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Effect of MMP-1 promoter polymorphisms on GCF MMP-1 levels and outcome of periodontal therapy in patients with severe chronic periodontitis

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Abstract

Aims: The aims of this study were to investigate (1) the matrix metalloproteinase-1 (MMP-1) promoter polymorphisms in severe chronic periodontitis (CP), (2) the relationship of periodontal therapy outcome with these genotypes, and (3) the gingival crevicular fluid (GCF) MMP-1 levels–MMP-1 genotype correlation.

Material and Methods: Genomic DNA was obtained from the peripheral blood of 102 patients with severe CP and 98 periodontally healthy subjects. MMP-1 −519A/G and −1607 1G/2G polymorphisms were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. 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-1 levels were analysed by enzyme-linked immunosorbent assay (ELISA).

Results: The distribution of MMP-1 genotypes did not significantly differ between the study groups. On the other hand, the −1607 2G allele frequency of severe CP patients was higher than that of healthy subjects. MMP-1 −519G allele carriers had higher GCF MMP-1 levels and percentage of sites with 4–6 mm clinical attachment level (CAL) compared with AA genotypes after non-surgical periodontal therapy ($p < 0.05$).

Conclusions: These data suggest that the −1607 2G polymorphic allele of the MMP-1 gene could be associated with susceptibility to severe CP in the Turkish population. It seems that −519AG and GG genotypes could play a role in the outcome of periodontal therapy.

Key words: GCF; gene polymorphism; MMP-1; periodontal therapy; periodontitis

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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Chronic periodontitis (CP), the most frequent form of periodontitis, is an infection-induced inflammatory disease that results from the interaction of periodontopathogenic bacteria and host immune response (Page et al. 1997, Reynolds & Meikle 1997, Tanner et al. 2007, Silva et al. 2008). There is a complex network of cytokines, prostaglandins, reactive oxygen species and proteolytic enzymes involved in the inflammatory and immune responses in the inflamed periodontal tissues during the progression of periodontitis (Reynolds & Meikle 1997, Okada & Murakami 1998, Hernández et al. 2007).

Matrix metalloproteinases (MMPs) are a family of host-derived genetically distinct but structurally related proteolytic enzymes involved in the physiological

and pathological degradation of extracellular matrix and basement membrane proteins (Birkedal-Hansen et al. 1993, Reynolds & Meikle 1997, Kinane 2000, Sorsa et al. 2004). Among the MMPs, MMP-1 is the most substrate specific, regarding its unique and selective capacity to degrade native interstitial collagens (Nagase et al. 2006). MMP-1 is expressed by fibroblasts, endothelial cells, macrophages, hepatocytes, chondrocytes, osteoblasts, tumour cells and migrating epidermal keratinocytes, but not neutrophils (Birkedal-Hansen et al. 1993, Pilcher et al. 1998). MMP-1 or interstitial collagenase is among the key proteolytic enzymes capable of degrading fibrillar collagens, especially types I and III, which are the predominant types of interstitial collagens in gingiva and are resistant to most proteinases (Matrisian 1990, Visse & Nagase 2003). Thus, MMP-1 exerts potential to initiate collagenolysis during periodontal inflammation (Birkedal-Hansen et al. 1993, Kinane 2000, Sorsa et al. 2004). It has been shown that MMP-1 levels significantly increased in the gingival crevicular fluid (GCF) and gingival tissue samples of patients with periodontitis compared with healthy controls (Meikle et al. 1994, Aiba et al. 1996, Ingman et al. 1996, Tüter et al. 2002). On the other hand, Haerian et al. (1995) have suggested that GCF MMP-1 levels do not correlate well with periodontal disease status.

The MMP-1 gene is defined on chromosome 11q22.3. The gene polymorphisms of this chromosome region have been shown to influence MMP gene expression and to be associated with susceptibility to several diseases (Ye 2000). Several polymorphisms in the promoters of a number of MMP genes, which are thought to affect the respective MMP production in an allele-specific manner, have been well characterized. A functional single nucleotide polymorphism (SNP) resulting from an insertion/deletion of a guanosine at nucleotide position -1607 has been identified in the promoter of the MMP-1 gene (Rutter et al. 1998). Two alleles have been detected, one allele having a single gaunosine (1G) and the other having two guanosines (2G) at the polymorphic site. The insertion of a G nucleotide at the -1607 position in the MMP-1 promoter SNP creates a new 5′-GGA-3′ core recognition sequence of the binding site for members of the ETS (erythroblast transformation specific) family of transcription factors, causing increased transcriptional activity. It has been demonstrated that the 2G allele binds substantially more recombinant Ets-1 transcription factor and has significantly higher transcriptional activities than the 1G allele (Rutter et al. 1998). A newly identified SNP at position -519 in the promoter region of the MMP-1 gene has been reported. This polymorphism consists of a guanine-to-adenine substitution (Jurajda et al. 2002). Two case–control studies have shown that an SNP in the MMP-1 promoter region of -1607 bp could be associated with severe CP in Chinese and Brazilian populations (de Souza et al. 2003, Cao et al. 2006). On the other hand, no association between -1607 1G/2G MMP-1 gene polymorphism and CP could be found in other populations (Holla et al. 2004, Itagaki et al. 2004, Astolfi et al. 2006). It has also been shown that -519A/G polymorphism of MMP-1 gene was not associated with susceptibility of CP in Czech and Brazilian populations (Holla et al. 2004, Astolfi et al. 2006).

Given the importance of MMP-1 in periodontal diseases, the investigation of genetic polymorphisms that affect its transcriptional activity may provide important information on its function in periodontal diseases. Studies investigating MMP-1 -519A/G and -1607 1G/2G gene polymorphisms in distinct populations gave conflicting findings (de Souza et al. 2003, Holla et al. 2004, Itagaki et al. 2004, Astolfi et al. 2006, Cao et al. 2006). To date, there is no study investigating how MMP-1 -519A/G and -1607 1G/2G gene polymorphisms affect GCF MMP-1 levels and what the possible influence is of the MMP-1 -519A/G and -1607 1G/2G gene polymorphisms on the outcomes of non-surgical periodontal therapy. Therefore, the aim of the present study was threefold: first, to investigate MMP-1 gene polymorphisms (-519A/G and -1607 1G/2G) in Turkish subjects with severe CP, second, to assess the association between polymorphisms of the MMP-1 gene (-519 and -1607) and GCF MMP-1 levels as well as periodontal status, and finally, to determine the effects of MMP-1 genotypes on the outcomes of non-surgical periodontal therapy.

Material and Methods

Study population

A total of 200 unrelated Turkish subjects from the Aegean region including 102 severe 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 comprised periodontally healthy volunteers from the staff and other subjects referred 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 or who smoked <10 cigarettes per day for <5 years were included in the present study. Exclusion criteria were as follows: oral diseases other than caries and periodontal disease, ongoing orthodontic therapy, a history of systemic or local disease with 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), as given below.

Severe CP group: The severe CP group included 39 females and 63 males ranging in age from 35 to 65, with a mean age 47.9 ± 7.6 years. Patients had at least 15 teeth. They had at least four sites with a clinical attachment level (CAL) of $\geqslant 5$ mm in at least two separate quadrants. They also had bleeding on probing (BOP) at >50% of the proximal sites. Diagnosis of CP was made if the CAL was commensurate with the amount of local factors of the patients.

Healthy control group: A total of 50 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 >3 mm probing depth (PD) and >2 mm CAL. Furthermore, they had a BOP score $<20\%$ at examination and no alveolar bone loss present in radiography (i.e., the distance between the cemento-enamel junction and bone crest was $\leqslant 3$ 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 the reference point in measurements of CAL. PD and CAL measurements were performed using a manual Williams probe. BOP (deemed positive if occurring within 15 s after periodontal probing) and supragingival plaque accumulation were recorded dichotomously.

Blood samples, DNA separation and MMP-1 genotyping

Two millilitres of a peripheral venous blood sample were collected in EDTA-anticoagulant tubes by the standard venipuncture method. Genomic DNA was prepared from whole blood using a genomic DNA isolation kit (NucleoSpin Blood L, Macherey-Nagel, Germany) according to the manufacturer's protocol. 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 an ethidium bromide-stained 1% agarose gel, by 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 system. All genotyping processes were performed using verified genomic DNA.

The -519A/G and -1607 1G/2G MMP-1 polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods as previously described (Jurajda et al. 2002). All genotyping was performed blindly with respect to clinical diagnosis by a single investigator (A. B.). Whenever the results were not clear, the analysis was repeated. If, after repetition, the result was still uncertain, no result was recorded for that polymorphism.

Collection of GCF samples and enzyme-linked immunosorbent assay (ELISA) for MMP-1

In the CP group, GCF samples were collected from two interproximal sites of non-adjacent teeth with $\geqslant 7$ mm PD. In the healthy group, GCF samples were collected from two approximal sites of 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 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.), and the strips were placed in a sterile polypropylene tube and kept at -40°C until being analysed. 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 a shaker. The eluted GCF samples were frozen until the ELISA.

GCF MMP-1 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-1 antibody, respectively. Any MMP-1 present would be bound to the wells; other components of the sample were removed by washing and aspiration. The second antibody to MMP-1 was horseradish peroxidase conjugated. The amount of peroxidase bound to each well was determined by the addition of tetramethylbenzidine (TMB) substrate. The reaction was stopped by adding an acid solution, and the resultant colour read at 450 nm in a microplate spectrophotometer (Labsystems Multiskan RC, VWR™ International, Espoo, Finland). The concentrations of MMPs in samples were determined by interpolation from standard curves. All determinations were carried out as duplicates. The incubation between each step was performed at room temperature on the shaker for 1–2 h according to the manufacturer's instructions, except for the TMB substrate, for which the incubation was performed in the dark without shaking for 30 min.

Periodontal treatment

Following the collection of clinical parameters, and venous blood and GCF samples, severe 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

Minimal sample size was determined to detect a 15% difference in the allele frequencies of severe CP and healthy groups with an 80% power and a $p = 0.05$ significance level. Chi-square (χ^2) analysis was used to test for deviation of genotype frequencies from the Hardy–Weinberg equilibrium. The distribution of the MMP-1 -519A/G and -1607 1G/2G genotypes and allele frequencies in severe CP and healthy control groups were also analysed by the χ^2-test. Allele frequencies were calculated from the observed numbers of genotypes. Haplotype frequencies of MMP-1 were calculated on severe CP patients and controls by direct counting. To calculate the frequency of MMP-1 haplotype by the direct counting method, all data were transferred from the statistical software to the spreadsheet programme. The χ^2-test was also used to examine differences in haplotype among study groups.

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), deep (⩾7 mm). Intra-group comparisons were made between baseline and follow-up examinations by repeated-measures ANOVA and the 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-1 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

The demographic characteristics of the study groups are presented in Table 1. The power calculation analysis revealed that the minimum required sample size was calculated as 87 patients with severe CP and 84 periodontally healthy subjects for the genotypic test. The frequencies of MMP-1 −519AG genotypes in the CP and healthy groups were found to be in agreement with the Hardy–Weinberg equilibrium ($p>0.05$, $\chi^2<3.84$). The distribution of MMP-1 −1607 1G/2G genotypes in both severe CP and healthy control subjects differed from the Hardy–Weinberg equilibrium ($p<0.05$, $\chi^2>3.84$).

Table 1. Demographic characteristics of the study subjects

	Severe CP	Healthy
Number of subjects	102	98
Age		
Mean ± SD (years)	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-smoking, *N* (%)	78 (76.5)	80 (81.6)

Distribution of MMP-1 genotypes, allele frequency and carriage of rare allele

One out of 102 severe CP patients could not be genotyped for −1607 1G/2G. One out of 98 control subjects could not be genotyped for both −519A/G and −1607 1G/2G.

Table 2 shows the MMP-1 −519AG and −1607 1G/2G genotype distributions and allele frequencies of the study groups. Distribution of the MMP-1 −519AA, AG and GG genotypes was not different between the study groups ($p=0.90$). The frequency of −519G allele carriage of the MMP-1 gene was similar in the CP and healthy groups ($p=0.95$). There was no significant difference in −519G and A allele frequencies between the study groups ($p=0.90$). There was no significant difference in the distribution of the MMP-1 −1607 1G/2G genotypes between the study groups ($p=0.27$). The frequency of −1607 2G allele carriage of the MMP-1 gene was similar in the CP and healthy groups ($p=0.14$). There was a marginal significant differ-

Table 2. Genotype distributions, allele frequencies and rare allele carriage of MMP-1 promoter polymorphisms in severe chronic periodontitis (CP) and healthy groups

−519A/G	Severe CP, *N* (%) *n* = 102	Healthy, *N* (%) *n* = 97	*p* (χ^2)
Genotype			
AA	53 (52)	50 (51.5)	0.90 (0.21)
AG	40 (39.2)	40 (41.3)	
GG	9 (8.8)	7 (7.2)	
G allele carriage	49 (48)	47 (48.5)	0.95 (0.003)
Allele frequency			
A	146 (71.6)	140 (72.2)	0.90 (0.02)
G	58 (28.4)	54 (27.8)	
−1607 1G/2G	*n* = 101	*n* = 97	
Genotype			
1G/1G	51 (50.5)	59 (60.8)	0.27 (2.62)
1G/2G	32 (31.7)	27 (27.8)	
2G/2G	18 (17.8)	11 (11.4)	
2G allele carriage	50 (49.5)	38 (39.2)	0.14 (2.14)
Allele frequency			
1G	134 (66.3)	145 (74.7)	0.06 (3.36)
2G	68 (33.7)	49 (25.3)	

Table 3. Multiple logistic regression analysis for the association between MMP-1 genotypes (−519A/G and −1607 1G/2G) and susceptibility to severe chronic periodontitis (CP), adjusting for confounding factors

	Adjusted odds ratio	95% confidence interval	*p*-value
MMP-1 −519A/G			
Age			
40–44	626.16	3.40–115260.38	**0.02**
45–49	118.32	1.29–10776.39	**0.04**
⩾50	401.01	3.56–45223.61	**0.01**
Gender	9.63	0.46–199.76	0.14
Smoking	0.37	0.02–6.52	0.50
Bleeding on probing	1.39	1.14–1.69	**0.001**
G allele carriage (AG and GG)	1.25	0.17–9.20	0.83
MMP-1 −1607 1G/2G			
Age			
40–44	814.33	3.54–187308.28	**0.02**
45–49	151.88	1.41–16423.66	**0.04**
⩾50	5.5.89	3.76–68161.82	**0.01**
Gender	8.85	0.50–157.10	0.14
Smoking	0.35	0.02–5.89	0.46
Bleeding on probing	1.40	1.14–1.72	**0.001**
2G allele carriage (1G/2G and 2G/2G)	0.55	0.08–3.86	0.55

p-values shown in bold indicate statistical significance ($p<0.05$).

ence between healthy controls and CP patients in the frequency of the −1607 2G allele ($p = 0.06$). Furthermore, the analysis of the subgroups did not reveal any significant influence of gender on the distributions of the genotype or allele frequency of these investigated SNPs (data not shown).

Four different haplotypes (−519G and −1607 2G, −519G and −1607 1G, −519A and −1607 2G, −519A and −1607 1G) were identified after analysing combinations of the genotype frequencies for the two MMP-1 gene polymorphisms. There was no significant difference in distribution of haplotypes between severe CP patients and healthy subjects ($\chi^2 = 2.482$ $p = 0.48$) (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 and score of BOP. The −519G and −1607 2G allele carrying genotypes of the MMP-1 gene were not found to be associated with severe CP (adjusted OR: 1.25, $p = 0.83$ and OR: 0.55, $p = 0.55$, respectively) (Table 3).

Clinical parameters in MMP-1 genotype subjects

In order to investigate whether carriage of the rare allele is associated with baseline clinical parameters, differences in clinical parameters between MMP-1 rare allele carrier and non-carrier subjects in the CP group were compared by an independent *t*-test. All clinical parameters of the rare allele carriers (−519GG and AG, and −1607 2G/2G and 1G/2G) were similar to those of non-carriers (−519AA and −1607 1G/1G), regarding the MMP-1 gene polymorphism ($p > 0.05$) (Table 4).

Clinical outcomes of periodontal treatment and GCF MMP-1 levels in MMP-1 genotype subjects

MMP-1 −519AG and −1607 1G/2G genotype distributions and allele frequencies of the severe CP patients who received non-surgical periodontal therapy ($n = 58$) were similar to those of the larger group ($n = 102$) (data not shown). We analysed the effects of non-surgical

Table 4. Baseline clinical parameters (mean ± SD) of severe chronic periodontitis (CP) patients distributed by rare allele carriers (AG and GG, 1G/2G and 2G/2G) and non-carriers (AA, 1G/1G)

MMP-1 −519A/G	AA ($n = 53$)	AG and GG ($n = 49$)	*p*-value
Mean PD (mm)	3.67 ± 0.82	4.00 ± 0.89	0.44
% sites with PD 4–6 mm	37.26 ± 13.71	33.97 ± 13.81	0.29
% sites with PD ⩾7 mm	8.80 ± 10.96	9.26 ± 9.99	0.86
Mean CAL (mm)	4.91 ± 1.49	5.34 ± 1.12	0.11
% sites with CAL 4–6 mm	45.71 ± 17.38	49.47 ± 14.84	0.31
% sites with CAL ⩾7 mm	23.49 ± 23.04	25.49 ± 21.35	0.69
Bleeding on probing (%)	73.97 ± 22.50	69.60 ± 23.16	0.32
Plaque (%)	81.89 ± 20.53	77.16 ± 22.13	0.26
MMP-1 −1607 1G/2G	1G/1G ($n = 51$)	1G/2G and 2G/2G ($n = 50$)	
Mean PD (mm)	3.92 ± 0.89	3.94 ± 0.83	0.95
% sites with PD 4–6 mm	34.86 ± 13.91	36.87 ± 13.88	0.50
% sites with PD ⩾7 mm	9.91 ± 11.42	7.79 ± 9.27	0.40
Mean CAL (mm)	5.16 ± 1.25	5.07 ± 1.44	0.61
% sites with CAL 4–6 mm	47.26 ± 16.27	47.87 ± 16.61	0.82
% sites with CAL ⩾7 mm	26.69 ± 21.38	21.77 ± 22.15	0.33
Bleeding on probing (%)	69.54 ± 23.13	73.68 ± 23.39	0.41
Plaque (%)	80.02 ± 20.76	79.35 ± 22.29	0.81

Table 5. Clinical parameters (mean ± SD) in severe chronic periodontitis (CP) patients (at baseline, and at 1 and 6 months after treatment) distributed by MMP-1 −519 rare allele carriers (AG and GG) and non-carriers (AA)

MMP-1 −519A/G	CP ($n = 58$)		*p*-value
	AA ($n = 28$)	AG and GG ($n = 30$)	
Mean PD (mm)			
Baseline	3.71 ± 0.60	3.76 ± 0.65	0.30
1 month	2.75 ± 0.52	2.93 ± 0.51	
6 months	2.63 ± 0.45	2.78 ± 0.41	
% sites with PD 4–6 mm			
Baseline	35.44 ± 14.23	33.74 ± 12.72	0.49
1 month	22.19 ± 11.93	22.02 ± 13.84	
6 months	18.49 ± 13.93	15.37 ± 9.76	
% sites with PD ⩾7 mm			
Baseline	7.96 ± 6.50	7.97 ± 7.12	0.79
1 month	3.06 ± 3.04	2.70 ± 3.67	
6 months	1.71 ± 2.28	2.90 ± 4.92	
Mean CAL (mm)			
Baseline	4.98 ± 1.25	5.13 ± 0.88	0.36
1 month	4.00 ± 1.17	4.27 ± 0.68	
6 months	4.05 ± 1.22	4.33 ± 0.72	
% sites with CAL 4–6 mm			
Baseline	44.82 ± 18.35	52.77 ± 13.02	0.003*
1 month	36.14 ± 15.02	46.99 ± 13.52	
6 months	34.89 ± 14.42	49.19 ± 17.86	
% sites with CAL ⩾7 mm			
Baseline	23.14 ± 20.90	23.44 ± 17.00	0.92
1 month	14.81 ± 15.48	13.87 ± 10.56	
6 months	1.71 ± 2.28	2.89 ± 4.92	
Bleeding on probing (%)			
Baseline	71.26 ± 24.51	64.29 ± 23.92	0.86
1 month	18.23 ± 20.04	15.64 ± 13.35	
6 months	20.12 ± 22.61	24.47 ± 22.44	
Plaque (%)			
Baseline	81.71 ± 22.22	75.05 ± 24.18	0.96
1 month	25.65 ± 22.36	21.48 ± 16.65	
6 months	21.50 ± 21.79	29.44 ± 27.83	

*The mean difference from the AA genotype is significant at <0.05 level, as revealed by ANCOVA.

Table 6. Clinical parameters (mean ± SD) in severe chronic periodontitis (CP) patients (at baseline, and at 1 and 6 months after treatment) distributed by MMP1 −1607 rare allele carriers (1G/2G and 2G/2G) and non-carriers (1G/1G)

MMP-1 −1607 1G/2G	CP ($n = 58$)		p-value
	1G/1G ($n = 32$)	1G/2G and 2G/2G ($n = 26$)	
Mean PD (mm)			
Baseline	3.73 ± 0.69	3.74 ± 0.53	0.74
1 month	2.85 ± 0.58	2.86 ± 0.43	
6 months	2.68 ± 0.40	2.74 ± 0.48	
% sites with PD 4–6 mm			
Baseline	32.85 ± 13.46	36.97 ± 13.41	0.29
1 month	20.96 ± 12.98	23.71 ± 12.99	
6 months	15.54 ± 10.91	18.35 ± 13.38	
% sites with PD ⩾7 mm			
Baseline	8.56 ± 7.57	7.21 ± 5.69	0.66
1 month	3.11 ± 3.87	2.64 ± 2.66	
6 months	2.41 ± 3.41	2.11 ± 4.55	
Mean CAL (mm)			
Baseline	4.98 ± 1.02	5.16 ± 1.16	0.74
1 month	4.15 ± 0.79	4.14 ± 1.15	
6 months	4.13 ± 0.81	4.26 ± 1.22	
% sites with CAL 4–6 mm			
Baseline	49.40 ± 15.89	48.42 ± 17.18	0.45
1 month	44.01 ± 16.41	39.12 ± 13.52	
6 months	43.19 ± 17.90	41.04 ± 18.06	
% sites with CAL ⩾7 mm			
Baseline	22.78 ± 17.86	23.99 ± 20.69	0.64
1 month	13.72 ± 10.67	15.19 ± 16.01	
6 months	2.41 ± 3.41	2.11 ± 4.55	
Bleeding on probing (%)			
Baseline	63.65 ± 24.15	71.49 ± 23.73	0.40
1 month	14.02 ± 13.37	20.29 ± 20.37	
6 months	22.11 ± 20.58	22.78 ± 25.45	
Plaque (%)			
Baseline	77.74 ± 22.66	79.15 ± 24.95	0.62
1 month	19.88 ± 16.87	28.49 ± 22.21	
6 months	26.61 ± 23.92	25.12 ± 27.40	

periodontal therapy on the clinical parameters and GCF MMP-1 levels between rare allele carriers and non-carriers. The mean PD, CAL scores, percentage of sites with BOP and plaque, percentage of sites with baseline PD 4–6 and ⩾7 mm and baseline CAL 4–6 and ⩾7 mm significantly decreased after non-surgical periodontal therapy (at 1 month and 6 months) in both carriers and non-carriers of MMP-1 −519G and −1607 2G alleles ($p < 0.001$) (Tables 5 and 6). There was a significant difference in the percentage of sites with baseline CAL 4–6 mm between the −519G allele carriers and non-carriers (−519AA) at baseline and after non-surgical therapy (at 1 month and 6 months) ($p = 0.003$) (Table 5). However, there were no significant differences in the other clinical parameters between carriers and non-carriers of MMP-1 −519G and −1607 2G alleles at baseline and after treatment (Tables 5 and 6).

Figure 1 shows the relation of MMP-1 genotype status with GCF MMP-1 levels as well as the effect of non-surgical periodontal therapy on these levels. MMP-1 −519G allele carrier subjects in the CP group had significantly higher GCF MMP-1 levels compared with non-carriers (−519AA) at baseline and 6 months after treatment ($p = 0.02$). However, GCF MMP-1 levels of −1607 2G allele carrier subjects were similar to those of −1607 2G allele non-carrier subjects at baseline and after treatment ($p > 0.05$). There were no significant differences in GCF MMP-1 levels after non-surgical periodontal therapy in both carriers and non-carriers ($p > 0.05$).

Discussion

The present study has demonstrated that severe CP and healthy subjects had similar MMP-1 −519AG and −1607 1G/2G genotypes in the Turkish population. On the other hand, subjects carrying the −1607 2G allele tended to be more susceptible to severe CP. Moreover, the clinical outcomes of non-surgical periodontal therapy were worse in −519G allele carriers (AG and GG) than in non-carriers. In addition, the GCF MMP-1 levels of −519G allele carriers were higher than those of non-carriers after non-surgical periodontal therapy.

MMPs play a central role in the regulation of periodontal tissue turnover in health and disease. Three important regulatory mechanisms capable of controlling the extent of MMP expression in the inflamed periodontium include (1) regulation of transcription levels and secretion, (2) activation of the proenzyme and (3) regulation of MMP activity by their endogenous inhibitors (Sorsa et al. 2006). Genetic variations in the MMP-1 promoter region can influence transcription activity, which is the key step in the regulation of MMP activity, and thereby increase the levels of MMP-1 protein expression (Rutter et al. 1998). On the other hand, other control mechanisms of MMP activity can 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-1 genotypes and severe CP might also suggest that an increase in the transcription of MMP-1 may not necessarily lead to an increased destructive effect of this enzyme on the periodontal tissues.

Currently, there are a limited number of studies evaluating the association between MMP-1 −519A/G polymorphism and CP. Similar genotype distributions and allele frequencies of the MMP-1 −519 gene were observed in the non-smoking Czech subjects with CP compared with periodontally healthy subjects (Holla et al. 2004). In a non-smoking Brazilian population, no association was found between genotype and allele distribution of this gene polymorphism and CP (Astolfi et al. 2006). The present study has shown that the frequencies of GG homozygote in severe CP (8.8%) and control subjects (7.2%) were lower than in those of Czech and Brazilian populations (Holla et al. 2004, Astolfi et al. 2006). The lack of association between MMP-1 −519A/G polymorphism and severe CP in the present study is consistent with previous data in Czech and Brazilian populations

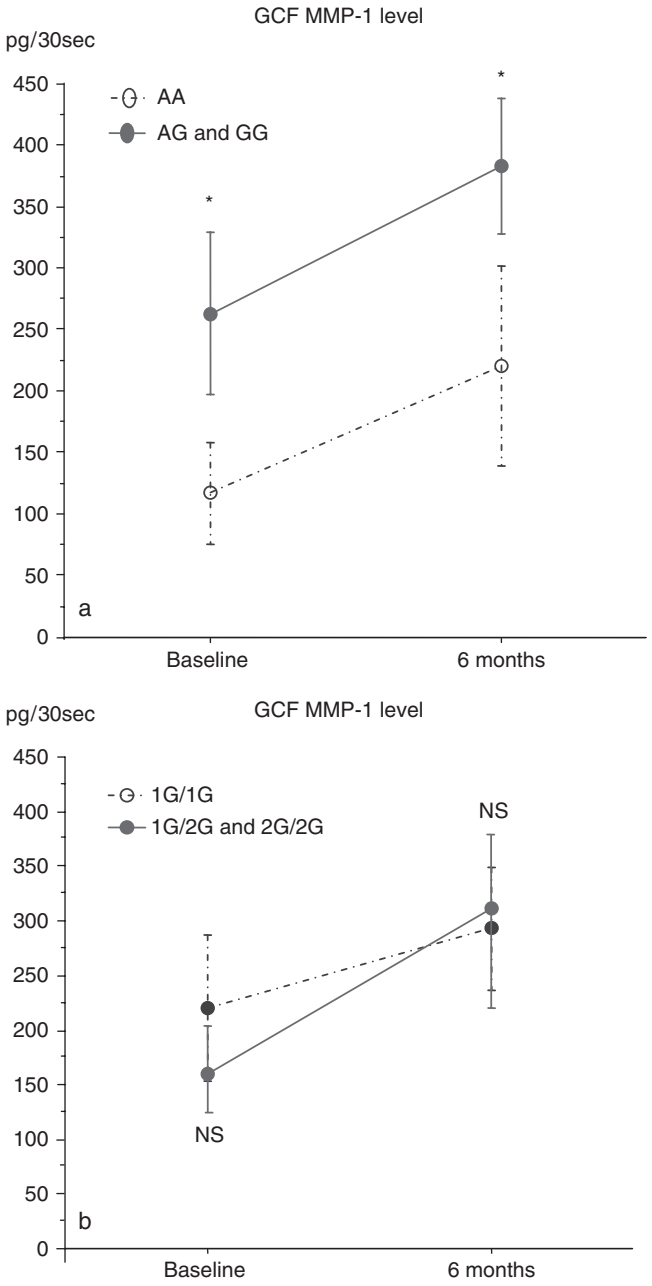

Fig. 1. (a) Changes in GCF MMP-1 levels (mean ± SD) in the MMP-1 −519 rare allele carriers (AG and GG) ($n = 30$) and non-carriers (AA) ($n = 28$) before and after non-surgical periodontal therapy in severe CP patients. *Significant differences from the AA genotype at baseline and after 6 months ($p = 0.02$). (b) Changes in GCF MMP-1 levels (mean ± SD) in the MMP-1 −1607 rare allele carriers (1G/2G and 2G/2G) ($n = 26$) and non-carriers (1G/1G) ($n = 32$) before and after non-surgical periodontal therapy. NS, no significant differences from the 1G/1G genotype at baseline and after 6 months ($p > 0.05$).

(Holla et al. 2004, Astolfi et al. 2006). On the other hand, the lower frequency of the MMP-1 −519A/G gene polymorphism in our CP and healthy groups compared with other populations (Holla et al. 2004, Astolfi et al. 2006) could be attributable to the ethnic differences between countries.

MMP-1 −1607 gene polymorphisms in CP patients have also been studied in different populations up to date. These studies have reported that different risk alleles for the MMP-1 gene could be associated with periodontitis in Chinese, Brazilian and Czech populations (de Souza et al. 2003, Holla et al. 2004, Cao et al. 2006), while others failed to show an association with these polymorphic alleles (Itagaki et al. 2004, Astolfi et al. 2006). In the present study, the frequency of the −1607 2G allele tended to be higher in the severe CP patients compared with the healthy subjects. The presence of different risk alleles for the same disease in different populations could be due to ethnic heterogeneity as well as methodological differences. Nevertheless, de Souza et al. (2003) reported that the MMP-1 2G allele was associated with severe CP in the non-smoking Brazilian population. In a more recent study that includes a larger group by the same investigators, severe CP patients tended to have higher 2G/2G genotype and 2G allele frequency compared with healthy subjects, although this difference does not seem to be associated with the susceptibility to periodontitis. It is possible that this discrepancy occurred due to methodological differences in both studies (de Souza et al. 2003, Astolfi et al. 2006) as well as the variation in the selection of the control group in different studies. In studies by Itagaki et al. (2004) and Astolfi et al. (2006), the control group consisted of subjects aged 25 or over, while Holla et al. (2004) included a reference control with unknown periodontal status. In the present study, age-matched periodontally healthy subjects were selected as a control group.

CP is a multifactorial disease whose manifestation and progression is influenced by a variety of factors such as genetic factors, smoking, 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 Loos et al. (2005) and 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, −519G and −1607 2G allele-carrying genotypes of the MMP-1 gene were not associated with susceptibility to CP. Smoking is known to be a well-established environmental risk factor for periodontitis, which influences the host inflammatory immune response by suppressing the activity of several mediators (Kinane & Chestnutt 2000, Palmer et al. 2005). In the present study, lack of association between smoking and MMP-1 gene polymorphism could be due to having a group of subjects who never smoked or who were light smokers.

In the present study, the mean change in clinical parameters at 6 months from

baseline of severe CP patients with the −1607 2G allele-carrying genotypes of the MMP-1 gene (1G/2G and 2G/2G) was similar to those of non-carriers (1G/1G). On the other hand, MMP-1 −519G allele carriers (AG and GG) had a higher percentage of sites with CAL 4–6 mm compared with non-carriers (AA) at baseline and after non-surgical therapy. MMP-1 −519G allele carriers had also elevated GCF MMP-1 levels at baseline and after non-surgical therapy. These findings suggest that non-surgical periodontal therapy has a limited effect on MMP-1 −519G allele carrier CP patients. We can suggest that MMP-1 −519AG and GG genotypes could play a role in the outcome of periodontal therapy. It can also be speculated that the presence of a higher percentage of sites with CAL 4–6 mm in CP patients with MMP-1 −519AG and GG genotypes might be at risk for further breakdown if they were untreated. On the other hand, because of the complexity of the disease, this does not necessarily show that the investigated allele is causatively related to the progression of the disease.

As a result, the 2G allele of the MMP-1 gene could be associated with susceptibility to severe CP. A higher percentage of sites with CAL 4–6 mm together with GCF MMP-1 levels in MMP-1 −519G allele carriers (AG and GG) at baseline and after non-surgical therapy might draw attention to the importance of regular maintenance in these patients for the prognosis of periodontal disease progression following periodontal therapy. To the best of our knowledge, this is the first study investigating the effect of MMP-1 −519 and −1607 gene polymorphisms on the clinical status and the GCF MMP-1 levels as well as on the non-surgical periodontal treatment outcomes in CP patients. We suggest that more extensive studies with larger patient groups and also other ethnic populations should be undertaken in order to analyse the putative relevance of MMP-1 −519 and −1607 gene polymorphisms in the pathogenesis of periodontitis, which would also be valuable in preventive, diagnostic and therapeutic strategies against the development of periodontitis.

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Clinical Relevance

Scientific rationale for the study: The investigation of genetic polymorphisms that affect the transcriptional activity of MMP-1 promoter may provide important information on its function in periodontal diseases. Therefore, we examined whether MMP-1 promoter polymorphisms constitute risk for severe chronic periodontitis and therapeutic responses in a Turkish population.

Principle findings: There was a significant difference between the study groups in the −1607 2G allele frequencies. MMP-1 −519G allele carriers had a higher percentage of sites with 4–6 mm CAL compared with AA genotypes after non-surgical therapy. The −519G allele carriers also had higher GCF MMP-1 levels compared with non-carriers after therapy.

Practical implications: The −1607 2G polymorphic allele of the MMP-1 gene appears to be related to severe CP. The MMP-1 −519G allele could be an important parameter in the planning of periodontal treatment.