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Association between lactoferrin gene polymorphisms and aggressive periodontitis among Taiwanese patients

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Background and Objective: A dramatic difference in the frequencies of the Lys/Arg single nucleotide polymorphism in the lactoferrin genotype between a small population of patients with localized juvenile periodontitis and healthy subjects has been reported. As the single nucleotide polymorphism could be associated with ethnicity, the present study aimed to investigate the association between polymorphisms of the lactoferrin gene and periodontitis.

Material and Methods: Sixty-five patients with aggressive periodontitis, 278 with chronic periodontitis and 88 healthy controls were genotyped for the Lys/Arg polymorphism of the lactoferrin gene at position 29 [reference sequence (rs) 1126478] in the N-terminal alpha-helical region.

Results: The frequencies of the GG genotype and the G allele were highest in the aggressive periodontitis group, followed by the chronic periodontitis group and then the healthy controls. The frequency of the G allele was significantly higher in aggressive periodontitis and chronic periodontitis groups than in healthy controls ($p = 0.0037$ and 0.0212). Although the difference of the GG genotype distribution between subjects with chronic periodontitis and healthy controls did not reach significance, the distribution of genotypes between aggressive periodontitis and healthy controls was significantly different. The association of the gene polymorphism and aggressive periodontitis still existed, even after adjusting for age, gender and smoking status by logistic regression analysis (GG/AG + AA: odds ratio = 2.16, 95% confidence interval = 1.09–4.35, $p = 0.0287$). After the study, subjects were further stratified by their smoking status; the GG genotype was still significantly associated with the risk of aggressive periodontitis in the nonsmoking group (odds ratio = 2.69, $p = 0.018$). However, there were no statistical differences between chronic periodontitis vs. healthy controls and aggressive periodontitis vs. healthy controls in the smoking group.

Conclusion: The present study revealed that the A/G polymorphism in the lactoferrin gene might be associated with aggressive periodontitis. The A allele might reduce the risk of development of aggressive periodontitis in a Taiwanese population. Our results also support the hypothesis that lactoferrin genetic polymorphisms could play a role in the risk for periodontitis separate from the smoking factor. The functionality of this gene's polymorphisms has to be further elucidated.

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Periodontitis is an inflammatory disease of the supporting tissues of the teeth that is caused by groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone. The primary etiological factor in the initiation of gingival inflammation and subsequent destruction of periodontal tissues is bacterial biofilms (1). It is generally thought that periodontitis occurs as a result of an imbalance between pathogenic microbes and local or systemic host responses (2). Polymorphonuclear neutrophils, monocytes and lymphocytes migrate from the bloodstream into the irritated or infected tissues during the inflammatory process and the activation of these cells secretes inflammatory mediators that guide an amplifying cascade of biochemical and cellular events (3,4). According to the progressive rate of the disease, periodontitis can be classified as chronic periodontitis and aggressive periodontitis. Beside the infection of specific pathogenic microorganisms, several investigators have shown that patients with aggressive periodontitis display functional defects of polymorphonuclear neutrophils (5–7). Thus, deficiency in neutrophil number (neutropenias), or neutrophil function may lead to increased susceptibility to infectious disease (8).

Lactoferrin is a member of the transferrin family of iron-binding proteins; it is present in most exocrine secretions, such as saliva, and plays an important role in mucosal defense (9). Lactoferrin is an antimicrobial agent that has presence in polymorphonuclear neutrophils (PMNs). The amount of lactoferrin is strongly correlated with the number of PMNs in gingival crevicular fluid, and provides a simple and effective marker of crevicular polymorphonuclear neutrophil numbers. Furthermore, lactoferrin showed better correlation with clinical indices than polymorphonuclear neutrophils (10). In addition, data have indicated that a genetic component may influence the predisposition to aggressive peridontitis. A previous study revealed a significant difference in the frequency of the Lys/Arg polymorphism of the lactoferrin gene between nine patients with localized juvenile periodontitis and 17 healthy subjects (11). A recent study analyzed the gene expression profile and reported that the lactoferrin gene, along with several other genes, showed differential expression with at least a two-fold difference between patients with refractory periodontitis and controls (12).

Age, smoking and oral hygiene are the risk factors for aggressive periodontitis, whereas oral hygiene is related to chronic periodontitis (13). Tobacco smoking has considerable negative effects on periodontal health (14) and has been found to be a risk factor for incomplete or delayed healing in patients following treatment for periodontal diseases (15). The aim of the present study was to investigate the influence of different lactoferrin genotypes on chronic and aggressive periodontitis. We also explored whether the smoking factor affected the impact of the lactoferrin polymorphism by increasing the risk for periodontitis.

Material and methods

Study population

Study subjects were recruited from patients who visited the Department of Periodontics of Kaohsiung Medical University Hospital, Taiwan, from January 2004 to May 2006. All subjects were of Chinese ancestry and were free from systemic diseases associated with destructive periodontal disease, such as diabetes mellitus, immunosuppression, human immunodeficiency virus infection, or polymorphonuclear and/or monocyte defects, via questioner and history review. Subjects who had taken antibiotics in the previous 3 mo, who were pregnant, currently breastfeeding, or in need of antibiotic prophylaxis before periodontal treatment, were excluded from this study.

On the basis of clinical examinations (probing depth and attachment loss) and radiographic patterns of alveolar bone destruction, each subject was diagnosed as having aggressive periodontitis or chronic periodontitis, or as a periodontally healthy control. The diagnostic criteria for aggressive periodontitis and chronic periodontitis were defined in accordance with the classification agreed at the World Workshop for Periodontics and The American Academy of Periodontology (1999) (16). Briefly, subjects of more than 35 years of age, with attachment loss $\geq$ 5 mm at more than one tooth and with more than three sites of probing depth $>$ 6 mm in more than one tooth distributed in each quadrant were diagnosed as having chronic periodontitis. Subjects who had more than eight teeth with attachment loss $>$ 5 mm and probing depth $>$ 6 mm, and at least three affected teeth that were not first molars or incisors, were diagnosed as having aggressive periodontitis. Subjects with no evidence of attachment loss at more than one site or pocket $>$ 3 mm were diagnosed as periodontally healthy and used as controls. The total number of participants in this study was 431: 65 with aggressive periodontitis (40 men and 25 women); 278 with chronic periodontitis (136 men and 142 women); and 88 healthy controls (51 men and 37 women). We also recorded the smoking status as nonsmoker or current smoker. Subjects who had never smoked or had quit smoking for at least 6 mo were recorded as being nonsmokers.

The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University, and written informed consent was obtained from each subject prior to taking part in the study.

Sample collection and DNA extraction

Twenty milliliters of heparin-anticoagulated peripheral blood was collected from each study subject. DNA was extracted from the peripheral blood leukocytes using the techniques of Blin & Stafford, with standard phenol/chloroform extraction and precipitation with ethanol (17). The DNA concentration was determined by ultraviolet-light spectrophotometry.

In this study we characterized a Lys/Arg polymorphism that occurs at position 29 (rs 1126478) in the N-terminal alpha-helical region of

human lactoferrin. This polymorphism results from an A/G transition in exon 1 of the human lactoferrin gene. Genotyping was carried out by using TaqMan technology. Polymerase chain reaction (PCR) primers and TaqMan MGB probes were designed and reactions were performed in 96-well microplates with ABI 9700 thermal cyclers (Applied Biosystems, Foster City, CA, USA). Reaction preparation was as follows: each well of 96-well plates contained 12.5 μL of ×2 TaqMan universal PCR Master Mix, 1.25 μL of ×20 SNP Genotyping Assay Mix and 10 ng of genomic DNA. The PCR program was as follows: an initial step for DNA polymerase activation by holding at 95°C for 10 min, and then 40 cycles at 92°C for 15 s and 60°C for 1 min. All the samples were genotyped once. The genotyping inconsistency in our laboratory was 0–2%, depending on different single nucleotide polymorphisms. Fluorescence was measured with an ABI 7500 Real Time PCR System and analyzed using its System SDS software (version 1.2.3).

Statistical analyses

All statistical analyses were performed using JMP 6.0 package (SAS, Cary, NC, USA). The two-sample *t*-tests or chi-square tests were used to compare means and proportions between the groups of control subjects and patients with periodontitis. To determine whether an association existed between lactoferrin genotype and allele frequency with periodontal disease, the significance of the difference in the distribution of genotypes and alleles between periodontal patients and control subjects was calculated by the chi-squared statistic and shown by the *p*-value. All *p*-values were two sided. A *p*-value of < 0.05 was considered to be statistically significant. Association of lactoferrin genotypes and periodontitis was analyzed by logistic regression of periodontal patients vs. controls. To control the potential confounding effects, gender, age and smoking status were used as independent variables for adjustment.

Table 1. Comparison of the demographic characteristics of patients with aggressive periodontitis (AgP) and chronic periodontitis (CP), and healthy controls (H)

	AgP, $n = 65$	CP, $n = 278$	H, $n = 88$	*p*-value
Age (years ± SD)	44.8 ± 9.6	49.9 ± 9.5	50.7 ± 11.2	0.0003
Gender, *n* (%)				
Male	40 (61.5)	136 (48.9)	51 (58.0)	0.0987
Female	25 (38.5)	142 (51.1)	37 (42.0)	
PPD (mm ± SD)	4.82 ± 3.47	4.13 ± 1.83	1.29 ± 0.47	< 0.0001
CAL (mm ± SD)	5.57 ± 2.24	4.51 ± 1.81	0.69 ± 0.60	< 0.0001
Smoking, *n* (%)				
Smokers	24 (36.9)	73 (26.3)	11 (12.5)	0.0273
Nonsmokers	41 (63.1)	205 (73.7)	77 (87.5)	

CAL, clinical attachment loss; PPD, probing pocket depth; SD, standard deviation.

Results

Table 1 provides a summary of the demographic characteristics of participants in the three groups. The mean age of the chronic periodontitis ($n = 278$) and the healthy control ($n = 88$) groups was similar (mean ± standard deviation = 49.9 ± 9.5 years for subjects with chronic periodontitis and 50.7 ± 11.2 years for healthy controls), but younger in the aggressive periodontitis ($n = 65$) group (mean ± standard deviation = 44.8 ± 9.6 years). There were more current smokers in the aggressive periodontitis group than in the chronic periodontitis and healthy control groups.

The allele frequency and genotype distributions for the lactoferrin polymorphisms among the three study groups are summarized in Table 2. The genotype distributions of this polymorphism were in Hardy–Weinberg equilibrium in our group of patients ($p = 0.5433$) and were similar to the report in the HapMap data (http://www.hapmap.org). The frequencies of the GG genotype and the G allele were highest in the aggressive periodontitis group (56.9% and 76.2%, respectively), followed by the chronic periodontitis (49.3% and 69.6%, respectively) and healthy control (36.4% and 60.2%, respectively) groups. By contrast, the frequencies of the AG and AA genotypes and of the A allele had the opposite order, with the lowest frequency occurring in the aggressive periodontitis group (38.5%, 4.6% and 23.8%, respectively) and the highest in the healthy controls (47.7%, 15.9% and 39.8%, respectively).

Tables 3 and 4 show the association of the lactoferrin genotype and allele distributions in each disease status. The frequencies of the G allele were significantly higher in aggressive periodontitis and chronic periodontitis groups than in healthy controls

Table 2. Genotype and allele frequencies of lactoferrin (LF) gene polymorphisms in patients with aggressive periodontitis (AgP) and chronic periodontitis (CP), and in healthy controls (H)

				p-value	
Genotype	AgP, *n* (%)	CP, *n* (%)	H, *n* (%)	AgP vs. H	CP vs. H
GG	37 (56.9)	137 (49.3)	32 (36.4)	0.0112*	0.0758
AG	25 (38.5)	113 (40.7)	42 (47.7)		
AA	3 (4.6)	28 (10.1)	14 (15.9)		
GG	37 (56.9)	137 (49.3)	32 (36.4)	0.0114*	0.0331*
AG + AA	28 (43.1)	141 (50.7)	56 (63.6)		
GG + AG	62 (95.4)	250 (89.9)	74 (84.1)	0.0212*	0.1471
AA	3 (4.6)	28 (10.1)	14 (15.9)		
G	99 (76.2)	387 (69.6)	106 (60.2)	0.0031*	0.0222*
A	31 (23.8)	169 (30.4)	70 (39.8)		

Significance was calculated using the chi-square test.
*$p < 0.05$.

Table 3. Genotype and allele frequencies of lactoferrin (LF) gene polymorphisms in patients with chronic periodontitis (CP) and in healthy controls (H)

Genotype	CP, *n* (%)	H, *n* (%)	*p*-value	Crude OR	Adjusted OR	*p*-value	95% CI
GG	137 (49.3)	32 (36.4)	0.263	1.71	1.63	0.2028	0.75–3.43
AG	113 (40.7)	42 (47.7)		1.26	1.24	0.5765	0.58–2.58
AA	28 (10.1)	14 (15.9)		1	1		
GG	137 (49.3)	32 (36.4)	0.1305	1.43	1.38	0.1863	0.86–2.24
AG + AA	141 (50.7)	56 (63.6)		1	1		
GG + AG	250 (89.9)	74 (84.1)	0.1376	1.69	1.64	0.1739	0.79–3.29
AA	28 (10.1)	14 (15.9)		1	1		
G	387 (69.6)	106 (60.2)	0.0212	1.51*	1.48*	0.0349	1.03–2.11
A	169 (30.4)	70 (39.8)		1	1		

OR, odds ratio of CP vs. H; adjusted by gender, smoking and age by logistic regression analysis.
*$p < 0.05$.
CI, confidence interval.

Table 4. Genotype and allele frequencies of lactoferrin (LF) gene polymorphisms in patients with aggressive periodontitis (AgP) and in healthy controls (H)

Genotype	AgP, *n* (%)	H, *n* (%)	*p*-value	Crude OR	Adjusted OR	*p*-value	95% CI
GG	37 (56.9)	32 (36.4)	0.0195	4.80*	6.73*	0.008	1.83–33.17
AG	25 (38.5)	42 (47.7)		2.59	3.97	0.0577	1.06–19.83
AA	3 (4.6)	14 (15.9)		1	1		
GG	37 (56.9)	32 (36.4)	0.0175	2.17*	2.16*	0.0287	1.09–4.35
AG + AA	28 (43.1)	56 (63.6)		1	1		
GG + AG	62 (95.4)	74 (84.1)	0.0386	3.91*	7.19*	0.0068	1.94–36.38
AA	3 (4.6)	14 (15.9)		1	1		
G	99 (76.2)	106 (60.2)	0.0037	2.11*	2.53*	0.001	1.47–4.46
A	31 (23.8)	70 (39.8)		1	1		

OR, odds ratio of AgP vs. H; adjusted by gender, smoking and age by logistic regression analysis.
*$p < 0.05$.
CI, confidence interval.

($p = 0.0037$ and 0.0212). Although the difference in genotype distributions between subjects with chronic periodontitis and healthy controls did not reach significance, the genotype distribution between subjects with aggressive periodontitis and healthy controls was significantly different. Using AA or AG + AA as the reference, the distribution of the GG genotype in subjects with aggressive periodontitis was significantly different ($p = 0.0195$ and 0.0175) from that of controls. The crude odds ratio was 4.80 for GG/AA and 2.17 for GG/AG + AA for aggressive periodontitis vs. healthy controls. The association of the gene polymorphism and aggressive periodontitis still existed, even after adjusting for age, gender and smoking status by logistic regression analysis (GG/AA: adjusted odds ratio = 6.73, 95% confidence interval = 1.83–33.17, $p = 0.0080$; GG/AG + AA: adjusted odds ratio = 2.16, 95% confidence interval = 1.09–4.35, $p = 0.0287$). This result indicated that patients with aggressive periodontitis have a higher distribution of GG-genotype or G-allele frequency than healthy controls.

The study subjects were further stratified by their smoking status to examine whether the smoking factor affects the impact of the lactoferrin polymorphism on the risk for periodontitis (Table 5). Distinct from the decreasing frequency of the GG genotype from the aggressive periodontitis to healthy controls in the nonsmoking group, the chronic periodontitis group had the highest GG genotype in the smoking group. Because the number of patients with the AA genotype was small after grouping, we combined AG and AA as the reference in this analysis. The lactoferrin gene (GG/AG + AA) polymorphism was not statistically different between either the chronic periodontitis vs. healthy controls or the aggressive periodontitis vs. healthy controls in the smoking group, while the distribution of the lactoferrin genotype of aggressive periodontitis in the nonsmoking group was significantly different from the healthy control group (crude odds ratio = 2.47, $p = 0.0145$). The GG genotype of lactoferrin was significantly associated with the risk of aggressive periodontitis in the nonsmoking group when adjusted for age and gender (aggressive periodontitis vs. healthy controls: adjusted odds ratio = 2.69, 95% confidence interval = 1.20–6.24, $p = 0.0180$).

Discussion

The present study shows that the lactoferrin polymorphism is related to the development of periodontitis. The risk allele, G (arginine), was more frequent in the aggressive periodontitis group (76.2%), followed by the chronic periodontitis group (69.6%) and then the healthy control group (60.2%). The increasing frequency is inconsistent with disease severity, which suggests a possible biological effect of this A/G polymorphism on periodontitis. Lactoferrin is an important part of the host's innate defenses (18). The N-terminal domain of lactoferrin is a cationic peptide called lactoferricin, which can cause damage to the outer membrane of gram-negative bacteria (19,20). When altering the amino acid composition within the region of the human lactoferrin gene that has been associated with functional importance, such single nucleotide polymorphisms may potentially affect the antimicrobial properties of lactoferrin and thus be important in disease caused by bacteria, including periodontitis (21). By replacing lysines on peptides derived from the lipopolysaccharide-binding domain of bactericidal/permeability-increasing protein, the bactericidal activity was inhibited (22). Lactoferrin containing lysine at position 29 has previously been reported to

Table 5. Comparison of lactoferrin (LF) genotypes in patients stratified by smoking status

					AgP vs. H				CP vs. H			
	LF	AgP, *n* (%)	CP, *n* (%)	H, *n* (%)	Crude OR	Adjusted OR[a]	*p*-value	95% CI	Crude OR	Adjusted OR[a]	*p*-value	95% CI
Nonsmoker	GG	24 (58.5)	94 (45.9)	28 (36.3)	8.57*	10.63*	0.0333	1.73–207.85	1.29	1.34	0.5101	0.55–3.12
	AG	16 (39.0)	85 (41.5)	39 (50.7)	4.10	5.81	0.1222	0.89–117.3	0.84	0.85	0.6948	0.36–1.90
	AA	1 (2.4)	26 (12.7)	10 (13.0)	1	1			1	1		
	GG	24 (58.5)	94 (45.9)	28 (36.3)	2.47*	2.69*	0.018	1.20–6.24	1.48	1.53	0.1273	0.89–2.68
	AG + AA	17 (41.5)	111 (54.1)	49 (63.7)	1	1			1	1		
	G	64 (78.0)	273 (66.6)	95 (61.7)	2.21*	2.44*	0.0066	1.30–4.73	1.24	1.27	0.2365	0.85–1.87
	A	18 (22.0)	137 (33.4)	59 (38.3)	1	1			1	1		
Smoker	GG	13 (54.2)	43 (58.9)	4 (36.4)	4.87	5.21	0.1814	0.50–83.49	16.12*	12.07*	0.0179	1.60–128.76
	AG	9 (37.5)	28 (38.4)	4 (36.4)	3.37	2.87	0.3204	0.33–34.54	10.50*	10.12*	0.0256	1.39–99.48
	AA	2 (8.3)	2 (2.7)	3 (27.2)	1	1			1	1		
	GG	13 (54.2)	43 (58.9)	4 (36.4)	2.08	2.21	0.3428	0.47–10.44	2.51	2.39	0.2106	0.63–9.98
	AG + AA	11 (45.8)	30 (41.1)	7 (63.3)	1	1			1	1		
	G	35 (72.9)	114 (78.1)	11 (50)	2.69	2.8	0.0654	0.94–8.64	3.56*	3.41*	0.0101	1.33–5.13
	A	13 (27.1)	32 (21.9)	11 (50)	1	1			1	1		

*$p < 0.05$.
[a]OR, odds ratio; (GG/AA, AG/AA) adjusted by age using logistic regression analysis.
[a]OR, odds ratio; (GG/AG + AA, G/A) adjusted by gender and age using logistic regression analysis.
Lf (GG/AG + AA)* smoking (CP/H), $p = 0.7518$; Lf* smoking (AgP/H), $p = 0.1757$.
CI, confidence interval.

exhibit significantly greater bactericidal activity against *Actinobacillus actinomycetemcomitans* than lactoferrin containing arginine (11). This suggests that allele A of lactoferrin may contribute to the host protection against microbial infections both by iron-dependent and iron-independent mechanisms (23).

Our results indicate that genetic variation of lactoferrin might play an important role in aggressive periodontal disease, which is consistent with the results of previous studies (11,24,25). The lactoferrin polymorphism analyzed in our study is the same as analyzed in Velliyagounder's study, but there were some differences in methodology and in the results. First, we obtained our data from a study population of 431, which comprised 65 subjects with aggressive periodontitis, 278 with chronic periodontitis and 88 healthy controls, whereas Velliyagounder's study was carried out in a small population comprising nine patients with localized juvenile periodontitis and 17 healthy subjects. Second, the G-allele frequencies of our results were 76.2%, 69.6% and 60.3% in aggressive periodontitis, chronic periodontitis and healthy controls groups, respectively; the aforementioned study's results were 28% and 76%, respectively, in patients with localized juvenile periodontitis and healthy subjects. The G allele occurred much less frequently in African–American patients with localized juvenile periodontitis than in Taiwanese patients with aggressive periodontitis (28% vs. 76%). This might be the result of different disease types and ethnicity. Some studies showed that ethnic factors are considered to be a major variable when evaluating the predisposition to aggressive periodontitis (26–29). One recent study has demonstrated the genetic associations between single nucleotide polymorphisms in the lactoferrin gene and aggressive periodontal disease in African–American, but not Caucasian, populations, and suggest that the polymorphism of human lactoferrin may be a marker for susceptibility to aggressive periodontitis between different ethnic populations (21). To our knowledge, there no previous study had been carried out on the lactoferrin genotype and periodontitis in Asia. This might be the first report on the lactoferrin polymorphism in association with aggressive periodontitis in a Taiwanese population. The finding that the lactoferrin polymorphism is a risk factor might have potential use in the future management of aggressive periodontitis.

Friedman *et al*. found that the concentration of lactoferrin increased twofold in gingival crevicular fluid in sites showing gingivitis, periodontitis and localized juvenile periodontitis (30). Our previous studies also showed that the total amount of lactoferrin was higher in periodontitis sites than in healthy sites, and was positively correlated with plaque index, gingival index, probing depth and probing attachment level ($p < 0.05$) (31). It has also been reported that the ratio of lactoferrin to lysozyme may be more representative and a useful diagnostic assay of periodontal inflammation. Lactoferrin originates from neutrophil degranulation, and the blood concentrations of lactoferrin increase during infection or inflammation (32). A previous study showed that lactoferrin had a better correlation with clinical indices than polymorphonuclear neutrophils (10). Thus, deficiency in neutrophil number (neutropenias), or neutrophil function may lead to increased susceptibility to infectious disease (8). The lactoferrin concentration in gingival crevicular fluid shows a quantitative correlation to disease severity, whereas the lactoferrin genotype may

contribute to host defense in a qualitative manner.

The present study shows that the lactoferrin gene A/G polymorphism is significantly associated with aggressive periodontitis, in both nonadjusted and adjusted models. In the study of prognostic factors in the treatment of generalized aggressive periodontitis, data showed that current smoking was strongly associated with nonresponding patients (odds ratio 3.8) (33). When our study subjects were further subgrouped by their smoking status to examine whether the smoking factor affects the impact of the lactoferrin polymorphism with a risk for periodontitis, there was no association found in the smoking group. However, as mentioned, there was an association in the nonsmoking group. Previous studies demonstrated that the levels of salivary lactoferrin were significantly lower in current smokers than in noncurrent smokers (34,35). Thus, the influence of lactoferrin on aggressive periodontitis was noticeable in the nonsmoking group but not in the smoking group; this was because of the low concentration of lactoferrin in the smoking group that weakened the preventive effect of lactoferrin on the development of periodontitis. When looking at the odds ratio of GG and AG, using AA as a reference, the association of GG with disease status was present, not only in subjects of the nonsmoking group with aggressive periodontitis but also in subjects of the smoking group with chronic periodontitis. The association of the AG genotype with the development of chronic periodontitis was also found in the smoking group. This implies that the smoking factor might reduce the function of the lactoferrin gene.

The data presented in Table 5 showed that there was no correlation between smoking and the lactoferrin polymorphism in relation to periodontal severity. This result was the same as reported in a previous study of genetic and smoking effect on oral precancerous lesions by Chung *et al.* (36). In the present study, we also found that the lactoferrin gene A/G polymorphism and cigarette smoking status had no synergistic or additive effect in relation to the risk of aggressive periodontitis. This suggests that smoking and genetic factors may be differently involved in the development of aggressive periodontitis, but the genetic effect seems to be weaker than the effect of smoking status in our study.

Conclusions

To our knowledge, this is the first report that the lactoferrin polymorphism is related to the development of periodontitis in a Taiwanese population. Our study showed that the lactoferrin gene A/G polymorphism could be significantly associated with the susceptibility to aggressive periodontitis both in nonadjusted and adjusted models. As the lactoferrin gene A/G polymorphism and cigarette smoking status had no synergistic or additive effect in relation to the risk of developing aggressive periodontitis, our results support the hypothesis that lactoferrin genetic polymorphisms could play a role in the risk for periodontitis separately from the smoking factor. The study also showed that the A allele of lactoferrin might reduce the risk for development of aggressive periodontitis and severity of the disease. Further investigations of these polymorphisms, combined with functional studies of gene and protein function, are needed to support this conclusion.

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