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Two Polymorphisms of Interleukin-4 Gene in Korean Adult Periodontitis

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Adult periodontitis is a multifactorial disease characterized by multple genetic and environmental factors. In view of the importance of interleukin-4 (IL-4) gene as a genetic factor for adult periodontitis, we investigated the relationship between two polymorphisms (-590 C → T polymorphism and 70 bp repeat polymorphism) of the human IL-4 gene and adult periodontitis in the Korean population. Genomic DNA was extracted from white blood cells of 32 adult periodontitis patients and 150 normal controls, respectively. There were no significant differences in the allele, genotype and haplotype distributions of two polymorphisms between normal controls and adult periodontitis group. Therefore, our results suggest that IL-4 gene locus contributes little to the interindividual susceptibility for adult periodontitis in Korean population.

Key words: Adult periodontitis, Cytokine, Korean population

INTRODUCTION

Adult periodontitis is a major public health issue of worldwide significance. It is a multifactorial chronic inflammatory disease of the supporting tissues of the teeth, starting with gingivae and progressing to gradual destruction of the body support and periodontal attachment of the teeth. This results in significant morbidity, with loosening and loss of teeth as the ultimate outcome.

Bacteria are essential though probably insufficient to cause adult periodontitis. The Gram-negative dark-pigmented coccobacillus *Porphyromonas gingivalis* and the capnophylic Gram-negative rod *Actinobacillus actinomycetemcomitans* have been implicated as specific pathogens in periodontitis (Haffajee and Socransky, 1994). Cigarette smoking (Bergström, 1989) and diabetes mellitus (Thorstensson and Hugoson, 1993; Collin *et al.*, 1998) are two well-documented risk factors for periodontitis. Furthermore, an individuals capacity to cope with stress has been associated with an increased severity of periodontal disease and has been suggested as a risk factor (Genco *et al.*, 1999).

Although periodontal bacteria are the major etiological factors of this disease, there are many variations in the severity of disease among the patients who have similar stimulating factors. Many researchers have agreed that the susceptibility to periodontal disease was at least partially genetically determined (Seysour, 1991).

Polymorphisms in cytokine genes have been associated with other diseases, which have an inflammatory pathogenesis. Genetic variations found within cytokine genes in adult periodontitis patients may represent a mechanism by which individuals are rendered susceptible to disease.

Interleukin-4 (IL-4) plays a crucial role in asthma, due to its action on B cells to switch the Ig isotype from IgM to IgE and to the maturation of T-helper cells to the Th2 phenotypes (Noguchi *et al.*, 1998; Hijazi and Haider, 2000). An increase of IgE has been observed in gingival tissue of individuals with adult periodontitis (Hyyppä, 1984). Due to these aspects, seemingly the immune mechanisms of allergic disorders may be similar to periodontal disease. Besides, recent studies have demonstrated an association between the genetic variation of IL-4 gene and immune diseases such as asthma and atopy in Caucasian and

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Japanese populations (Rosenwasser *et al.*, 1995; Noguchi *et al.*, 1998). In this regard, IL-4 gene is a good candidate for the etiology of adult periodontal disease.

Therefore, we investigated the association between two polymorphisms of IL-4 gene and adult periodontitis in ethnically homogeneous Koreans.

MATERIALS AND METHODS

Study subjects

A total of 32 cases of adult periodontitis (24 male and 8 female; age range 31~67 year; mean age 48 year) were recruited from Dr. Chois Dental Office, Seoul, Korea, and 150 periodontically healthy control subjects (76 male, 73 female and 1 unknown; age range 27~86 year; mean age 56 year) collected from Clinical Pathology, Seoul Hygiene Hospital Seoul, Korea. Informed consent was obtained from all subjects. The baseline clinical parameters for the patients with adult periodontitis are presented in Table I.

DNA analysis

Genomic DNA was extracted using Wizard® Genomic DNA purification kit (Promega Co. Ltd., Madison, WI, USA) from whole blood. Polymerase Chain Reaction (PCR) techniques were used for 70 bp repeat polymorphism in intron 2 and C → T polymorphism in 590 promoter region of IL-4 gene (Gonzáles *et al.*, 2001). 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 two polymorphisms studied were:

(a) For C → T polymorphism

sense, 5'-ACTAGGCCTCACCTGATACG-3', anti-sense, 5-GTTGTAATGCAGTCCTCCTG-3 (Wallney and Cookson, 1996)

(b) For 70 bp repeat polymorphism

sense, 5'-TAGGCTGAAAGGGGGAAAGC-3', anti-sense, 5-CTGTTCACCTCAACTGCTCC-3 (Mout *et al.*, 1991)

Table I. 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	42.0 ± 23	10 - 100
gingival redness	38 ± 19	5 - 100
bleeding on probing	32 ± 20	7 - 100
% Current smokers	37.5	

[1]Standard deviation

Amplification was carried out with DNA thermocycler. For C → T polymorphism, the reactions were denatured at 94 °C for 1 min, at 57 °C for 1 min and at 72 °C for 1 min for a total 40 cycles. For 70 bp repeat polymorphism, the reactions were denatured at 94 °C for 1 min, at 55 °C for 1 min and at 72 °C for 1 min for a total 40 cycles. Ten μL of PCR product for the C → T polymorphism was restriction-digested overnight with 10 units of *Bsm* FI (New England Biolab, Schwalbach/Taunus, Germany) at 37°C. The resulting products were visualized by 2% agarose gel electrophoresis and ethidium bromide staining.

Statistical analysis

Differences in genotype and allele frequencies were assessed by χ^2-test. The heterozygosity and polymorphism information content (PIC) values were calculated as previously described (Bostein *et al.*, 1980). The relative ratio of adult periodontitis associated with allelic variation was expressed in terms of an odds ratio (OR) with 95% confidence interval (CI). Expectation maximization (EM) algorithm was used for pair-wise linkage disequilibrium test and haplotype analysis of IL-4 gene polymorphisms. A Monte-Carlo simulation using the Clump (version 1.6) program was performed to test the statistical significance of the association between the haplotype distribution and adult periodontitis (Sham and Curtis, 1995). The degree of non-random association was determined by calculation of the delta (Δ) (Hill and Robertson, 1968) and D' (Lewontin, 1964) between the polymorphic sites in the IL-4 gene. To test the significance of linkage disequilibrium, $n\Delta^2$ value was used as the χ^2 distribution with 1 df (degree of freedom). A p-value of less than 0.05 indicated statistical significance. All statistical analyses were performed with the computer program of SPSSWIN (version 10.0).

RESULTS

-590 C → T polymorphism

The -590 C → T polymorphism of IL-4 gene was detected by digestion with restriction enzyme *Bsm* FI after PCR amplification. C allele yielded two fragments of 192 bp and 60 bp, and T allele gave a band of 252 bp (Fig. 1).

The genotype and allele frequencies of -590 C → T polymorphism were shown in Table II. The genotype frequencies of CC, CT and TT were 3, 36 and 61% in controls, and 9, 38 and 53% in adult periodontitis group, respectively. The heterozygosity and PIC values of this polymorphism represented the values of 0.3292 and 0.2750 for controls, and 0.4043 and 0.3226 for adult periodontitis group, respectively. According to the heterozygosity and PIC values, -590 C → T polymorphism showed the relatively high degree of polymorphism in the both groups. The observed genotype distributions were not significantly deviated from

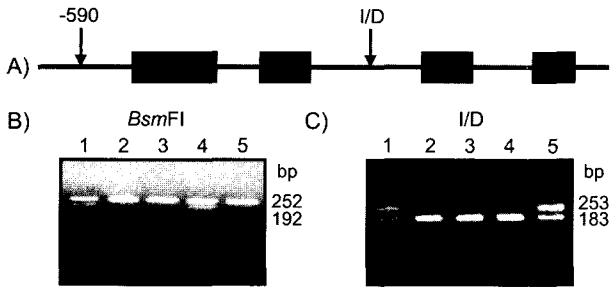

Fig. 1. Schematic diagram of IL-4 gene. A) Restriction map of IL-4 gene. A) -590C/T polymorphism of IL-4 gene. Lane 1 and 4, CT genotypes; lane 2, 3 and 5, TT genotypes. B) I/D polymorphism of IL-4 gene. Lane 1 and 5, I/D genotypes; lane 2~4, D/D genotypes.

Table II. Genotype and allele frequencies of the 590C/T polymorphism of the IL-4 gene in controls and adult periodontitis group

	Genotype No. (%)			Allele No. (%)			
	CC	CT	TT	C	T	H^1	PIC^2
Control	4(3)	51(36)	87(61)	59(21)	225(79)	0.3292	0.2750
Periodontitis	3(9)	12(38)	17(53)	18(28)	46(72)	0.4043	0.3226
χ^2		3.1000		1.6380			
P		0.2120		0.2010			
Odds ratio (CI)[3]		1.49(0.812.76)					

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

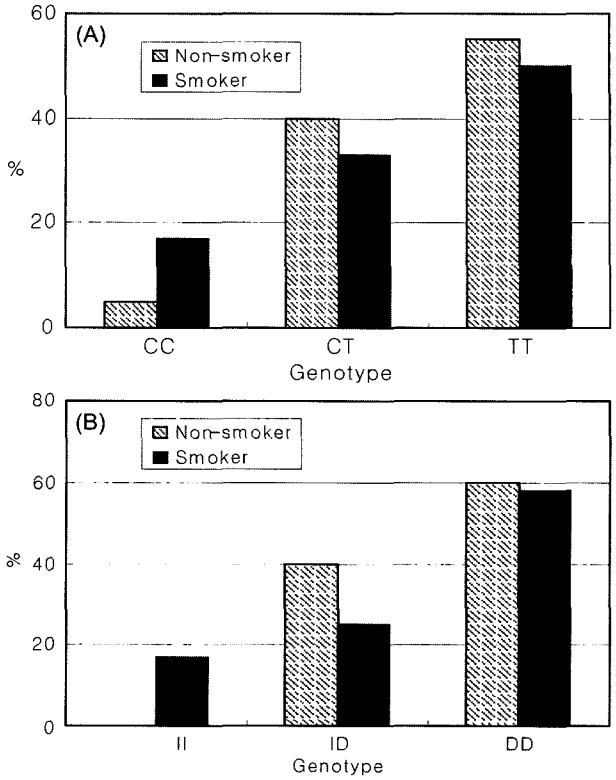

Fig. 2. The polymorphic patterns of IL-4 gene by smoking status in adult periodontitis group. A) -590C/T polymorphic patterns. B) I/D polymorphic patterns. There were no significant differences in genotype distribution in the both cases (P > 0.05).

those expected for Hardy-Weinberg equilibrium.

There were no significant differences in genotype and allele frequencies between controls and adult periodontitis group by case-control comparison. When case group was stratified by smoking status, there was also no significant association between the genotype distribution of -590 C → T polymorphism and smoking status (Fig. 2).

70 bp repeat polymorphism

The 70 bp repeat polymorphism of IL-4 gene was detected by direct electrophoresis after PCR amplification. I allele yielded a band of 253 bp, while D allele gave a band of 183 bp (Fig. 1).

The genotype and allele frequencies of 70 bp repeat polymorphism were shown in Table III. The genotype frequencies of II, ID and DD were 2, 33 and 65% in controls, and 6, 35 and 59% in adult periodontitis group, respectively. The heterozygosity and PIC values of this polymorphism represented the values of 0.2994 and 0.2546 for controls, and 0.3589 and 0.2945 for adult periodontitis group, respectively. According to the heterozygosity and PIC values, 70 bp repeat polymorphism showed the relatively low degree of polymorphism in the both groups compared with -590 C → T polymorphism. The observed genotype

Table III. Genotype and allele frequencies of the 70 bp repeat polymorphism of the IL-4 gene in controls and adult periodontitis group

	Genotype No. (%)			Allele No. (%)			
	II	ID	DD	I	D	H^1	PIC^2
Contol	3(2)	49(33)	98(65)	55(18)	245(82)	0.2994	0.2546
Periodontitis	2(6)	11(35)	19(59)	15(23)	49(77)	0.3589	0.2945
χ^2		1.9030		0.8850			
P		0.3862		0.3470			
Odds ratio (CI)[3]		1.36(0.712.61)					

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

distributions were not significantly deviated from those expected for Hardy-Weinberg equilibrium.

Likewise 590 C → T polymorphism, there were no significant differences in genotype and allele frequencies between two groups by case-control comparison. When smoking status stratified case group, II genotype was only observed in smokers. However, there was no significant association in overall genotype distribution between the 70 bp repeat polymorphism and smoking status (Fig. 2).

Table IV. Haplotype frequencies and linkage disequilibrium statistics (D′, Δ) between pairs of two polymorphisms in the IL-4 gene

Haplotype		Contol	Periodontitis
-590C/T	70 bp Ins/Del		
C	I	0.014773	0.000000
C	D	0.173171	0.234375
T	I	0.776008	0.718750
T	D	0.036049	0.046875
Total chromosomes		282	64
Δ		0.842567	0.884229
D'		0.900934	0.999697
χ^2		200.197200	50.039099
P		<0.00001	<0.00001

There were no significant differences in haplotype frequencies between control and adult periodontitis group (Monte-Carlo simulation, T_4 = 1.5642 df = 1, P = 0.5512, simulation number = 10,000). The significant linkage disequilibrium was detected in the both groups (P<0.05).

Haplotype analysis

The haplotype distribution and the linkage disequilibrium statistic reflecting the extent or significance of pair-wise nonrandom association between two polymorphic sites studied were shown in Table IV. There was no significant difference in haplotype distribution between two groups. However, the significant pair-wise linkage disequilibrium between two polymorphic sites studied was detected in both groups by χ^2-test (P < 0.05).

DISCUSSION

Genetic factors are known to play an important role in the pathogenesis of periodontal diseases. A recent study has suggested a positive effect of IL-4 gene in early-onset periodontitis (Gonzáles *et al.*, 2001). However, there is no report on the relationship between the genetic variations of IL-4 gene and adult periodontitis.

IL-4 is a potent down regulator of macrophage function (Shapira *et al.*, 1992), and inhibits of PGE2 (prostaglandin E2) and cytokines by macrophages (Te Velde *et al.*, 1990; Corcoran *et al.*, 1992). IL-4 is also a down regulator of CD14 receptor (Lauener *et al.*, 1990) and induces the apoptosis of monocyte. Thus, upper data are based on the hypothesis of Shapira *et al.*, (1992) that the absence of IL-4 triggers periodontal disease.

The human IL-4 gene is located on chromosome 5q23-31, in close vicinity to the IL-3, IL-5, IL-13, GM-CSF and IRF1 cluster of cytokine genes (Le Beau *et al.*, 1989; van Leeuwen *et al.*, 1989) and consists of 4 exons separated by 3 introns. Some studies have identified several molecular variants (-590 C → T and 70 bp repeat polymorphisms) of IL-4 gene, and used as genetic markers for clinical association studies (Mout *et al.*, 1991; Wallney and Cookson, 1996). Until now, genetic variations of IL-4 gene was significantly associated with asthma and atopy (Walley and Cookson, 1996), multiple sclerosis (Vandenbroeck *et al.*, 1997), autoimmune thyroid disease (Hunt *et al.*, 2000) and early-onset periodontitis (Gonzáles *et al.*, 2001).

The present study revealed that there were no significant differences between controls and adult periodontitis group in genotype and allele frequencies of -590 C → T and 70 bp repeat polymorphisms in the IL-4 gene. Therefore, it is unlikely that these two polymorphisms are significantly associated with the ethiology of adult periodontitis among Koreans.

Smoking is an important risk factor of periodontal disease (Bergström, 1989), and we, therefore, considered as confounding factor in this genetic association study. When patients were subdivided by smoking status, the genotype distributions of these polymorphisms was also similar between two groups. It is unlikely that significant gene and environment interaction was existed in our subjects.

By pair-wise haplotype analysis, the significant linkage disequilibrium between two polymorphic sites was detected. This finding suggests that the haplotype occurred by two polymorphisms decreases the information content for linkage analysis, while it did not require the large sample size to perform the association study. Therefore, association study may be better than linkage analysis to discover the disease susceptibility gene in the case of two polymorphisms in the IL-4 gene.

Within the limitations of the present study of about 150 controls and 32 adult periodontitis group, we failed to demonstrate the association between two polymorphisms of IL-4 gene, and the occurrence of adult periodontitis. It could be however, argued that the relatively small number of subjects may give a low probability of detecting a small effect of the polymorphism (a small gene effect is expected in the case of a disease as complex as adult periodontitis). Moreover, these types of study design are prone to type II error (that is, failing to reject the null hypothesis that there is no differences in allelic distributions between two groups when it is false). The lack of association indicates that the particular DNA changes causing the polymorphisms are not responsible for adult periodontitis, and that polymorphisms are not in linkage disequilibrium with other loci that are responsible. Negative finding generated by retrospective case-control studies can in no way be advocated to rule out the gene effects in clinical phenotype under investigation. That is why we cannot exclude the possibility that IL-4 gene is somehow involved in the pathogenesis of adult periodontitis.

Nevertheless, there were no significant differences in allele, genotype and haplotype distributions of two poly-

morphisms in the IL-4 gene between controls and adult periodontitis patients in Koreans. This might suggest that these polymorphisms are not useful as genetic marker to investigate the relationship between the IL-4 gene and adult periodontitis in Koreans.

Taking into account the genetic heterogeneity of adult periodontitis, further studies should test for association with other candidate genes involved in the pathogenesis of adult periodontitis.

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