Polymorphisms in an interferon-γ receptor-1 gene marker and susceptibility to periodontitis*

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Chronic marginal periodontitis is an inflammatory condition in which the supporting tissues of the teeth are destroyed. Interferon (IFN)-γ is a cytokine that plays a pivotal role in the defense against infection, and mutations in the gene coding for the ligand binding chain (α, R1) of the IFN-γ receptor (IFNGR1) confer susceptibility on infections caused by poorly virulent mycobacteria. Using an intronic $(CA)_n$ polymorphic microsatellite marker within the IFNGR1 gene we investigated whether genetic polymorphisms are associated with periodontitis. In 62 periodontitis patients and 56 healthy controls we found a total of 13 polymorphisms, 11 of which were found in the periodontitis patients and 9 in the controls. Although we observed a trend towards an association with disease for allele 192, there were no significant differences in allele frequency between patients and controls. We therefore cannot find any evidence to suggest that IFNGR1, as a single dominant gene, contributes to susceptibility to periodontitis. However, in combination with the environmental risk factor, smoking, the same allelic marker was significantly associated [OR = 5.56 (1.16<OR<36.31), $P = 0.014$, $P_{corr} = 0.027$] with periodontitis. Our results support the multigene-environment interaction model of disease susceptibility to periodontitis. □ *Genotyping; IFN-γ receptor; IFNGR1; periodontitis; polymorphism; smoking*

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Chronic marginal periodontitis is an inflammatory disease of the supporting tissues of the teeth. The condition is characterized by inflammation of the periodontal tissues, periodontal attachment loss, destruction of alveolar bone, and the loss of teeth (1). It is triggered by pathogenic bacteria in dental plaque, but the connective tissue and alveolar bone destruction mainly associated with periodontitis is mediated by the local release of pro-inflammatory mediators, especially cytokines (2). Severe periodontitis develops in about 10–15% of the population (3). Studies in twins have shown that there is a distinct genetic component involved in the pathogenesis of the disease (4) and it is now accepted that the disease develops in genetically susceptible subjects in whom unfavorable environmental conditions prevail (5). Indeed, although specific periodontal bacteria such as *Porphyromonas gingivalis* are believed to be major etiological agents, some individuals harbor these microorganisms, but do not have any signs of disease progression (6). Accordingly, in some studies focusing on the role of genetic predisposition, several polymorphisms in host defense genes have been found associated with periodontitis (5).

Interferon (IFN)-γ is a cytokine produced by activated $CD4^+$ T lymphocytes and NK cells and is a major macrophage activating factor. IFN-γ is believed to play an important role in periodontitis, although it is as yet unclear whether this role is protective or pathogenic in nature (7). It has been proposed that IFN-γ is associated with stable periodontal conditions, while Th2 type cytokines such as interleukin (IL)-4 are associated with disease progression (8, 9). Others have reported that IFN-γ is involved in destructive periodontal lesions, whereas Th2 type cytokines would be protective (10).

The cellular effects of cytokine signaling are dependent upon the expression of functional cytokine receptors on the target cells. Mutations in the gene coding for the R1 chain of the IFN-γ receptor (IFNGR1) have previously been reported to confer dominant or recessive susceptibility to infections caused by poorly virulent *mycobacteria* via impaired signaling (11, 12). We have also recently shown that a particular allelic marker in the IFNGR1 is associated with susceptibility to tuberculosis, but not in a Mendelian fashion (13). In light of the possible relationship between genetic variations in the receptor for IFN-γ and infections, we examined whether polymorphisms within the IFNGR1 gene are associated with adult periodontitis. We did this by assessing allelic frequencies of polymorphisms in an intronic gene marker. The marker is a microsatellite consisting of multiple repeats of CA dinucleotides located in the penultimate (5th) intron of the IFNGR1 gene (12). The reported sequence around this

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region (FA1, Genebank accession number U84721) has 21 CA repeats.

Methods

Study population

A total of 62 consecutive patients (22 M, 40 F) attending the Academic Center of Dentistry Amsterdam (ACTA) for treatment of periodontitis participated in this study. The referent population consisted of 56 (25 M, 31 F) unrelated subjects residing in the same geographic area who did not have periodontitis. All subjects were >21 years of age. On the basis of interview and the birthplace of both parents, only subjects of strictly Northern European Caucasian heritage were included in the study. All persons were informed both verbally and in writing about the purpose of the study and signed an informed consent document prior to participation. An institutional review board approved the study. None of the volunteers suffered from any systemic disease. Smoking habits were recorded and pack years calculated as number of packs/day times years smoking. Patients were classified as non-smokers, former smokers or current smokers; former smokers were those whose number of years of smoking cessation was <2 times their individual number of pack years (14). The possible systemic component of the periodontal infection in patients was measured by the levels of C-reactive protein (CRP) in plasma; CRP levels in controls were also determined (15). Median values for populations were used as a measure of high and low CRP carriers.

Diagnosis of periodontitis

A set of full mouth dental radiographs was taken for each periodontitis patient and the number of teeth present was determined. Subsequently, for each patient all teeth were radiographically examined on the mesial and distal aspects. The number of teeth without bone loss (distance cemento-enamel junction to the bone crest $\leq$2 mm), as well as numbers of teeth within the following bone loss categories were determined: (1) bone loss beyond the coronal 1/3 of the root length (bone loss >1/3); and (2) bone loss $\geq$50% of the root length.

Bacteriological samples

A pooled subgingival plaque sample was taken from all subjects to assess the prevalence and proportions of the following bacteria: *A. actinomycetemcomitans*, *P. gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Bacteroides forsythus*, and *Peptostreptococcus micros*. Sampling was performed as described previously (16). In control subjects, samples were taken from the mesio- and distobuccal sites of the first molars; when these were not present the second molars were used for sampling. Subgingival samples were taken from the deepest bleeding site per quadrant. The culturing and identification procedures of the samples were performed according to previously described standard procedures (16).

DNA isolation

Venous blood samples were obtained by venipuncture in the antecubital fossa. The blood was collected into an EDTA containing vacuum tube and transported to the laboratory for DNA extraction. DNA isolation was performed following a miniprep procedure as described previously (14). The integrity of DNA preparations was checked on 0.6% agarose minigels. Purified DNA samples were stored at 4°C in sealed microtubes.

DNA analysis

A region of approximately 200 bp surrounding the $(CA)_n$ microsatellite was amplified by means of the polymerase chain reaction (PCR) with the use of the primers 5′ TTGTACTCATGCTTTGATG 3′ and FAM-labeled 5′ TTAGTGTTTGTTCTGGCTC 3′. PCR products were genotyped by electrophoresis on 6% polyacrylamide gels with the use of an ABI 370 sequencer (Applied Biosystems) and the computer software Genescan (Perkin-Elmer).

Statistical evaluation

Contingency table analyses and chi-square tests were carried out and significance values were calculated. We analyzed allelic frequencies using the Statcalc program. Differences of $P < 0.05$ were considered statistically significant. Correction was done by the Bonferroni method.

Results

A description of patient characteristics is given in Table 1. The mean age of both periodontitis patients and control subjects was 43 years. There were more females in both the patient and control groups, but there was no significant difference between groups. There was a significant difference in smoking status between patients and controls ($P < 0.001$): more patients were classified as former smokers or current smokers, while there was a higher proportion of non-smokers among the control subjects. Mean levels of CRP between patients (2.0 ± 3.0 mg/L) and controls (2.9 ± 4.8 mg/L) did not statistically differ. In the patient population, on average, 26.3 teeth were present; the mean number of teeth in the control group was 27.5. Among the patients, a mean of 7.5 teeth without bone loss was recorded. Furthermore, in the periodontitis patients a mean of 9.0 teeth showed bone loss beyond the coronal 1/3 of the root length, 6.0 teeth showed bone loss $\geq$50% of the root length (Table 1).

The results of the analyses of the subgingival plaque samples are presented in Table 2. A significantly higher prevalence of periodontopathogens in periodontitis versus

Table 1. Demographic characteristics of study population. Values are means ± standard deviations, numbers (%) of subjects, or mean numbers (%) of teeth

	Control ($n = 56$)	Periodontitis ($n = 62$)
Age	43.0 ± 12.3	43.4 ± 10.4
Gender		
Male	25 (45%)	22 (36%)
Female	31 (55%)	40 (65%)
Smoking*		
Non-smoker	35 (63%)	15 (24%)
Former smoker	3 (5%)	2 (3%)
Smoker	18 (32%)	45 (73%)
CRP (mg/L)	2.0 ± 3.0	2.9 ± 4.8
Number (%) of teeth		
Total	27.5 ± 1.5	26.3 ± 3.2
Without bone loss (%)	27.5 (100%)	7.5 (28%)
With bone loss in the following categories:		
>1/3 bone loss (%)	0	9.0 (35%)
≥50% bone loss (%)	0	6.0 (23%)

* Significantly different distribution between groups: $P < 0.001$ (chi-square test).

Table 3. Allele frequencies of polymorphisms in the IFNGR1 gene in periodontitis patients ($n = 62$) and healthy controls ($n = 56$) (percentage in parentheses)

Allele	Controls ($n = 112$)*	Patients ($n = 124$)*†
174	29 (26%)	25 (20%)
176	0	2
182	1	3
184	0	1
186	11 (10%)	16 (13%)
188	0	2
190	17 (15%)	12 (10%)
192‡	12 (11%)‡	23 (19%)‡
194	25 (22%)	26 (21%)
196	13 (12%)	13 (10%)
198	3	0
200	1	0
202	0	1
Grouped (<5%)†	5 (4%)	9 (7%)

* Total alleles.
† A selection of the above listed alleles analyzed as a single group. The group comprises all alleles from the above list without indicated percentage. Each member had a frequency of <5% in both periodontitis patients and controls.
‡ $P < 0.15$.

controls was observed, except for *A. actinomycetemcomitans* and *F. nucleatum*. Proportions of cultured bacteria in culture-positive subjects were not different between the two groups, except for *B. forsythus* ($P = 0.027$).

Out of a total of 38 detected genotypes of IFNGR1 polymorphic genetic marker, periodontitis patients had 26 different genotypes, whereas the control population had 22. Interestingly, smokers with periodontitis had 23 diverse genotypes. This was more than observed for control smokers, who numbered 15. Uniformity in genotypes for control smokers was also evident from IFNGR1 allele marker analysis for homozygosity. One-third of control smokers were homozygous, compared with one-fifth of smokers with periodontitis.

A total of 13 polymorphisms were found in patients and controls combined; 11 in the periodontitis patients and 9 in the healthy controls. Alleles 174, 186, 190, 192, 194, and 196 were the most common and their frequencies are given in Table 3. The largest variation in frequency between patients and controls was observed for allele 192 (19% versus 11%, respectively), although this difference was not statistically significant ($P = 0.14$). The other alleles had similar frequencies in the patient and control groups. Alleles 176, 182, 184, 188, 198, 200, and 202 had low frequencies in both patients and controls (<5%) and were grouped for statistical analyses.

Within the patient group, we further assessed whether there was an association between IFNGR1 gene polymorphisms and disease severity. As can be seen from Table 4, we graded severity in a total of 60 patients, 15 of whom had severe disease, while 45 were classified as having moderate disease. We did not find any significant differences between the groups. Allele frequencies for the IFNGR1 gene were not different between aggressive periodontitis patients and controls, and between chronic periodontitis and controls.

We subsequently included smoking status in the analysis. As indicated in Table 5, the carriage rate of

Table 2. Results of the analyses of the subgingival microbial samples for control and periodontitis. The left column per group represents prevalence, i.e. number (n) of subjects culture positive (in parentheses), while the right column per group represents the mean values for proportion ± standard deviation of the microorganism of the cultivable anaerobic microflora in culture positive subjects

	Control ($n = 56$)		Periodontitis ($n = 62$)		
	n (%)	Proportions	n (%)	Proportions	P values*
A. actinomycetemcomitans	6 (11)	2.1 ± 3.9	13 (21)	3.0 ± 5.5	0.136
P. gingivalis-	4 (7)	29.2 ± 31.3	33 (53)	23.4 ± 21.1	<0.001
P. intermedia	38 (68)	7.2 ± 9.7	53 (85)	6.4 ± 6.6	0.012
B. forsythus	30 (54)	4.4 ± 6.0	55 (89)	8.7 ± 9.6	<0.001
F. nucleatum	48 (86)	4.7 ± 5.0	57 (92)	6.3 ± 7.6	0.117
P. micros	41 (73)	6.6 ± 8.4	55 (89)	6.0 ± 6.6	0.013

* P values from statistical analysis for prevalence of a bacterial species between controls and patients.

Table 4. Allele frequencies of polymorphisms in the IFNGR1 gene in periodontitis patients ($n = 60$; for 2 patients, radiological data were unavailable, and they could not be classified) according to disease severity (percentage in parentheses)

Allele	Moderate ($n = 90$)	Severe ($n = 30$)*
174	16 (18%)	8 (27%)
176	2 (2%)	0
182	2 (2%)	1 (0.3%)
184	0	1 (0.3%)
186	13 (14%)	2 (7%)
188	2 (2%)	0
190	10 (11%)	2 (7%)
192	15 (17%)	7 (23%)
194	20 (22%)	6 (20%)
196	10 (11%)	2 (7%)
202	0	1 (0.3%)

* Total alleles.

Table 5. Allele frequencies of polymorphisms in the IFNGR1 allele-marker frequencies in periodontitis patients ($n = 60$) and healthy controls ($n = 56$) divided into smokers and non-smokers (as described in the Methods section). The smoking history was not available for 2 patients, who were thus not classified

	Controls ($n = 70$)†		Patients ($n = 28$)†		Controls ($n = 42$)†		Patients ($n = 92$)†	
	Non-smoking		Non-smoking		Smoking		Smoking	
Allele	No	%	No	%	No	%	No	%
174	17	24	9	32	12	29	15	16
176	0	0	0	0	0	0	2	2
182	1	1	1	4	0	0	2	2
184	0	0	0	0	0	0	1	1
186	9	13	4	14	2	5	12	13
188	0	0	2	7	0	0	0	0
190	8	11	1	4	9	21	10	11
192#	10	14	2	7	2	5#	20	22#
194	15	21	6	21	10	24	19	21
196	10	14	3	11	3	7	10	11
198	0	0	0	0	3	7	0	0
200	0	0	0	0	1	2	0	0
202	0	0	0	0	0	0	1	1

† Total alleles.
# $P = 0.0138$, $P_{corr} = 0.027$; OR 5.56 (1.16<OR<36.31).

allele 192 was significantly higher in smoker patients (22%) compared to smoker controls (5%) (OR = 5.56, $P = 0.0138$, $P_{corr} = 0.027$). No differences in allele frequencies were seen between non-smoking patients and controls.

The current periodontitis patients were previously examined for the presence of periodonto-pathogenic bacteria in dental plaque (16), and for plasma levels of C-reactive protein (CRP) (15). When we divided the patient group into persons with and persons without *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, or *Fusobacterium nucleatum* in their subgingival plaque samples, we found no significant differences in polymorphisms of IFN-γ allele marker. Similarly, no differences in allele frequencies were observed between the patient group and the control group, who were selected on the basis that their cultures for both *A. actinomycetemcomitans* and *P. gingivalis* were negative. Likewise, in persons with higher and lower than median values of plasma CRP (median = 1.2), we could not find any clear associations with IFNGR1 gene polymorphisms in relation to susceptibility. Interestingly, carriers of allelic marker 192 in smokers with periodontitis with high CRP were more numerous than their counterpart in the control population (23% versus 14%). Although the numbers of persons in subpopulations analyzed for CRP values were too small for statistical analyses, we consider these differences as trends. Taken altogether, we find no association of IFN-γ allelic marker polymorphisms with carriage of various periodonto-pathogenic bacteria in dental plaque. Similarly, CRP values were not significantly associated with polymorphisms in IFN-γ allelic markers.

Discussion

Polymorphisms in any number of genes which play a role in IFN-γ dependent inflammatory responses could be important in determining susceptibility or resistance to periodontitis. As the dominant macrophage-activating factor, IFN-γ dependent inflammatory responses can play an important role in tissue destruction. Strong IFN-γ signaling can stimulate macrophages in periodontal tissue to produce excessive amounts of IL-1, IL-6 and tumor necrosis factor (TNF)-α, with subsequent activation of osteoclasts, resulting in destruction of supporting bone (2). IL-1 in particular is believed to act as a major mediator of tissue destruction in periodontal disease and analyses of gingival tissues have identified increased concentrations of both IL-1α and -β at the protein and/or mRNA level (17). However, the specific role played by IFN-γ in periodontitis is unclear, because it has also been reported that mitogen-stimulated peripheral blood mononuclear cells from periodontitis patients show reduced IFN-γ production and mRNA expression (9, 18). In addition, we have also previously shown a reduced IFN-γ production in *ex vivo* T-cell cultures from patients with periodontitis after stimulation with HSP antigens (19).

We presently chose to analyze the IFNGR1 gene, as some mutations have been shown to be associated with impaired IFN-γ signaling due to a lack of the intracytoplasmic domain of the receptor [for review see (20)]. However, we did not find alleles that could serve as genetic markers significantly associated with the occurrence of periodontitis or its severity. We recently reported that the IFNGR1 allele 192 occurred with a significantly lower frequency in tuberculosis patients as compared to controls, suggesting a protective association (13). Interestingly, and in contrast to tuberculosis patients, we found a trend that the same allele (192) occurred with an increased frequency in periodontitis patients as compared to controls (19%

versus 11%, respectively), although the difference was non-significant (Table 3). It was, however, significant in the smoker patients (OR = 5.56, $P = 0.013$, $P_{corr} = 0.027$) (Table 5). This may suggest that this allele is associated with a functional hypermorphic variation, i.e. stronger IFN-γ-dependent inflammatory responses. However, a functional assessment is needed to confirm this.

In theory, a relative deficiency of IFN-γ signaling secondary to IFNGR1 mutations could facilitate the establishment of periodontopathic bacteria, such as *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, and *F. nucleatum*. These in turn might cause gingival tissue and bone damage, as seen in this form of periodontitis. However, we could not find any association between IFNGR1 alleles and the presence or absence of any of these pathogens. Alternatively, increased IFN-γ signaling might lead to extensive macrophage activation that could hypothetically also cause gingival tissue and bone damage, as either the reaction to infection or perhaps as autoimmune disease. Activated macrophages secrete cytokines like IL-1, IL-6, and TNF-α, which induce the release of acute phase proteins by the liver. We searched for an association between IFNGR1 gene polymorphisms and levels of serum CRP. The analyses did not show any significant associations. When periodontitis patients were subdivided into smokers and non-smokers, the allelic IFN-γ marker 192 showed only a trend for association with higher CRP values. However, CRP as a systemic factor is probably a less reliable marker for local tissue changes, such as the activation of macrophages. Interestingly, IL-1 genotype composite polymorphism analysis (the type of analysis we were unable to perform in the present work because of insufficient numbers) showed an increased risk of periodontal disease for IL-1 genotype-positive smokers. Subjects who never smoked showed no increased risk for specific IL-1 genotype (21). Along the same line of thought, severe chronic periodontitis might be associated with a polymorphism in the IL-10 gene (G to A transition at the –1087 position) in the Swedish Caucasian population. This association was increased in smokers (22). IL-10 is a T-helper 2-response polarizing cytokine; the action of this cytokine is thought to counteract the actions of IFN-γ and Th1-type cytokines such as IL-12, IL-18, and IL-23. Thus, hypothetically, a less efficient allele of IL-10 might skew the Th1/Th2 balance, for example, in immune responses to periodonto-pathogenic bacteria in favor of Th1 type, including IFN-γ, resulting in greater bone destruction. These reports, along with our findings, concur with the suggestion that there is an interaction between multigenetic and environmental risk factors in susceptibility to periodontitis.

In summary, our results do not provide evidence that polymorphisms in the IFNGR1-gene marker are associated in Mendelian fashion with chronic marginal periodontitis. In smokers, we found that the frequency of allelic marker 192 was significantly associated with periodontitis, compared to controls. This allele may not itself have an etiological role in periodontitis, but instead act as a response modifier to smoking and perhaps other environmental factors.

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References

1. Offenbacher S. Periodontal diseases: pathogenesis. Ann Periodontol 1996;1:821–78.
2. Okada H, Murakami S. Cytokine expression in periodontal health and disease. Crit Rev Oral Biol Med 1998;9:248–66.
3. Gjermo P. Epidemiology of periodontal diseases in Europe. J Parodontol Implantol Orale 1998;17:111–21.
4. Michalowicz BS, Aeppli D, Virag JG, Klump DG, Hinrichs JE, Segal NL, et al. Periodontal findings in adult twins. J Periodontol 1991;62:293–9.
5. Loos BG, Van der Velden U. Genetics in relation to periodontitis. In: Lindhe J, Karring T, Lang NP, editors. Clinical periodontology and implant dentistry. London/Copenhagen: Blackwell/Munksgaard; 2003. p. 387–99.
6. Seymour GJ. Importance of the host response in the periodontium. J Clin Periodontol 1991;18:421–6.
7. Seymour GJ, Gemmell E. Cytokines in periodontal disease: where to from here? Acta Odontol Scand 2001;59:167–73.
8. Tokoro Y, Matsuki Y, Yamamoto T, Suzuki T, Hara K. Relevance of local Th2-type cytokine mRNA expression in immunocompetent infiltrates in inflamed gingival tissue to periodontal diseases. Clin Exp Immunol 1997;107:166–74.
9. Petit MD, Hovenkamp E, Hamann D, Roos MT, van der Velden U, Miedema F, et al. Phenotypical and functional analysis of T cells in periodontitis. J Periodontal Res 2001;36: 214–20.
10. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. Periodontol 2000;5:112–41.
11. Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondaneche MC, Dupuis S, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nat Genet 1999;21:370–8.
12. Altare F, Jouanguy E, Lamhamedi-Cherradi S, Fondaneche MC, Fizame C, Ribierre F, et al. A causative relationship between mutant IFNgR1 alleles and impaired cellular response to IFNgamma in a compound heterozygous child. Am J Hum Genet 1998;62:723–6.
13. Fraser DA, Bulat-Kardum L, Knezevic J, Babarovic P, Matakovic-Mileusnic N, Dellacasagrande J, et al. Interferon-gamma receptor-1 gene polymorphism in tuberculosis patients from Croatia. Scand J Immunol 2003;57:480–4.
14. Craandijk J, van Krugten MV, Verweij CL, van der Velden U, Loos BG. Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. J Clin Periodontol 2002;29:28–34.
15. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol 2000;71:1528–34.
16. van Winkelhoff AJ, Loos BG, van der Reijden WA, van der Velden U. Porphyromonas gingivalis, Bacteroides forsythus and other putative periodontal pathogens in subjects with and without periodontal destruction. J Clin Periodontol 2002; 29: 1023–8.
17. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontology 2000;14:216–8.
18. Sigusch B, Klinger G, Glockmann E, Simon HU. Early-onset and adult periodontitis associated with abnormal cytokine production by activated T lymphocytes. J Periodontol 1998;69: 1098–104.

19. Petit MD, Wassenaar A, van der Velden U, van Eden W, Loos BG. Depressed responsiveness of peripheral blood mononuclear cells to heat-shock proteins in periodontitis patients. J Dent Res 1999;78:1393–400.
20. Casanova JL, Abel L. Genetic dissection of immunity to mycobacteria: the human model. Annu Rev Immunol 2002;20: 581–620.
21. Meisel P, Siegemund A, Grimm R, Herrmann FH, John U, Schwahn C, et al. The interleukin-1 polymorphism, smoking, and the risk of periodontal disease in the population-based SHIP study. J Dent Res 2003;82:189–93.
22. Berglundh T, Donati M, Hahn-Zoric M, Hanson LA, Padyukov L. Association of the-1087 IL 10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians. J Clin Periodontol 2003;30:249–54.

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