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Genetic variations in the matrix metalloproteinase-1 promoter and risk of susceptibility and/or severity of chronic periodontitis in the Czech population

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Abstract

Objectives: Matrix metalloproteinase-1 (MMP-1) is a potent enzyme degrading extracellular matrix that was implicated in the pathogenesis of chronic periodontitis. Therefore, the aim of our study was to examine the association between three promoter polymorphisms of the MMP-1 gene and chronic periodontitis susceptibility and/or severity in a Czech population.

Materials and Methods: A total of 329 Caucasian subjects were enrolled in this study. They were 133 patients with mild to severe chronic periodontitis and 196 unrelated control subjects. MMP-1 promoter polymorphisms (− 1607 1G/2G, − 519A/G, and − 422A/T) were genotyped using standard polymerase chain reaction–restriction fragment length product methods.

Results: Genotype analysis of the three single nucleotide polymorphisms across 27 different combinations showed significant association with chronic periodontitis ($p < 0.05$). Analyses of individual polymorphisms showed no differences in distribution of the − 519A/G and − 422A/T variants between periodontitis and control groups. However, a trend to increased frequency of the − 1607 1G allele was observed in patients with chronic periodontitis compared with the controls ($p = 0.054$). When the groups were further stratified by smoking status, the 1G allele was associated with chronic periodontitis among non-smokers but not among smokers ($p = 0.033$). On the contrary, the distribution of genotype frequencies of the MMP-1 − 422A/T polymorphism was different between the patient and control smokers with respect to heterozygotes (73.91% versus 50.91%; $p = 0.017$).

Conclusions: Our results demonstrate that the polymorphisms in the MMP-1 promoter may have only a small effect on the etiopathogenesis of chronic periodontitis.

Key words: gene; haplotype; matrix metalloproteinase-1; periodontitis

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Chronic periodontitis is the most common form of periodontal disease, which affects over 30% of the adult population. Both genetic and environmental factors are involved in this inflammatory disease aetiology of which is influenced by interaction of periodontal pathogens and host responses (Offenbacher 1996). There is much evidence for the role of matrix metalloproteinases (MMPs) in the destructive processes of periodontal disease linked with the unbalanced production between MMPs and their endogenous tissue inhibitors (TIMPs) (Birkedal-Hansen 1993,

Kinane 2000). The MMP family includes a growing family of zinc-dependent endopeptidases that were classified according to their substrate specificity into collagenases, gelatinases, stromelysins, and membrane-bound type (Birkedal-Hansen 1995). MMPs play an important role in physiological and pathological events during embryonic development, morphogenesis, angiogenesis and tissue repair, and they are also responsible for excessive breakdown of connective tissue in inflammatory disorders, e.g. periodontitis (Birkedal-Hansen 1993, Ingman et al. 1996). The evidence for the role of MMPs in periodontal destruction has accumulated for more than three decades. Specifically, these enzymes are responsible for the degradation of the collagen fibres attached to the root surface, allowing the apical migration and lateral extension of the pocket epithelium. Loss of attachment and the formation of periodontal pockets are clinical sequelae of the pathological increase in collagen destruction (Kinane 2000).

MMP-1 or interstitial collagenase is most widely expressed MMP possessing proteolytic activities against fibrillar collagens (collagens types I, II, and III), the most abundant class of extracellular matrix proteins in interstitial connective tissue (Matrisian 1990). In most normal cells, physiologic levels of MMP-1 are low, but in pathological states, such as inflammation, there may be dysregulation of MMP-1 because the expression of MMP-1 is potently upregulated by cytokines and growth factors (Vincenti et al. 1996, Aho et al. 1997, Rutter et al. 1997).

The MMP-1 gene is located in 11q22 (Pendas et al. 1996) and is translated in a wide variety of cells, e.g. fibroblasts, macrophages, endothelial, and epithelial cells (Birkedal-Hansen et al. 1993b, Shin et al. 2002, Bar-Or et al. 2003). The level of MMP-1 expression can be influenced by the polymorphism in the promoter region of the MMP-1 gene resulting from a guanine insertion at –1607 bp (i.e., the GG versus G variants), which results in the creation of a binding site for the Ets family of transcription factors as well as in the increased transcription of the MMP-1 gene and increased enzyme activity (Rutter et al. 1998). In fact, a recent study showed that a promoter polymorphism − 1607 1G/2G in the MMP-1 locus was associated with periodontitis severity in the Brazilians (de Souza et al. 2003). However, other studies were not done to replicate these findings.

The present study was conducted to explore the association of the MMP-1 gene promoter polymorphisms with chronic periodontitis susceptibility and/or severity risk in a Czech population and to compare the results with the previous findings in the Brazilians (de Souza et al. 2003).

Material and Methods

Study subjects

All subjects were Caucasians of exclusively Czech nationality, free of all systemic diseases (especially cardiovascular disorders – coronary heart disease (CHD) and hypertension, diabetes mellitus or allergy) and were not taking any medication. Phenotype status was assigned without knowledge of genotypes by two independent investigators. One hundred and thirty-three Czech patients with chronic periodontitis (68 males and 65 females; age range from 35 to 50 years; mean age 42.9 ± 7.2 SD) referred to the Periodontal Clinic of the Masaryk University St Anne Hospital were included in the study. The control population consisted of 196 unrelated Caucasian subjects (94 males and 102 females; mean age 47.1 ± 14.1 SD) residing in the same geographic area as the included patients, who did not have clinical history of periodontal disease. Subjects were screened using a WHO probe and the CPITN (Community Periodontal Index of Treatment Needs) was assessed (Ainamo et al. 1982). All patients fulfilled the diagnostic criteria defined by the International Workshop for a Classification of Periodontal Diseases and Conditions for chronic periodontitis (Armitage 1999).

Clinical assessments

Clinical assessments of the patients were performed by the same investigator at the patient's first visit as described previously (Izakovicova Holla et al. 2002b). The assessed clinical parameters were: probing pocket depth (PPD), clinical attachment loss (CAL), tooth mobility, and radiographs. PPD (from the free gingival margin to the bottom of the pocket) and CAL (from the cement–enamel junction to the bottom of the pocket) of all teeth were assessed by using a probe at four sites/tooth: mesiobuccal (mb), distobuccal (db), mesiolingual (ml), and distolingual (dl). The loss of alveolar bone was determined radiographically. Full-mouth radiographs of diagnostic quality were evaluated by a single calibrated reader for interproximal bone loss. We used the index according to Mühlemann & Mazor (1955) to evaluate decreases in alveolar bone level. In nearly all patients extensive alveolar bone loss ($\geqslant$25%) was determined.

Patients were classified according to the severity of their periodontal disease, on the basis of the amount of CAL (Armitage 1999), into one of the three disease categories. ''Mild'' ($n = 11$) classification required no CAL $>$ 2 mm and no sites with bone loss $>$ 25%. ''Moderate'' ($n = 52$) classification required patients with teeth exhibiting $\geqslant$3 and $\leqslant$6 mm CAL. ''Severe'' ($n = 70$) classification required patients with teeth exhibiting $>$6 mm CAL.

Smoking history

In order to adjust for the effect of smoking history on periodontal disease, the subjects (patients and controls) were classified into the following groups:

(1) subjects who never smoked (referred to as non-smokers);
(2) subjects who were former smokers for $\geqslant$5 pack years (referred to as former smokers) or current smokers (smokers):
   (a) light smokers: individuals smoking $\leqslant$10 cigarettes per day for $<$5 pack years;
   (b) moderate smokers: individuals smoking $>$10 cigarettes per day for $\geqslant$5 pack years and $<$10 pack years;
   (c) severe smokers: individuals smoking $>$10 cigarettes per day for $\geqslant$10 pack years.

Pack years were calculated by multiplying the number of years of smoking by the average number of cigarette packs smoked per day (McDevitt et al. 2000).

Informed consent was obtained from all participants. The study was performed with the approval of the ethics committee of the Medical Faculty, Masaryk University Brno.

Genotype identification

The genomic DNA was isolated from peripheral blood leucocytes by a standard method using the proteinase K digestion of cells (Sambrook et al.

1989) and genotyped for MMP-1 (− 519A/G) by polymerase chain reaction–restriction fragment length product (PCR—RFLP) as previously described (Jurajda et al. 2002).

The − 1607 1G/2G polymorphism was determined by newly developed mismatch PCR technique with primers (5′-GAAATTGTAGTTAAATCCTTAG AAAG-3′ and 5′-TATGGATTGCTGT TTTCTTTCTGC-3′) as described previously (Jurajda et al. 2001).

The analysis of the polymorphism − 422A/T in the promoter of the MMP-1 gene was performed by PCR–RFLP modified original method described elsewhere (Thiry-Blaise et al. 1995).

Statistical analysis

Allele frequencies were calculated from the observed numbers of genotypes. The significance of differences in allelic frequencies between each of the two groups was determined by Fisher's exact test. χ^2 Analysis was used to test for a deviation of genotype distribution from Hardy–Weinberg equilibrium and for a comparison of differences in genotype combinations among groups. The haplotype frequencies were calculated from the observed genotype frequencies assuming that during zygote formation haplotypes combine at random according to their frequencies in the set (the assumption being analogous to Hardy–Weinberg equilibrium). This assumption was verified by a reversed calculation of the expected genotype frequencies from the calculated haplotype frequencies. Differences between the control and the patient groups were tested by a simulation using the Monte-Carlo method. Contingency table analysis, odds ratio (OR), 95% confidence intervals, and significance values were estimated with the use of the programme package Statistica v 3.0 (Statsoft Inc., Tulsa, OK, USA).

Results

Table 1 shows the baseline clinical parameters of the subject population. There was no significant difference in age and sex between the groups.

Table 2 presents the MMP-1 (− 1607 1G/2G, − 519A/G, and − 422A/T) allele and genotype frequencies for cases and controls overall, and stratified by smoking history. For all polymorphisms, genotype distributions were not significantly different from Hardy–Weinberg equilibrium in the control group. The frequency of the –1607 1G allele was marginally increased in the patients with chronic periodontitis compared with the reference group ($p = 0.054$). No statistically significant differences in allele or genotype frequencies of the MMP-1 gene were found for –519A/T and/or –422A/G polymorphisms between cases and the control subjects.

Table 1. Demographic data in patients with chronic periodontitis and controls

	Controls	Patients with periodontitis
no. of subjects	196	133
mean age (years) ± SD	47.1 ± 14.1	42.9 ± 7.2
age range (years)	33–60	35–50
sex (M/F)	94/102	68/65
% smokers	28.1	34.6

Table 2. Allele and genotype distributions of the matrix metalloproteinase-1 (MMP-1)$^{-1607}$, MMP-1^{-519}, and MMP-1^{-422} in patients with chronic periodontitis and in controls

	Total		Non-smokers		Smokers	
	controls	cases	controls	cases	controls	cases
MMP-1 (− 1607 1G/2G)						
1G/1G (%)	59 (30.1)	50 (37.6)	42 (29.8)	36 (41.4)	17 (30.9)	14 (30.4)
1G/2G (%)	93 (47.4)	62 (46.6)	65 (46.1)	36 (41.4)	28 (50.9)	26 (56.5)
2G/2G (%)	44 (22.4)	21 (15.8)	34 (24.1)	15 (17.2)	10 (18.2)	6 (13.1)
1G allele	0.538	0.609	**0.528**	**0.621**	0.564	0.587
2G allele	0.462	0.391	**0.472**	**0.379**	0.436	0.413
	$p = 0.054$		$\boldsymbol{p = 0.033}$			
MMP-1 (− 519A/G)						
AA (%)	65 (33.2)	43 (32.3)	45 (31.9)	24 (27.6)	20 (36.4)	19 (41.3)
AG (%)	86 (43.9)	59 (44.4)	64 (45.4)	40 (46.0)	22 (40.0)	19 (41.3)
GG (%)	45 (23.0)	31 (23.3)	32 (22.7)	23 (26.4)	13 (23.6)	8 (17.4)
A allele	0.551	0.545	0.546	0.506	0.564	0.620
G allele	0.449	0.455	0.454	0.494	0.436	0.380
MMP-1 (− 422A/T)						
AA (%)	42 (21.4)	28 (21.1)	32 (22.7)	20 (23.0)	10 (18.2)	8 (17.4)
AT (%)	92 (46.9)	73 (54.9)	64 (45.4)	39 (44.8)	28 (50.9)	34 (73.9)
TT (%)	62 (31.6)	32 (24.1)	45 (31.9)	28 (32.2)	17 (30.9)	4 (8.7)
					$\boldsymbol{p = 0.017}$	
A allele	0.449	0.485	0.454	0.454	0.436	0.543
T allele	0.551	0.515	0.546	0.546	0.564	0.457

Because smoking is a known risk factor for periodontitis, non-smoking and smoking subjects were analysed separately. Interestingly, when only non-smoking subjects were included in the analysis, the difference between the allelic frequencies of the 1G/2G polymorphism in the chronic periodontitis patients and control population became significant ($p = 0.033$). However, no significant differences were detected in the allelic and/or genotype distributions between smoking patients and control smokers for this variant. Conversely, as shown in Table 2, there were differences in the distribution of the –422A/T polymorphism between a subgroup of smoking patients versus smoking controls ($p = 0.017$).

Finally, we investigated the possibility that the risk of chronic periodontitis susceptibility and/or severity may be modified by a combination of the three variants. The genotypes of the three polymorphisms studied yielded 27 possible combinations, one of them was absent from both controls and patients. The overall representation of the combinations in the patients differed significantly from that found in the controls ($p < 0.05$). This difference was, for example, influenced by the decreased frequency of the 1G2G (− 1607), AA (− 519), TT (− 422) and increased frequency of the 1G2G (− 1607), AA (− 519), AT (− 422) combinations in patients, and to a lesser extent by many other combinations (evaluation based on their contribution to the overall variation, Table 3).

In a further haplotype analysis, eight possible haplotypes were deduced from

Table 3. Composite genotype distributions of the matrix metalloproteinase-1 (MMP-1) polymorphisms

	Genotypes			Controls		Patients	
	− 1607	− 519	− 422	*N*	%	*N*	%
1	1G1G	AA	AA	5	2.55	3	2.22
2	1G2G	AA	AA	3	1.53	5	3.70
3	2G2G	AA	AA	3	1.53	2	1.48
4	1G1G	AA	AT	6	3.06	3	2.22
5	**1G2G**	**AA**	**AT**	**6**	**3.06**	**13**	**9.63**
6	2G2G	AA	AT	12	6.12	11	8.15
7	1G1G	AA	TT	0	0.00	2	1.48
8	**1G2G**	**AA**	**TT**	**15**	**7.65**	**1**	**0.74**
9	2G2G	AA	TT	15	7.65	5	3.70
10	1G1G	AG	AA	10	5.10	4	2.96
11	1G2G	AG	AA	10	5.10	4	2.96
12	2G2G	AG	AA	1	0.51	1	0.74
13	1G1G	AG	AT	13	6.63	10	7.41
14	1G2G	AG	AT	33	16.84	21	15.56
15	2G2G	AG	AT	1	0.51	0	0.00
16	1G1G	AG	TT	2	1.02	4	2.96
17	1G2G	AG	TT	12	6.12	13	9.63
18	2G2G	AG	TT	4	2.04	2	1.48
19	1G1G	GG	AA	6	3.06	8	5.93
20	1G2G	GG	AA	4	2.04	1	0.74
21	1G1G	GG	AT	10	5.10	13	9.63
22	1G2G	GG	AT	8	4.08	4	2.96
23	2G2G	GG	AT	3	1.53	0	0.00
24	1G1G	GG	TT	7	3.57	3	2.22
25	1G2G	GG	TT	2	1.02	2	1.48
26	2G2G	GG	TT	5	2.55	0	0.00
				$\chi^2 = 38.110$		$p = 0.045$	

Table 4. Haplotype distributions of the matrix metalloproteinase-1 (MMP-1) polymorphisms

	Haplotypes			Relative frequency of haplotypes			
				non-smokers		smokers	
	− 1607	− 519	− 422	controls	patients	controls	patients
1	1G	A	A	0.113	**0.099**	0.124	**0.177**
2	1G	G	A	0.202	0.232	0.205	0.189
3	1G	A	T	0.075	0.075	0.127	0.074
4	1G	G	T	**0.138**	**0.214**	0.108	0.146
5	2G	A	A	0.107	0.105	**0.065**	**0.177**
6	2G	G	A	0.032	0.017	0.042	0.000
7	2G	A	T	0.251	0.226	0.248	0.191
8	2G	G	T	0.082	0.031	0.081	0.045
				$p > 0.05$			

the observed genotype frequencies as presented in Table 4. A trend to increased relative frequency of the 1G (− 1607)/G (− 519)/T (− 422) haplotype (0.214) in non-smoking patients compared with control non-smoking (0.138), as well as frequency of the 2G/A/A haplotype (0.177) in smoking patients compared with referent smokers (0.065) was observed. Using a Monte-Carlo model calculation, differences among all subgroups were non-significant ($p > 0.10$).

Conclusions

Chronic periodontitis is regarded as multifactorial disease onset and severity of which are influenced by both genetic and environmental factors (Hart & Kornman 1997). This study represents an investigation of the role of the MMP-1 in chronic periodontitis in the Czech population.

Firstly, we analysed a possible association of the 1G/2G promoter variant of the MMP-1 gene. The results of previous studies suggest that this polymorphism may have a functional consequence as it affects the functions of the MMP-1 enzyme (Rutter et al. 1998) and hence represents a candidate polymorphism of a strong biological relevance to the development of chronic inflammatory diseases (Vincenti et al. 1996). There is only one study, by de Souza et al. (2003), examining this polymorphism with respect to the pathophysiology of chronic periodontitis. The authors found marginally significant association of the 2G allele and severity of chronic periodontitis in the non-smoking Brazilians. The 2G/2G genotype was observed in 12/26 (46.15%) severe chronic periodontitis patients, in 6/24 (25.0%) moderate patients, and in 9/37 (24.3%) controls. In our study, we were able to detect only a borderline association of inverse 1G allele with chronic periodontitis in the Czech population. The association between 1G/2G polymorphism and chronic periodontitis was observed most strongly in non-smoking, and not in smoking subjects. We have shown in another study (Izakovicova Holla et al. 2002a) that genetic susceptibility to periodontitis can easily be evincible in non-smokers because degradation of connective tissues in smokers may occur as a consequence of smoking, thus possibly masking the influence of individual variants of the MMP-1 polymorphisms. Furthermore, the frequency of 1G/1G homozygotes in a subgroup of severe periodontitis non-smoking patients (40.4%) was increased in relation to non-smoking controls (29.8%), and also in comparison with smoking patients (30.4%) or smoking healthy subjects (30.9%), but it was similar to frequency of the 1G/1G genotype in mild/moderate non-smoking periodontitis patients (42.5%). These data are at odds with those reported by de Souza and co-workers but these discrepancies may reflect some involvement of variable combinations of risk alleles in different population (Moraes et al. 2003). Nevertheless, our study found similar frequencies of this polymorphism among controls composed of the Brazilian population.

Secondly, comparison of distribution of genotype combinations between periodontitis and referent groups revealed significant differences although 27 possible combinations were analysed. Combined genotype in the − 1607, − 519, and − 422 position (1G/2G-

AA-AT) was overbalanced in the periodontitis group, while the other significant contributing combination (1G/2G-AA-TT) was more frequent in the reference group. This finding affirmed the associations of these polymorphisms ascertained separately. It cannot be discerned definitively from the data presented whether these associations represent a simple additive effect of the individual substitutions.

Thirdly, the simulation haplotype model allowed us to observe eight haplotypes of the MMP-1 gene. To our knowledge, this is the first study examining MMP-1 gene haplotypes in relation to chronic periodontitis. The study shows a difference in occurrence of the relative haplotype frequencies between non-smoking and smoking subjects. However, it is interesting that haplotype 1G(−1607)-G(−519)-T(− 422) preponderated in non-smoking patients with periodontitis, while inverse haplotype 2G-A-A preponderated in smoking periodontitis subjects, even if the differences were statistically non-significant. It seems that there is a trend to increased frequency of different haplotype combinations in patients with chronic periodontitis in relation to smoking status.

In conclusion, although this study should be considered only as a pilot study, our results suggest that the polymorphisms in the MMP-1 gene promoter may have only a small effect on the development of chronic periodontitis. Our results contrast with those reported by de Souza et al. (2003), and thus do not lend support to a general association between the MMP-1 gene variants and severity of chronic periodontitis. The study of genetic associations is difficult, and is confounded by many factors as sample selection, genetic admixture, regional differences in gene frequencies, and gene–environment interactions. However, the increasing interest to find genetic markers for periodontal disease is essential. It could advance our understanding of the pathogenesis of this common complex disease, facilitate the early identification of individuals at high risk, and may provide new targets for the design of novel preventive and therapeutic measures.

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