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Association of matrix metalloproteinase-1 promoter polymorphism with generalized aggressive periodontitis in a Chinese population

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Background: A single nucleotide polymorphism in the promoter region of −1607 bp of the human matrix metalloproteinase-1 (MMP-1) gene has been found to be associated with an increased risk of various inflammatory diseases and cancer metastasis.

Objectives: The present study aimed to examine the distribution of MMP-1 genotypes in a group of Chinese subjects with generalized aggressive periodontitis and a group of periodontally healthy subjects, and to evaluate the possible association of the MMP-1 promoter polymorphism with aggressive periodontitis.

Methods: Genomic DNA was obtained from whole blood samples in 40 Chinese subjects with generalized aggressive periodontitis and 52 periodontally healthy subjects as controls. MMP-1 promoter fragment was amplified by polymerase chain reaction, and the polymorphisms were analyzed by restriction endonuclease cleavage. The alleles were detected by polyacrylamide gel electrophoresis and visualized with ethidium bromide.

Results: The detection frequency of 2G allele was significantly higher in the subjects with generalized aggressive periodontitis (68.7%) than in the control subjects (49%) ($p < 0.01$). The genotype of 2G/2G was found in 52.5% of the patients, which was significantly greater than that of control subjects (23.1%) ($p < 0.05$).

Conclusion: The present study suggests that a single nucleotide polymorphism in the MMP-1 promoter region of −1607 bp may be associated with generalized aggressive periodontitis in Chinese population.

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Human periodontitis is initiated and perpetuated by a group of predominantly Gram-negative and anaerobic bacteria that colonize the subgingival environment. The severity of periodontitis is dependent on a dynamic equilibrium of interactions between the microbial challenge and host immuno-inflammatory responses (1). Degradation of periodontal tissues is mainly mediated by matrix metalloproteinases (MMPs), a family of metal-dependent proteolytic enzymes that degrade extracellular matrix and basement membranes. Currently, at least

28 MMPs have been characterized (2, 3), many of which have been identified in inflamed periodontal tissues and gingival crevicular fluid. These enzymes are thought to play a crucial role in the destruction of periodontal tissues (4, 5).

MMP-1 is a 55 kDa enzyme and it is synthesized and secreted ubiquitously by connective tissue cells and macrophages (6), as well as by numerous tumor cells (7, 8). It is the major proteolytic enzyme that can cleave native interstitial collagens type I and III, which are the most abundant protein components of extracellular matrix in periodontium. Normally, the expression levels of MMP-1 in most cells are low, but it is readily induced by phorbol esters, growth factors and inflammatory cytokines.

A genetic variation in the MMP-1 promoter region can influence the levels of MMP-1 transcription, and hence this gene may be crucial in mediating connective tissue degradation in the pathogenesis of periodontitis. This variation is a single nucleotide polymorphism located at −1607 bp, where an additional guanine (G) creates an Ets binding site, 5′-GGA-3′, instead of 5′-GAT-3′, the core binding site for members of the Ets transcription factors (9). It has been shown that the 2G allele significantly increased the transcription activity of MMP-1 as compared to the 1G allele (9). The 2G allele displays heightened MMP-1 transcription both in tumor cells and in normal fibroblasts, and the levels of MMP-1 expression may result from the presence of the 2G allele and/or from elevated expression of the transcription factors that bind to this site. The presence of the 2G allele has recently been associated with severe chronic periodontitis (10) and the development of breast cancer and ovarian cancer (7, 8).

Evidence of studies on population and family has indicated that inter-individual genomic variations might have a strong influence on the susceptibility to aggressive periodontitis (11–13). Polymorphisms in the interleukin-1 cluster have been the main focus of attention in recent years. In contrast to genetic association in chronic periodontitis, studies of aggressive periodontitis have not yielded consistent results (14). There are reports on the association of interleukin-1 genotypes with risk of aggressive periodontitis (15, 16), whereas others failed to find such an association (17, 18). These different results may be related to the heterogeneity of the disease, variations in sample selection and ethnic backgrounds, and variable design of the reported studies (14). Further study is therefore necessary to identify specific genetic markers of aggressive periodontitis. The present study was to examine the distribution of MMP-1 genotypes in a group of Chinese subjects with generalized aggressive periodontitis and a group of periodontally healthy subjects, and to evaluate the possible association of the MMP-1 promoter polymorphism with generalized aggressive periodontitis.

Materials and methods

Subjects

Ninety-two Chinese adults were recruited for the study, consisting of 40 subjects with generalized aggressive periodontitis from the patient pool of the Periodontology Clinic at the School of Stomatology, Wuhan University and 52 subjects with healthy periodontal conditions as controls. The full-mouth clinical parameters recorded included plaque index, bleeding on probing, probing pocket depth and clinical attachment loss at six sites per teeth. The subject data are presented in Table 1.

The diagnosis of generalized aggressive periodontitis was made on the basis of past dental history, clinical parameters and radiographic patterns of alveolar bone loss, following the criteria defined by the American Academy of Periodontology in 1999 (19). All subjects with generalized aggressive periodontitis were below the age of 35 years and presented with > 5 mm of attachment loss in at least one site on more than eight teeth and three of these teeth were other than first molars and incisors. The periodontally healthy subjects did not show any sites with probing pocket depth > 3 mm and clinical attachment loss > 1 mm in any quadrant or radiographic evidence of bone loss. The general health of all subjects recruited was good. Among the 40 patients with generalized aggressive periodontitis, 10 were smokers who smoked less than five cigarettes per day for less than 5 years. None of the control subjects were smokers. The nature and procedures of the study were explained and informed consents were obtained from all recruits, and the Ethics Committee, School of Stomatology, Wuhan University approved the study protocol.

Sampling

Samples of peripheral blood were obtained by direct venipuncture from the arm vein of each subject. The 20-ml blood samples collected were transferred to EDTA tubes and were stored at −70°C until used for extraction of DNA.

Extraction of DNA

DNA was extracted using a genetic extracting kit (Takara Biotechnology (Dalian) Co. Ltd, Dalian, China). DNA was dissolved in Tris-EDTA buffer (10 mM Tris pH 7.8, 1 mM EDTA). The DNA concentration was estimated by measurement of OD_{260}. The final preparation was stored at −20°C until further analysis.

Table 1. Subjects data

Subject groups	*n*	Age (years) Mean ± SD	Gender		Probing depth (mm)	Clinical attachment loss (mm)
			Male	Female		
GAgP	40	28.7 ± 5.3	18 (45%)	22 (55%)	4.3 ± 1.2	5.5 ± 1.4
Control	52	26.5 ± 4.2	28 (54%)	24 (46%)	2.0 ± 0.3	0.0

n, number of subjects; GAgP, generalized aggressive periodontitis.

Polymerase chain reaction (PCR)

The sequences of the PCR primers were: forward, 5′ –TCGTGAGA-ATGTCTTCCCATT 3′; reverse, 5′-TCTTGGATTGATTTGAGATAA-GTGAAATC-3′. The reverse primer was specially designed to introduce a recognition site of restriction enzyme *Xmn*I, according to the protocol employed in previous studies (10, 20). The 1G allele has this recognition site, whereas the 2G allele destroys the recognition site by inserting a guanine (G). PCR was carried out in a total volume of 25 μl, containing 100 ng genomic DNA, 10 mM Tris-His (PH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 pM of each primer and 2 U Taq DNA polymerase (Biostar International, Toronto, ON, Canada). The PCR cycling conditions were 1 min at 95°C followed by 35 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C, with a final extension of 72°C for 5 min.

Restriction endonuclease cleavage

A 15 μl aliquot of PCR products was mixed with 4 μl of solution containing 2 μl 10 × nuclear extract (NE) buffer, 2 μl bovine serum albumin (1 mg/ml) and 1 μl of *Xmn*I (10 U/ml, Promega, Madison, WI, USA). The reaction took place at 37°C over night.

Gel electrophoresis

The total amount of the digestion aliquot was mixed with 2 μl of loading buffer. The electrophoresis was performed on a 12% vertical non-denaturing polyacrylamide gel at 50 V for 4 h and the gel was visualized with ethidium bromide.

Statistical analysis

The difference in genotype distribution between the patient group and control group was assessed by the chi-squared test. A p-value of < 0.05 was considered statistically significant. The risk associated with individual alleles and genotype was calculated as the odds ratio (OR) with 95% confidence intervals (95% CI). Statistical analyses were performed using the SPSS 11.0 statistical package (SPSS Inc., Chicago, IL, USA).

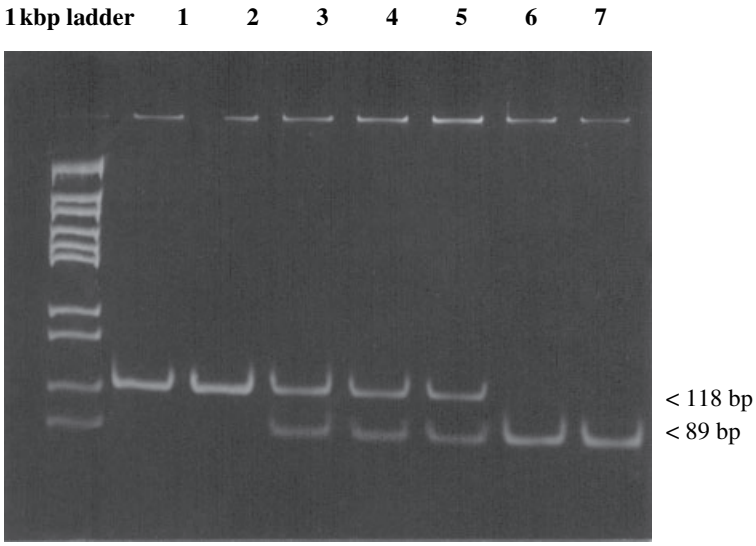

Fig. 1. Electrophoresis results of matrix metalloproteinase-1 −1607 1G/2G polymorphisms performed by *Xmn*I. Two mismatches were introduced in the reverse primer annealed sequence (5′-GAANNNNTTC-3′) for the restriction endonuclease *Xmn*I site. Thus, *Xmn*I digested the 1G allele, creating two fragments of 89 bp and 29 bp (the 29 bp fragment was too small to display in the electrophoresis figure). So 1G/1G homozygotes displayed a single 89 bp band, 2G/2G homozygotes showed a single 118 bp band, and heterozygotes exhibited two bands: 118 bp and 89 bp. The size marker is 1000 bp ladder (pGEM-7Zf(+)DNA/*Hae*III markers) shown at 657 bp, 458 bp, 434 bp, 328 bp, 289 bp, 267 bp, 174 bp, 142 bp, 102 bp, 80 bp, etc. Lane 1, polymerase chain reaction products; lane 2, 2G/2G homozygote; lanes 3–5, 1G/2G heterozygote; lanes 6–7, 1G/1G homozygote.

Results

Two mismatches were introduced into the reverse primer annealed sequence (5′-GAANNNNTTC-3′) for the restriction endonuclease *Xmn*I site. Thus, *Xmn*I digested the 1G allele, creating two fragments of 89 bp and 29 bp (Fig. 1). The polymorphism demonstrated Hardy–Weinberg equilibrium.

As shown in Table 2, the detection frequency of 2G allele was significantly higher in the subjects with generalized aggressive periodontitis (68.7%) than in the control subjects (49.0%) ($p < 0.01$). Individuals with the 2G allele seem to be approximately twice at greater risk for developing the generalized aggressive periodontitis ($p = 0.007$, OR = 2.286, 95% CI = 1.243–4.205). Overall, there was a significant difference in the detection frequency of genotypes between the patient and control groups ($\chi^2 = 8.513$, $p = 0.014$) (Table 3). The genotype of 2G/2G was found in 52.5% of the patients, which was significantly greater than that of control subjects (23.1%) ($p < 0.05$). Individuals with the 2G/2G genotype seem to be three times more at greater risk for developing the disease than those who are 1G/1G and 1G/2G genotypes ($p = 0.004$, OR = 3.684, 95% CI = 1.505–9.018) (Table 3).

Discussion

Human periodontitis is initiated and perpetuated by a group of predominantly gram-negative and anaerobic bacteria and it characterized by inflammatory destruction of connective tissues and alveolar bone. It is generally agreed that although bacteria are essential for disease initiation, they are insufficient for the disease progression and outcomes. The severity of periodontal

Table 2. Distribution of the matrix metalloproteinase-1 alleles in the control and subjects with generalized aggressive periodontitis

	Controls		GAgP			
Alleles	*n*	%	*n*	%	*p*-value	OR (95%CI)
1G	53	51.0	25	31.3	0.007	
2G	51	49.0	55	68.7		2.286 (CI = 1.243–4.205)

n, number of alleles; GAgP, generalized aggressive periodontitis; OR, odds ratio; CI, confidence interval.

Table 3. Distribution of the matrix metalloproteinase-1 genotypes in the control and subjects with generalized aggressive periodontitis (GAgP). N = number of subjects

	Controls		GAgP		
Genotypes	*n*	%	*n*	%	*p*-value
1G/1G	13	25.0	6	15.0	0.014
1G/2G	27	51.9	13	32.5	
2G/2G	12	23.1	21	52.5	

destruction is rather dependent on a dynamic equilibrium of bacteria–host interactions that could be significantly modified by multiple environmental and acquired risk factors and genetic risk factors (1, 21). Recent studies provided evidence that nearly half of the variance in clinical profiles of chronic periodontitis could be attributed to genetic factors (22, 23). In recent years, genetic polymorphisms in cytokines and inflammatory mediators have been the main focus of biomedical research. Genetic polymorphisms are known to affect both qualitative and quantitative aspects of host responses to microbiological challenge. Further investigation into the linkage of these genetic polymorphisms with stable phenotypic characteristics of periodontitis patients may provide the framework for identification of molecular biomarkers to be incorporated into individual risk profiles and may also help to set the foundation for developing new treatment strategies (24).

Much of the evidence for a genetic role in periodontitis has come from the extensive studies on aggressive periodontitis (24). It is noted that studies on this particular form of periodontitis may likely identify the genetic variation of the disease and provide invaluable information for better clinical management of the patients.

The present study, for the first time, reported the distribution of MMP-1 polymorphism at −1607 bp in the MMP-1 promoter in a group of Chinese subjects with generalized aggressive periodontitis and in Chinese subjects with a healthy periodontal condition. We found that the MMP-1 polymorphism was associated with aggressive periodontitis in the Chinese population investigated. The detection frequency of 2G allele was significantly higher in the subjects with generalized aggressive periodontitis (68.7%) than in the control subjects (49.0%). Individuals with the 2G allele seemed to be approximately twice at greater risk for developing the generalized aggressive periodontitis with an odds ratio of 2.3. This single nucleotide polymorphism in the MMP-1 promoter is not a mutation or genetic variation found in tumor cells. In a previous study, Rutter *et al.* (9) showed that the distribution of the single nucleotide polymorphism of MMP-1 promoter in a normal population was approximately 30% (1G homozygous), 30% (2G homozygous) and 40% (1G/2G heterozygous). However, in tumor cells cultured *in vitro*, the incidence of the 2G allele rose to 62.5%, suggesting the MMP-1 polymorphism may correlate with more aggressive matrix degradation and facilitate cancer progression (9).

Our current study found that the prevalence of the MMP-1 genotype of 2G/2G in these patients with generalized aggressive periodontitis (52.5%) was significantly greater than that of healthy control subjects (23.1%), and the individuals with this genotype appear to be three times at greater risk for developing the disease than individuals who have 1G/1G and 1G/2G genotypes. Our findings are consistent with previous studies on cancer that have linked the MMP-1 2G/2G genotype with an increase risk of development of breast cancer (7) and ovarian cancer (8), as well as colorectal cancer progression (25). The genetic polymorphism could augment transcription activity and increase the levels of protein expression of MMP-1 (9). Given the strong link between increased MMP-1 expression and the presence of the 2G allele, it is possible that this genetic polymorphism may provide a useful and potentially important mechanism for the increased degradation of periodontal tissues that is observed in patients with aggressive periodontitis. A recent study in a Brazilian population showed that the MMP-1 promoter polymorphism was associated with the severe chronic periodontitis phenotype (10). Holla *et al.* (26) recently demonstrated that the association between 1G/2G polymorphism of MMP-1 and chronic periodontitis was observed most strongly in non-smokers but not in smokers, and the polymorphisms in the MMP-1 promoter may have a limited effect on the etiopathogenesis of chronic periodontitis. In contrast, a recent report from a Japanese population showed no significant differences in genotype distributions, allele frequencies, carriage rates and haplotype frequencies in the MMP-1 gene promoter polymorphisms among different groups of subjects with aggressive periodontitis, chronic-periodontitis or those with healthy periodontal conditions (27), which was different from those previously reported in Brazilian (10) or Czech populations (26). Although the distribution of certain genetic polymorphism may vary with different ethnic groups, our present study substantiates that 1G/2G polymorphism could be a risk factor for generalized aggressive periodontitis in Chinese population. Taken together, these studies suggest that this MMP-1 promoter polymorphism could serve as a potential genetic marker for aggressive periodontitis. As human periodontitis is a multifactorial inflammatory disease with a complex pathogenesis, the relationship between a genetic polymorphism and the severity of periodontal destruction is deemed to be complex and should be interpreted with caution. Further longitudinal study

with a large sample is warranted to confirm our initial findings.

In conclusion, the present study for the first time reported the distribution of MMP-1 polymorphism at −1607 bp in the MMP-1 promoter in a group of Chinese subjects with generalized aggressive periodontitis and in Chinese subjects with a healthy periodontal condition as controls. A single nucleotide polymorphism in the MMP-1 promoter region of −1607 bp may be associated with generalized aggressive periodontitis in Chinese population.

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