1–2 h according to the manufacturer's instructions, except for the TMB substrate; the incubation time was performed without shaking in the dark for 30 min.

Periodontal treatment

After collection of clinical parameters, and venous blood and GCF samples, CP patients received full-mouth non-surgical periodontal therapy. Periodontal therapy included motivation, instructions in oral hygiene and full-mouth scaling and root planing under local anaesthesia. All patients were treated by a single periodontist (D. P.). The therapy was administrated blind as to genotype status and no antibiotics were prescribed following therapy. After non-surgical therapy, follow-up appointments were scheduled at months 1, 3 and 6 for supportive periodontal therapy. Clinical periodontal measurements were repeated at 1 and 6 months. GCF sampling was also repeated at 6 months.

Statistical analysis

Chi-square (χ^2) analysis was used to test for deviation of genotype frequencies from Hardy–Weinberg equilibrium. The distributions of MMP-13 -77A/G and 11A/12A genotypes and allele frequencies in CP and healthy control groups were also analysed by Fisher's exact test or the χ^2 test. Allele frequencies were calculated from the observed numbers of genotypes. Differences in baseline clinical parameters between subjects with rare allele carriers and non-carriers in the CP group were compared by the independent *t*-test. The relationship between genotype and disease status was analysed by multiple logistic regression analysis while adjusting for confounders such as age, gender, smoking status and score of BOP. Sites were also subgrouped according to baseline PD and CAL category: moderate (4–6 mm), and deep ($\geqslant 7$ mm). Intragroup comparisons were made between baseline and follow-up examinations by repeated measures ANOVA and post hoc Bonferroni test. Inter-group comparisons were made between rare allele carriers and non-carriers at baseline and after treatment by ANCOVA. Differences in GCF MMP-13 levels at baseline and 6 months between subjects with rare allele carriers and non-carriers in the CP group were compared by repeated measures ANOVA. The *p*-values <0.05 were considered statistically significant. All data analysis was performed using a statistical package (SPSS 14.0, SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the demographic characteristics of the study groups. Minimal sample size was determined to detect a 15% difference in the allele frequencies of CP and healthy groups with 75% power and $p = 0.05$ significance level. The power calculation analysis revealed that the minimum required sample size was calculated as 98 patients with CP and 94 periodontally healthy subjects for the genotypic test and as 77 patients and 74 healthy controls for the allele frequency test. The frequencies of MMP-13 -77AG genotypes in the CP and healthy groups were found to be in agreement with Hardy–Weinberg equilibrium ($p > 0.05$, $\chi^2 < 3.84$).

Table 1. Demographic characteristics of study subjects

	CP patients	Healthy subjects
Number of subjects	102	98
Age (years)		
Mean ± SD	47.9 ± 7.6	43.7 ± 7
Range	35–65	35–70
Gender		
Male, *N* (%)	63 (61.8)	48 (49)
Female, *N* (%)	39 (38.2)	50 (51)
Smoking habit		
Non-smoker, *N* (%)	78 (76.5)	80 (81.6)
Light smokers, *N* (%)	24 (23.5)	18 (18.4)

CP, chronic periodontitis; SD, standard deviation.

Distribution of MMP-13 genotypes, allele frequency and carriage of rare allele

Three out of 102 CP patients and three out of 98 control subjects could not be genotyped for both −77A/G and 11A/12A.

The MMP-13 −77AG and 11A/12A genotype distributions and allele frequencies of the study groups are given in Figs 1 and 2. The distribution of the MMP13 −77AA, AG and GG genotypes was similar in the CP and healthy groups ($p = 0.89$) (Fig. 1). The frequency of the −77G allele carriage of the MMP-13 gene was not different between the study groups ($p = 0.68$) (Fig. 1). There was also no significant difference in −77G and A allele frequencies between the study groups ($p = 0.64$) (Fig. 2).

The mutant genotype 12A/12A at position −291 was not present in CP patients and healthy subjects. A total of 2% of the CP patients and 1.1% of the healthy subjects were heterozygous (11A/12A). The distribution of the MMP-13 11A/12A genotypes was similar in the CP and healthy groups ($p = 1.00$) (Fig. 1). The frequency of the rare allele, 12A, was 1% in CP patients and 0.5% in healthy subjects (Fig. 2), with no significant difference between study groups ($p > 0.05$). No further statistical analysis could be performed in the 11A/12A polymorphism due to the low prevalence of rare allele 12A.

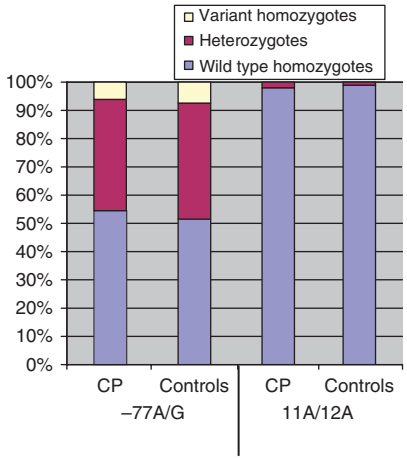

Fig. 1. Distribution of single-nucleotide polymorphism genotypes in chronic periodontitis (CP) patients ($N = 99$) and controls ($N = 95$). Variant homozygotes: −77GG and 12A/12A Heterozygotes: −77A/G and 11A/12A, Wild-type homozygotes: −77AA and 11A/11A.

The subgroup analysis did not reveal any significant influence of gender regarding the distributions of the genotype or the allele frequency of these investigated SNPs (data not shown).

Multiple logistic regression analysis

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, gender, smoking status (light smokers) and score of BOP. The −77G allele carrying the genotype of the MMP-13 gene was not found to be associated with severe generalized CP (adjusted OR: 0.11, $p = 0.11$) (Table 2).

Clinical Parameters in MMP-13 Genotype Subjects

In order to investigate whether carriage of the rare allele is associated with baseline clinical parameters, differences in clinical parameters between the MMP-13 rare allele carrier and non-carrier subjects in the CP group were compared by the independent *t*-test. All clinical parameters of the rare allele carrier (−77GG and AG) were similar to those of non-carrier (−77AA) regarding the MMP-13 gene polymorphism ($p > 0.05$) (Table 3).

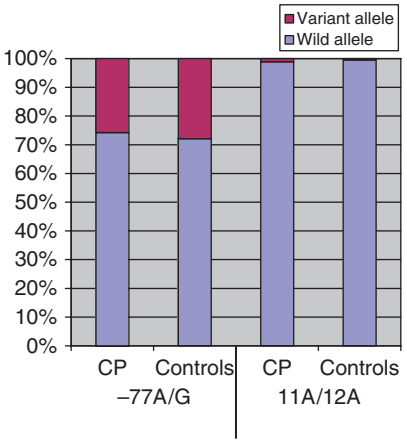

Fig. 2. Frequency of wild-type alleles (−77A and 11A) and variant alleles (−77G and 12A) in severe chronic periodontitis (CP) patients ($N = 99$) and controls ($N = 95$).

Clinical outcomes of periodontal treatment and GCF MMP-13 levels in MMP-13 genotype subjects

MMP-13 −77AG genotype distributions and allele frequencies of the CP patients who received non-surgical periodontal therapy ($n = 58$) were similar to the larger group ($n = 102$) (data not shown). We analysed the effects of non-surgical periodontal therapy on the clinical parameters and GCF MMP-13 levels between a rare allele-carrier and a non-carrier. The mean PD, CAL scores, percentage of sites with BOP and plaque, percentage of sites with baseline PD 4–6 mm and ⩾7 mm and baseline CAL 4–6 mm and ⩾7 mm significantly decreased after non-surgical periodontal therapy (at 1 and 6 months) in both carrier and non-carrier of the MMP-13 −77G allele ($p < 0.001$) (Table 4). There were no significant differences in all clinical parameters between an MMP-13 −77G allele carrier and a non-carrier at baseline and after treatment (Table 4).

Figure 3 shows the relation of MMP-13 genotype status to GCF MMP-13 levels as well as the effect of non-surgical periodontal therapy on these levels. GCF MMP-13 levels of −77G allele carrier (AG and GG) subjects were similar to those of −77G allele non-carrier (AA) subjects at baseline and after treatment ($p = 0.13$). There

Table 2. Multiple logistic regression analysis for the association between MMP-13 −77A/G genotypes and susceptibility to chronic periodontitis adjusting for confounding factors

MMP-13 −77A/G	Adjusted odds ratio	95% confidence interval	*p*
Age (years)			
40–44	447.06	$3.42–5.8 \times 10^4$	**0.01**
45–49	108.71	$1.43–8.3 \times 10^3$	**0.03**
⩾50	187.15	$2.41–1.5 \times 10^4$	**0.01**
Gender	10.36	$0.5–2.1 \times 10^2$	0.13
Smoking status	0.26	0.01–5.76	0.39
Bleeding on probing	1.41	1.15–1.72	**0.001**
G allele carriage (AG and GG)	0.11	0.01–1.59	0.11

p-values in bold face indicate statistical significance ($p < 0.05$).
MMP, matrix metalloproteinase.

Table 3. Baseline clinical parameters (mean ± SD) of chronic periodontitis (CP) patients distributed by rare allele carriers (AG and GG) and non-carriers (AA)

MMP-13 −77A/G	CP patients		
	AA ($n = 54$)	AG and GG ($n = 45$)	p
Mean PD (mm)	3.94 ± 0.87	3.92 ± 0.85	0.923
% sites with PD 4–6 mm	35.50 ± 14.14	36.30 ± 13.49	0.753
% sites with PD ⩾7 mm	8.74 ± 10.72	9.66 ± 10.26	0.636
Mean CAL (mm)	5.08 ± 1.37	5.17 ± 1.35	0.737
% sites with CAL 4–6 mm	46.86 ± 16.55	46.87 ± 15.94	0.812
% sites with CAL ⩾7 mm	24.68 ± 21.28	24.65 ± 22.55	0.988
Bleeding on probing (%)	69.42 ± 25.21	75.04 ± 20.19	0.216
Plaque (%)	78.80 ± 21.38	80.24 ± 21.96	0.720

CAL, clinical attachment loss; MMP-13, matrix metalloproteinase-13; PD, probing depth; SD, standard deviation.

Table 4. Clinical parameters (mean ± SD) in chronic periodontitis (CP) patients (at baseline, 1 and 6 months) distributed by MMP-13 −77 rare allele carriers (AG and GG) and non-carriers (AA)

MMP-13 −77A/G	CP patients ($n = 58$)		
	AA ($n = 34$)	AG and GG ($n = 24$)	p^*
Mean PD (mm)			
Baseline	3.76 ± 0.64	3.71 ± 0.60	0.63
1 month	2.93 ± 0.51	2.73 ± 0.51	
6 months	2.68 ± 0.42	2.75 ± 0.45	
% sites with PD 4–6 mm			
Baseline	34.58 ± 13.39	34.53 ± 13.65	0.42
1 month	21.22 ± 12.36	23.36 ± 13.65	
6 months	14.89 ± 10.56	19.70 ± 13.41	
% sites with PD ⩾7 mm			
Baseline	7.81 ± 6.62	8.17 ± 7.09	0.62
1 month	2.65 ± 2.86	3.19 ± 3.99	
6 months	2.19 ± 4.16	2.51 ± 3.57	
Mean CAL (mm)			
Baseline	5.00 ± 1.00	5.13 ± 094	0.75
1 month	4.16 ± 0.94	4.12 ± 0.99	
6 months	4.13 ± 1.03	4.29 ± 0.96	
% sites with CAL 4–6 mm			
Baseline Eline	49.39 ± 17.11	48.27 ± 15.10	0.82
1 month	42.42 ± 16.93	40.81 ± 12.53	
6 months	42.07 ± 18.01	42.72 ± 17.63	
% sites with CAL ⩾7 mm			
Baseline	23.86 ± 18.40	22.50 ± 19.75	0.96
1 month	13.89 ± 12.20	15.01 ± 14.43	
6 months	2.19 ± 4.16	2.51 ± 3.57	
Bleeding on probing %			
Baseline	64.64 ± 26.04	71.92 ± 21.26	0.89
1 month	18.92 ± 20.83	14.00 ± 7.91	
6 months	20.70 ± 25.12	24.73 ± 18.22	
Plaque %			
Baseline	77.60 ± 23.19	79.21 ± 23.90	0.82
1 month	23.54 ± 22.38	23.43 ± 15.13	
6 months	25.77 ± 21.22	25.39 ± 22.59	

*ANCOVA.

CAL, clinical attachment loss; MMP-13, matrix metalloproteinase-13; PD, probing depth; SD, standard deviation.

were marginally significant difference between GCF MMP-13 levels at baseline and after non-surgical periodontal therapy in both carriers and non-carriers ($p = 0.07$).

Discussion

The present study has demonstrated that MMP-13 gene polymorphisms are not associated with the susceptibility to severe, generalized CP in a Turkish population. Two CP patients and one healthy subject had an 11A/12A genotype whereas none of the study subjects had mutant genotype 12A/12A. Moreover, the clinical outcomes of non-surgical periodontal therapy were not different in −77G allele carriers (AG and GG) than those of non-carriers. In addition, the GCF MMP-13 levels of MMP-13 −77G allele carriers were similar to those of non-carriers after non-surgical therapy.

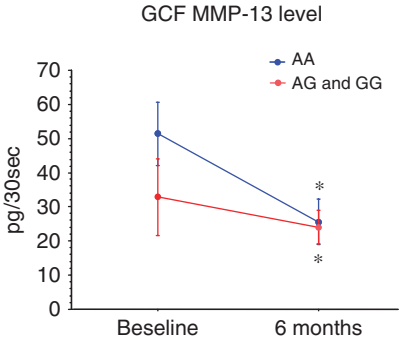

Fig. 3. Changes in gingival crevicular fluid (GCF) matrix metalloproteinase-13 (MMP-13) levels (mean ± standard error) in the MMP-13 −77 rare allele carriers (AG and GG) ($n = 24$) and non-carriers (AA) ($n = 34$) before and after non-surgical periodontal therapy in chronic periodontitis patients. *Marginal significant differences from baseline in both carriers and non-carriers ($p = 0.07$).

Genetic variations in the MMPs promoter region can influence transcription activity, which is one of the key steps in the regulation of MMPs activity, and thus increase the levels of MMP expression (Rutter et al. 1998). On the other hand, other control mechanisms of MMP activity may also be important in periodontal tissues. Nevertheless, in an in vitro study, it has been previously shown that MMP activation, but not the gene expression of these molecules, resulted in collagen degradation (Zhou & Windsor 2006). In the present study, the lack of association between MMP-13 gene polymorphisms and severe, generalized CP might also suggest that an increase in the MMP-13 transcription may not necessarily lead to an increase in the destructive effect of this enzyme on the periodontal tissues.

Promoter polymorphisms of the MMP genes could be associated with predisposition to various diseases (Ye 2000). In general, cells carrying genetic variations in MMPs could over-produce

MMPs leading to enhanced prevalence of systemic collagenolytic diseases (Sorsa et al. 2006). However, periodontitis patients are usually, as in the present study, systemically rather healthy. To date, the MMP-13 −77A/G gene polymorphism has been reported to be associated with coronary artery disease or aorta atherosclerosis (Yoon et al. 2002). Moreover, it has been stated that MMP-13 genotypes may play a role in determining the functional status of rheumatoid arthritis (Ye et al. 2007). In this respect, it has been suggested that cardiovascular diseases and rheumatoid arthritis could be linked to periodontitis. The relationship between coronary heart disease and periodontal disease may be dependent on the genetic factors that both diseases have in common (Syrjänen 1990). However, there were no studies evaluating the association between the MMP-13 −77A/G and the −291 11A/12A polymorphism and CP.

CP is a multifactorial disease whose manifestation and progression is influenced by a variety of factors such as genetic factors, smoking status, age and gender (Albandar 2002, Heitz-Mayfield 2005). In the present study, logistic regression analysis was performed to evaluate the genotype effects on an increased severity of CP while adjusting these confounding factors as was suggested by others (Loos et al. 2005, Shapira et al. 2005). In this model, patient age and BOP were found to be significant confounders to the increased susceptibility to CP. On the other hand, the −77G allele carrying genotypes of the MMP-13 gene was not associated with susceptibility to severe, generalized CP. Smoking is known to be a well-established environmental risk factor for periodontitis that influences host inflammatory immune response by suppressing the activity of several mediators (Kinane & Chestnutt 2000, Palmer et al. 2005). In the present study, lack of an association between smoking status and susceptibility to severe, generalized CP could be due to having a group of subjects who had never smoked or were light smokers.

In the present study, the mean change in clinical parameters at 6 months from baseline of CP patients with the −77G allele carrying (AG and GG) genotypes of the MMP-13 gene was similar to those of non-carriers (AA). In addition, MMP-13 −77G allele carriers and non-carriers had similar GCF MMP-13 levels at baseline and after non-surgical therapy. Therefore, the present data do not provide evidence for the use of this polymorphism as a severity factor for CP. Based on the present findings, we can suggest that MMP-13 −77AG and GG genotypes seemingly do not play a role in the disease prognosis and the periodontal therapy outcome. Therefore, other candidate genes might be associated with the irreversible tissue damage in destructive periodontal disease.

In conclusion, MMP-13 −77A/G gene polymorphisms do not appear to have a significant influence on the susceptibility to severe, generalized CP and are also not associated with the clinical severity of CP as well as outcome of periodontal therapy and GCF MMP-13 levels. On the other hand, due to the low frequency of the 11A/12A polymorphism (2%) in severe, generalized CP group, it is impossible to interpret its relationship with the severity of periodontitis. To the best of our knowledge, this is the first study investigating MMP-13 −77A/G gene polymorphisms on the clinical status and GCF MMP-13 levels as well as the effect of these polymorphisms on non-surgical periodontal treatment outcomes in severe, generalized CP patients. The present study has some limitations. Differences in genetic variants between cases and controls may have been affected by population heterogeneity due to selection of subjects from a metropolitan region. Furthermore, only two polymorphisms of the MMP-13 gene were evaluated in the present study. The other variants of this gene and the effect of the haplotype combinations have not been evaluated. We suggest that more extensive studies in other ethnical populations should be undertaken in order to analyse the putative relevance of the MMP-13 −77A/G and 11A/12A gene polymorphisms in the pathogenesis of periodontitis. Thus, the importance of the MMP-13 gene polymorphism could be confirmed in terms of preventive, diagnostic and therapeutic strategies against the development of periodontitis.

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Clinical Relevance

Scientific rationale for the study: Genetic polymorphisms in the promoter region of the MMP genes might provide important information on its function in periodontal diseases. Therefore, whether MMP-13 promoter polymorphisms constitute a risk for severe, generalized CP and therapeutic responses in a Turkish population has been investigated.
Principal findings: There were no significant differences among the severe, generalized CP and healthy groups in −77AG genotypes and allele frequencies. The 11A/12A genotype frequency was 2% in CP patients and 1% in healthy subjects. No significant effects of the −77G allele on clinical periodontal parameters and GCF MMP-13 levels were observed after non-surgical therapy.
Practical implications: The present data failed to demonstrate a clinically relevant relationship between the MMP-13 −77A/G polymorphism and periodontal status.