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Interleukin-10 (–592 C/A) and interleukin-12B (+16974 A/C) gene polymorphisms and the interleukin-10 ATA haplotype are associated with periodontitis in a Taiwanese population

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Background and Objective: Single nucleotide polymorphisms are assumed to be associated with the differential production of cytokines. We evaluated gene polymorphisms of interleukin-10 (−592C > A, −819C > T and −1082G > A) and interleukin-12B (+16974) in patients with chronic periodontitis ($n = 145$) and generalized aggressive periodontitis ($n = 65$) in comparison with healthy controls ($n = 126$).

Material and Methods: Gene promoter polymorphisms were analyzed by polymerase chain reaction with sequence-specific primers. Genotype and allele frequencies were analyzed using the chi-square test and logistic regression analysis.

Results: The interleukin-10 −592 polymorphism showed significant differences among the three groups ($p = 0.0330$). The genotype frequencies of the −592 locus between the chronic periodontitis and healthy control groups were significantly different (AC vs. AA: odds ratio = 0.33). The combination ATA/ATA seemed to be associated with susceptibility to generalized aggressive periodontitis ($p = 0.0276$). Patients with the composite ATA/ACC were less likely to develop chronic periodontitis ($p = 0.0248$). The CC genotype of interleukin-12B (+16974) was related to chronic periodontitis (CC vs. AA, $p = 0.0211$; CC vs. AA + AC, $p = 0.0187$). The AC heterozygosity of interleukin-12B was significantly lower in chronic periodontitis vs. healthy controls ($p = 0.0500$).

Conclusion: The interleukin-10 gene polymorphism at position −592C > A may be associated with a lower risk for development of chronic periodontitis. The interleukin-10 haplotype ATA is associated with generalized aggressive periodontitis. On the other hand, interleukin-12B genetic variants at position +16974 are associated with susceptibility to chronic periodontitis.

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Periodontitis is a multifactorial disease that is caused mainly by gram-negative microorganisms present in the plaque adjacent to the gingiva, resulting in stimulation of host cells to produce molecules important in the immuno-inflammatory response (1). The local inflammatory response is inevitably bound to the destruction of alveolar bone and connective tissue (2,3). Cytokines play a pivotal role in the regulation of the type and magnitude of immune response, and the polymorphic nature of the cytokine genes may confer flexibility on the immune response (4–6).

Interleukin-10 is the most potent anti-inflammatory cytokine as it downregulates the production of pro-inflammatory cytokines and chemokines by activated monocytes, polymorphonuclear leukocytes and eosinophils, prevents antigen-specific T-cell activation, inhibits T-cell expansion and intensifies the release of the inflammatory modulator interleukin-1ra (7). Moreover, interleukin-10 is a pleiotropic cytokine with strong anti-inflammatory properties, regulating B-cell proliferation and differentiation and exhibiting immunoregulatory activities, such as expansion of interleukin-4-producing T cells (8). The biological significance of interleukin-10 is extensive and diverse because of its role in many diseases. Interleukin-10 is known to play a substantial role in inflammation and immune processes (9,10). Interleukin-10 has been found to have a beneficial effect in some diseases (i.e. rheumatoid arthritis) (11). On the other hand, interleukin-10 has been found to have a detrimental action in other diseases, such as systemic lupus erythematosus and systemic sclerosis (12,13). Genotypic variations in the human interleukin-10 promoter may account for individual variation in the production of interleukin-10 and susceptibility to particular diseases (14–16). Periodontitis lesions demonstrated a significantly higher messenger ribonucleic acid expression for interleukin-10 than autologous peripheral blood mononuclear cells (17). The gene encoding interleukin-10 was mapped to chromosome 1q31-32 (18). Three promoter single nucleotide polymorphisms have been described in this gene: (−1082) G/A; (−819) C/T; and (−592) C/A (19). The three single nucleotide polymorphisms from the transcriptional start site have been associated with the altered synthesis of interleukin-10 in response to inflammatory stimuli (20,21). The −1082 single nucleotide polymorphism is a G to A substitution and lies within a putative Ets transcription factor binding site (19). This allele is known to be associated with high *in vitro* production of interleukin-10 (18). The −819 single nucleotide polymorphism presents a dimorphic polymorphism, a C to T substitution, and may affect an estrogen responsive element (14). The −592 single nucleotide polymorphism is a C to A substitution and lies within a region with a negative regulatory function (19). These three dimorphisms exhibit strong linkage disequilibrium and may be potential haplotypes (21).

Interleukin-12 is a heterodimeric pro-inflammatory and immunoregulatory cytokine, composed of the two disulphide-bonded polypeptide chains p35 and p40, which is critical to the orchestration of cell-mediated immune responses in both the innate and adaptive immune systems (22–24). Recently, complete genomic sequence analysis of the interleukin-12 gene encoding its p40 subunit (interleukin-12B) identified several intronic polymorphisms, a single nucleotide polymorphism at position +16974 (or 1188) in the 3′-untranslated region of interleukin-12B (24,25) and a promoter polymorphism (interleukin-12Bpro) (26). Interleukin-12B is located at chromosome 5q31-33. A wide array of studies further demonstrated differences in genotype and allele frequencies of cytokine gene polymorphisms that were dependent on ethnicity and race (27,28). The A/C base pair substitution at position +16974 (or 1188) is associated with interleukin-12B mRNA expression levels and these reports are somewhat controversial (29–32).

The genetic factors of polymorphisms in cytokine genes have recently been described in susceptibility to periodontitis. From the PubMed search, a limited number of publications reported interleukin-10 gene polymorphisms in patients with chronic periodontitis or aggressive periodontitis. Mellati *et al.* (33), Berglundh *et al.* (20), Scarel-Caminaga *et al.* (34), Sumer *et al.* (35) and Reichert *et al.* (36) suggested that the interleukin-10 gene polymorphisms were associated with susceptibility to periodontitis. By contrast, Kinane *et al.* (37), Babel *et al.* (38), Yamazaki *et al.* (39) and Gonzales *et al.* (40) failed to demonstrate associations between periodontitis and polymorphisms in the interleukin-10 gene. As the single nucleotide polymorphisms −1082 G → A, −819 C → T and −592 C → A, which have been associated with interleukin-10 production, are strongly associated with ethnicity, determination of the ethnic distribution of interleukin-10 promoter single nucleotide polymorphisms and their haplotype frequencies is necessary for understanding the potential importance of periodontitis association studies.

Although interleukin-12 is a key factor in cell-mediated immunity, and an association between the severity of periodontal disease and interleukin-12 levels has been indicated (41), no study on the association of interleukin-12 gene polymorphisms with periodontal disease was found in the PubMed search, despite nearly 100 reports on interleukin-12 gene polymorphisms being indexed by PubMed.

In this study of a Taiwanese population, we investigated whether three polymorphisms at positions −1082, −819 and −592 in the promoter of the interleukin-10 gene and a single nucleotide polymorphism in the 3′-untranslated region of interleukin-12B (+16974 or 1188 *Taq*I) are involved in the susceptibility to chronic periodontitis and generalized aggressive periodontitis.

Material and methods

Study population

One-hundred and forty five patients with chronic periodontitis, 65 patients with generalized aggressive perio-

dontitis and 126 unrelated healthy controls were recruited from the Chung-Ho Memorial Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan and from Chi-Mei Medical Center, Tainan, Taiwan. All subjects were of Han Chinese ethnicity and from Taiwan.

Participants had at least 18 teeth when they were enrolled into the present study. They were classified into three groups: chronic periodontitis; generalized aggressive periodontitis; and healthy controls. The clinical diagnosis was based on periodontal examinations (probing pocket depth and clinical attachment loss) and radiographic patterns of alveolar bone destruction. The diagnostic criteria for generalized 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). Briefly, subjects of more than 35 years of age, with clinical attachment loss ≥ 5 mm at more than one tooth, with more than three sites of probing pocket depth > 6 mm, and lesions distributed at more than two teeth in each quadrant, were diagnosed with chronic periodontitis. Subjects who had more than eight teeth with clinical attachment loss > 5 mm and probing pocket depth > 6 mm, and at least three affected teeth that were not first molars or incisors, were diagnosed with generalized aggressive periodontitis. The age at diagnosis was below 35 years in most patients with generalized aggressive periodontitis. Subjects with no evidence of attachment loss (clinical attachment loss ≤ 1 mm) at more than one site, probing depth less than 3 mm and no history of previous periodontal disease were defined as periodontally healthy controls. The recruited healthy controls were older than 35 years, which may have helped to avoid misclassification. Smoking status was recorded as nonsmoker and smoker. Current and former smokers were included in the smoker group.

The experimental protocol of the present study was approved by our university's Ethics Committee, and signed informed consent was obtained from all participants before they were recruited into the present study.

Single nucleotide polymorphisms

Polymorphisms in the promoter region of interleukin-10 and interleukin-12 were identified using the National Center for Biotechnology Information single nucleotide polymorphism database (42).

Genomic DNA and polymerase chain reaction primers

Genomic DNA was extracted from the buffy coat using a commercial DNA isolation kit (Qiagen, Hilden, Germany). The three single nucleotide polymorphisms in the interleukin-10 gene promoter were detected by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion. The primer pairs of interleukin-10 and interleukin-12 single nucleotide polymorphisms were modified from those described by Stanilova & Miteva (43) and Edwards-Smith *et al*. (44) and are presented in Table 1. Amplification reactions were performed with 200 ng of genomic DNA, 1.0 μM each primer, 1× reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP and 1.5 U Taq polymerase (Supertherm, London, UK).

Thermal cycler conditions

The PCR amplification conditions of interleukin-10 −1082 were an initial denaturation at 95°C for 10 min, followed by 40 cycles at 94°C (35 s), 60°C (35 s) and 72°C (45 s), with a final extension step at 72°C (10 min); the PCR amplification conditions of interleukin-10 −819 were an initial denaturation at 94°C for 10 min, followed by 35 cycles at 94°C (30 s), 55°C (30 s) and 72°C (7 min), with a final extension step at 72°C (10 min); and the PCR amplification conditions of interleukin-10 −592 were an initial denaturation at 94°C for 10 min, followed by 35 cycles at 94°C (15 s), 60°C (15 s) and 72°C (1 min), with a final extension step at 72°C (7 min).

The PCR amplification conditions of interleukin-12 +16974 were an ini-

Table 1. Oligonucleotide primers used in the polymerase chain reaction (PCR) and restriction enzymes used for the digestion of PCR products

	Forward primer	Reverse primer	Size of PCR product (size of RFLP products)	Restriction enzyme
Interleukin-10 −592	5′-GAC TAC TCT TAC CCA CTT CC-3′	5′-GGA TTG AGA AAT AAT TGG GTC C-3′	319 bp (189 bp; 130 bp)	*Rsa*I
Interleukin-10 −819	5′-TCA TTC TAT GTG CTG GAG ATG-3′	5′-GAA GTG GGT AAG AGT AGT CTG-3′	204 bp (120 bp; 84 bp)	*Mae*III
Interleukin-10 −1082	5′-CTCGCTGCAACCCAACTGGC-3′	5′-TCTTACCTATCCCTACTTCC-3′	139 bp (106 bp; 33 bp)	*Mnl*I
Interleukin-12 +16974	5′- TTT GGA GGA AAA GTG GAA GA-3′	5′- AAC ATT AAC TAC ATC CGG C-3′	299 bp (168 bp; 131 bp)	*Taq*I

RFLP, restriction fragment length polymorphism.

tial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C (1 min), 54.3°C (1 min) and 72°C (2 min), with a final extension step at 72°C (7 min).

Restriction enzyme digestion

Ten microlitres of PCR product was digested with *Mnl*I for interleukin-10 −1082 single nucleotide polymorphisms, with *Mae*III for interleukin-10 −819 single nucleotide polymorphisms, with *Rsa*I for interleukin-10 −592 single nucleotide polymorphisms and with *Taq*I for interleukin-12 + 16974 single nucleotide polymorphisms (Table 1). After electrophoresis in a 3% Nusieve agarose gel, digested fragments were visualized under ultraviolet light to determine the genotypes.

Statistical analysis

Statistical analysis was performed using JMP statistical software (SAS, Cary, NC, USA). The chi-square test was used to test for deviation of genotype frequencies from Hardy–Weinberg equilibrium (45) and to compare the genotype distributions among subjects with chronic periodontitis, generalized aggressive periodontitis and healthy controls. Odds ratios and 95% confidence intervals were calculated to determine the strength of the association. Multivariate logistic regression was utilized to assess the relationship of genotype to disease status while adjusting for potential confounders, such as smoking history. A p-value of < 0.05 was considered significant.

Results

Table 2 provides a summary of the demographic characteristics of participants in the three groups. The mean age of the 145 chronic periodontitis patients (85 men and 60 women) was 53.39 ± 8.62 (standard deviation) years. The mean age of generalized aggressive periodontitis patients (n = 65; 39 men and 26 women) was 38.46 ± 7.85 years. In the healthy controls (n = 126; 59 men and 67 women), the mean age was 49.00 ± 10.58 years. The percentage of smokers was higher in the chronic periodontitis and the generalized aggressive periodontitis groups (26.9% and 23.1% respectively) than in the healthy control group (13.5%).

The allele frequencies and genotype distributions for the three interleukin-10 gene polymorphisms and the interleukin-12 gene polymorphism among the three study groups are summarized in Table 3. The genotype frequencies were in agreement with the Hardy–Weinberg equilibrium.

Distribution of interleukin-10 polymorphisms

The distributions of the interleukin-10 gene polymorphisms at position −592 were statistically different among the three groups (χ^2 = 10.484, p = 0.0330)

Table 3. Genotype distribution in chronic periodontitis (CP), generalized aggressive periodontitis (AgP) and healthy control (H) groups

	CP (n = 145)		AgP (n = 65)		H (n = 126)			
Genotype	n	%	n	%	n	%	χ^2	p-value
Interleukin-10 −592								
AA	86	59.3	38	58.5	62	49.2	10.484	0.0330
AC	32	22.1	21	32.3	48	38.1		
CC	27	18.6	6	9.2	16	12.7		
Interleukin-10 −819								
CC	16	11.0	6	9.2	12	9.5	2.692	0.6105
CT	52	35.9	22	33.9	55	43.7		
TT	77	53.1	37	56.9	59	46.8		
Interleukin-10 −1082								
AA	132	91.0	60	92.3	115	91.3	0.095	0.9537
AG	13	9.0	5	7.7	11	8.7		
GG	0	0	0	0	0	0		
Interleukin-12B + 16974								
AA	46	31.7	17	26.2	42	33.3	6.812	0.1462
AC	70	48.3	37	56.9	72	57.2		
CC	29	20	11	16.9	12	9.5		

Table 2. The demographic characteristics of participants

	CP (n = 145)		AgP (n = 65)		H (n = 126)			
	n	%	n	%	n	%	χ^2	p-value
Age	53.39 ± 8.62		38.46 ± 7.85		49.00 ± 10.58			< 0.0001
Gender								
Male	85	58.6	39	60.0	59	46.8	4.776	0.0900
Female	60	41.4	26	40.0	67	53.2		
PPD (mm)	3.70 ± 1.66		4.70 ± 3.32		1.29 ± 0.45			< 0.0001
CAL (mm)	4.57 ± 1.81		5.04 ± 2.20		0.29 ± 0.46			< 0.0001
Smoking status								
Smokers	39	26.9	15	23.1	17	13.5	7.452	0.0241
Nonsmokers	106	73.1	50	76.9	109	86.5		

AgP, generalized aggressive periodontitis; CAL, clinical attachment loss; CP, chronic periodontitis; H, healthy control; PPD, probing pocket depth.

(Table 3). Significant differences in genotype frequencies of the −592 locus between chronic periodontitis and healthy control groups were confirmed through odds ratio and 95% confidence intervals in total subjects (odds ratio = 0.33, 95% confidence interval = 0.15–0.70, in AC vs. AA genotype). No statistically significant difference in the distribution of interleukin-10 polymorphisms at −1082, −819 and −592 was found between generalized aggressive periodontitis and healthy control groups (Table 4).

The distribution of haplotypes arranged as alleles showed no significant difference in the total samples when comparing chronic periodontitis and generalized aggressive periodontitis groups with the healthy control group. The dominant haplotype was ATA in the three groups (63.4% in the chronic periodontitis group, 64.3% in the healthy control group and 73.9% in the generalized aggressive periodontitis group) (Table 5). Individuals with the homozygous ATA/ATA haplotype seemed to be more than twice as likely to develop generalized aggressive periodontitis than individuals with other haplotypes (odds ratio = 2.52, 95% confidence interval = 1.14–5.98, $p = 0.0276$) and the carriers of the combined ATA/ACC polymorphisms were less likely to develop chronic periodontitis than individuals with other haplotypes (odds ratio = 0.46, 95% confidence interval = 0.23–0.90, $p = 0.0248$) (Table 5).

Distribution of interleukin-12 polymorphisms

The polymorphic interleukin-12 genotype at position +16974 was found to be more frequent in all subjects with chronic periodontitis than in healthy controls (20% vs. 9.5%) (Table 3). The CC genotype was associated with chronic periodontitis as compared with AA and AA + AC genotypes ($\chi^2 = 5.32$, odds ratio = 3.11, 95% confidence interval = 1.22–8.53, $p = 0.0211$ and $\chi^2 = 5.53$, odds ratio = 2.37, 95% confidence interval = 1.18–5.04, $p = 0.0187$ respectively) (Table 4). There was a higher frequency of the AC genotype in healthy controls and patients with generalized aggressive periodontitis (57.2% and

Table 4. Frequencies of interleukin-10 −592, −819, −1082 and interleukin-12B +16974 single nucleotide polymorphisms in chronic periodontitis (CP), generalized aggressive periodontitis (AgP) and healthy control (H) groups

Genotype	H $n = 126$ (%)	CP $n = 145$ (%)	AgP $n = 65$ (%)	CP development, OR (95% CI)	AgP development, OR (95% CI)
Interleukin-10 −592					
AA	62 (49.2)	86 (59.3)	38 (58.5)	1.00	1.00
AC	48 (38.1)	32 (22.1)	21 (32.3)	0.33* (0.15–0.70)*	0.88** (0.34–2.36)**
CC	16 (12.7)	27 (18.6)	6 (9.2)	2.12** (0.89–5.35)	0.65** (0.16–2.31)
AC + CC	64 (50.8)	59 (40.7)	27 (41.5)	0.66** (0.41–1.07)	0.69** (0.37–1.26)
AA + AC	110 (87.3)	118 (81.4)	59 (90.8)	1.00	1.00
CC	16 (12.7)	27 (18.6)	6 (9.2)	1.57** (0.81–3.13)	0.70** (0.24–1.80)
A allele	172 (68.3)	204 (70.3)	97 (74.6)	1.00	1.00
C allele	80 (31.7)	86 (29.7)	33 (25.4)	0.91** (0.63–1.31)	0.73** (0.45–1.17)
Interleukin-10 −819					
CC	12 (9.5)	16 (11.0)	6 (9.2)	1.00	1.00
CT	55 (43.7)	52 (35.9)	22 (33.9)	0.65** (0.32–1.35)	0.64** (0.24–1.70)
TT	59 (46.8)	77 (53.1)	37 (56.9)	1.22 (0.59–2.50)	1.57 (0.64–4.01)
TT + CT	114 (90.5)	129 (89.0)	59 (90.8)	0.85** (0.38–1.86)	1.04** (0.38–3.10)
CC + CT	67 (53.2)	68 (46.9)	28 (43.1)	1.00	1.00
TT	59 (46.8)	77 (53.1)	37 (56.9)	1.28** (0.80–2.08)	1.50** (0.82–2.76)
C allele	79 (31.3)	84 (29.0)	34 (26.2)	1.00	1.00
T allele	173 (68.7)	206 (71.0)	96 (73.8)	1.12** (0.77–1.62)	1.29** (0.81–2.09)
Interleukin-10 −1082					
AA	115 (91.3)	132 (91.0)	60 (92.3)	1.00	1.00
AG	11 (8.7)	13 (9.0)	5 (7.7)	1.03** (0.44–2.43)	0.87** (0.26–2.51)
GG	–	–	–	–	–
A allele	241 (95.6)	277 (95.5)	125 (96.2)	1.00	1.00
G allele	11 (4.4)	13 (4.5)	5 (3.8)	1.03** (0.45–2.38)	0.88** (0.27–2.47)
Interleukin-12B + 16974					
AA	42 (33.3)	46 (31.7)	17 (26.2)	1.00	1.00
AC	72 (57.2)	70 (48.3)	37 (56.9)	0.50* (0.25–0.99)	0.80** (0.34–1.87)
CC	12 (9.5)	29 (20)	11 (16.9)	3.11* (1.22–8.53)	2.53** (0.76–8.35)
AC + CC	84 (66.7)	99 (68.3)	48 (73.8)	1.07** (0.65–1.79)	1.41** (0.73–2.80)
AA + AC	114 (90.5)	116 (80.0)	54 (83.1)	1.00	1.00
CC	12 (9.5)	29 (20.0)	11 (16.9)	2.37* (1.18–5.04)	1.94** (0.79–4.69)
A allele	156 (61.9)	162 (55.9)	71 (54.6)	1.00	1.00
C allele	96 (38.1)	128 (44.1)	59 (45.4)	1.28** (0.91–1.81)	1.35** (0.88–2.07)

Smoking status, age and gender were adjusted by multivariate logistic regression analysis..
CI, confidence interval; OR, odds ratio.
*$p < 0.05$, **$p > 0.05$.

Table 5. Distribution of interleukin-10 locus haplotypes found in chronic periodontitis (CP), generalized aggressive periodontitis (AgP) and healthy control (H) groups

Haplotype −1082 −819 −592	CP n = 290 n (%)	H n = 252 n (%)	AgP n = 130 n (%)	H n = 252 n (%)
GCC	10 (3.5)	10 (4.0)	5 (3.9)	10 (4.0)
ACC	55 (19.0)	60 (23.8)	28 (21.5)	60 (23.8)
ATA	184 (63.4)	162 (64.3)	96 (73.9)	162 (64.3)
GTA	1 (0.3)	1 (0.4)	–	1 (0.4)
ATC	21 (7.2)	10 (4.0)	–	10 (4.0)
GCA	2 (0.7)	–	–	–
ACA	17 (5.9)	9 (3.5)	1 (0.7)	9 (3.5)
ATA	184 (63.4)	162 (64.3)	96 (73.8)	162 (64.3)
Others	106 (36.6)	95 (35.7)	34 (26.2)	95 (35.7)
p-value/OR/95% CI*	0.8324/1.04/0.72–1.51		0.1051/0.64/0.37–1.09	
ACC	55 (19.0)	60 (23.8)	28 (21.5)	60 (23.8)
Others	235 (81.0)	192 (76.2)	102 (78.5)	192 (76.2)
p-value/OR/95% CI*	0.2359/1.30/0.84–2.01		0.8849/1.04/0.58–1.90	
Haplotype	n = 126	n = 126	n = 65	n = 126
−1082 −819 −592/−1082 −819 −592	n (%)	n (%)	n (%)	n (%)
ATA/ATA	69 (47.6)	53 (42.1)	37 (56.9)	53 (42.1.)
Others	76 (52.4)	73 (57.9)	28 (43.1)	73 (57.9)
p-value/OR/95% CI*	0.8007/0.93/0.53–1.63		0.0276/2.52/1.14–5.98	
ATA/ACC	24 (16.6)	37 (29.4)	18 (27.7)	37 (29.4)
Others	121 (83.4)	89 (70.6)	47 (72.3)	89 (70.6)
p-value/OR/95% CI*	0.0248/0.46/0.23–0.90		0.1937/1.82/0.75–4.65	

Smoking status, age and gender were adjusted by multivariate logistic regression analysis.
*CI, confidence interval; OR odds ratio.

56.9%) compared to patients with chronic periodontitis (48.3%) (Table 3). The AC heterozygous genotype was significantly lower in patients with chronic periodontitis than in healthy controls (χ^2 = 3.84, odds ratio = 0.50, 95% confidence interval = 0.25–0.99, p = 0.0500 in all chronic periodontitis patients).

In the analysis of chronic periodontitis vs. generalized aggressive periodontitis, there were no significant differences between these four polymorphisms in the present study.

Discussion

There is a marked difference in the distribution of interleukin-10 polymorphisms among ethnic groups. The haplotype frequencies of interleukin-10 gene promoters in Taiwanese subjects are quite different from those of Caucasians (21) but are racially close to those of southern Chinese and Japanese subjects (39,46). The frequency of the ATA haplotype was 0.64 in the present study, 0.64 in southern Chinese subjects, 0.71 in Japanese subjects and 0.21 in Caucasians. Moreover, in the present study we found no subject with the −1082 GG genotype, but in studies of western populations, more than 10% had the GG genotype (34% in English subjects, 12% in Italian subjects and 16% in Greek subjects) (47). Most recently, Donati *et al*. (48) reported that interleukin-10-positive cells in the peripheral area of periodontitis lesions were significantly larger in subjects with the −187 interleukin-10 GG genotype than in subjects with the AG or AA genotype.

Recent studies have found no significant association between the interleukin-10 −1087 G/A gene promoter polymorphisms and susceptibility to chronic periodontitis in Japanese and Brazilian subjects, but these polymorphisms might be associated with the severity of chronic periodontitis (odds ratio = 2.58) in Swedish Caucasians (20,34,39). The −819 C/T and −592 C/A loci were correlated with susceptibility to chronic periodontitis in Brazilian subjects but not in Japanese or German Caucasian subjects (34,39,41). In the present study, subjects with the AC genotype of the interleukin-10 −592 locus had a lower susceptibility to chronic periodontitis. This was similar to the results of the Brazil investigation, in which the C allele at the −592 position of interleukin-10 seems to 'protect' individuals from chronic periodontitis (34).

It is known that the interleukin-10 ATA/ATA genotypes are associated with lower interleukin-10 production upon stimulation with microbial lipopolysaccharide than other genotypes (15,39). The amount of interleukin-10 secreted in cell cultures indicated that the GCC haplotype is associated with an increased production of interleukin-10, whereas the ACC and the ATA haplotypes are associated with intermediate and decreased production of interleukin-10 (49). In German subjects, the combination of ATA/ATA was found only in patients with generalized aggressive periodontitis (36). In our study, the incidence of ATA was 63.4% in subjects with chronic periodontitis, 64.3% in healthy controls and 73.9% in subjects with generalized aggressive periodontitis. Analysis of the interleukin-10 haplotype demonstrated that subjects with the ATA/ATA genotype showed a significantly higher incidence of generalized aggressive periodontitis and individuals with the ATA/ACC genotype were less susceptible to chronic periodontitis. Individuals in the CP and AgP groups with the ATA haplotype would have lower levels of interleukin-10, and this could explain the high level of inflammatory cytokines found in periodontitis.

In ethnic comparisons, differences were found in interleukin-10 and interleukin-12 gene polymorphisms between eastern and western populations. In the present study, the incidence of the +16974(AC) genotype of interleukin-12B was 57% (Table 4). This is similar to the incidence found in Hong Kong Chinese (53%) (50) and Japanese (52%) subjects (51) but different from that found in Australian (27%) (52) and Bulgarian (32%) subjects (43). In this study, patients with the interleukin-12 CC genotype had a higher susceptibility to chronic periodontitis (odds ratio = 3.11, p = 0.0211). Several single

nucleotide polymorphisms in both p40 and p35 subunits of interleukin-12 have been identified (24,25). Disease associations with the +16974 (or 1188) *Taq*I polymorphism at the 3′-untranslated region of the interleukin-12B gene have also been reported (24,25). It was found that that individuals with the CC genotype in the interleukin-12B 3′-untranslated region had significantly higher interleukin-12 secretion levels *in vitro* than individuals with AC or AA genotypes (29). Individuals who are high producers of interleukin-12 might be more susceptible to chronic periodontitis as a result of the pro-inflammatory role of interleukin-12 (22–24). The AC genotype frequency was lower in subjects with chronic periodontitis, suggesting that the AC genotype may have a lower susceptibility for the development of chronic periodontitis. This was the same as the study result of silicosis (53) in which the AC genotype was approximately five times more frequent in patients with mild disease than in patients with severe disease.

In particular, the AC genotype was found to be more protective in nonsmokers with chronic periodontitis than in all patients ($p = 0.0282$, odds ratio = 0.41 and $p = 0.0500$, odds ratio = 0.50). This means that smoking may interfere with the genetically determined susceptibility or resistance to periodontits.

In conclusion, within the limitations of ethnicity, sample selection and number in this study, the interleukin-10 gene polymorphism at position −592 seems to be associated with a lower risk for developing chronic periodontitis. The genotype frequencies of interleukin-10 analysis suggested that ATA homozygotes may be associated with aggressive periodontitis. On the other hand, the distribution of interleukin-12B genetic variants at position +16974 is associated with susceptibility to chronic periodontitis.

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