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Genetic polymorphisms in the MMP-1 and MMP-3 gene may contribute to chronic periodontitis in a Brazilian population

Claudia Maria Astolfi[1], André Luís Shinohara[1], Rodrigo Augusto da Silva[1], Maria Cristina Leme Godoy Santos[2], Sergio Roberto Peres Line[2], Ana Paula de Souza[1]

[1]Oral Biology Program, University of Sagrado, Coração-Bauru, SP, Brazil; [2]Department of Morphology, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil

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Abstract

Objectives: Matrix metalloproteinase-1 and -3 (MMP-1, MMP-3) represent proteinases that degrade macromolecules of the extracellular matrix. These enzymes play a fundamental role during destruction of periodontal tissues. Genetic polymorphisms were characterized in the promoter region of the MMP-1 and MMP-3 genes. The aim of this study was to investigate the relationship between these genetic variations with chronic periodontitis in a Brazilian population.

Material and Methods: Non-smoking subjects ($n = 114$) exhibiting sites $\geqslant 5$ mm clinical attachment loss were recruited for study. Control subjects ($n = 109$) should not exhibit clinical signals of periodontitis. MMP-1 (− 1607 1G/2G, − 519 A/G) and MMP-3 (− 1612 5A/6A) gene promoter polymorphisms were genotyped using PCR-RFLP methods.

Results: Analysis of polymorphisms showed no differences in distribution of the − 1607 1G/2G and − 519 A/G variants in the MMP-1 gene between the healthy and periodontitis group ($p>0.05$). However, the distribution of genotype frequencies of the − 1612 5A/6A polymorphism in the MMP-3 gene showed that the 5A/5A genotype was significantly more frequent in the periodontitis group ($p = 0.008$). The same was not observed in the 5A/6A genotype once only one 5A allele is carried. We also observed a trend to increase the frequency of the MMP-1/MMP-3 haplotype (2G/5A) in the periodontitis group ($p = 0.08$).

Conclusion: On the basis of the results, no significant association is found for the MMP-1 polymorphisms with susceptibility of periodontitis, while the MMP-3 gene polymorphism may contribute to periodontal tissue destruction during periodontitis in Brazilian subjects.

Key words: MMP-1; MMP-3; haplotype; genetic polymorphism; periodontitis

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Inflammatory destruction of periodontal attachment apparatus is the hallmark of periodontal disease. The inflammatory reaction associated with periodontitis may damage the surrounding cells and connective tissue structures, including alveolar bone, causing tooth loss (Page 1991, 1992, Genco 1992). During this event, the most important component of the periodontium lost is collagen type I, which is found in the periodontal ligament and alveolar bone organic matrix. Four distinct pathways may be involved in this destruction: plasminogen dependent, phagocytic, osteoclastic and matrix metalloproteinase (MMP) pathway (Birkedal-Hansen 1993). A wide range of evidences have indicated that the most important pathway is the one that involves MMPs (Birkedal-Hansen 1993), as active collagenases and gelatinases are found in the crevicular gingival fluid of patients with periodontitis in much larger amounts than in control subjects (Makela et al. 1994).

MMPs represent a family of dependent metal ion endopeptidases that are capable of degrading all extracellular matrix components, including several types of collagen and basement membrane components (Birkedal-Hansen 1993, Woessner & Nagase 2000). MMPs are metal dependent since all members of this

family have a zinc- and a calcium-binding catalytic domain. They are secreted as inactive pro-enzymes (zymogens) and their activation occurs in the tissue by cleavage of the N-terminal propeptide domain by other proteinases (Murphy & Knäuper 1997). MMPs are present in both active and latent forms in chronically inflamed gingival tissues and gingival crevicular fluid.

DNA polymorphisms have been found in the promoter regions of several MMPs. Basal and inducible levels of MMP gene expression can be influenced by these genetic variations that may influence the development and progression of several diseases, including periodontal disease (Ye 2000). An insertion/deletion of a guanine at position −1607 of the human MMP-1 gene creates two different alleles: one having a single guanine (1G) and the other having two guanine (2G) (Rutter et al. 1998). A case–control study performed in 87 nonsmoking Caucasian subjects showed a positive association between the 2G allele and the severity of chronic periodontal disease (de Souza et al. 2003). This same allele was also associated with early dental–implants failure (Santos et al. 2004). Other studies performed in a Czech and Japanese population have found no association between this MMP-1 polymorphism and periodontitis (Hollá et al. 2004, Itagaki et al. 2004). Recently, polymorphism at position −519 of the MMP-1 gene promoter corresponding to an A/G change was described (Jurajda et al. 2002).

A 5A/6A functional polymorphism at position in the −1612 of the MMP-3 (stromelysin-3) gene promoter has been associated with atherosclerosis in a number of genetic epidemiological studies (Ye et al. 1995, de Maat et al. 1999). The frequency of the 5A allele is significantly higher in affected individuals than in control subjects, and the risk of acute myocardial infarction in individuals carrying one or two copies of the 5A allele was estimated to be 2.25-fold (Terashima et al. 1999, Ye 2000). MMP-3 is capable of degrading a wide range of extracellular matrix proteins, promoting the cleavage of atherosclerotic plaque. These data indicate that the 5A/6A repeats are of potential interest in studies of DNA polymorphism–periodontal disease association.

The aim of the present work was to study the association of MMP-1 and MMP-3 gene polymorphisms and periodontal disease in a Brazilian population. We also investigated the association of MMP-1 and MMP-3 haplotypes with chronic periodontitis.

Material and Methods

Subject selection

A convenience sample of unrelated nonsmoking subjects >25 years old were recruited for the study. The patients were from the Southeastern region of Brazil. All subjects were in good general health and had at least 20 teeth in the mouth. Subjects did not have any of the following exclusion criteria: diseases of the oral hard or soft tissues, except caries and periodontal disease; use of orthodontic appliances; need for pre-medication for dental treatment; chronic usage of anti-inflammatory drugs; a history of diabetes, hepatitis or HIV infection; immunosuppressive chemotherapy; a history of any disease known to severely compromise immune function; present acute necrotizing ulcerative gingivitis; or current pregnancy or lactation. Subjects completed personal medical and dental history questionnaires, and within a protocol approved by an Institutional Review Board, signed a consent form after being advised of the nature of the study. Diagnosis and classification of disease severity were performed on the basis of clinical parameters and consisted of physical examination, medical and dental history, probing depth, assessment of clinical attachment loss (CAL), tooth mobility and observation of gingival bleeding on probing. Measurements of probing depth and attachment level were recorded at six points around each tooth. Subjects were included in clinical categories according to periodontal disease severity:

(1) *Healthy group* (*never smoked*) ($n = 109$): subjects found to exhibit no signs of periodontal disease as determined by the absence of CAL and no sites with probing depth >3 mm.
(2) *Periodontitis group* (*never smoked*) ($n = 114$): subjects with at least three teeth exhibiting sites ⩾5 mm CAL, in at least two different quadrants.

Genotype identification

The genomic DNA was isolated from epithelial buccal cells as previously described (Trevilatto & Line 2000). The −1607 1G/2G and −519 A/G MMP-1 polymorphisms were determined by PCR-RFLP as previously described (Jurajda et al. 2002, de Souza 2003, respectively).

MMP-3 genotypes were determined using specific forward primers for 5A/6A alleles and a common reverse primer that amplified DNA fragments of 95 bp (allele 5A) and 92 bp (allele 6A). PCR was carried out in a total volume of 25 μl, containing 400 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1 μM of each primer, 200 μM each dATP, dCTP, dGTP and dTTP and 2.5 U *Taq* DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). The solution was incubated for 3 min. at 95°C, followed by 35 cycles of 1 min. at 95°C, 1 min. at 41°C (6A allele)/1 min. at 44°C (5A allele) and 1 min. at 72°C, with a final extension of 72°C for 7 min. The DNA primer sequences, restriction enzymes and fragment lengths (bp) are shown in Table 1.

Genotype analysis and gel electrophoresis

Digestion was carried out in a total volume of 20 μl containg 15 μl DNA, 0.2 μl restriction enzyme (1 U), 0.3 μl bovine serum albumin (BSA), 2.5 μl water and was mixed with 3 μl of loading buffer and electrophoresed on a 10% vertical non-denaturing polyacrylamide gel at 20 mA. The gel was silver stained using the method described by Sanguinetti et al. (1994).

Statistical analysis

Statistical significance of the differences in observed frequencies of the polymorphism among healthy and periodontitis groups was determined by the χ^2 test ($p < 0.05$) (BioEstat ver.2.0). The odds ratio (OR) was calculated using BioEstat ver.2.0 software. The haplotype frequencies were calculated using Arlequin ver.2000 (software for population genetics data analysis). Analysis of Hardy–Weinberg equilibrium was used to test deviation of genotype distribution.

Results

Table 2 shows the characteristics of the subject population. The genotype distributions for MMP-1 and MMP-3 polymorphisms were with Hardy–Weinberg equilibrium. Tables 3 and 4 show the MMP-1 (−1607 1G/2G, −519 A/G) allele and genotype frequencies for

Table 1. Primers, restriction enzymes and fragments

SNP	Primers (5′–3′)	PCR	Restriction enzyme	Fragment (bp)
MMP-1 (− 1607)	F: 5′-TCG TGA GAA TGT CTT CCC ATT-3′ R: 5′-TCT TGG ATT GAT TTG AGA TAA GTG AAA TC-3′	55°C (1 min.)	*Xmn*I (37°C)	118 (29+89)
MMP-1 (− 519)	F: 5′-CAT GGT GCT ATC GCA ATA GGG T-3′ R: 5′-TGC TAC AGG TTT CTC CAC ACA C-3′	49°C (1 min.)	*Kpn*I (37°C)	200 (176+24)
MMP-3 (allele 5A)	F: 5′-GAC AAG ACA TGG TTT TTC-3′ R: 5′-AGA CAT GGG TCA CGG-3′	41°C (1 min.)	X	95
MMP-3 (allele 6A)	F: 5′-AAG ACA TGG TTT TTT-3′ R: 5′-AGA CAT GGG TCA CGG-3′	44°C (1 min.)	X	92

MMP-1, matrix metalloproteinase-1

healthy and periodontitis groups. The 2G allele in the MMP-1 gene, which was shown to increase MMP-1 transcription (Rutter et al. 1998, Noll et al. 2001), was not observed at a higher frequency in the periodontitis groups. The 2G allele was observed at a frequency of 54.1% and 58.8% in the healthy and periodontitis groups, respectively ($p = 0.3717$) (Table 3). The 2G/2G genotype was found at frequencies of 30.3% and 36%, 8% in the healthy and periodontitis groups, respectively ($p = 0.5785$). No statistically significant differences in the distribution of the − 519A/G polymorphism in the MMP-1 gene were found between the healthy and periodontitis groups (Table 4). The frequency of the haplotype distribution of MMP-1 polymorphisms was also not different in the groups. We observed a linkage between the polymorphisms − 519 A/G and − 1607 1G/2G polymorphisms in the MMP-1 promoter gene. The A allele − 519 and 2G allele − 1607 are often found, while the G allele − 519 is frequently found with the 1G allele − 1607 ($p < 0.001$).

The frequencies for healthy and periodontitis groups of the different alleles and genotypes of the MMP-3 gene (− 1612 5A/6A) are shown in Table 5. The results indicated that there was an association between the 5A/5A genotype with the periodontitis group ($p = 0.008$). Individuals with the 5A/5A genotype are approximately three times more likely to develop periodontitis ($p = 0.008$; OR = 3.18%, 95% confidence interval (CI) = 1.23–8.44). However, when we analysed the genotypes 5A/5A against 5A/6A, we observed that individuals carrying only one 5A allele do not show risk to periodontitis and the 6A allele may be protective of the disease ($p = 0.0164$).

MMP-1 and MMP-3 haplotype analysis shows a trend to weeds an increased relative frequency of the 2G/5A haplotype in the periodontitis group ($p = 0.06$) (Table 6). The 1G/2G+5A/5A and 2G/2G+5A/5A composite genotypes were also increased in the periodontitis group (Table 7).

Table 2. Characteristics of the study population

	Healthy ($n = 109$)	Periodontitis ($n = 114$)
Age (years)	38.12	42.58
Mean (± SD)	± 13.8	± 9.9
Female	78 (71%, 5%)	87 (76%, 3%)
Male	31 (28%, 5%)	27 (23%, 7%)
Caucasoid	94 (86%, 2%)	82 (72%)
Afro-American/Mulatto	12 (11%, 0%)	32 (28%)
Japanese	3 (2.8%)	0 (0.0%)
Periodontal status		
At least 5 mm CAL (%)	0.0	100

CAL, clinical attachment loss.

Table 3. Alleles and genotype distribution of MMP-1 $^{-1607}$

MMP-1 (− 1607)	*n* (%)		*p* value	OR
	healthy ($n = 109$)	periodontitis ($n = 114$)		
Alleles				
1G	100 (45.9)	94 (41.2)	0.3717	
2G	118 (54.1)	134 (58.8)		
Genotype				
1G/1G	24 (22.0)	22 (19.3)		
1G/2G	52 (47.7)	50 (43.9)	0.5785	0.74 ($p = 0.37$)
2G/2G	33 (30.3)	42 (36.8)		

MMP-1, matrix metalloproteinase-1; OR, odds ratio.

Table 4. Alleles and genotype distribution of MMP-1 $^{-519}$

MMP-1 (− 519)	*n* (%)		*p* value	OR
	healthy ($n = 94$)	periodontitis ($n = 109$)		
Alleles				
A	94 (50.00)	119 (54.4)	0.4104	
G	94 (50.00)	99 (45.6)		
Genotype				
A/A	17 (18.0)	22 (24.7)		
A/G	60 (64.0)	65 (59.7)	0.50	0.74 ($p = 0.32$)
G/G	17 (18.0)	17 (15.6)		

MMP-1, matrix metalloproteinase-1; OR, odds ratio.

Discussion

The purpose of the present study was to investigate the association of the MMP-1 (1G/2G − 1607; A/G − 519) and MMP-3 (5A/6A − 1612) polymorphisms with susceptibility to chronic periodontitis. Previously, we reported that the MMP-1 2G allele was associated with severe chronic periodontitis in Brazilian subjects (de Souza et al. 2003). Now, although the 2G/2G genotype tends to be more frequent in the

Table 5. Alleles and genotype distribution of MMP-3 $^{-1612}$

MMP-3	*n* (%)			
	healthy ($n = 103$)	periodontitis ($n = 90$)	*p* value	OR (95% CI)
Alleles				
5A	86 (41.7)	90 (50.0)	0.1281	
6A	120 (58.3)	90 (50.0)		
Genotype				
5A/5A	08 (7.8)	19 (21.1)	0.008*	3.18 (CI = 1.23–8.44)
6A/6A	25 (24.3)	19 (21.1)		
5A/6A	70 (67.9)	52 (57.8)		

MMP-3, matrix metalloproteinase-3; OR, odds ratio.
*Statistically significant.

Table 6. Haplotype distribution of MMP-1 $^{-1607}$ and MMP-3 $^{-1612}$ polymorphisms

Haplotype	Healthy	Periodontitis	*p* value
1G-5A	0.29	0.21	0.32
1G-6A	0.16	0.20	0.61
2G-5A	0.09	0.20	0.06*
2G-6A	0.45	0.37	0.43
–	–	–	0.08*

MMP-1, Matrix metalloproteinase-1; MMP-3, matrix metalloproteinase-3.
*Statistically significant.

Table 7. Composite genotype distribution of MMP-1 $^{-1607}$ and MMP-3 $^{-1612}$

Genotypes	Healthy			Periodontitis			*p* value
	5A/5A	5A/6A	6A/6A	5A/5A	5A/6A	6A/6A	
1G/1G	6	12	1	4	7	5	
1G/2G	2	34	11	8	20	10	0.1319
2G/2G	2	10	17	5	10	16	

MMP-1, matrix metalloproteinase-1; MMP-3, matrix metalloproteinase-3.
*Statistically significant.

periodontitis group compared with the healthy group, we observed that the MMP-1 genotype distribution is similar in both healthy and periodontitis groups and it does not seem to be associated with susceptibility to periodontitis. It is possible that the discrepancy occurred due to methodological differences, as the sample size in the periodontitis group was nearly twice as large as in the previous work and because the periodontitis group had not been classified by disease severity.

Studies suggested that the -1607 MMP-1 polymorphism may affect gene transcription. The 2G allele, together with an adjacent adenosine, creates a core binding site (5′-GGA-3′) for the Ets family of transcription factors immediately adjacent to an AP-1 site, causing a 37-fold increase in transcription activity (Kanamori et al. 1999, Ye 2000, Sternlicht & Werb 2001). Tumours bearing the 2G allele can secrete higher levels of MMP-1, and the presence of this allele was associated with the development of ovarian cancer (kanamori et al. 1999). Moreover, an unusually large proportion of metastatic melanomas with loss of heterozygosity at this site retains the high-expressing 2G allele (Noll et al. 2001).

The -1612 MMP-3 (stromelysin-3) gene promoter is also a functional polymorphism and has been associated with atherosclerosis in a number of genetic epidemiological studies (Ye et al. 1995, de Maat et al. 1999). The frequency of the 5A allele is significantly higher in affected individuals than in control subjects, and the risk of acute myocardial infarction in individuals carrying one or two copies of the 5A allele was estimated to be 2.25-fold (Terashima et al. 1999, Ye 2000).

Polymorphisms in MMP-1 and MMP-3 genes were investigated with chronic periodontitis in Japanese and Czech populations. Hollá et al. (2004) found an association of MMP-1 1G/1G genotype in a subgroup of severe nonsmoking chronic periodontitis in Czech subjects. Itagaki et al. (2004) did not find differences in both MMP-1 and MMP-3 allele and genotype distributions between healthy and chronic periodontitis groups.

Contradictory results on genes polymorphisms–disease association studies are often found, and this may occur due to several reasons. Distinct races and/or populations may have different risk alleles for a same disease (Itagaki et al. 2004). Some studies reported risk genotypes for interleukin-1β, interleukin-1α and periodontitis in a Caucasian population (Kornman et al. 1997, Lopez et al. 2005), while others failed to show an association with these polymorphic alleles (Konig et al. 2005).

At least three important regulatory mechanisms may control the action of MMPs on the components of the extracellular matrix: regulation of transcription levels, activation of the pro-enzyme into the extracellular matrix (including the plasmin-dependent or MMP-dependent pathway) and the inhibition of MMPs by their tissue inhibitors of MMPs (TIMPs). Control of transcription has been considered the key step in the regulation of MMPs activity. However, the control of protein activity is of key importance. MMPs activity is controlled by endogenous activators, inhibitors and factors that influence MMP secretion, cell surface localization and MMP degradation and clearance (Sternlicht & Werb 2001). Therefore, the lack of association between MMP-1 polymorphisms and periodontal disease suggests that an increase in the mRNA transcription may not necessarily lead to an increased effect of this enzyme on the extracellular matrix.

MMP genes are expressed when required in physiological extracellular matrix remodelling or during pathological tissue destruction (Matrisian 1990, Fini et al. 1998, Ye 2000). Normal or inflammatory cells carrying the MMP-1 2G allele or the MMP-3 5A allele will only overexpress these MMPs, in relation to the MMP-1 1G allele or MMP-3 6A alleles, when the cell metabolism is somehow altered in a specific tissue. This explains why chronic periodontitis subjects carrying risk alleles do not have systemic collagenolytic diseases such as cancer metastases and rheumatoid arthritis.

Finally, we believe that MMP gene polymorphisms may be important modulators of periodontal disease. On the basis of the results, no significant association was found for the MMP-1 polymorphisms with susceptibility of periodontitis, while the MMP-3 gene polymorphism may contribute to periodontal tissue destruction in periodontitis in Brazilian subjects.

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Address:
Ana Paula de Souza
Oral Biology Program
PRPPG-USC
Rua Irmã Arminda 10-50
17011-160, Bauru, SP
Brazil
E-mail: anapaula_pardo@yahoo.com.br