J. Toxicol. Pub. Health
Vol. 20, No. 4, pp. 299-305 (2004)

Official Journal of
Korean Society of Toxicology

Available online at http://www.toxmut.or.kr

Association Study Between Genetic Polymorphisms in Interleukin-1 Gene Family and Adult Periodontitis in Korean

Byung Yong Kang[1], Chin Yang Kang[2] and Kang Oh Lee[3]

[1]Research Institute for Life Science, [2]Department of Pharmacy,
[3]Department of Life Science, Sahmyook University, Seoul 139-742, Korea

Received August 16, 2004; Accepted November 10, 2004

ABSTRACT. Adult periodontitis (AP) is a chronic inflammatory disease whose etiology is not well defined. Some studies suggested that the clinical characteristics of this disease may be in part explained by genetic factors, and some attempts to find genetic markers for this disease were successful. The interleukin-1 (IL-1) gene family as one of genetic factors may influence the expression of adult periodontitis. The aim of present study is to investigate the frequencies of genetic polymorphisms in the IL-1 gene family encoding three genes (IL-1A, IL-1B and IL-1RN) in Korean AP patients and periodontically healthy controls. There were no significant differences in genotype and allele frequencies of these polymorphisms between two groups, respectively. However, -511 polymorphism of IL-1B gene was significantly associated with mean pocket depth (MPD, mm) value in AP patients ($P<0.05$). Therefore, our results suggest that -511 polymorphism in the IL-1B gene may be useful as a genetic marker for the severity of AP in Koreans.

Keywords: Adult periodontitis, Interleukin-1, Korean population.

INTRODUCTION

Adult periodontitis (AP) is an infectious disease of the tooth-supporting tissues that is characterized by gingival inflammation and alveolar bone loss (Laine *et al.*, 2001). While uncomplicated gingivitis is a common concomitant of inadequate oral hygiene, progression to AP with loss of supporting structures is influenced by several factors, including genetic predisposition (Hassel and Harris, 1995; Hart and Kornman, 1997). The search for genetic markers of AP has been generally disappointing, since no consistent disease associations have emerged (Galbraith *et al.*, 1999). However, recent studies of cytokine gene polymorphisms in patients with AP have yielded more encouraging results (Gore *et al.*, 1998; Kornman and di Giovine, 1998).

The proinflammatory cytokine interleukin-1 (IL-1) and its relation with the pathways of periodontal tissue and bone destruction has been well established, while several studies have shown higher levels of IL-1A and IL-1B in gingival cervical fluid (GCF) and gingival tissues in periodontally diseased subjects (Offenbacher, 1996; Tatakis, 1993).

A family of genes regulating the production of these cytokines has been identified on the long arm of chromosome 2. This gene family consists of the IL-1A, IL-1B and IL-1RN (receptor antagonist) genes (Nicklin *et al.*, 1994). Genetic polymorphisms in the IL-1 gene family have been associated with a variety of polygenic diseases including systemic lupus erythematosus (Blakemore *et al.*, 1994), juvenile rheumatoid arthritis (McDowell *et al.*, 1995), alopecia areata (Tarlow *et al.*, 1994), diabetic nephropathy (Blackemore *et al.*, 1996), inflammatory bowel disease (Bioque *et al.*, 1995), myasthenia gravis (Huang *et al.*, 1998) and AP (Kornman *et al.*, 1997).

The first study of cytokine gene polymorphisms in AP was reported by Kornman *et al.* (1997) who found a significant association between genetic polymorphisms in the IL-1 gene family and adult periodontitis. However, further studies for other ethnic groups have yielded controversial results about the relationship between genetic polymorphisms in the IL-1 gene family and AP (Amitage

Correspondence to: Kang Oh Lee, Department of Life Science, Sahmyook University, San 26-21, Kongreung-2 Dong, Nowon-Gu, Seoul 139-742, Korea
E-mail: leeko@syu.ac.kr

et al., 2000; Galbraith *et al.*, 1999; McDevitt *et al.*, 2000). So far, no existing data referring to subjects of Korean origin are available in this context. Thus, we investigated the prevalence of the genetic polymorphisms in the IL-1 gene family from a Korean population of healthy periodontal status, and compared this prevalence with the one from a group of patients with AP.

MATERIALS AND METHODS

Study subjects

A total of 32 cases of AP (24 male and 8 female; age range 31~67 year; mean age 48.0 year) were recruited from Dr. Choi's Dental Office, Seoul, Korea, and 149 healthy control subjects (73 male, 75 female and 1 unknown; age range 27~80 year; mean age 56.0 year) collected from Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea. Clinical characteristics of AP group were shown in Table 1.

Biochemical assay

Blood samples were obtained in EDTA tubes from the subjects who had been fasting for 12~16 h. Serum concentrations of total cholesterol (TC), triglyceride (TG) and glucose were measured by enzymatic colorimetric methods with a commercial kit (Boehringer Mannheim, Germany) and chemistry analyzer. Serum high-density lipoprotein (HDL) cholesterol concentration was determined by measuring cholesterol in the supernatant after precipitation of the serum with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automatic analyzer with reagents and calibrators from Boehringer Mannheim. Serum lipoprotein(a) concentration was measured by the immunoprecipitation method (SPQ test System, Incstar Corporation, Stillwater, Minn., USA), and serum apolipoprotein AI concentration was determined by the immunoturbidimetric method (Cobas Integra, Roche Diagnostics, USA). Serum low density lipoprotein cholesterol (LDL) concentration was calculated using the formula by Friedewald *et al.* (1972). The mean pocket depth (MPD, mm) was evaluated by several dentists.

Table 1. Baseline clinical parameters of the patients with adult periodontitis (n=32)

	Mean ± SD[1]	Range
Age (year)	48.0 ± 8.8	31~67
Missing teeth	1.4 ± 2.0	0~10
Mean pocket depth (mm)	5.0 ± 0.8	3.5~7.2
% sites with:		
plague	42. ± 23	10~100
gingival redness	38 ± 19	5~100
bleeding on probing	32 ± 20	7~100
% Current smokers	37.5	

[1]Standard deviation.

DNA analysis

Genomic DNA was extracted using automatic nucleic acid isolation & purification system (BioNex Inc., Seoul, Korea) from whole blood. The polymorphisms investigated in this study were a bi-allelic -889 polymorphism within the promoter region of the IL-1A gene (Kornman *et al.*, 1997), a bi-allelic -511 polymorphism within the promoter region of the IL-1B gene (di Giovine *et al.*, 1992), a bi-allelic +3954 polymorphism within the exon 5 of the IL-1B gene (Kornman *et al.*, 1997) and a multiallelic variable number of tandem repeat (VNTR) polymorphism within the second intron of the IL-1RN gene (Tarlow *et al.*, 1993). Briefly, total 50 μl of the reaction mixture contained 200~400 ng of genomic DNA, 100 ng of each primer, 200 μM of each dNTP, and buffers recommended by the manufacturer. The sequences of the primer for each polymorphism were described in Table 2.

Amplification was carried out with DNA thermocycler: one cycle at 94°C for 5 min, 35 cycles at 94°C for 1 min, at 55~65°C for 1 min and at 72°C for 1 min with a final polymerization at 72°C for 10 min. Amplified PCR products were digested by proper restriction enzymes and visualized by agarose or polyacrylamide gel electrophoresis. In the case of a VNTR polymorphism within the second intron of the IL-1RN gene, PCR product was directly genotyped by agarose gel electrophoresis

Table 2. Polymorphic sites and primer sequences for the PCR amplification of IL-1 gene family

Gene	Polymorphism	Primer sequence	Reference
IL-1A	-889	5'-AAGCTTGTTCTACCACCTGAACTAGGC-3' 5'-TTACATATGAGCCTTCCATG	Kornman *et al.*, 1997
IL-1B	-511	5'-TGGCATTGATCTGGTTCATC-3' 5'-GTTTAGGAATCTTCCCACTT-3'	di Giovine *et al.*, 1992
	+3954	5'-CTCAGGTGTCCTCGAAGAAATCAAA-3' 5'-GCTTTTTTGCTGTGAGTCCCG-3'	Kornman *et al.*, 1997
IL-1RN	VNTR	5'-CTCAGCAACACTCCTAT-3' 5'-TCCTGGTCTGCAGGTAA-3'	Tarlow *et al.*, 1993

without the digestion by a restriction enzyme.

Statistical analysis

Differences in 2 × N contingency table analyses were assessed by $\chi 2$-independence test, while 2 × 2 contingency table analyses by Fisher's exact test. For the VNTR polymorphism with more than two alleles, the statistical significance of the association between the genetic marker and AP was estimated with a Monte Carlo simulation using the Clump (version. 1.6) program (Sham and Curtis, 1995). The heterozygosity and polymorphism information content (PIC) values were calculated as previously described (Bostein *et al.*, 1980). Differences in means according to each genotype were tested by non-parametric Mann-Whitney U test or Kruskal-Wallis test. A p-value of less than 0.05 indicated statistical significance. All statistical analysis was performed using the computer program of SPSS-WIN (version 11.0).

RESULTS

Polymorphic pattern

(1) IL-1A(-889). A -889 polymorphism in the IL-1A gene was detected by digestion with restriction enzyme *Nco*I after PCR amplification (Fig. 1A). Examination of the polyacrylamide gels after *Nco*I digestion showed two fragments of 83 bp + 16 bp (N1 allele) and a single 99 bp fragment (N2 allele). The genotype frequencies of the IL-1A(-889) in controls and AP patients were presented in Table 3. The absence of N2N2 genotype in the IL-1A(-889) was found in our AP group. No statistically significant differences were found between two groups for any of the genotype frequencies investigated (P>0.05). The heterozygosity and PIC values of IL-1A(-889) represented the values of 0.1588 and 0.1462 for controls, and 0.0689 and 0.0665 for AP patients, respectively. According to the heterozygosity and PIC values, IL-1A(-889) showed indicated a relatively low degree of polymorphism in both groups.

(2) IL-1B(-511). A -511 polymorphism in the IL-1B gene was detected by digestion with restriction enzyme *Ava*I after PCR amplification (Fig. 1B). *Ava*I digestion of the amplified product and subsequent agarose gel electrophoresis yielded 191 bp + 113 bp fragments (A1 allele) and a single 304 bp fragment (A2 allele). Results of the prevalence of genotypes for controls and AP patients were presented in Table 3. Statistical analysis revealed no significant differences in genotype frequencies between the two groups, respectively (P>0.05).

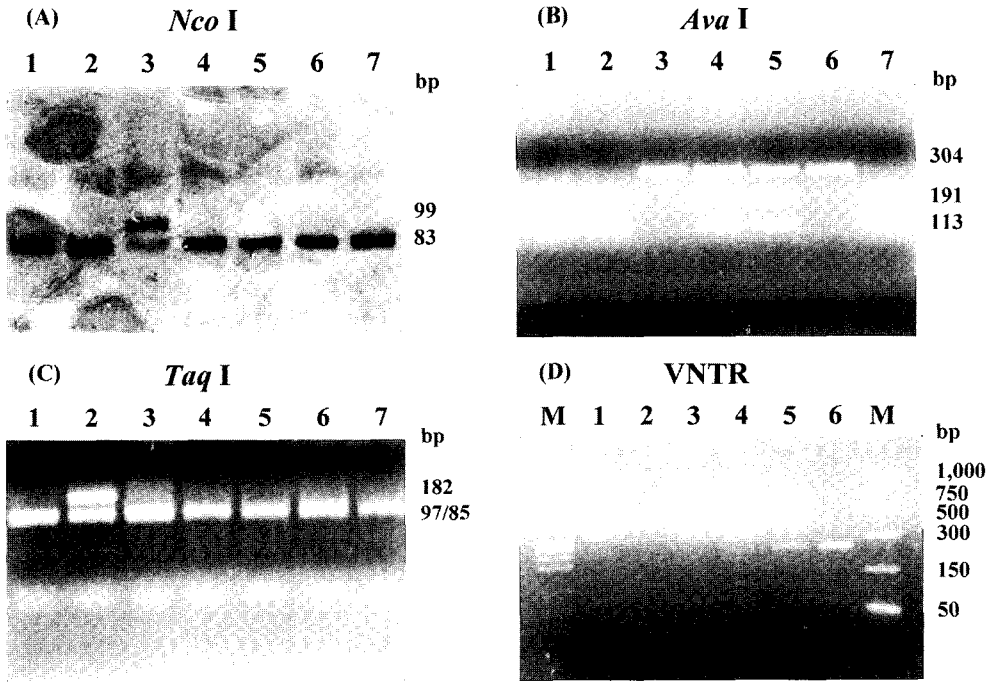

Fig. 1. Polymorphic patterns of IL-1 gene family. A) -889 genotypes of IL-1A gene. Lane 1, 2 and 4~7, N1N1 genotypes; lane 3, N1N2 genotype. B) -511 genotypes of IL-1A gene. Lane 1, 2 and 7, A2A2 genotypes; lane 3 and 6, A1A1 genotypes; lane 4 and 5, A1A2 genotypes. C) +3954 genotypes of IL-1A gene. Lane 1, 3~7, T1T1 genotypes; lane 2, T1T2 genotype. D) VNTR genotypes of IL1RN gene. Lane M, size markers; lane 1 and 2, V1V3 genotypes; lane 3, V1V1 genotypes; lane 4, V1V4 genotype; lane 5, V1V2 genotype; lane 6, V2V2 genotype.

Table 3. Comparison of genotype frequencies for four polymorphisms within the IL-1 gene family in normal controls and adult periodontitis group

Site	Genotypes	Control			Adult Periodontitis		
		Freq.[1]	H[2]	PIC[3]	Freq.	H	PIC
IL-1A							
-889	N1N1	0.85	0.1588	0.1462	0.93	0.0689	0.0665
	N1N2	0.13			0.07		
	N2N2	0.02			0.00		
IL-1B							
-511	A1A1	0.25	0.5000	0.3750	0.20	0.4800	0.3648
	A1A2	0.51			0.40		
	A2A2	0.24			0.40		
+3954	T1T1	0.95	0.0555	0.0540	0.94	0.0605	0.0587
	T1T2	0.04			0.06		
	T2T2	0.01			0.00		
IL-1RN							
VNTR	V1V1	0.87	0.1264	0.1207	0.91	0.1223	0.1177
	V1V2	0.11			0.03		
	V1V3	0.01			0.03		
	V1V4	0.01			0.00		
	V2V2	0.00			0.03		

[1]Frequency, [2]heterozygosity and [3]polymorphism information content.

The heterozygosity and PIC values of IL-1B(-511) represented the values of 0.5000 and 0.3750 for controls, and 0.4800 and 0.3648 for AP patients, respectively. According to the heterozygosity and PIC values, IL-1A (-889) showed indicated a reasonably high degree of polymorphism in both groups.

(3) IL-1B(+3954). A +3954 polymorphism in the IL-1B gene was detected by digestion with restriction enzyme *Taq*I after PCR amplification (Fig. 1C). *Taq*I digestion of the amplified DNA yielded two fragments of 97 bp and 85 bp for T1 allele and a single fragment of 182 bp for T2 allele The genotype frequencies of IL-1B(+3954) in controls and AP patients were shown in Table 3. The absence of T2T2 genotype in the IL-1B(+3954) was found in our AP group. No significant differences were observed in the genotype frequencies between the two groups, respectively (P>0.05). The heterozygosity and PIC values of IL-1B(+3954) represented the values of 0.0555 and 0.0540 for controls, and 0.0605 and 0.0587 for AP patients, respectively. According to the heterozygosity and PIC values, IL-1A(+3954) showed indicated a very low degree of polymorphism in both groups.

(4) IL-1RN(VNTR). Intron 2 in the IL-1RN gene contains a VNTR polymorphism of an 86 bp length of DNA, and this polymorphism was directly detected by PCR and agarose gel electrophoresis in our study. The PCR products were determined by electrophoresis on a 3% agarose gel stained with ethidium bromide. V1 allele (4 repeats) was 410 bp in size, V2 allele (2 repeats) was 240 bp, V3 allele (5 repeats) was 500 bp, V4 allele (3 repeats) was 325 bp, and V5 allele (6 repeats) was 595 bp (Fig. 1D). The genotype distributions of IL-1RN(VNTR) was displayed in Table 3. V1V1 genotype were most frequent in our subjects. V1V4 genotype was observed in only control group, while A2A2 genotype in AP group. There was no significant difference in overall genotype distribution between the two groups, (P> 0.05). Also, The heterozygosity and PIC values of IL-1RN(VNTR) represented the values of 0.1264 and 0.1207 for controls, and 0.1177 and 0.0587 for AP patients, respectively. According to the heterozygosity and PIC values, IL-1RN(VNTR) showed indicated relatively low degree of polymorphism in both groups.

Distribution of the IL-1A(-889)/Il-1B(+3954) composite genotype

The composite genotype of IL-1A(-889)/IL-1B(+3954) described by Kornman *et al.* (1997) was investigated in controls and AP patients, respectively. No significant difference was found between controls and AP patients

Table 4. Distribution of composite IL-1A(-889)/IL-1B(+3954) genotype in controls and patients

Genotype	Subject number (%)		Total
	Controls	Cases	
Negative[1]	106(98.1)	28(93.3)	134(97.1)
Positive[2]	2(1.9)	2(6.7)	4(2.9)
Total	108(100.0)	30(100.0)	138(100.0)

[1]Subjects carrying both N2 allele of IL-1A(-889) and T2 allele of IL-1B(+3954) and [2]subjects not carrying both N2 allele of IL-1A(-889) and T2 allele of IL-1B(+3954).

Table 5. The comparison of the anthropometric data and intermediate phenotypes according to the genetic polymorphisms of the IL-1 gene family in Korean adult periodontitis patients

	P-value			
	IL-1A(-889)	IL-1B(-511)	IL-1B(+3954)	IL-1RN(VNTR)
Age (year)	0.964	0.261	0.907	0.196
TC (mg/dl)[1]	0.304	0.248	0.403	0.272
TG (mg/dl)[2]	0.153	0.684	0.226	0.443
HDL-chol (mg/dl)[3]	0.108	0.475	0.198	0.205
LDL-chol (mg/dl)[4]	0.422	0.088	0.488	0.840
Lp(a) (mg/dl)[5]	0.448	0.823	0.532	0.288
ApoAI (mg/dl)[6]	0.246	0.736	0.444	0.172
Glucose (mg/dl)	0.228	0.361	0.226	0.173
MPD (mm)[7]	0.789	**0.044**[a]	0.734	0.798
Smoking status	0.146	0.196	0.133	0.415

[1]Total cholesterol, [2]triglyceride, [3]HDL-cholesterol, [4]LDL-cholesterol, [5]lipoprotein(a), [6]apolipoprotein AI, and [7]mean pocket depth.
[a]Statistically significant difference (Kruskal-Wallis test, $P<0.05$).

for IL-1A(-889)/IL-1B(+3954) composite genotypes ($P>0.05$).

Relationship with clinical characteristics

Table 5 presented the comparison of clinical characteristics according to each polymorphism in AP group. By statistical analysis, -511 polymorphism in the IL-1B gene was significantly associated with MPD value in AP group ($P< 0.05$).

DISCUSSION

AP is believed to has a multifactorial origin, and genetically inherited factors, which may contribute by conferring disease susceptibility, are likely to be expressed through variations in the host's inflammatory-immune response to periodontal pathogens (Genco, 1994; Page, 1991). The molecular mechanisms of how these genetic factors function have been reported more commonly and ultimately may become the focus of future therapeutic interventions. The impact of the genetic factor on AP has been incorporated into periodontology as a new line of research. Given the multifactorial aetiology of this disease and the novelty of this approach, a lot of information and data are required in order to demonstrate and establish a clear relationship between genetic variants, the pathogenetic process and the final clinical outcome.

Cytokines are regulatory proteins of low molecular weight, which are active in very small amounts (femtomolar to picomolar concentrations) (Bendtzen, 1994). They transmit information between cells and form a complex network of interactions. Most cytokines are pleiotrophic. They may act synergistically or may inhibit the action of other cytokines (Kjeldsen *et al.*, 1993). Cytokines are produced by activated cells, and mainly exert their effects locally by binding to high affinity receptors on the surface of a variety of cell types. Stable interindividual differences in cytokine secretion patterns have been reported (Mølvig *et al.*, 1988). The proinflammatory cytokines, IL-1, play a central role in the pathogenesis of many autoimmune and infectious diseases, including periodontitis (Roberts *et al.*, 1997a, b).

The genes encoding IL-1A, IL-1B and IL-1RN lie on the long arm of human chromosome 2 (Nicklin *et al.*, 1994). The investigation of genetic polymorphisms in this IL-1 gene family is intuitively interesting, since it is recognized that cytokines such as IL-1A and IL-1B may play an important role in the pathogenesis of periodontitis (Offenbacher, 1996), and several of the known polymorphisms within the IL-1 gene family appear to regulate gene expression level (Pociot *et al.*, 1992).

The present study revealed no associations between four genetic polymorphisms within the IL-1 gene family and AP in Korean population. This lack of any associations between genetic polymorphisms within the IL-1 gene family and AP in the population presented here, bring into doubt the usefulness of these candidate genes as markers of susceptibility to this form of periodontitis.

Kornman *et al.* (1997) first reported that the composite genotypes of IL-1A(-889)/Il-1(+3954) was significantly associated with AP in Caucasians. Further studies on the associations between the composite genotype of IL-1A(-889)/Il-1(+3954) and susceptibility of AP were conflicting in subjects with various ethnic background (Amitage *et al.*, 2000; Anusaksathien *et al.*, 2003; Meisel *et al.*, 2002; Sakellari *et al.*, 2003). In the present study, there was a low prevalence of genotype-positive subjects as compared with those found in other ethnic groups. The majority of the subjects exhibited homozygous allele 1 of the IL-1A(-889)/IL-1B(+3954) with only

2.9% of the subjects genotype positive. Our results were similar to those reported by Amitage *et al.* (2000) who showed that only 2.3% of the Chinese subjects were genotype positive and by Anusaksathien *et al.* (2003) who showed that only 1.6% of the Thai subjects were genotype positive. On the other hand, the distribution of the genotype-positive subjects from the Caucasian population varied from 29~46% depending on the geographic locations (Cullinan *et al.*, 2001; Hodge *et al.*, 2001; Kornman *et al.*, 1997). Hispanic and African-American subjects were approximately 26% and 14% genotype positive, respectively (Caffesse *et al.*, 2002; Walker *et al.*, 2000). Taken together, the results have clearly shown that the distribution of IL-1A(-889)/IL-1B(+3954) genetic polymorphisms varies with population due to different ethnic and geographic backgrounds.

The distribution of genotype-positive cases was so small that the establishment of an association between the composite genotype of IL-1A(-889)/IL-1B(+3954) and AP was not meaningful. Our results were also similar to those reported by Armitage *et al.* (2000) and Anusaksathien *et al.* (2003) in which it was demonstrated that the prevalencies of genotype-positive cases in the other Asian populations were so low that no relationship with the susceptibility of AP could be established. The application of the composite genotype of IL-1A(-889)/IL-1B(+3954) may not be valuable in predicting the susceptibility of AP in the Asian population.

One of the most valuable finding of the study reported here was the detection of an significant association between a genetic polymorphism of IL-1B(-511) and MPD value in our AP group. Analysis of variance revealed that individuals (5.0 ± 0.8 mm) carrying allele 2 of IL-1B(-511) indicated higher MPD value than individuals (4.5 ± 0.8) carrying only 1 allele. The Guzman *et al.* (2003) reported that this polymorphism was significantly associated with periodontal disease in a diabetic population of Caucasian heritage. With smoking (Meisel *et al.*, 2002, 2004), diabetic status is an important risk factor for periodontal diseases (Emrich *et al.*, 1991). Although our study could not include any information on the diabetic status of our study samples, these observations on the association between the genotype of IL-1B(-511) and the severity of AP detected by 2 studies may be meaningful to maintain periodontal health. Knowledge of genetic factors, such as the genotype of IL-1B(-511) that are significantly associated with the severity of AP, should therefore enhance the clinicians ability to estimate the future course of disease for a specific patient. In addition, such information may be used to modify the patients risk and to guide prevention and therapy.

However, the results presented here should be interpreted cautiously as there are a few subjects in some of the strata. Most of the studies published so far consist of low number of subjects who are mostly convenience samples, an obstacle also in the present study. Therefore, additional studies using larger sample size are needed to further address these issues, although our results suggest that a genetic polymorphism of IL-1B(-511) may affect the severity of AP in Korean population.

REFERENCE

Amitage, G.C., Wu, Y., Wang, H.-Y., Sorrell, J., di Giovine, F.S. and Duff, G.W. (2000): Low prevalence of a periodontitis associated interleukin-1 composite genotype in individuals of chinese heritage. *J. Periodontol.*, **71**, 164-171.

Anusaksathien, O., Sukboon, A., Sitthiphong, P. and Teanpaisan, R. (2003): Distribution of interleukin-1β+3954 and IL-1α-889 genetic variations in a Thai population group. *J. Periodontol.*, **74**, 1796-1802.

Bendtzen, K. (1994): Cytokines and natural regulators of cytokines. *Immunol. Lett.*, **43**, 111-123.

Bioque, G., Crusius, J.B.A., Koutroubakis, I., Bouma, G., Kostense, P.J., Meuwissen, S.G.M. and Pena, A.S. (1995): Allelic polymorphism in the IL-1α and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. *Clin. Exp. Immunol.*, **102**, 379-383.

Blakemore, A.I.F., Cox, A., Gonzalez, A.M., Maskil, J.K., Hughes, M.E., Wilson, R.M., Ward, J.D. and Duff, G.W. (1996): Interleukin-1 receptor antagonist allele (IL1RN*2) associated with nephropathy in diabetes mellitus. *Hum. Genet.*, **97**, 369-374.

Blakemore, A.I.F., Tarlow, J.K., Cork, M.J., Gordon, C., Emery, P. and Duff, G.W. (1994): Interleukin-1 receptor antagonist gene polymorphisms as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum.*, **37**, 1380-1385.

Bostein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980): Construction of a genetic map using restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **32**, 314-331.

Caffesse, R.G., De LaRosa, M.R., De LaRosa, M.G. and Mota, L.F. (2002): Prevalence of interleukin 1 periodontal denotype in a Hispanic dental population. *Quintessence Int.*, **33**, 190-194.

Cullinan, M.P., Westerman, B., Hamlet, S.M., Palmer, J.E., Faddy, M.J., Lang, N.P. and Seymour, G.J. (2001): A longitudinal study of interleukin-1 gene polymorphisms and periodontal disease in a general adult population. *J. Clin. Periodontol.*, **28**, 1137-1144.

di Giovine, F.S., Takhsh, E., Blakemore, A.I.F. and Duff, G.W. (1992): Single base polymorphism at -511 in the human interleukin-1β gene (IL1β). *Hum. Mol. Genet.*, **1**, 450.

Emrich, L.J., Shlossman, M. and Genco, R.J. (1991): Periodontal disease in non-insulin dependent diabetes mellitus. *J. Periodontal.*, **62**, 123-130.

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, **18**, 499-502.

Galbraith, G.M.P., Hendley, T.M., Sanders, J.J., Palesch, Y. and Pandey, J.P. (1999): Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. *J. Clin. Periodontol.*, **26**, 705-709.

Genco, R.J. (1994): Assessment of risk or periodontal disease. *Compend. Suppl.*, **18**, S714-S717.

Gore, E.A., Sanders, J.J., Pandey, J.P., Palesch, Y. and Galbraith, G.M. (1998): Interleukin-1β +3953 allele 2: Association with disease status in adult periodontitis. *J. Clin. Periodontol.*, **25**, 781-785.

Guzman, S., Karima, M., Wang, H.-Y. and Van Dyke, T.E. (2003): Association between interleukin-1 genotype and periodontal disease in a diabetic population. *J. Periodontol.*, **74**, 1183-1190.

Hart, T.C. and Kornman, K.S. (1997): Genetic factors in the pathogenesis of periodontitis. *Periodontal 2000*, **14**, 202-215.

Hassell, T.M. and Harris, E.L. (1995): Genetic influences in caries and periodontal diseases. *Crit. Rev. Oral. Biol. Med.*, **6**, 319-342.

Hodge, P.J., Riggio, M.P. and Kinane, D.F. (2001): Failure to detect an association with IL1 genotypes in european caucasians with generalised early onset periodontitis. *J. Clin. Periodontol.*, **28**, 430-436.

Huang, D., Pirskanen, R., Hjelmstrom, P. and Lefvert, A.K. (1998): Polymorphisms in IL-1β and IL-1 receptor antagonist genes are associated with myasthenia gravis. *J. Neuroimmunol.*, **81**, 76-81.

Kjeldsen, M., Holmstrup. P. and Bendtzen, K. (1993): Marginal periodontitis and cytokines: a review of the literature. *J. Periodontol.*, **64**, 1013-1022.

Kornman, K.S., Crane, A., Wang, H.Y., di Giovine, F.S., Newman, M.G., Pirk, F.W., Wilson, T.G., Higginbottom, F.I. and Duff, G.W. (1997): The interleukin 1 genotype as a severity factor in adult periodontal disease. *J. Clin. Periodontol.*, **42**, 72-77.

Kornman, K.S. and di Giovine, F.S. (1998): Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. *Ann. Periodontal.*, **3**, 327-338.

Laine, M.L., Farre, M.A., Garcia-Gonzalez, M.A., van Dijk, L.J., Ham, A.J., Winkel, E.G., Crusius, J.B.A., Vandenbroucke, J.P., van Winkelhoff, A.J. and Pena, A.S. (2001): Polymorphisms of the interleukin-1 gene family, oral microbial pathogens and smoking in adult periodontitis. *J. Dent. Res.*, **80**, 1695-1699.

McDevitt, M.J., Wang, H.-Y., Knobelman, C., Newman, M.G., di Giovine, F.S., Timms, J., Duff, G.W. and Kornman, K.S. (2000): Interleukin-1 genetic association with periodontitis inclinical practice. *J. Periodontol.*, **71**, 156-163.

McDowell, T.L., Symons, J.A., Ploski, R., Forre, O. and Duff, G.W. (1995): A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1á polymorphism. *Arthritis Rheum.*, **38**, 221-228.

Meisel, P., Schwahn, C., Gesch, D., Bernhardt, O., John, U. and Kocher, T. (2004): Dose-effect relation of smoking and the interleukin-1 gene polymorphism in periodontal disease. *J. Periodontol.*, **75**, 236-242.

Meisel, P., Siegemund, A., Dombrowa, S., Sawaf, H., Fanghaenel, J. and Kocher, T. (2002): Smoking and polymorphisms of the interleukin-1 gene cluster (IL-1α, IL-1β and IL-1RN) in patients with periodontal disease. *J. Periodontol.*, **73**, 27-32.

Mølvig, J., Baek, L., Christensen, P., Manogue, K.R., Vlassara, H., Platz, P., Nielsen, L.S., Svejgaard, A. and Nerup, J. (1988): Endotoxin-stimulated human monocyte secretion of interleukin 1, tumour necrosis factor alpha and prostaglandin E2 shows stable interindividual differences. *Scand. J. Immunol.*, **27**, 705-716.

Nicklin, M.J.H., Weith, A. and Duff, G.W. (1994): A physical map of the region encompassing the human interleukin-1α, interleukin-1β and interleukin-1 receptor antagonist genes. *Genomics*, **19**, 382-384.

Offenbacher, S. (1996): Periodontal diseases: Pathogenesis. *Ann. Periodontol.*, **1**, 821-878.

Page, R.C. (1991): The role of inflammatory mediators in the pathogenesis of periodontal disease. *J. Periodont. Res.*, **26**, 230-242.

Pociot, F., Mølvig, J., Wogensen, L., Worsaae, H. and Nerup, J. (1992): A Taq I polymorphism in the human interleukin-1β gene correlates with IL-1β secretion *in vitro*. *Eur. J. Clin. Invest.*, **22**, 396-402.

Roberts, F.A., Hockett, R.D. Jr., Bucy, R.P. and Michalek, S.M. (1997a): Quantitative assessment of inflammatory cytokine gene expression in chronic adult periodontitis. *Oral Microbiol. Immunol.*, **12**, 336-344.

Roberts, F.A., McCaffery, K.A. and Michalek, S.M. (1997b): Profile of cytokine mRNA expression in chronic adult periodontitis. *J. Dental Res.*, **76**, 1833-1839.

Sham, P.C. and Curtis, D. (1995): Monte Carlo tests for associations between diseases and alleles at highly polymorphic loci. *Ann. Hum. Genet.*, **59**, 97-105.

Sakellari, D., Koukoudetsos, S., Arsenakis, M. and Konstantinidis, A. (2003): Prevalence of IL-1A and IL-1B polymorphisms in a Greek population. *J. Clin. Periodontol.*, **30**, 35-41.

Tarlow, J.K., Blakemore, A.I.F., Lennard, A., Solari, R., Hughes, H.N., Steinkasserer, A. and Duff, G.W. (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.

Tarlow, J.K., Clay, F.E., Cork, M.J., Blakemore, A.I.F., McDonagh, A.J., Messenger, A.G. and Duff, G.W. (1994): Severity of alopecia aerata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. *J. Invest. Dermatol.*, **103**, 387-390.

Tatakis, D.N. (1993): Interleukin-1 and bone metabolism: A review. *J. Periodontol.*, **64(Suppl 5)**, 416-431.

Walker, S.J., Van Dike, T.E., Rich, S., Kornman, K.S., di Giovine, F.S. and Hart, T.C. (2000): Genetic polymorphisms of the IL-1 and IL-1 genes in african american LJP patients and an african-american control population. *J. Periodontol.*, **71**, 723-728.