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Investigation of interleukin-13 gene polymorphisms in individuals with chronic and generalized aggressive periodontitis in a Taiwanese (Chinese) population

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Background and Objective: The interleukin-13 (IL-13) −1112 C/T polymorphisms have been analyzed previously in a North European population of patients with aggressive periodontitis. The present study was carried out to investigate the association of polymorphisms in the IL-13 gene with susceptibility to periodontitis in a Taiwanese population.

Material and Methods: The genotyping of IL-13 −1112 C/T polymorphisms in 60 patients with aggressive periodontitis, 204 patients with chronic periodontitis and 95 healthy controls was carried out using the polymerase chain reaction–restriction fragment length polymorphism technique. Genotypes and allele frequencies among study groups were compared using Fisher's exact test ($p < 0.05$). Pearson's chi-square test was used for analysis of the Hardy–Weinberg equilibrium.

Results: The distributions of CC genotypes and C alleles between patients with aggressive periodontitis and healthy controls were significantly different ($p = 0.034$ and 0.046). After adjustment for age, gender, betel nut chewing and smoking status using logistic regression analysis, the odds ratio (OR) was 6.45 [95% confidence interval (CI) = 1.99–23.72, $p = 0.003$] for aggressive periodontitis. However, the CC genotype was only significantly associated with the risk of aggressive periodontitis in the nonsmoking group (OR = 4.48, 95% CI = 1.31–16.93, $p = 0.020$).

Conclusion: The CC genotype or C allele appears to increase the risk of developing aggressive periodontitis in Taiwanese subjects.

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Periodontitis is an inflammatory disease involving connective tissue destruction, attachment loss and alveolar bone resorption (1,2). Specific gram-negative anaerobic bacteria and their products have been implicated as the causative agents of periodontitis. However, recent studies have suggested that the host response, in which many

immune cell types including polymorphonuclear leukocytes, macrophages, lymphocytes and fibroblasts were involved, is responsible for most of the pathology of periodontitis (3). *Porphyromonas gingivalis* is capable of inducing high levels of inflammatory mediators, including interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, tumor necrosis factor-α, interferon-γ, IFN-γ-inducible protein 10, monocyte chemoattractant protein-1, regulated on activation, normal T-cell-expressed and secreted (RANTES) and prostaglandin E_2, in an *ex vivo* human whole-blood model. However, a high interindividual variability in the levels of secreted mediators was observed (4). Cytokines produced by a number of T cells are characterized by their pleiotropism and pluripotentiality. Among these cytokines, IL-4 and IL-13 are produced by T helper type 2 (Th2) lymphocytes. Tsai *et al*., Ebersole & Taubman and Giannopoulou *et al*. (5–7) have concluded that Th2 cytokines, for example, IL-4, are associated with the remission or improvement of periodontal inflammation (disease). Both genes encoding IL-4 and IL-13 are located on chromosome 5q31-33 and may predispose individuals to asthma and atopy. Although IL-4 and IL-13 share many biological activities, some are unique to IL-13 (8,9). It has been proposed that the predominant inflammatory lymphocytic cell type in gingivitis is the T cell and in periodontitis is the B cell (10,11). By activating the production of transforming growth factor-β by macrophages, IL-13 may act indirectly on fibroblasts (12). Many other cells, including B cells, smooth muscle, endothelium and epithelium, can also be targets regulated by IL-13. Cytokines, including IL-4, IL-6, IL-10 and IL-13, may be associated with the increased replication of mast cells. Interleukin-13 expression has also been found in periodontitis lesions (13–16). Interleukin-13 mRNA was detected in 21% of periodontitis samples but in none of the biopsies from healthy tissue. However, mRNA for transforming growth factor-β was detected in all tissues (17). Recently, several different single nucleotide polymorphisms in the IL-13 promoter region have been described (18–21). The C/T exchange at position −1112 leads to the overproduction of IL-13 in Th2 lymphocytes and therefore it may be associated with allergic asthma (22,23), Graves' disease (24) and mastocytosis (25). The −1112 C/T and –1512 A/C polymorphisms have been analyzed in patients with aggressive periodontitis in a North European population. The results showed that the genotype and allele frequencies did not differ between aggressive periodontitis and healthy control groups (26). However, at present there are no data on the frequency and role of IL-13 gene polymorphisms in periodontitis among Taiwanese (Chinese) of Han-ethnicity.

Based on the plausible role of IL-13 in periodontitis and the ethnic variants of genetic polymorphisms, investigation of the IL-13 gene polymorphism that affects IL-13 transcription may provide important information on their association with periodontitis. This study compared the frequency of the IL-13 −1112 C/T polymorphisms and the distribution of the genotypes among patients with chronic periodontitis and aggressive periodontitis, and in healthy controls, in a Taiwanese population.

Material and methods

Study subjects

Study subjects were recruited from patients who visited the Department of Periodontics of Kaohsiung Medical University Hospital, Taiwan, from January 2004 to May 2008. All subjects were of Taiwanese Han-ethnicity and were free from systemic diseases that correlate with destructive periodontal disease, such as diabetes mellitus, immunosuppression, HIV infection, or polymorphonuclear leukocyte and/or monocyte defects, via questionnaire and review of history.

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 being periodontally healthy. 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) (27). Briefly, subjects older than 35 years of age, with attachment loss ≥ 5 mm at more than one tooth site and with more than three sites of probing depth > 6 mm involving more than one tooth distributed in each quadrant, were diagnosed as having chronic periodontitis. Subjects who had more than eight teeth with attachment loss of ≥ 5 mm and probing depth of ≥ 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 depth of ≥ 3 mm were diagnosed as periodontally healthy and used as controls. The total number of participants in this study was 359, comprising 60 with aggressive periodontitis (31 men and 29 women), 204 with chronic periodontitis (111 men and 93 women) and 95 who were periodontally healthy (52 men and 43 women). The smoking status of participants was recorded as nonsmoker or current smoker. Subjects who had never smoked or had quit smoking for at least 6 mo before the start of the study were recorded as nonsmokers. The betel nut chewing status of participants was recorded as for smokers (i.e. subjects who had never chewed betel nut or had quit chewing betel nut for at least 6 mo before the start of the study were recorded as non-betel nut chewers).

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

Blood samples and DNA extraction

Twenty millilitres of heparin anticoagulated peripheral blood was collected from each study subject. DNA was extracted from the peripheral blood leucocytes using standard phenol/chloroform extraction techniques and

precipitation with ethanol (28). The DNA concentration was determined using ultraviolet spectrophotometry.

Analysis of the IL-13 genotype

The IL-13 genotype at position −1112 from the transcription start site was determined using the polymerase chain reaction–restriction fragment length polymorphism method (29). For analysis of the IL-13 −1112 C/T polymorphism, the following primers were used: 5′-GGAATCCAGCATGCC-TTGTGAGG-3′ and 5′-GTCGCC-TTTTCCTGCTCTTCCCGC-3′. The reaction conditions and cycling parameters were as follows: 100 ng of genomic DNA (1 μL) was used for PCR amplification in a reaction mixture containing 2.5 μL of 10× reaction buffer, 2.0 μL of 2.5 mM dNTP, 0.3 μL of *Taq* polymerase (*Taq* DNA polymerase, 5 U/μL; Supertherm, Bristol, UK), 18.7 μL of distilled water and 0.25 μL of each primer. The PCR conditions were as follows: samples were denatured at 95°C for 10 min. followed by 40 cycles of 95°C for 45 s, 55°C for 30 s, 72°C for 50 s and then a final extension for 10 min at 72°C. Restriction digestion was accomplished using *Bst*UI (NEB, Schwalbach am Taunus, Germany) at 60°C for 1 h. For the IL-13 −1112 C/T genotype, a 224 bp PCR fragment was generated for allele C and a 247 bp fragment was generated for allele T (30). Sequencing was performed on approximately 25% of randomly selected samples, to confirm the genotype results.

Statistical analyses

All statistical analyses were performed using the 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 control and the periodontitis groups. To determine whether an association existed between periodontal disease and the IL-13 genotype and allele frequency, the significance of the difference in the distribution of genotypes and alleles between periodontitis patients and control subjects was calculated using chi-square statistics and validated by the *p*-value. All *p*-values were two-sided. A *p*-value of < 0.05 was considered to be statistically significant. Association of IL-13 genotypes and periodontitis was analyzed by logistic regression of periodontitis patients vs. controls. To control the potential confounding effects, gender, age, smoking status and betel nut chewing habit were used as independent variables for adjustment.

Results

Demographical data and clinical parameters

A total of 60 patients with generalized aggressive periodontitis, 204 patients with chronic periodontitis and 95 healthy controls took part in the study. Clinical characteristics, smoking, betel nut chewing, age and gender distribution are summarized in Table 1. The values of the clinical parameters (probing pocket depth and clinical attachment loss) were higher in aggressive periodontitis and chronic periodontitis groups. The mean age was significantly younger in the aggressive periodontitis group than in the other groups.

Distribution of genotypes

The distribution of genotypes in periodontitis patients and the healthy control group, as well as the results of Fisher's exact test, are shown in Table 2. Homozygosity for the IL-13 C allele at position −1112 was found in 78.3% of the patients with aggressive periodontitis, in 57.4% of the patients with chronic periodontitis and in 54.7% of the healthy controls. A total of 21.7% individuals with aggressive periodontitis, 41.2% of individuals with chronic periodontitis and 43.2% of healthy controls were heterozygous (C/T). The prevalence of the T allele homozygote was very low in our study population: none was found in patients of the aggressive periodontitis group, and the prevalence was 1.5% in patients of the chronic periodontitis group and 2.1% in the healthy control group. The distribution of the genotypes (CC/CT + TT) between patients with aggressive periodontitis and healthy controls was significantly different ($p < 0.05$). The frequency of the CC genotype and C allele were highest among the patients with aggressive periodontitis, followed by those with chronic periodontitis and then healthy controls. The distribution of IL-13 −1112 genotypes in patients and controls did not differ from Hardy–Weinberg equilibrium.

Association of genotypes and periodontitis

Tables 3 and 4 display the association of the IL-13 −1112 genotype and allele distributions in each disease status.

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

	AgP	CP	H	
	n = 60	*n* = 204	*n* = 95	*p*-value
Age (years ± SD)	36.6 ± 7.4	51.0 ± 6.2	42.5 ± 5.3	< 0.0001
Gender (*n*, %)				
Male	31 (51.7)	111 (54.4)	52 (54.7)	0.921
Female	29 (48.3)	93 (45.6)	43 (45.3)	
Smoking (*n*, %)				
Smokers	16 (26.7)	69 (33.8)	18 (18.9)	0.24
Nonsmokers	44 (73.3)	135 (66.2)	77 (81.1)	
Betel nut chewing (*n*, %)				
Yes	11 (18.3)	40 (19.6)	5 (5.3)	0.041
No	49 (81.7)	164 (80.4)	90 (94.7)	
PPD (mm ± SD)	4.53 ± 0.71	3.66 ± 1.30	2.50 ± 0.31	< 0.0001
CAL (mm ± SD)	5.15 ± 0.84	4.70 ± 1.61	0.67 ± 0.40	< 0.0001

CAL, clinical attachment loss; PPD, probing pocket depth.
p value: comparison between groups.

Table 2. Genotype and allele frequencies of interleukin-13 (IL-13) polymorphisms in patients with aggressive periodontitis (AgP) and chronic periodontitis (CP), and in healthy controls (H)

	AgP	CP	H	AgP vs. H	CP vs. H
Genotype	*N*(%)	*N*(%)	*N*(%)	*p* value	*p* value
CC	47 (78.3)	117 (57.4)	52 (54.7)	0.009*	0.802
CT	13 (21.7)	84 (41.2)	41 (43.2)		
TT	0 (0.0)	3 (1.5)	2 (2.1)		
CC	47 (78.3)	117 (57.4)	52 (54.7)	0.034*	0.671
CT + TT	13 (21.7)	87 (42.6)	43 (45.3)		
C	107 (89.2)	318 (77.9)	145 (76.3)	0.046*	0.658
T	13 (10.8)	90 (22.1)	45 (23.7)		

Chi-square test, *$p < 0.05$.

Table 3. Analysis of genotype and allele frequencies of interleukin-13 (IL-13) 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
CC	117 (57.4)	52 (54.7)	0.802	1.43	3.45	0.411	0.18–66.67
CT	84 (41.2)	41 (43.2)		1.31	3.00	0.709	0.55–2.43
TT	3 (1.5)	2 (2.1)		1	1		
CC	117 (57.4)	52 (54.7)	0.671	1.11	1.20	0.632	0.57–2.51
CT + TT	87 (42.6)	43 (45.3)		1	1		
C	318 (77.9)	145 (76.3)	0.658	1.10	1.18	0.587	0.65–2.13
T	90 (22.1)	45 (23.7)		1	1		

OR, odds ratio of CP vs. H; adjusted by age, gender, smoking and betel nut chewing by logistic regression analysis.
CI, confidence interval.

Table 4. Genotype and allele frequencies of interleukin-13 (IL-13) 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
CC	47 (78.3)	52 (54.7)	0.111				
CT	13 (21.7)	41 (43.2)					
TT	0 (0.0)	2 (2.1)					
CC	47 (78.3)	52 (54.7)	0.002*	2.98*	6.45*	0.003*	1.99–23.72
CT + TT	13 (21.7)	43 (45.3)		1	1		
C	107 (89.2)	145 (76.3)	0.005*	2.55*	3.58*	0.015*	1.35–10.89
T	13 (10.8)	45 (23.7)		1	1		

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

There was no significant difference between patients with chronic periodontitis and healthy controls. The CC genotype of the patients with aggressive periodontitis was significantly higher than in healthy controls (78.3:54.7%). Using T or CT + TT as the reference, the crude odds ratio (OR) was 2.55 for C/T and 2.98 for CC/CT + TT in comparison with patients with aggressive periodontitis and healthy controls. The association of the IL-13 −1112 gene polymorphism and aggressive periodontitis still existed, even after adjusting for age, gender, smoking and betel nut chewing status by logistic regression analysis [C/T: adjusted OR = 3.58, 95% confidence interval (CI) = 1.35–10.89, $p = 0.015$; CC/CT + TT: adjusted OR = 6.45, 95% CI = 1.99–23.72, $p = 0.003$]. The results indicated that patients with aggressive periodontitis had a higher distribution of the CC genotype and C allele frequency than healthy controls.

Impact of the IL-13 polymorphism on the risk for periodontitis

The study subjects were further stratified by their smoking status to examine whether the smoking factor would augment the impact of the IL-13 polymorphism on the risk for periodontitis (Table 5). As the frequency of the TT genotype was very small after grouping, we combined CT and TT as the reference in this analysis. A significant difference of the IL-13 genotype between aggressive periodontitis and healthy controls was only found in the nonsmoking group. This significant association still remained after adjustment for age, gender and betel nut chewing (aggressive periodontitis vs. healthy controls: crude OR = 3.08, $p = 0.007$; adjusted OR = 4.48, 95% CI = 1.31–16.93, $p = 0.020$).

Discussion

Several different factors may contribute to the pathogenesis of periodontitis. Recent data suggest that the genetic background may also play a potential role in disease manifestation and evolution (31–36). However, so far, little is known about the *IL-13* gene and its exact role in periodontitis. The results of our study show that the genetic variant of periodontitis is associated with the −1112T polymorphism (genotype CC) of the *IL-13* gene. To our knowledge, this is the first study investigating the IL-13 −1112 C/T polymorphism in Chinese patients with periodontitis.

Several reports have indicated the presence of IL-13 in periodontal lesions (13–16,26). However another study only detected IL-13 in patients with gingivitis and in subjects with healthy gingiva (37). It has been proposed that the shift from gingivitis to periodontitis may be caused by an imbalance between Th1 and Th2 cells (38,39). Many other studies also point out that IL-13 shares biological fea-

Table 5. Comparison of interleukin-13 (IL-13) genotypes in patients studied according to smoking status

	IL-13 −1112 genotype			
	CT/TT	CC	95% CI	*p*-value
Chronic periodontitis				
Nonsmoking				
Crude OR	1	1.45	(0.82, 2.54)	0.197
Adjusted OR	1	1.59	(0.70, 3.61)	0.263
Smoking				
Crude OR	1	2.86	(0.85, 9.52)	0.08
Adjusted OR	1	∞	–	0.186
Aggressive periodontitis				
Nonsmoking				
Crude OR	1	3.08*	(1.39, 7.19)	0.007*
Adjusted OR	1	4.48*	(1.31, 16.93)	0.020*
Smoking				
Crude OR	1	2	(0.33, 16.16)	0.463
Adjusted OR	1	∞	–	

OR, odds ratio of chronic periodontitis vs. healthy controls or aggressive periodontitis vs. healthy controls; adjusted by age, gender and betel nut chewing by logistic regression analysis.
CI, confidence interval.*$p < 0.05$.

tures with IL-4 in controlling differentiation to Th2 cells, which respond with the progression from gingivitis to periodontitis (40–45). As the gingival concentrations of IL-13 were nearly 10 times greater than those of IL-4 within the tissues examined, IL-13 may be more important than IL-4 for maintaining the Th2 response (46).

The results of our present study are different from the data of Gonzales *et al*. (26) in which the genotype and the allele frequencies of the IL-13 polymorphisms were not different between the aggressive periodontitis and control groups of Caucasians. The IL-13 −1112C polymorphism (genotype CC) is known to lead to a lower transcription rate for IgE production (25). In our present study, the CC polymorphism was detected at a higher frequency in patients with aggressive periodontitis than in patients with chronic periodontitis or in healthy controls. The difference might be caused by different ethnicity. Our previous studies showed that ethnic factors were considered to be a major variable for evaluating the predisposition to aggressive periodontitis (47–49). The distribution of the IL-13 −1112C/T genotype of our result is similar to the results of studies of the Chinese Han Nationality in China (50–52). The authors concluded that the IL-13 −1112C/T was associated with an increased serum IgE level and might be an important candidate gene for asthma. The results of this study showed that patients with aggressive periodontitis had a higher distribution of the CC genotype or a higher C-allele frequency than healthy controls. When considered together, we suggest that the IL-13 polymorphism −1112 CC genotype, or the C allele, may be a predisposing factor in the development of aggressive periodontitis in Taiwanese people of Han-ethnicity.

The association of the IL-13 polymorphism −1112 CC genotype or C allele and aggressive periodontitis is demonstrated both in nonadjusted and adjusted models. From a study of prognostic factors in the treatment of generalized aggressive periodontitis, data showed that current smoking was strongly associated with nonresponding patients (OR 3.8) (53). According to the results, smokers had more lymphocytes and higher levels of IFN-γ and IL-13. The authors suggest that the increased Th cell activity, and specifically an elevated Th2 profile in smokers, may constitute a risk for patients who smoke that may induce conversion of periodontal stability into progressive disease (54). When our study subjects were further subgrouped by their smoking status to examine whether the smoking factor synergistically impacted the IL-13 −1112 polymorphism, increasing the risk for periodontitis, no association was found in the smoking group. However, as mentioned earlier, there was an association in the nonsmoking group. That means that the influence of the IL-13 −1112 polymorphism on aggressive periodontitis was noticeable in the nonsmoking group, but not in the smoking group. This seems to suggest that the influence of smoking on the development of aggressive periodontitis was stronger than the impact of the IL-13 gene polymorphism. The data also agree with our previous finding that smoking and genetic factors may influence the progression of aggressive periodontitis in different ways. However, the impact of genetics seems to be less than the impact of the smoking status (48).

Conclusions

To our knowledge, this is the first report that the IL-13 −1112 C/T polymorphism is related to the development of periodontitis in Taiwanese people of Han-ethnicity. Our data also demonstrate that the CC genotype of the IL-13 −1112 C/T polymorphism may play a role in the development of aggressive periodontitis in a Taiwanese population. The present results support the hypothesis that IL-13 genetic polymorphisms could play a role in the risk for periodontitis separately from the smoking factor. Further investigations of the combination effect of IL-4/IL-13 on the relative risk for developing aggressive periodontitis are needed.

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