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Association analysis between the −2518MCP-1 (A/G) polymorphism and generalized aggressive periodontitis in a Chinese population

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Background and Objective: It has been suggested that aggressive periodontitis has a genetic basis. Monocyte chemoattractant protein 1 (MCP-1) plays a critical role in the recruitment of monocytes and the development of periodontitis. The −2518MCP-1(A/G) polymorphism has been implicated as a risk or susceptibility factor for a variety of autoimmune conditions and inflammatory diseases. The intent of this investigation was to study whether the −2518MCP-1(A/G) polymorphism is associated with generalized aggressive periodontitis in the Chinese population.

Material and Methods: One hundred and twenty-four patients with generalized aggressive periodontitis and 94 healthy subjects were included in this case–control study. Genomic DNA was isolated from a peripheral blood sample obtained from each subject. Gene polymorphisms of −2518MCP-1(A/G) were analyzed by a standard polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay. A logistic regression analysis was performed to test the association between the −2518MCP-1(A/G) genotype (alleles) and generalized aggressive periodontitis with adjustment of the major covariates (gender, age and smoking status).

Results: There was no significant association of the −2518MCP-1(A/G) polymorphism with generalized aggressive periodontitis in the unstratified subjects. However, when patients were stratified by gender, the frequency of the G^+ genotype was significantly lower in female patients with generalized aggressive periodontitis compared with female controls ($p = 0.036$, adjusted odds ratio = 0.3, 95% CI: 0.1–0.9). In female patients with generalized aggressive periodontitis, the probing pocket depth was larger in subjects with the AA genotype than in subjects with the G^+ genotype (5.07 mm vs. 4.30 mm; $Z = -2.470$, $p = 0.014$).

Conclusion: The polymorphisms of −2518MCP-1 may play an important role in determining generalized aggressive periodontitis susceptibility in this cohort of Chinese women.

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Aggressive periodontitis is a subgroup of periodontal diseases characterized by significant and relatively rapid destruction of the periodontal supporting tissues in otherwise healthy adolescents and young adults (1). While periodontitis is a multifactorial disease with microbes as the initiator, the manifestation and progression are the outcome of complex interactions between oral bacteria and the host response, often modulated by behavioral factors. The results of population and familial studies in aggressive periodontitis (2), as well as a twin study in chronic periodontitis (3), indicate that genetic factors seem to have a strong influence on susceptibility to periodontitis.

Monocytes are an important component of the host defence system, playing a pivotal role in the maintenance of host–parasite homeostasis in periodontitis (4,5). Monocyte chemoattractant protein 1 (MCP-1, also called CCL2), a potent mediator of both monocyte recruitment and activation, is expressed in the chronic inflammatory infiltrate and along the basal layer of the oral epithelium in periodontitis, and monocytes/macrophages were demonstrated to be present in the same areas. In human inflamed gingival tissue, the numbers of cells expressing MCP-1 are related to the degree of inflammation (4,6,7). Patients with chronic periodontitis and aggressive periodontitis have significantly higher levels of MCP-1 in the gingival crevicular fluid compared with periodontally healthy subjects, and the MCP-1 levels in gingival crevicular fluid are positively correlated with both probing depth and clinical attachment loss (8–10). All of the above suggest that MCP-1 plays an important role in the recruitment of monocytes and amplification of inflammatory signals in bacterially induced periodontitis.

Recently, a bi-allelic A/G polymorphism has been found in the MCP-1 distal gene-regulatory region at position −2518 (the number indicates the nucleotide position relative to the major transcription start site) that affects the level of MCP-1 expression in response to an inflammatory stimulus. Monocytes from individuals carrying a G allele at position −2518 produce more MCP-1 after treatment with interleukin (IL)-1beta than monocytes from A/A homozygous subjects (11,12). The −2518MCP-1(A/G) polymorphism has been implicated as a risk or susceptibility factor for a variety of autoimmune conditions and inflammatory diseases, such as asthma (13), rheumatoid arthritis (14), coronary artery disease (12) and systemic lupus erythematosus (15).

The effect of the −2518MCP-1(A/G) polymorphism on periodontitis has not been investigated until now. Considering that there might be a genetic basis for aggressive periodontitis, the present study was designed to explore the association of the −2518MCP-1(A/G) polymorphism with generalized aggressive periodontitis in the Chinese population.

Material and methods

Study population

One hundred and twenty-four patients with generalized aggressive periodontitis and 94 healthy subjects were included in the case–control study. The patients with generalized aggressive periodontitis were recruited from the periodontic clinics at Peking University, School and Hospital of Stomatology. The diagnostic criteria for generalized aggressive periodontitis were based on the 1999 International Classification of The Periodontal Diseases and Conditions (1). Diagnoses were confirmed by periodontal examination and full-mouth periapical radiographs. The following clinical criteria were used: (i) under 35 years of age; (ii) at least six teeth (at least three of which were not first molars or incisors) with a probing depth of ≥ 5 mm and clinical attachment of ≥ 3 mm; (iii) no periodontal treatments within the past 12 mo; (iv) female patients were not pregnant or lactating; and (v) clinically healthy, except for the presence of periodontitis.

Some of the healthy control subjects were selected from staff and students at the School and Hospital of Stomatology, and others were volunteers visiting the same place for regular dental check-ups. None of the healthy subjects had previous or existing clinical evidence of periodontitis (probing depth ≤ 3 mm; clinical attachment distances ≤ 1 mm from the cemento–enamel junction; and < 10% of sites with a bleeding index of ≥ 2). None had a familial history of severe periodontitis or a known systemic disorder that could affect the periodontal conditions. All clinical examinations and diagnoses were performed by two skilled practitioners. Smoking status was also recorded. All subjects were Han Chinese people and were unrelated. The study protocol was approved by the Ethics Committee of Peking University Health Science Center. Informed consent was obtained from all participants, in accordance with the Declaration of Helsinki.

Isolation of genomic DNA

A 2-mL EDTA-anticoagulated peripheral blood sample was obtained from each subject by venipuncture. Genomic DNA was isolated from each sample using a blood DNA mini kit (Watson Biotechnologies, Inc., Shanghai, China) following the manufacturer's instructions. DNA integrity was checked and DNA quantified using agarose-gel electrophoresis. Plasma was also separated and stored frozen at −70°C until required for analysis.

−2518MCP-1(A/G) genotyping

A standard polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was utilized for −2518MCP-1(A/G) genotyping, as described previously (12). PCR amplification was performed at an optimum annealing temperature in a thermocycler (PTC-200; MJ Research, Inc., Watertown, MA, USA). PCR products were checked by electrophoresis on a 2% [weight in volume (w/v)] agarose gel, and the target fragment was digested by the corresponding restriction endonuclease (New England Biolabs, Beverly, MA, USA) according to the manufacturer's instructions (Table 1). Digested products were detected by gel electrophoresis. Position

Table 1. Genotyping of the −2518MCP-1(A/G) polymorphism

Gene	Variant	Primer sequences (5′ → 3′)	PCR		Digestion	
			Size (bp)	T_m[a]	Enzyme	T[b]
MCP-1	A/G	*Forward: TCTCTCACGCCAGCACTGACC *Reverse: GAGTGTTCACATAGGCTTCTG	234 bp	62°C	*Pvu*Π; G = *Pvu*Π(+) A = *Pvu*Π(−)	37°C

[a]Annealing temperature.
[b]Incubation temperature.
*These two primers were described by Szalai *et al.* (12).
MCP-1, monocyte chemoattractant protein 1; PCR, polymerase chain reaction.

−2518 in the *MCP-1* gene has an adenine (A) to guanine (G) substitution, and the G allele is a complete cleavage site of *Pvu*Π. Digestion of the target fragment (234 bp) with *Pvu*Π yields fragments of 159 and 75 bp when G is at position −2518. The digested products were separated by electrophoresis on a 3% (w/v) agarose gel.

Measurement of plasma MCP-1

The plasma MCP-1 levels were detected using a commercially available ELISA (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

Quality control

To confirm the genotyping results, direct sequencing of the PCR products was performed in 20% of the heterozygous subjects at the polymorphism loci. The restriction site was confirmed by direct DNA sequencing. After purification using a gel extraction kit (E.Z.N.A.; Omega Bio-tek, Inc, Doraville, GA, USA), PCR products were sequenced using a genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Quantitative variables (age) were expressed as mean ± standard deviation, and categorical variables (gender and smoking status) were presented as counts and percentages. Differences between the patient group and the control group were tested with parametric tests (*t*-tests or chi-square tests). A logistic regression analysis was performed to test the association between the −2518MCP-1(A/G) genotype (alleles) and generalized aggressive periodontitis with adjustment of the major covariates (gender, age and smoking status).

The plasma MCP-1 concentrations and clinical parameters (probing pocket depth and attachment loss) were not normally distributed and therefore the between-group differences of these variables were tested using the Mann–Whitney *U*-test. Spearman correlation analysis was used to test the relationships between plasma MCP-1 concentration and clinical parameters.

All p values were two-sided and defined as $p < 0.05$ for statistical significance. The strength of the associations was determined using an odds ratio (OR) calculation and 95% confidence interval (95% CI).

Results

Study population characteristics are shown in Table 2. The age and gender distribution was reasonably similar in patient and control groups. The mean ages of patients with generalized aggressive periodontitis and control subjects were 28.9 and 30.7 years, respectively. There was a significant difference in smoking status between patients and controls (18.5% vs. 5.3%, $\chi^2 = 8.36$, $p = 0.004$).

The genotype and allele distributions of the −2518MCP-1(A/G) polymorphism in patients with generalized aggressive periodontitis and healthy controls are shown in Table 3. The MCP-1 genotype distributions satisfied the criteria for Hardy–Weinberg equilibrium in each group. There

Table 2. Study population characteristics

Characteristic	GAgP patients (n = 124)	Healthy controls (n = 94)
Age (years)	28.9 ± 6.7	30.7 ± 8.2
Gender		
Male	58 (46.8)	45 (47.9)
Female	66 (53.2)	49 (52.1)
Smoking status*		
Nonsmoker	101 (81.5)	89 (94.7)
Current smoker	23 (18.5)	5 (5.3)

Data are given as mean ± standard deviation or as n (%).
*Significantly different distribution between patients with generalized aggressive periodontitis (GAgP) and healthy controls: $\chi^2 = 8.36$, $p = 0.004$.

Table 3. Genotype and allele frequency distributions of the −2518MCP-1(A/G) polymorphism in patients with generalized aggressive periodontitis (GAgP) and in healthy controls

Polymorphism	Frequency distributions		
	GAgP patients (n = 124)	Healthy controls (n = 94)	p
Genotype			
A/A	32 (25.8)	18 (19.1)	
A/G	56 (45.2)	42 (44.7)	0.390
G/G	36 (29.0)	34 (36.2)	
Allele			
A	120 (48.4)	78 (41.5)	
G	128 (51.6)	110 (58.5)	0.152

Data are given as n (%), unless indicated otherwise.

was no significant difference in the distributions of the genotypes and alleles when the patients with generalized aggressive periodontitis as a whole were compared with the controls, even after adjusting for age, gender and smoking status in the multivariate logistic regression (Table 4). However, when each of the two groups was separated into male and female subgroups, a significant decrease in the frequency of the G^+ genotype was found in female patients with generalized aggressive periodontitis compared with healthy female controls (71.2% vs. 85.7%, adjusted OR: 0.3, 95% CI: 0.1–0.9, $p = 0.036$). A difference was also noted in the distribution of the G allele in female subjects (48.5% vs. 59.2%, adjusted OR: 0.6, 95% CI: 0.4–1.1, $p = 0.093$), although the difference was not significant. No significant association of the −2518MCP-1(A/G) polymorphism with generalized aggressive periodontitis was found in male subjects (Table 4).

The values of probing pocket depth and attachment loss in female patients with generalized aggressive periodontitis are shown in Fig. 1. The probing pocket depth was higher in AA subjects than in G^+ subjects (5.07 mm vs. 4.30 mm; $Z = -2.470$, $p = 0.014$). There was no significant difference in attachment loss between the two groups (4.84 mm vs. 4.15 mm; $Z = -1.739$, $p = 0.082$).

The plasma levels of MCP-1 in patients with generalized aggressive periodontitis were higher than in healthy controls (154.07 pg/mL vs. 91.66 pg/mL; $Z = -4.087$, $p = 0.000$, Fig. 2), but not associated with probing pocket depth and attachment loss ($r = -0.027$, $p = 0.866$ and $r = -0.159$, $p = 0.328$, respectively). Furthermore, there were no significant differences in plasma MCP-1 levels between the G^+ genotype and the AA genotype, either in all patients ($Z = -0.835$, $p = 0.417$; Fig. 3) or in the female subjects ($Z = -0.952$, $p = 0.368$; Fig. 4).

In addition, the results of direct sequencing were coincident with those of restriction enzyme reaction in all selected subjects. The coincidence rate was 100%.

Discussion

In the present study, the gene polymorphism of MCP-1 was analyzed. The genotype and allele frequencies were similar to those of previously reported studies in healthy Chinese subjects (16,17), and those in Japanese (18) and Korean (19) subjects. The genotype and allele frequencies in

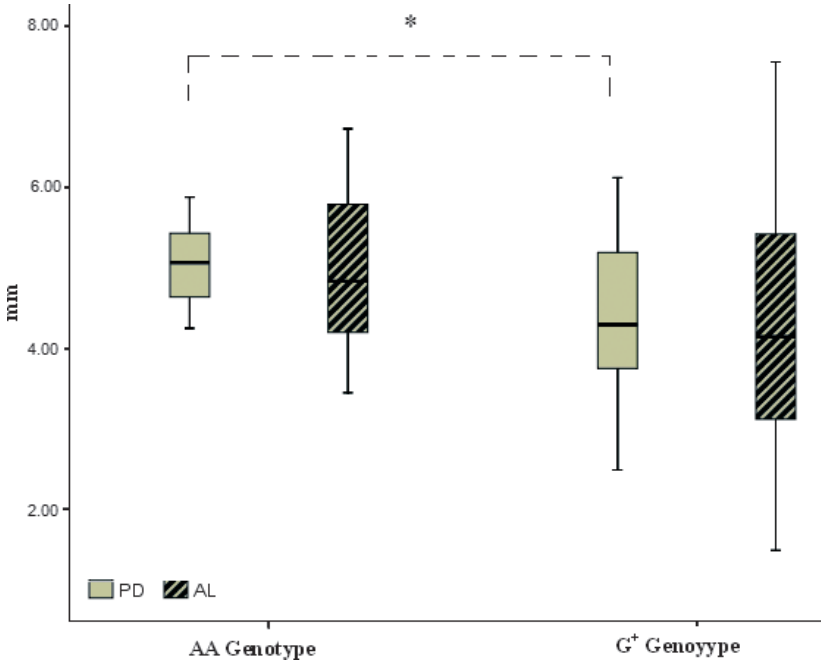

Fig. 1. Probing pocket depth and attachment loss in female patients with generalized aggressive periodontitis. Differences between probing pocket depth and attachment loss were tested between AA subjects and G^+ subjects using the Mann–Whitney *U*-test. *The probing pocket depth was higher in AA subjects than in G^+ subjects ($p < 0.05$).

Table 4. Adjusted associations between the −2518MCP-1(A/G) genotype/allele and generalized aggressive periodontitis (GAgP)

	Total study population[a]					Male subjects[b]					Female subjects[b]				
	Control	GAgP	GAgP vs. control			Control	GAgP	GAgP vs. control			Control	GAgP	GAgP vs. control		
MCP-1	*n* (%)	*n* (%)	OR	95% CI	*p*	*n* (%)	*n* (%)	OR	95% CI	*p*	*n* (%)	*n* (%)	OR	95% CI	*p*
Genotype															
AA	18 (19.1)	32 (25.8)	1			11 (24.4)	13 (22.4)	1			7 (14. 3)	19 (28.8)	1		
G^+	76 (80.9)	92 (74.2)	0.6	0.3–1.2	0.122	34 (75.6)	45 (77.6)	0.9	0.3–2.4	0.861	42 (85.7)	47 (71.2)	0.3	0.1–0.9	0.036
Allele															
A	78 (41.5)	120 (48.4)	1			38 (43.3)	52 (44.8)	1			40 (40.8)	68 (51.5)	1		
G	110 (58.5)	128 (51.6)	0.7	0.5–1.0	0.077	52 (56.7)	64 (55.2)	0.8	0.4–1.4	0.450	58 (59.2)	64 (48.5)	0.6	0.4–1.1	0.093

[a]Multiple logistic regression model adjusted for gender (male, female), age (tertile) and smoking status (no, yes).
[b]Multiple logistic regression model adjusted for age (tertile) and smoking status (no, yes).
95% CI, 95% confidence interval; MCP-1, monocyte chemoattractant protein 1; OR, odds ratio.

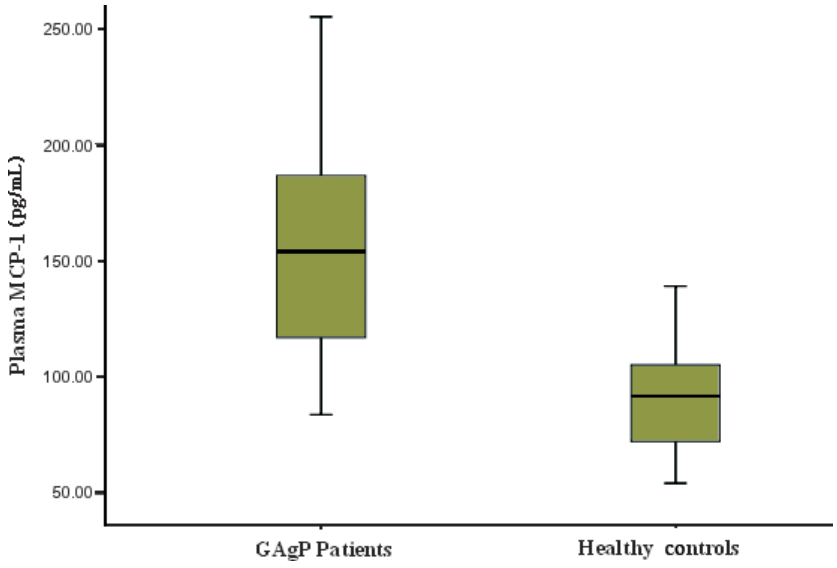

Fig. 2. The plasma monocyte chemoattractant protein 1 (MCP-1) levels of patients with generalized aggressive periodontitis and healthy controls. *Statistically significant difference between the two groups ($p < 0.01$; Mann–Whitney *U*-test).

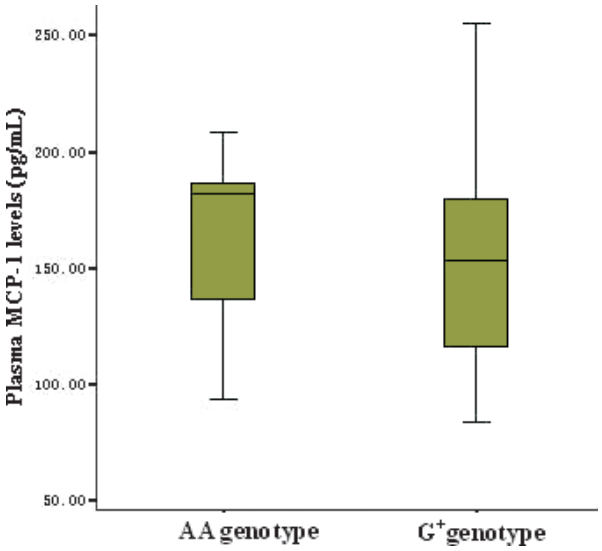

Fig. 3. Comparison of plasma monocyte chemoattractant protein 1 (MCP-1) levels between patients with generalized aggressive periodontitis and the AA genotype and patients with generalized aggressive periodontitis and the G^+ genotype. There were no significant differences in the plasma MCP-1 levels between patients with the AA genotype and those with the G^+ genotype ($p > 0.05$; Mann–Whitney *U*-test).

Chinese subjects were obviously different from those of African–American, Caucasian and Hispanic subjects (11,12,20); the G-allele frequency was increased in Chinese subjects.

The frequency of the G^+ genotype at −2518MCP-1 was significantly decreased in female patients with generalized aggressive periodontitis in the present study. An investigation on gender-dependent HLA associations with periodontitis revealed some gender-determined differences in HLA deviations in relation to aggressive periodontitis (21). Li *et al.* found that the polymorphisms of IL-1A +4845 and IL-1B −511 may play an important role in determining generalized aggressive periodontitis susceptibility in Chinese men (22). Zhang *et al.* (23) reported that the XX genotype of the estrogen receptor-alpha (ER-alpha) gene might be a risk indicator for chronic periodontitis in the female Han Chinese population. It is interesting that the DNA samples for the present study were selected from the same subjects as in the studies of Li *et al.* and Zhang *et al.* All these associations indicate that the combined effect of gene polymorphisms and gender may play a role in individual predisposition to periodontitis.

The A/G polymorphism in the *MCP-1* distal gene-regulatory region at position −2518 affected the level of *MCP-1* expression in response to an inflammatory stimulus. Monocytes from individuals carrying a G allele at position −2518 produced more MCP-1 after treatment with IL-1β than did monocytes from A/A homozygous subjects (11,13). The molecular basis for the effect of the −2518 G allele on MCP-1 transcription is not yet clear. Rovin *et al.* (11) speculated that this polymorphism did not alter the known transcription factor-binding sites of the MCP-1 distal regulatory region, but might affect a previously unidentified site. Validating this concept, recent studies suggested that the G allele might represent a genetic susceptibility to a variety of autoimmune conditions and inflammatory diseases, and the frequencies of the G^+ genotype and G allele were increased significantly in patients. However, the frequency of the G^+ genotype was decreased significantly in female patients in the present study. It was reported that the GA/GA haplotype genotype might be a two-edged genetic risk factor in HIV infection (24). The expression of MCP-1 appeared to provide partial protection from viral infection; once inflammation had been established, MCP-1 expression served primarily as a mediator of inflammation and leukocyte recruitment rather than as an inhibitor of HIV infection. With respect to the two-edged effect of MCP-1 expression, the decreased G^+ genotype may be related to insufficient MCP-1 expression and to a lower protective effect in the present study. Therefore,

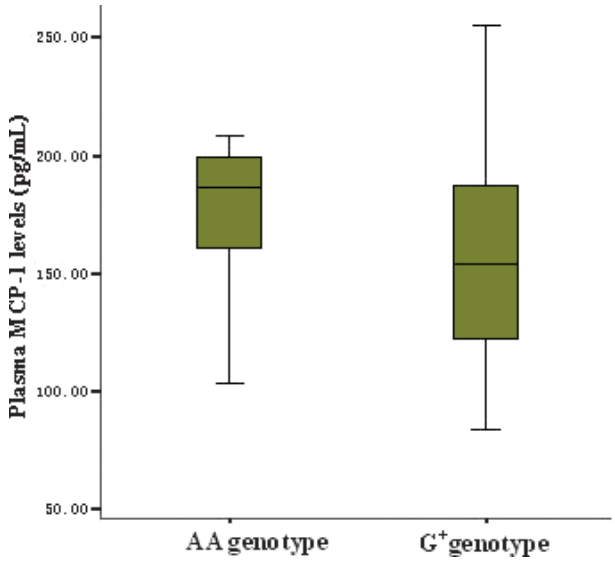

Fig. 4. Comparison of plasma monocyte chemoattractant protein 1 (MCP-1) levels between female patients with generalized aggressive periodontitis and the AA genotype and female patients with generalized aggressive periodontitis and the G^+ genotype. There were no significant differences in the plasma MCP-1 levels between patients with the AA genotype and those with the G^+ genotype ($p > 0.05$; Mann–Whitney *U*-test).

the female patients with the AA genotype were probably more susceptible to aggressive periodontitis. Consistent with the above assumption, an association was observed between MCP-1 genotype and the mean probing depth in female patients with generalized aggressive periodontitis. The probing pocket depth was higher in AA subjects than in G^+ subjects ($Z = -2.470$, $p = 0.014$).

The plasma levels of MCP-1 were increased in patients with generalized aggressive periodontitis compared with healthy controls ($Z = -4.087$, $p = 0.000$). The possible reason for the increase of plasma levels of MCP-1 in patients with generalized aggressive periodontitis could be either spillover from the gingival crevicular fluid or gingival tissues to the peripheral circulation or it could be caused by a systemic inflammatory response to progressive disease in the periodontal pocket. However, the association was not observed between plasma MCP-1 levels and MCP-1 genotype, either in all patients ($Z = -0.835$, $p = 0.417$) or in female patients ($Z = -0.952$, $p = 0.368$).

It should be noted that genetic factors play a role in aggressive periodontitis and it is more likely that many loci with small effects contribute to aggressive periodontitis, with possibly the influence of environmental factors (25). A recent study of the MMP1-1607 single nucleotide polymorphism in chronic periodontitis demonstrated a limited role of gene polymorphism, where extensive chronic antigenic challenge overcame the genetic control and played a major role in the determination of MMP-1 expression (26). The present study shows the results of a preliminary analysis only, which should be confirmed by further investigations.

To the best of our knowledge, this is the first association study between the −2518MCP-1(A/G) polymorphism and generalized aggressive periodontitis susceptibility in a relative large population. The study indicated the association of a −2518MCP-1(A/G) polymorphism with a subgroup (female) of patients with generalized aggressive periodontitis. This marker might be used to identify subgroups at higher risk of aggressive periodontitis in Chinese patients.

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