Gene Polymorphisms of TNF-α-308(G/A), IL-10-1082(G/A), IL6-174(G/C) and IL1Ra(VNTR) in Egyptian Cases with Adult and Early Onset Periodontitis

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Citation

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Abstract

Background: Periodontitis is an inflammatory destruction of periodontal tissue associated with cytokine dysfunctions.

Objectives:To check for the association of polymorphisms of cytokine genes with the susceptibility and severity of Periodontitis in cases from Egypt.

Subjects: 46 cases with periodontitis in addition to 98 healthy unrelated controls. Cases were classified into aggressive or early onset periodontitis (EOP) (17 cases, 36.9%) and chronic or adult onset periodontitis (AOP) (29 cases, 63.1%).

Methods: DNA was amplified using PCR-SSP for detection of polymorphisms related to TNF-α $^{-308}$ (G/A), IL-10 $^{-1082}$ (G/A), IL-6 $^{-174}$ (G/C) and IL-1Ra (VNTR).

Results: Total cases showed high significant frequency of homozygous IL-10 $^{-1082}$ (A/A) (OR=3.97, P<0.05), TNF-α $^{-308}$ (G/G) (OR=6.71, P<0.001), IL-6 $^{-174}$ C/C (OR=8.18, P<0.001) and IL-1Ra (A_1/A_1) (OR=3.42, P<0.05) genotypes. These were considered risk genotypes for disease susceptibility. The same was shown AOP cases while EOP cases showed only high significant frequency of homozygous IL-6 $^{-174}$ (C/C) (OR=8.36, P<0.05) and TNF-α $^{-308}$ (G/G) (OR=4.72, P<0.05) genotypes. On the other hand, combined heterozygosity for IL-10 $^{-1082}$ (G/A) with TNF-α $^{-308}$ (G/A) showed lowest significant frequency among all cases (P<0.001) and were considered low risk genotypes.

Conclusions: Cytokine gene polymorphisms may be used as a marker for periodontitis susceptibility, and severity helping for early diagnosis and induction of prophylaxis to affected families.

ABBREVIATIONS

TNF; tumor necrosis factor,
IL; interleukin,
IL-1Ra; IL-1 receptor antagonist,
PCR-SSP: polymerase chain reaction with sequence specific primers.

INTRODUCTION

Periodontitis is a chronic bacteriogenic infection leading to rapid destruction of the tissues supporting the teeth in otherwise healthy individuals. The most common form of this disorder has been reported to affect about 30– 40% of an adult population and approximately 10% of these subjects exhibit severe disease ([1], [2]). Genetic factors may account for 50% of the variations seen in periodontal disease expression in humans and also contribute to the variance in clinical symptoms of periodontitis as detected from studies in twins ([2], [3]).

Cytokines play an important role in the pathogenesis of periodontal diseases by promoting periodontal tissue inflammation and destruction through the stimulation of prostaglandin production and the induction of collagenase and other proteases([4]).

IL-10 mRNA expression in periodontitis lesions was found significantly higher than that in autologous peripheral blood

mononuclear cells in spite of large variation between patients ([5]). These variations of expression may be due to another mechanism, possibly regulatory network between inflammatory and anti-inflammatory cytokines. These led to speculate that the expression of mRNA and subsequent protein production are influenced by the gene polymorphisms, and that specific polymorphisms may be associated with the inflammatory response in periodontal disease ([6], [7]). One of these polymorphisms of IL-10 at -1082 was shown to affect in vitro IL-10 production in peripheral blood of healthy subjects ([8]).

Interleukin-1 is known as an important cytokine that had been shown to play a role in the earliest stages of gingival inflammation ([9], [10]). One of the important polymorphisms at the IL-1 gene cluster is that of IL-IRN-VNTR caused by variable numbers of an 86 bp tandem repeat located in the second intron of the gene. The most common alleles have been termed allele one (A_1, 4 repeats), allele two (A_2, 2 repeats) in addition to other 3 alleles ([11]).

Furthermore, IL-1 and TNF-α were reported to stimulate many cells to produce matrix metalloproteinase, prostaglandins and proinflammatory cytokines, as well as affecting bone metabolism, all of which in turn contribute to the pathogenesis of periodontitis. TNF synthesis may be influenced by the presence of certain gene polymorphisms ([12],[13], [14]).

IL-6 is a multifunctional cytokine that regulates immune responses and its effects overlap those of IL-1 and TNF. It is widely distributed among cells of the periodontium and is thought to play a role in the elevated B-cell response observed in the gingival tissues of patients with chronic periodontitis. A significant higher expression of IL-6 mRNA was reported in tissues of periodontitis patients ([15], [16]).

Taking into consideration that cytokine gene polymorphisms are population specific, we were interested to test for the association of these polymorphisms with periodontitis among Egyptian cases. In a case control study, we attempted testing the association of susceptibility and severity of periodontitis with polymorphisms of 2 pro-inflammatory cytokines (TNF-α at position -308 and IL-6 at position -174) and 2 anti-inflammatory cytokines (IL-10 genes at position -1082 and of IL-1Ra VNTR).

SUBJECTS AND METHODS

This work included a random sample of 50 cases presenting with generalized form of periodontitis recruited from the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University, which is the main referral site in the Nile Delta Region of Egypt. Four smoker cases were excluded from the study to nullify the effect of smoking on disease severity and clinical behavior. Studied cases (46) included 26 men and 20 women with a mean age of 40.85 ± 13.22 years (range: 19-65 years). According to clinical presentation, they were classified into aggressive or EOP (17 cases, 36.9%) and chronic or AOP (29 cases, 63.04%). Positive family history was given in 14 (30.4%) of these cases.

Cases genotypes were compared to 98 healthy unrelated adult volunteers with negative family history of the disease from the same locality. These included 52 males and 46 females and their mean age was 44.9 ± 6.7 years.

DNA EXTRACTION AND PURIFICATION

After obtaining informed consent from all cases and controls, venous blood samples (3 ml) were collected on EDTA (ethylenediamine tetraacetate) containing tubes, DNA was extracted promptly using DNA extraction and purification kit (Gentra Systems, USA) according to manufacturer's instructions and then stored at -20 C till use.

PCR AMPLIFICATION

Three single nucleotide polymorphisms (SNPs) were analyzed including promoter sites TNF-α $^{-308}$ (G/A), IL-10 $^{-1082}$ (G/A) and IL-6 $^{-174}$ (G/C) as well as IL-1Ra ^{VNTR} as previously described ([17], [18],[19], [20]). For TNF-α, IL-6 and IL-10 SNPs identification, PCR with sequence-specific primers (PCR-SSP) in two reactions employing a common forward and 2 reverse primers was used, and for IL-1Ra VNTR polymorphism, a single PCR reaction employing a forward and a reverse primers was used. All primers, Taq polymerase, dNTP, and $MgCl_2$ were purchased from QiaGene (QiaGene, USA). The assay was performed in Techne-Genius thermal-cycler (England). Briefly, 100-500 ng of genomic DNA was added to 25 µ1 of reaction mixture containing 1 µM of each common/specific primer, 200 µM of each dNTP, and 1 U of Taq DNA polymerase. We were careful to have master mixes for multiple cases and also for different polymorphisms at the same sitting with confirmation of the negative amplification to obtain accurate subject genotyping.

DETECTION OF AMPLIFIED PRODUCTS

The entire reaction volume plus 5 µ1 of bromophenol blue track dye were loaded into 2% agarose gel (Boehringer Mannheim) containing ethidium bromide. Gels were electrophoresed for 20 minutes at 200 V, photographed under UV light (320 nm) and then scored for the presence or absence of an allele specific band. Figure (1) shows the amplified PCR products of TNF-α $^{-308}$ (G/A), IL-10 $^{-1082}$ (G/A) and IL-6 $^{-174}$ (G/C) compared to size marker whereas figure 2. shows amplified alleles of IL-1Ra VNTR region in intron 2 of the gene.

Statistical analysis; Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 10.0). The frequency of studied allelic polymorphisms among cases was compared to that of controls describing number and percent of each and tested for positive association using Fisher's exact test (modified Chi square test) and Odds ratio with a minimum level of significance of <0.05.

RESULTS

Analysis of IL-10 $^{-1082}$ (G/A) polymorphism among cases compared to controls (table 1), showed that homozygous form A/A was found significantly high in total cases (OR=3.97, P<0.05) and AOP cases (OR=4.29, P<0.05) while the heterozygous form G/A was found significantly low among the same groups (P<0.05 in both groups). Analysis of IL-6 $^{-174}$ (G/C) polymorphism (table 2), showed that homozygous form C/C was found significantly high in total cases (OR=8.18, P<0.001), in EOP(OR=8.36, P<0.05) and AOPcases (OR=8.079, P<0.001), while the heterozygous form G/A was found significantly lower among the same groups (P<0.001 in all).

Figure 1

Table 1: Frequency of IL-10 (G/A) genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

	Genotypes			Alleles	
	G/G	G/A	A/A	G	A
Control(98) N(%)	5(5.1)	85(86.7)	8(8.2)	95 (48.5)	101 (51.5)
Total cases(46) N(%)	4(8.7)	**30(65.2)** *	**12(26.1)** *	38 (41.3)	54 (58.7)
OR (95%CI)	1.77 (0.5-6.9)	**0.28 (0.12-0.7)**	**3.97 (1.5-10.5)**	0.75 (0.6-1.24)	1.34 (0.8-2.2)
Clinical types					
EOP(17) N(%)	1(5.9)	12(70.6)	4(23.5)	14 (41.2)	20 (5.9)
OR (95%CI)	1.16 (0.12-9.6)	0.36 (0.11-1.2)	3.46 (0.9-13.14)	0.7 (0.4-1.6)	1.34 (0.6-2.8)
AOP(29) N(%)	3(10.3)	**18(62.1)** *	**8(27.6)** *	24 (41.4)	34 (58.6)
OR (95%CI)	2.15 (0.5-9.6)	**0.25 (0.98-0.7)**	**4.29 (1.4-12.4)**	0.8 (0.4-1.4)	1.33 (0.7-2.4)

*P<0.05 significant ** P<0.001 extremely significant
OR = Odds ratio, (95%CI) = 95% Confidence interval
EOP : early onset periodontitis AOP : adult onset periodontitis

Figure 2

Table 2: Frequency of IL-6 (G/C) genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

	Genotypes			Alleles	
	G/G	G/C	C/C	G	C
Control(98) N(%)	5(5.1)	87(88.8)	6(6.1)	97(49.5)	99(50.5)
Total cases(46) N(%)	6 (13.0)	**24 (52.2)****	**16 (34.8)****	36 (39.1)	56 (60.9)
OR (95%CI)	2.79 (0.8-9.7)	**0.14 (0.1-0.3)**	**8.18 (2.9-2.8)**	0.66 (0.4-1.1)	1.5 (0.9-2.5)
Clinical types					
EOP(17) N(%)	2 (11.8)	**9 (52.9)****	**6 (35.3)***	13 (38.2)	21 (61.8)
OR (95%CI)	2.48 (0.4-13.9)	**0.14 (0.5-0.44)**	**8.36 (2.3-0.5)**	0.6 (0.3-1.33)	1.6 (0.8-3.34)
AOP(29) N(%)	4 (13.8)	**15 (51.7)****	**10 (34.5)****	23 (39.7)	35 (60.3)
OR (95%CI)	2.98 (0.7-11.9)	**0.14 (0.1-0.4)**	**8.07 (2.6-4.89)**	0.67 (0.4-1.2)	1.49 (0.8-2.7)

*P<0.05 significant ** P<0.001 extremely significant
OR = Odds ratio, (95%CI) = 95% Confidence interval
EOP : early onset periodontitis AOP : adult onset periodontitis

Analysis of TNF-α $^{-308}$ (G/A) polymorphism (table 3), showed that homozygous form G/G was found significantly high in total cases (OR=6.71, P<0.001), in EOPcases (OR=4.7, P<0.05) and AOPcases (OR=4.0, P<0.05), while the heterozygous form G/A was found significantly low among the group of AOPcases only (P<0.05). Analysis of IL-1Ra VNTR polymorphism (table 4), showed that homozygous form A_1/A_1 was found significantly high in total cases (OR=3.42, P<0.05) and AOPcases (OR=2.7, P<0.001), while the heterozygous form A_1/A_2 was found significantly low among total cases and AOPcases (P<0.05 and <0.001 respectively).

Figure 3

Table 3: Frequency of TNF-α (G/A) genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

	Genotypes			Alleles	
	G/G	G/A	A/A	G	A
Control(98) N(%)	6 (6.1)	81 (82.7)	11(11.2)	93(47.4)	103(52.6
Total cases(46) N(%)	**10(21.7)****	31(67.4)	5(10.9)	51(55.4)	41(44.6)
OR (95%CI)	**6.71 (2.4-8.9)**	0.4 (0.2-0.97)	0.96 (0.3-2.95)	1.38 (0.8-2.3)	0.7 (0.4-1.2)
Clinical types					
EOP(17) N(%)	**4(23.5)** *	11(64.7)	2(11.8)	19(55.9)	15(44.1)
OR (95%CI)	**4.7 (1.2-8.98)**	0.4 (0.12-1.2)	1.06 (0.2-5.2)	1.4 (0.7-2.9)	0.7 (0.3-1.5)
AOP(29) N(%)	**6(20.7)** *	20(69.0)	3(10.3)	32(55.2)	26(44.8)
OR (95%CI)	**4.0 (1.2-3.6)**	0.47 (0.2-1.2)	0.91 (0.24-3.5)	1.4 (0.76-2.5)	0.7 (0.4-1.3)

*P<0.05 significant ** P<0.001 extremely significant
OR = Odds ratio, (95%CI) = 95% Confidence interval
EOP : early onset periodontitis AOP : adult onset periodontitis

Figure 4

Table 4: Frequency of IL-1Ra VNTR genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

	Genotypes		Alleles	
	A1/A1	A1/A2	A1	A2
Control(98) N(%)	58(58.8)	40(41.2)	156(79.6)	40(20.4)
Total cases(46) N(%)	**38(82.6)** *	**8(17.4)** *	**84(91.3)** *	**8(8.7)***
OR(95%CI)	**3.42 (1.4-8.1)**	**0.3(0.13-0.7)**	**2.7(1.2-6)**	**0.4(0.2-0.8)**
Clinical types				
EOP(17) N(%)	11(64.6)	6 (35.3)	28(82.4)	16(17.6)
OR(95%CI)	1.32(0.5-3.9)	0.79(0.3-2.3)	1.2(0.5-3.1)	0.8(0.3-2.2)
AOP(29) N(%)	**27(93.1)** **	**2(6.9)** **	**56(96.6)** **	**2(3.8)****
OR(95%CI)	**2.7(2.2-43.2)**	**0.1(1.02-0.5)**	**7.2(1.7-30.7)**	**0.14(0.03-0.6)**

*P<0.05 significant ** P<0.001 extremely significant
OR (95%CI) = Odds ratio (95% Confidence interval)
EOP : early onset periodontitis AOP : adult onset periodontitis

Analysis of the frequency of combined phenotypes (table 5), showed that the combined genotypes [TNF-α $^{-308}$ G/G with IL-10 $^{-1082}$ A/A] was of highest significant frequency among cases (OR=31.62, P<0.001) followed by [TNF-α $^{-308}$ G/G with IL-6 $^{-174}$ C/C] (OR= 20.85, P<0.05) followed by [IL-6 $^{-174}$ (C/C) with IL-10 $^{-1082}$ (A/A)] (OR= 11.83, P<0.001) followed by [IL-6 $^{-174}$ (C/C) with IL-1Ra (A_1/A_1)] (OR= 10.28, P<0.001). On the other hand, maximum significant lower frequency was found among cases with combined heterozygosity genotypes of [IL-10 $^{-1082}$ (G/A) with TNF-α $^{-308}$ (G/A)] (P<0.001).

Figure 5

Table 5: Frequency of combined genotypes of different cytokines among cases compared to controls with their statistical significance.

Combined Genotype	Cases N(%)	Control N(%)	OR (95%CI)
TNF-α^{-308}G/G with IL-10^{-1082}A/A	**6(13.04)****	0(0.0)	**31.62 (1.74-57.81)**
TNF-α^{-308}G/G with IL-6^{-174}C/C	**4(8.7)***	0(0.0)	**20.85 (1.09-396-35)**
IL-6^{-174}C/C with IL-10^{-1082}A/A	**5(10.86)***	1(0.0)	**11.83 (1.34-104.48)**
IL-6^{-174}C/C with IL-1Ra VNTR A_1/A_1	**14(30.43)****	4(2.04)	**10.28 (3.15-33.52)**
IL-10^{-1082}G/A with TNF-α^{-308}G/A	**21(45.7)****	73(67.35)	**0.29 (0.14-0.61)**

*P<0.05 significant ** P<0.001 extremely significant
OR (95%CI) = Odds ratio (95% Confidence interval)

Interestingly, no significant difference was found in the frequencies of all studied alleles except for IL-1Ra A_1 that showed significant higher frequency among total and AOP cases, while the other allele A_2 has shown significant lower frequency among same groups.

DISCUSSION

The observation of a familial occurance of periodontitis is indicative of its genetic basis ([21]). Similarly, positive family history was obtained in approximately one third of the currently studied cases supporting this consept.

It has been suggested that individual cytokine gene polymorphism may be considered a useful indicator or a diagnostic marker for inflammatory disorders including periodontal diseases among affected families ([22], [23]).

Polymorphisms in the IL-1 gene cluster are correlated with the severity of adult periodontal disease ([24]). On the other hand, IL-1RN gene showed 5 allelic polymorphisms that have been found associated with a variety of human diseases, primarily of epithelial cells or tissues. A_1 allele containing four repeats, was the most common allele and was found in 73.6% of the population, while A_2 allele containing two repeats, was found in 21.4% of the population ([25]). The same ratio was nearly found in our studied controls. Thus, A_1 and A_2 alleles were found in 79.6% and 20.4% of controls respectively. Analysis of IL-1Ra VNTR polymorphism among our studied cases showed the presence of high signifant frequency of homozygous form A_1/A_1 with a significant low frequency of heterozygous form A_1/A_2 in total cases and AOPcases. These results were different from that reported previously of association of variable number tandem repeat (VNTR) polymorphisms in IL-1 receptor antagonist with generalized early-onset periodontitis ([26]).

Although this study showed that ILRN allele A_2 was found

protective in cases of periodontitis, other authors reported increased severity of other immune conditions like systemic lupus erythematosus and Sjögren's syndrome in Japanese populations carrying the same allele. Others reported that the IL-1Ra intron 2 polymorphism did not influence the susceptibility to or severity of rheumatoid arthritis ([27], [28]).

Besides the immunoregulatory effects of IL-10, the ability to enhance proliferation of B cells and autoreactive B cells, in particular, is of interest. Regarding the association between the -1082 IL-10 gene polymorphism and severe AOP, it was suggested that IL-10 related functions on autoreactive B cells are involved in the pathogenesis of periodontal disease ([8], [29], [30]).

The association of an IL-10 gene polymorphisms, with periodontitis in a Japanese population was studied and showed that none of the haplotypes could be linked to subjects with periodontitis and that the occurrence of the G allele at position -1082 in such patients was in relative terms low ([31]). However, in the current study, a significant higher frequency of A IL-10 $^{-1082}$ A/A genotype was found in cases compared to controls that may be considered a risk genotype for periodontitis susceptibility. On the other hand, significant lower frequency of G/A heterozygosity genotype was found among total cases compared to controls that may be considered a protective genotype against susceptibility to periodontitis. These results were in contrast with what previously reported that the proportion of subjects that exhibited the G/G genotype was significantly larger in subjects with severe periodontitis than in periodontally healthy individuals ([32]). This conflict can be explained on the basis of racial difference or association with other interactive types of cytokines or genetic markers predisposing for the disease in the studied cases.

Conflicting results have been reported regarding the association of -308 TNF-α gene polymorphism and periodontitis. Authors reported no differences in the distribution of TNF-α alleles between patients adult periodontitis and in healthy matched controls or between patients with different disease severity ([24], [33], [34]). Our studied cases however showed a significant higher frequency of homozygous (G/G) genotype compared to controls that may be considered a risk genotype for periodontitis susceptibility

In the current study, total cases showed a significant higher frequency of homozygous genotype IL-6-174 (C/C) compared to controls. Thus, IL-6 $^{-174}$ C/C genotype may be considered a risk genotype for periodontitis susceptibility. On the other hand, significant lower frequency was observed with heterozygous genotype G/C that was considered a low risk or protective genotype. This result was in contrast to what was reported in caucasian Brazilians that genotype G/G was statistically associated with susceptibility to AOP ([35]). The finding that the frequency of heterozygous individuals (G/C) decreases the degree of severity of the disease was conforming with the current study although it was speculated to be due to a protective function of allele C, whose presence may reduce IL-6 production. Similarly, a reduction in the frequency of the C/C genotype in patients with systemic-onset juvenile arthritis was found suggesting that this genotype confers a protective influence against the development of the disease ([36]).

Analyzing studied Egyptian cases for combined genotypes, a certain pattern could be found to play a role in periodontitis disease susceptibility and/or severity. These included (IL-10 $^{-1082}$ A/A with TNF-α $^{-308}$ G/G; OR= 31.62), (TNF-α $^{-308}$ G/G with IL-6 $^{-174}$ C/C; OR= 20.85), (IL-10 $^{-1082}$ A/A with IL-6 $^{-174}$ C/C; OR= 11.83) and (IL-6 $^{-174}$ C/C with IL-1Ra VNTR A_1/A_1; OR= 10.28). On the other hand, maximum protection genotypes included heterozygous genotypes (IL-10 $^{-1082}$ G/A with TNF-α $^{-308}$ G/A; OR= 0.29).

CONCLUSION

Cytokine gene polymorphisms may be used as a marker for periodontitis susceptibility, clinical behavior and severity helping for early diagnosis and induction of prophylaxis to other family members of affected probands against disease progression.

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References

1. Papapanou PN and Lindhe J (2003): Epidemiology of periodontal disease. In: Lindhe J, Karring T and Lang NP(eds). Clinical Periodontology and Implant Dentistry, 4th ed. UK: Blackwell Munksgaard.
2. Borrell LN. and Papapanou P N. (2005): Analytical epidemiology of periodontitis. J Clin Periodontol, 32 (Suppl. 6): 132-158.
3. Kinane DF, Berglundh T and Lindhe J (2003): Host-parasite interactions in periodontal disease. In: Lindhe J,

Karring T and Lang NP (eds). Clinical Periodontology and Implant Dentistry, 4th ed. UK: Blackwell Munksgaard.
4. Kinane DF, Shiba H, and Hart TC (2005): The genetic basis of periodontitis Periodontology 2000 39 : 91
5. Yamazaki K, Tabeta K, Nakajima T, Ohsawa Y, Ueki K, Itoh H and Yoshie H (2001): Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. J Clin Periodontol., 28:828-832.
6. Aoyagi T, Yamazaki K, Kabasawa-Katoh Y, Nakajima T, Yamashita N, Yoshie H and Hara K (2000): Elevated CTLA-4 expresion on CD4 T cells from periodontitis patients stimulated with Prophyromonas gingivalis outer membrane antigen. Clin Exp Immunol., 119:280-286.
7. Takashiba S and Naruishi K (2006): Gene polymorphisms in periodontal health and disease Periodontology 2000, 40 : 94
8. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ and Hutchinson IV (1997): An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet., 24:2134-2138
9. Honig J, Rordorf-Adam C, Siegmund C, Wiedemann W and Erard F (1989): Increased interleukin-1 beta (IL-1beta) concentration in gingival tissue from periodontitis patients. J Periodontal Res., 24:362-367.
10. Heasman PA, Collins JG and Offenbacher S (1993): Changes in crevicular fluid levels of interleukin-1 beta, leukotriene B4, prostaglandin E2, thromboxane B2 and tumor necrosis factor alpha in experimental gingivitis in humans. J Periodontal Res., 28:241-247.
11. Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A and Duff GW (1993): Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet., 91:403-404.
12. Alexander MB and Damoulis PD (1994): The role of cytokines in the pathogenesis of peridontal disease. Curr Opin in Periodontol., 2:39-53.
13. Duff GW (1994): Molecular genetics of cytokines. In: Thomson A(ed). The Cytokine Handbook, 2nd ed., pp. 21-30. London: Academic Press.
14. Wilson AG, di Giovine FS and Duff GW (1995): Genetics of TNF-alpha in autoimmune, infectious and neoplastic disease. Journal of Inflammation, 45:1-12.
15. Fujihashi K, Kono Y, Beagley KW, Yamamoto M, McGhee JR, Mestecky J and Kiyono H (1993): Cytokine and periodontal disease: immunopathological role of interleukins for B cell response in chronic inflamed gingival tissues. J Periodontol., 64: 400-406.
16. Prabhu A, Michalowicz BS and Mathur A (1996): Detection of local and systemic cytokines in adult periodontitis. J Periodontol., 67:515-522.
17. Sargen K, Demaine AG and Kingsnorth AN (2000): Cytokine gene polymorphisms in acute pancreatitis. Pancreas, 1:24-35.
18. Cavet J, Middleton PG, Segall M, Noreen H, Davies SM and Dickinson AM (1999): Recipient tumor necrosis factor. and interleukin-10 gene polymorphisms associate with early mortalilty and acute graft-versus-host diseases severity in HLA-matched sibling bone marrow transplants. Blood, 94: 3941-3946.
19. Cavet J, Dickinson AM, Norden J, Taylor PR, Jackson GH and Middleton PG (2001): Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. Blood, 98:1594-1600.
20. Wilkinson RJ, Patel P, Llewelyn M, Hirsch CS, Pasvol G, Snounou G, Davidson RN and Toossi (1999): Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1? on tuberculosis. J Exp Med., 189: 1863-1874.
21. Page RC, Sims TJ, Geissler F, Altman LC and Baab DA (1984): Abnormal leukocyte mobility in patients with early onset periodontitis. J Periodontal Res., 19:591-594.
22. Cullinan MP, Westerman B, Palmer JE, Faddy MJ and Seymor GJ (2000): IL-1 genotype and progression of periodontal disease in an Australian population. J Dent Res., 79.
23. Scarel-Caminaga RM, Trevillatto PC, Souza AP, Brito RB Jr and Line SR (2002): Investigation of an IL-2 polymorphism in patients with different levels of chronic periodontitis. J Clin Periodontal., 29:587-591.
24. Lin L, Pan YP ,and Yin LY.(2003): Study on the correlation of cytokine gene polymorphism with chronic periodontitis. Shanghai Kou Qiang Yi Xue., 12(6): 456-459.
25. Tountas NA, Casini-Raggi V, Yang HD, Giovine FS, Vecchi M, Kam L, Melani L, Pizarro TT, Rotter JI and Cominelli F (1999): Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. Gastroenterology, 117:806-813.
26. Tai H, Endo M, Shimada Y, Gou E, Orima K, Kobayashi T, Yamazaki K and Yoshie H (2002): Association of interleukin-1 receptor antagonist gene polymorphisms with early-onset periodontitis in Japanese. J Clin Periodontol., 29:882-888.
27. Blakemore AI, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW (1994): Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. Arthritis Rheum., 37:1380-1385.
28. Perrier S, Coussediere C, Dubost JJ, Albuisson E and Sauvezie B (1998): IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjögren's syndrome and rheumatoid arthritis. Clin Immunol Immunopathol., 87:309-313.
29. Berglundh T, Liljenberg B, Tarkowski A and Lindhe J (2002): The presences of local and circulating autoreactive B cells in advanced periodontitis. J Clin Periodontol., 29:281. 286.
30. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I and Woo P (1999): Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum., 42: 1101-1108.
31. Yamazaki K, Nakajima T, Kubota Y, Gemmell E, Seymour GJ and Hara K (1997): Cytokine messenger RNA expression in chronic inflammatory periodontal disease. Oral Microbiol Immunol., 12:281-287.
32. Berglundh T, Donati M, Hahn-Zoric M, Hanson LA and Padyukov L (2003): Association of the -1087 IL-10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians. J Clin Periodontol., 30:249-254.
33. Craandijk J, van Krugten MV, Verweij CL, van der Velden U and Loos BG (2002): Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. J Clin Periodontol., 29:28-34.
34. Donati M, Berglundh T, Hytonen AM, Hahn-Zoric M, Hanson L-A and Padyukov L (2005): Association of the -159 CD14 gene polymorphism and lack of association of the -308 TNFA and Q551R IL-4RA polymorphisms with severe chronic periodontitis in Swedish Caucasians. J Clin Periodontol, 32: 474-479.
35. Trevilatto PC, Scarel-Caminaga RM, de Brito RB Jr, de Souza AP and Line SR (2003): Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. J Clin

Periodontol., 30:438-442.
36. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudin JS, Humphries S and Woo P (1998): The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest., 102: 1369-1376.

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