

IL-1 gene polymorphisms in Korean periodontitis patients

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I. Introduction

The primary cause for developing periodontitis is bacteria, but host factors such as diabetes, smoking, and genetics could contribute to the clinical appearance as well as the extent and severity of periodontal destruction. The host inflammatory response to the bacterial challenge is one of the factors contributing to the destruction of periodontal tissue^{1,2}. The role of proinflammatory cytokines has been studied extensively in periodontal diseases. Involvement of interleukin-1 (IL-1) in the pathogenesis of periodontal disease has been suspected because of association between the known biological effects of the cytokine and the manifestations of the disease. IL-1 level has been reported to be elevated in inflamed periodontal tissues and gingival crevicular fluid associated with periodontitis^{3,4}. Masada et al.³ reported that IL-1 might serve as a marker of periodontal tissue destruction because IL-

1 was produced and released locally in periodontal disease at concentrations sufficient to mediate tissue inflammation and bone resorption. IL-1 enables ingress of inflammatory cells into sites of infection; stimulates eicosanoid release by monocytes and fibroblasts; stimulates matrix metalloproteinases; and contributes to the inflammatory cascade of the microbial immune response^{5,6}.

Proinflammatory cytokine IL-1 exists in 2 forms, IL-1 α and IL-1 β . There are 3 genes that regulate the production of IL-1 variants: IL-1A, IL-1B, and IL-1 receptor antagonist (IL-1 RN)⁷. These genes are located close to each other on chromosome 2q13. Genes IL-1A and IL-1B control the production of proinflammatory proteins, IL-1 α and IL-1 β respectively. IL-1 RN controls the synthesis of an antagonist protein (IL-1ra) that impedes IL-1 α and IL-1 β ^{8,9}. The specific forms alleles of each IL-1A and IL-1B gene present in an individual vary. The term "genetic polymorphism" refers to a change in the sequence

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of nucleotides comprising a gene. A polymorphism in the IL-1A promoter region at 889, which is 99-100% concordant with IL-1A+4845^{10,11}. In the IL-1B, 2 biallelic base change polymorphisms have been reported to influence the protein production¹². One is in the promoter region at position 511, and the other is in exon 5 at +3954. IL-1 RN intron 2 contains various number of tandom repeat(VNTR) of 86-bp sequence and 5 different alleles were reported¹³.

Kornman et al.¹⁴ reported that the presence of specific alleles at two IL-1 gene polymorphisms was associated with increased severity of periodontal disease in adult non-smokers of Northern European ancestry. Specifically individuals carrying the “2” allele of the bi-allelic restriction fragment length polymorphism of the IL-1B+3953(after +3954) and the IL-1A-889 loci in either the heterozygous or homozygous state at both loci (so called “genotype-positive”) were observed to have a significantly greater risk for developing severe periodontitis when compared to a mild periodontitis group. In the population of Caucasian Northern Europeans studied so far, it was reported that approximately 36% of the individuals carried this genotype. Of the non-smokers aged 40 to 60 years in this population who had severe periodontitis, 78% were genotype-positive¹⁴. It has also been reported that those who were positive for the IL-1 composite genotype showed 2.7 times higher risk of tooth loss than genotype-negative patients in treated periodontitis patients under long-term maintenance care¹⁵.

Since the frequency of many genetic alleles varies among ethnic groups, it is necessary to establish allele frequencies in populations. Several investigations showed the frequency that individuals were genotype positive in different ethenic groups. Armitage et al.¹⁶ reported the prevalence of both IL-1A and IL-1B polymorphisms were dramatically lower in Chinese than those reported for

Europeans. Tai et al.¹⁷ reported that there was no significant difference in the IL-1A+4845 and IL-1B+3954 genotypes and allele frequencies between generalized early onset periodontitis (G-EOP) patients and healthy controls in Japanese. However, the frequency of IL-1RN (intron 2 VNTR) polymorphic allele was found to be significantly increased in G-EOP patients (odds ratio=3.40).

The aim of this study was to evaluate the distribution of IL-1 gene cluster polymorphisms in groups of periodontitis patients with different forms and severities in Korea.

II. Materials and methods

1. Subjects and clinical assessments

The subjects in this study were recruited from periodontal patients who were examined and treated at the Dept. of Periodontics, Chonnam National University Hospital. Each subject had no history of systemic diseases, and had not taken any medications. The probing depth and clinical attachment loss were recorded at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual). Full mouth periapical radiographs or a panoramic radiograph taken within 1 year were evaluated for interproximal bone loss measurements from the cemento-enamel junction of the tooth to the bone crest, expressed in % of the total root length. The reference group consisted of 92 children, from the Dept. of Pediatric Dentistry. The patients over 40 years of age were classified by their periodontal status¹⁴. Mild and moderate chronic periodontitis (CP) patients were diagnosed if they exhibited no pockets deeper than 3 mm and no sites with bone loss more than 15% of root length, and if they exhibited less than 4 interproximal sites with \geq 50% bone loss and total mean bone loss of 17 to

28%, respectively. Severe CP patients were those who exhibited more than 7 interproximal sites with $\geq 50\%$ bone loss. Inclusion criteria for generalized aggressive periodontitis (GAgP) patients were usually affecting persons under 30 years of age, but patients may be older, pronounced episodic nature of the destruction of attachment and alveolar bone, and generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors¹⁸).

2. Sample collection and extraction of DNA

From each subject, buccal swab was performed by rubbing buccal mucosa 10 times using sterile foam tipped applicator (Hardwood product company, USA) after thorough mouth-washing and then stored at -20°C . Genomic DNA was obtained from buccal swab by extracting DNA with heating at 95°C for 5 min in 200 μl of 50 mM NaOH and neutralizing with 20 μl Tris (pH 8.0).

3. Analysis of the IL-1A+4845, IL-1B+3954, and IL-1B-511 polymorphisms

DNA samples were amplified by multiplex PCR. PCR amplification was done using 10 μl sample DNA in 50 μl volume of the reaction mixture containing 10X reaction buffer, 1.5 mM MgCl_2 , 0.2 mM dNTP, 3 pairs of 0.75M primer, 1.5U Taq polymerase (DynazymeTM, Finnzymes, Finland) and DDW. The following primers were used: for determination of IL-1A+4845 genotype, sense 5' -ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA-3', antisense 5'-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT-3'¹⁹); for IL-1B+3954, sense 5'-CTC AGG TGT CCT CGA AGA AAT CAA A-3', antisense 5'-GCT TTT TTG CTG TGA GTC CCG-3'¹⁴); for IL-1B-511, sense 5'-TGG CAT TGA TCT GGT TCA TC-3', antisense 5'-GTT TAG GAA TCT TCC CAC TT-3'.¹⁴) Amplification was performed under following conditions in thermal cycler (Geneamp[®] PCR system 2700, Applied Biosystems, USA): at 95°C for 15 min, 10 cycles at 95°C for 30 sec and 58°C for 2 min, 20 cycles at 95°C for 10 sec, 53°C for 50 sec, and 72°C for 30 sec, and finally at 72°C for 10 min²⁰). The PCR products were checked by 2% agarose gel electrophoresis (Figure 1). The resulting products were 240 bp (IL-1A+4845), 190 bp (IL-1B+3954), and 304 bp (IL-1B-511).

The resulting PCR products were digested with

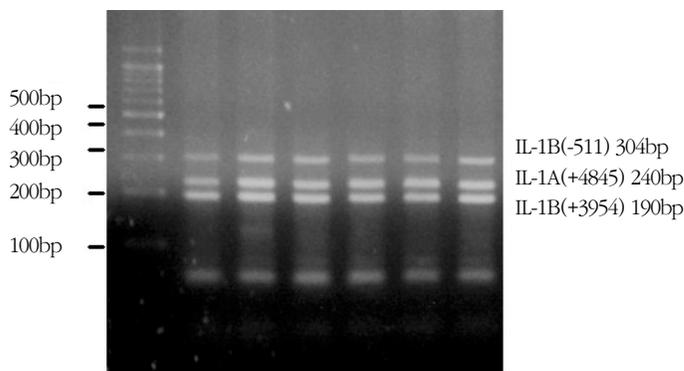


Figure 1. Amplicons of multiplex PCR of IL-1A+4845, IL-1B +3954 and IL-1B-511.

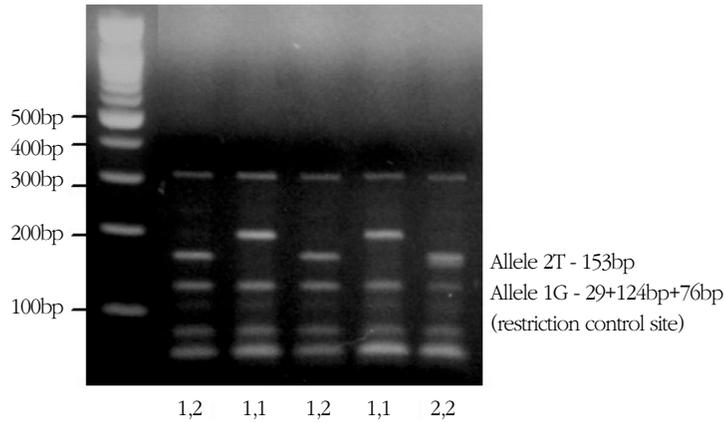


Figure 2. IL-1A+4845 alleles and genotype determination.

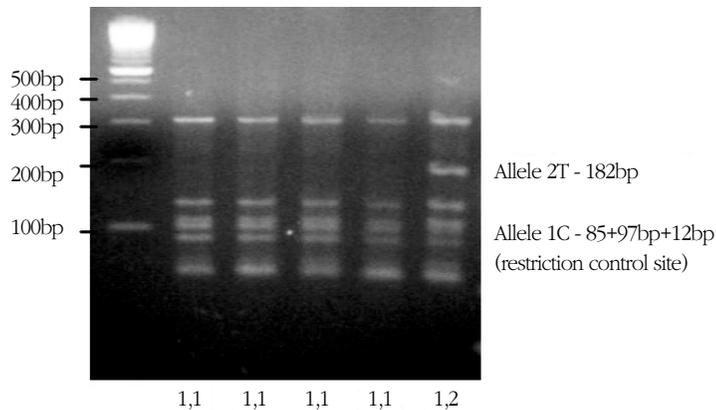


Figure 3. IL-1B+3954 alleles and genotype determination.

restriction enzymes, as follows:

1. IL-1A+4845 : Ten microliters of the PCR products were digested with Fnu 4H1 (New England BioLabs, Hitchin, UK) at 37°C for 1 hr. The restriction fragments were determined on 2% agarose gel electrophoresis stained with 0.1% ethidium bromide. The resulting products of 124 + 29 bp (allele 1G), 153 bp (allele 2T), and a constant 76 bp (restriction control site) are diagnostic (Figure 2).
2. IL-1B+3954 : Ten microliters of the PCR products were digested with 3 units of Taq I (Gibco

BRL, Life Technologies Inc., Rockville, USA) at 65°C for 1 hr. The restriction fragments were determined on a 3% agarose gel stained with 0.1% ethidium bromide. Allele 1 gave products of 85 + 97 bp, while allele 2 gave products of 182 bp and 12 bp (Figure 3).

3. IL-1B-511 : Ten microliters of the PCR products were digested with 3 units of Ava I (New England BioLabs.) at 37°C for 2 hr. The resulting products of 190 + 114 bp (allele 1) and 304 bp (allele 2) are diagnostic (Figure 4).

The simultaneous presence of specific polymor-

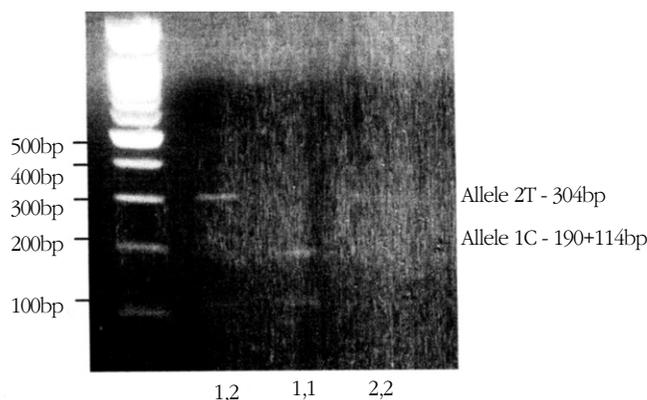


Figure 4. IL-1B-511 alleles and genotype determination.

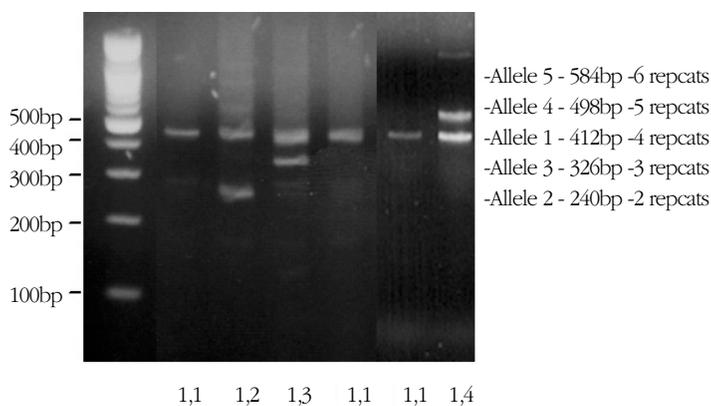


Figure 5. IL-1RN alleles and genotype determination.

phisms, allele 2 of the IL-1A+4845 and IL-1B+3954 loci, was evaluated. Individuals possessing a combination of these genetic polymorphisms were referred to being genotype-positive according to Komman et al.¹⁴.

4. Analysis of the IL-1 RN (intron 2 VNTR) polymorphism

The IL-1 RN intron 2 contains a VNTR of an 86 bp length of DNA. The primers used were as follows: sense 5'-CTC AGC AAC ACT CCT AT-3', antisense 5'-TCC TGG TCT GCA GGT AA-3'¹⁴. The reaction conditions used were as follows: four microliters of

sample DNA was amplified in 25 μ l volume of the reaction mixture containing 10X reaction buffer, 1,5 mM MgCl₂, 0,4 mM dNTP, a pair of 0,75M of each primer, 1,5U Taq polymerase, and DDW. The PCR conditions comprised a denaturing step for 10 min at 95°C, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 70°C for 2 min, and finally at 70°C for 5 min. Genotype was determined by molecular size analysis of PCR products on 2% agarose gel stained with 0,1% ethidium bromide. Allele 1 (4 repeats) was 412 bp in size; allele 2 (2 repeats) was 240 bp; allele 3 (3 repeats) was 326 bp; allele 4 (5 repeats), 498 bp; and allele 5 (6 repeats), 584 bp (Figure 5).

5. Statistical Analysis

Data were analyzed using the SPSS 12,0 software (SPSS Inc, USA). One-way analysis of variance was performed to test the differences of the clinical parameters among each group of patients. The distribution of IL-1 genetic polymorphisms in the patients were calculated using descriptive statistical analysis. The χ^2 test was used to analyse the data for the association between periodontitis severities and the IL-1 α IL-1 β and IL-1ra genotypes. The test results were considered statistically significant at the P value less than 0,05.

III. Results

1. Clinical assessments

The group characteristics and clinical parameters are summarized in Table 1. Ten patients were categorized as having mild chronic periodontitis with mean bone loss of $13,5 \pm 2,9\%$ (related to the root length). Thirty eight patients were categorized as having moderate chronic periodontitis with mean bone loss of $23,4 \pm 6,4\%$. Twenty seven patients were categorized as having severe chronic periodontitis with mean bone loss of $26,3 \pm 12,6\%$. Twenty five patients were categorized as having generalized aggressive periodontitis with mean bone loss of $31,0 \pm 7,9\%$.

There was no significant difference in the mean age among different periodontitis groups. Out of

Table 1. Summary of clinical parameters in different periodontitis groups

Group	N	Age	Smoking (%)	Probing Depth (mm)	Attachment Loss (mm)	Interproximal Bone Loss (% to root length)
Mild CP	10	47,7 \pm 4,6	20,0	2,4 \pm 0,5	2,5 \pm 0,5	13,5 \pm 2,9
Moderate CP	38	50,9 \pm 8,0	21,1	2,6 \pm 0,7	2,7 \pm 0,6	23,4 \pm 6,4
Severe CP	27	50,4 \pm 6,5	11,1	3,1 \pm 0,9	3,4 \pm 1,0	26,3 \pm 12,6
GAgP	25	40,5 \pm 7,0	8,0	3,5 \pm 1,0	3,4 \pm 1,2	31,0 \pm 7,9

Mean \pm SD. CP ; Chronic periodontitis, GAgP ; Generalized aggressive periodontitis

Table 2. Distribution (%) of IL-1A+4845, IL-1B+3954 and IL-1B-511 genotype according to the disease severity

Genotype	Mild CP	Moderate CP	Severe CP	GAgP
IL-1A+4845				
(1,1)	60,0	47,4	37,0	20,0
(1,2)	30,0	42,1	55,6	72,0
(2,2)	10,0	10,5	7,4	8,0
IL-1B+3954				
(1,1)	90,0	92,1	77,8	88,0
(1,2)	10,0	7,9	22,2	12,0
IL-1B-511				
(1,1)	20,0	33,3	25,9	8,0
(1,2)	30,0	38,9	40,7	60,0
(2,2)	50,0	27,8	33,3	32,0

CP ; Chronic periodontitis, GAgP ; Generalized aggressive periodontitis

Table 3. Distribution (%) of IL-1 RN genotype according to the disease severity

Genotype	Mild CP	Moderate CP	Severe CP	GAgP
(1,1)	80,0	51,5	61,5	80,0
(1,2)	20,0	21,1	26,9	8,0
(2,2)		10,5		4,0
(1,3) or (3,4)		2,6		
(1,4) or (4,4)		2,6	7,7	8,0
(1,5)		2,6	3,8	

CP ; Chronic periodontitis, GAgP ; Generalized aggressive periodontitis

Table 4. Allele 2 carriage rate(%) in periodontitis subjects and reference group

	N	IL-1A+4845	IL-1B+3954	IL-1B-511	IL-1 RN
Reference group	92	76,9	7,7	62,2	19,1
Periodontitis group	100	61,0	13,0	76,6	34,0
Mild CP	10	40,0**	10,0	80,0	20,0
Moderate CP	38	52,6**	7,9	65,7	45,5
Severe CP	27	63,0	22,2*	74,0	38,5
GAgP	25	80,0	12,0	92,0**	20,0

CP ; Chronic periodontitis, GAgP ; Generalized aggressive periodontitis

* statistically significant $p < 0,05$ by χ^2 - test compared to the reference group.

** statistically significant $p < 0,01$ by χ^2 - test compared to the reference group.

subjects with severe chronic periodontitis, 11,1% were smokers, compared with 20% and 21,1% in mild and moderate chronic periodontitis groups, respectively.

2. Analysis of IL-1 gene polymorphism

The distribution of polymorphisms in the genes for IL-1 α IL-1 β and IL-1 receptor antagonist was evaluated in 4 groups of patients with periodontitis. Genotype status of CP and GAgP group are shown in Table 2 and 3.

The distribution of genotype with allele 2 was 61%, 13%, 76,6%, and 34% for IL-1A+4845, IL-1B+3954, IL-1B-511, and IL-1 RN respectively in periodontitis patients, and 76,9%, 7,7%, 62,2%, and 19,1% in the reference group (Table 4). For IL-1A+4845, allele 2 carriage rate was 40%, 52,6%, 63%,

and 80% in mild, moderate, severe CP and GAgP group, respectively. The allele 2 carriage rate of IL-1A+4845 gene in mild and moderate CP was significantly lower than the reference group ($p < 0,01$)(Table 4, Figure 6). For IL-1B+3954, allele 2 carriage rate was 10%, 7,9%, 22,2%, and 12% in mild, moderate, severe CP and GAgP patients, respectively. The allele 2 carriage rate of IL-1B+3954 gene in severe CP was significantly higher than the reference group (22,2% vs 7,7%, $p < 0,05$). All of the carriers of IL-1+3954 allele 2 also have IL-1A+4845 allele 2 and were considered as composite genotype-positive. Thirteen out of the 100 patients (13%) of the periodontitis group were genotype-positive, and 7,7% of the reference group were genotype-positive. Periodontitis group tended to be higher in the frequency of the genotype-positive than the reference group.

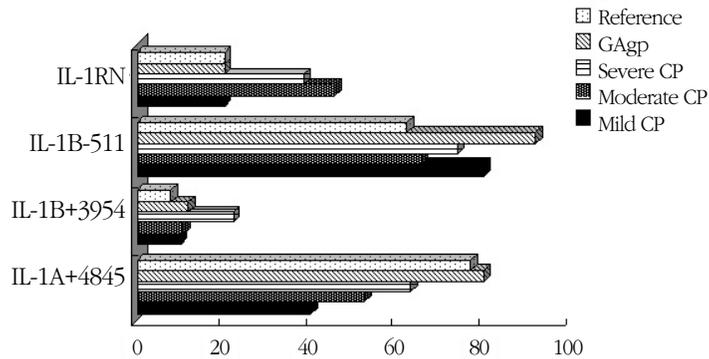


Figure 6. Allele 2 carriage rate(%) of IL-1A+4845, IL-1B+3954, IL-1B-511, and IL-1RN in periodontitis groups and reference group.

Table 5. The distribution(%) of composite genotype positive of IL-1A+4845 and IL-1B+3954 in each group

Group	Genotype-positive	Genotype-negative
Reference group	7.7	92.3
Periodontitis group	13.0	87.0
Mild CP	10.0	90.0
Moderate CP	7.9	92.1
Severe CP	22.2*	77.8
GAgP	12.0	88.0

CP ; Chronic periodontitis, GAgP ; Generalized aggressive periodontitis

* statistically significant $p < 0,05$ by χ^2 - test compared to the reference group.

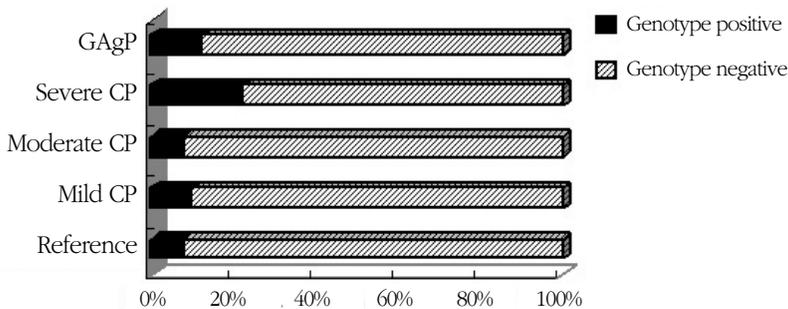


Figure 7. The distribution (%) of composite genotype positive of IL-1A+4845 and IL-1B+3954 in periodontitis groups and reference group.

The frequency of IL-1B-511 gene polymorphism in periodontitis group tended to be higher than the reference group (76,6% vs 62,2%). The frequency of the carriage of allele 2 in the GAgP group (92%) was significantly higher than the reference group

($p < 0,01$). The frequency of allele 2 carriage for IL-1 RN in mild, moderate and severe CP groups and reference group was 20%, 45,5%, 38,5%, and 20% and it tended to be higher in moderate to severe CP groups than the reference group (Table 4, Figure 6).

Table 6. Allele 2 carriage rates(%) according to ethnic group

Ethnic group	IL-1A+4845	IL-1B+3954	IL-1B-511	IL-1RN
Caucasians ^{28,29)}	37.5 ²⁸⁾	61.6 ²⁹⁾	59.4 ²⁸⁾	48.6 ²⁹⁾
African-American ^{18,30)}	26.9 ¹⁸⁾	27.0 ¹⁸⁾		20.4 ³⁰⁾
Japanese ¹⁶⁾	18.6	9.3	68.0	8.2
Korean ³¹⁾		10.8	75.4	13.8
Korean (this study)	76.9	7.7	62.2	19.1

The distribution of composite genotype-positive of IL-1A+4845 and IL-1B+3954 in mild, moderate, and severe CP groups, GAgP group and reference group was 10%, 7.9%, 22.2%, 12%, and 7.7%, respectively. The frequency of IL-1 geno-positive in severe CP was significantly higher than the reference group and the other forms of periodontitis subjects ($p < 0.05$) (Table 5, Figure 7).

IV. Discussion

Since it was reported first by Komman et al.¹⁴⁾ on the relationship between the IL-1 genotype and adult periodontitis, this association has been confirmed in other studies^{1,21,22,23)} sometimes with controversial conclusions²⁴⁾. McDevitt et al.²⁵⁾ demonstrated that patients bearing the positive genotype show an increased risk of moderate and progressive periodontitis when compared to those with negative genotypes. On the other hand, contradicting results were shown regarding the role of IL-1 haplotypes for prognosis of periodontal diseases^{26,27)}.

The several studies revealed the different frequency of IL-1A, IL-1B SNPs, and IL-1 RN (intron 2 VNTR) polymorphisms in various ethnic groups. Allele 2 carriage rates in Caucasian were 37.5% for the IL-1A+4845; 59.4%, IL-1B-511; 61.6%, IL-1B+3954; 48.6%, IL-1 RN (VNTR) polymorphisms^{28,29)}. Moreover, in African-Americans, carriage rates were 26.9% for the IL-1A+4845, 27.0% for the IL-1B+3954, and 20.4% for the IL-1 RN^{18,30)}. And

Tai et al.¹⁶⁾ reported that allele 2 carriage rates were 18.6% for the IL-1A+4845, 9.3% for IL-1B+3954, 68.0% for the IL-1B-511, and 8.2% for IL-1 RN polymorphisms respectively, in Japanese. Our results demonstrated that allele 2 carriage rates were 76.9% for the IL-1A+4845, 7.7% for IL-1B+3954, 62.2% for the IL-1B-511, and 19.1% for IL-1 RN, respectively in Korean reference group. In particular, the allele 2 carriage rates of IL-1B+3954, and IL-1 RN were lower in Korean than Caucasian, whereas the rate of IL-1A+4845 was higher in Korean than the Caucasian and the Japanese data (Table 6).

In the present study on Korean subjects, the prevalence of genotype-positive subjects was low compared with those found in other ethnic groups. The majority of the subjects exhibited homozygous allele 1 of the IL-1B+3954 and IL-1A+4845 genes with only 13% of the periodontitis subjects and 7.7% of healthy reference group showing genotype-positive. However, the prevalence of genotype-positive subjects in this study was higher than the other Asian ethnic groups reported by Armitage et al.¹⁶⁾ who showed that only 2.3% of the Chinese subjects were genotype-positive. On the other hand, the distribution of the genotype-positive subjects from the Caucasian population varied from 29% to 46.4% depending on the geographic locations. Hispanic³²⁾ and African American subjects¹⁸⁾ were approximately 26% and 14% genotype-positive, respectively. In this study, none of periodontitis subjects and none of reference groups exhibited allele 2 homozygous

genotype at IL-1B+3954 loci. These results were in agreement with those of Tai and coworkers¹⁷⁾ who reported no allele 2 homozygosity of IL-1B+3954 in Japanese subjects. Armitage et al.¹⁶⁾ also found that the prevalence of the allele 2 homozygous of IL-1B+3954 and IL-1A-889 loci were 0% and 0.67% in the Chinese population, respectively. In addition, allele 2 homozygous IL-1B+3954 gene was not detected in the Taiwanese Chinese subjects³³⁾ and Thai groups³⁴⁾. Thus, the prevalence of allele 2 at both the IL-1B+3954 and IL-1A+4845 loci is much less in the Asian population compared with those reported for the other ethnic groups. Taken together, the results have clearly shown that the distribution of IL-1B+3954 and IL-1A+4845 genetic polymorphisms varies with population due to different ethnic and geographic backgrounds.

The distribution of composite genotype positive pattern of IL-1B+3954 and IL-1A+4845 in mild, moderate, severe chronic periodontitis, generalized aggressive periodontitis patients, and the reference group was 10%, 7.9%, 22.2%, 12%, and 7.7%. In the severe chronic periodontitis group, the frequency of geno-positive pattern was higher than the reference and the other groups. In this study, the reference group consisted of 92 children from the Dept. of Pediatric Dentistry. As a children group could represent the future general population, we used this group as the reference group, instead of the periodontally healthy control group.

In this study, the prevalence of IL-1A+4845 gene polymorphism was 76.9% in the reference group, 61.0% in periodontitis group. The prevalence was higher compared to the other ethnic group (Table 6) and was significantly lower in mild and moderate periodontitis groups compared to the reference group. Anusaksathien et al.³⁴⁾ studied the distribution of IL-1A-889 genetic polymorphism for periodontitis patient in Thai ethnic group and it was

23.3% in healthy, 13.8% in mild and moderate CP, 4% in severe CP and 15.4% in AgP. The prevalence of IL-1A+4845 genetic polymorphism was lower than our results, the pattern of the distribution of IL-1A+4845 allele 2 in periodontitis patients was decreased to the severity of periodontitis.³⁴⁾ On the other hand, in our results, it was 40.0% in mild CP, 52.6% in moderate CP, 63.0% in severe CP and 80.0% in GAgP. The IL-1A+4845 gene polymorphism was more frequent in severe CP and GAgP, compared to mild CP. The association between GAgP and the IL-1A+4845 gene polymorphism needs further research.

IL-1B-511 gene polymorphism in periodontitis groups was higher than the reference group in this study. Guzman et al.³⁵⁾ has studied the association of IL-1 genotypes with periodontal disease in a diabetic population, and they evaluated the prevalence of IL-1A+4845, IL-1B+3954, IL-1B-511, and IL-1RN+1028 polymorphisms. There was a tendency suggesting that allele 1 at IL-1B-511 and IL-1B+3954 was overrepresented among diabetics with periodontal disease. They suggested that the IL-1 genotype be associated with risk for severe periodontitis in the diabetic population. In our study, the frequency of allele 2 at IL-1B-511 was higher than allele 1 in periodontitis groups, especially generalized aggressive periodontitis group. Tseung et al.³³⁾ reported IL-1B-511T allele (allele 2) was significantly higher among Taiwanese compared with Caucasian (77% verse 50%). Based on these studies, the frequency of IL-1B-511 allele 2 seems to be different by the ethnic groups and severity of periodontitis.

In the present study, the IL-1 gene polymorphisms in GAgP group was not different from the reference group except in IL-1B-511. This suggests that IL-1B-511 gene polymorphisms could be significantly associated with aggressive periodontitis in Korean and this IL-1 gene polymorphisms could

contribute to the different pathogenesis of chronic periodontitis and aggressive periodontitis

The prevalence of IL-1 RN intron 2 (VNTR) gene polymorphism in mild, moderate and severe CP, and GAgP groups and reference group was 20%, 45.5%, 38.5%, 20% and 19.1%, and the prevalence of gene polymorphism tended to be higher in moderate to severe CP groups than the reference group. Parkhill et al.²⁹⁾ and Tai et al.¹⁷⁾ suggested that IL-1 RN polymorphisms could be associated with generalized early onset periodontitis and supported the role for genetic and environmental factors in susceptibility to early onset periodontitis. However, IL-1 RN polymorphisms in GAgP were lower than the other periodontitis groups in the present study. The IL-1 RN allele 2 was associated with increased IL-1ra production both *in vitro* and *in vivo*.^{8,36)} Only a few studies have investigated the relationship between IL-1ra production and periodontal disease. Rawlinson et al.³⁷⁾ revealed the relationship between the severity of periodontitis and the increasing GCF levels of IL-1 β and decreasing levels of IL-1ra. Ishihara et al.³⁸⁾ reported that total amount of IL-1 (IL-1 α + IL-1 β)/IL-1ra ratio was correlated with the severity of periodontitis, but IL-1ra itself was not significant. Perrier et al.³⁹⁾ found that the IL-1 RN allele 2 was associated with Sjögren's syndrome. Patients with IL-1 RN allele 2 generally had lower salivary IL-1ra levels and higher serum levels than patients without the allele 2. They suggested that this discrepancy between local and systemic IL-1ra production levels may result from different effects in different cells, since the sources of IL-1ra are epithelial cells in the oral mucosa and keratinocytes in the skin, while circulating IL-1ra comes from mononuclear cells. Thus, the relationship among these IL-1 families in inflammatory diseases such as periodontitis remains still unclear.

Further studies are needed to clarify the relation-

ship between IL-1B SNPs, especially IL-1B-511, and IL-1 RN (VNTR) polymorphisms in Korean and the local and systemic protein levels of these IL-1 families in periodontitis patients. So far, most studies including this study have characterized the relationship between being genotype-positive and the presence of periodontitis, but did not provide valuable predictive information. In most studies, patients have not been monitored longitudinally. The ability of the genetic susceptibility test to predict the onset and progression of periodontitis remains to be confirmed. Thus, prospective studies are needed to evaluate of the IL-1 gene polymorphisms as the risk factors in the progression of the periodontal disease.

V. Conclusion

This study was aimed to investigate the distribution of IL-1A+4845, IL-1B+3954, IL-1B-511, and IL-1 RN intron 2 (VNTR) gene polymorphisms in group of Korean subjects based on their periodontal status, including mild, moderate, severe chronic periodontitis, and generalized aggressive periodontitis and to compare to the reference group. A total of 100 periodontitis patients were included in the periodontitis group and the reference group consisted of 92 children, from the Dept. of Pediatric Dentistry, Chonnam National University Hospital. Genomic DNA was obtained from buccal swab. The IL-1A+4845, IL-1B+3954 and IL-1B-511 genes were genotyped by amplifying the polymorphic region using multiplex polymerase chain reaction (PCR), followed by restriction enzyme digestion and gel electrophoresis. IL-1 RN polymorphism was detected by PCR amplification and fragment size analysis in agarose gel.

From this study, following results were obtained.

1. The distribution of genotype having allele 2

was 61%, 13%, 76.6%, and 34% for IL-1A+4845, IL-1B+3954, IL-1B-511, and IL-1 RN respectively in periodontitis group, 76.9%, 7.7%, 62.2%, and 19.1% in the reference group.

2. The distribution of composite genotype positive pattern of IL-1B+3954 and IL-1A+4845 in mild, moderate, severe chronic periodontitis, and generalized aggressive periodontitis group was 10%, 7.9%, 22.2%, and 12% respectively, and 13% for the periodontitis group combined. The severe chronic periodontitis group showed significantly higher rate of geno-positive pattern than the reference group and the other forms of periodontitis group ($p < 0.05$).
3. The frequency of IL-1B-511 gene polymorphism in periodontitis group was higher than the reference group. The frequency of the allele 2 carriage in the generalized aggressive periodontitis patients was higher than reference group ($p < 0.01$).
4. The frequency of allele 2 carriage for IL-1 RN in mild, moderate and severe chronic periodontitis, generalized aggressive periodontitis group and reference group was 20%, 45.5%, 38.5%, 20% and 19.1%, and the frequency was higher in moderate and severe chronic periodontitis group than the other groups.

From these results, it was suggested that the polymorphism of IL-1A+4845, IL-1B+3954, IL-1B-511 and IL-1 RN intron 2 (VNTR) gene could be associated with severity of chronic periodontitis and different forms of periodontitis in Korean population.

VI. REFERENCES

1. Genco RJ. Assessment of risk of periodontal disease. *Compend Suppl* 1994;18:S714-S717.
2. Page RC. The role of inflammatory mediators in

the pathogenesis of periodontal disease. *J Periodont Res* 1991;26:230-242.

3. Masada MP, Persson R, Kenny JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1 α and -1 β in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *J Periodont Res* 1990;26:156-163.
4. Preiss DS, Meyle J. Interleukin-1 beta concentration of gingival crevicular fluid. *J Periodontol* 1994;65:423-428.
5. Offenbacher S. Periodontal diseases: pathogenesis. *Ann Periodontol* 1996;1:821-78.
6. Reynolds JJ, Meikle MC. Mechanisms of connective tissue matrix destruction in periodontitis. *Periodontol* 2000 1997;14:144-157.
7. Nicklin MJH, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, beta and the interleukin-1 receptor antagonist genes. *Genomics* 1994;19:382-384.
8. Danis VA, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1ra) gene polymorphism. *Clini Exp Immunol* 1995;99:303-310.
9. Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol* 1998;16:457-499.
10. Cox A, Camp NJ, Nicklin MJ, di Giovine FS, Duff GW. An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers. *Am J Hum Genet* 1998;62:1180-1188.
11. Komman KS, di Giovine FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. *Ann Periodontol* 1998;3:327-338.
12. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A Taq I polymorphism in the human

- interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest* 1992;22:396-402.
13. Tarlow JK, Blackmore AIF, Lennard A, Solari R, Hughes HN, Steinkasser A, Duff GW. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Human Genetics* 1993;91:403-404.
 14. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW, Wilson TG Jr, Higginbottom FL, Duff GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;24:72-77.
 15. McGuire MK, Nunn ME. Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival. *J Periodontol* 1999;70:49-56.
 16. Armitage GC, Wu Y, Wang HY, Sorrell J, di Giovine FS, Duff GW. Low prevalence of a periodontitis-associated interleukin-1 composite genotype in individuals of Chinese heritage. *J Periodontol* 2000;71:164-171.
 17. Tai H, Endo M, Shimada Y, Gou E, Orima K, Kobayashi T, Yamazaki K, Yoshie H. Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. *J Clin Periodontol* 2002;29:882-888.
 18. Consensus report: Aggressive periodontitis. *Ann Periodontol* 1999;4:1-53.
 19. Walker SJ, Van Dyke TE, Rich S, Kornman KS, di Giovine FS, Hart TC. Genetic polymorphisms of the IL-1 α and IL-1 β genes in African-American LJP patients and an African-American control population. *J Periodontol* 2000;71:723-728.
 20. Becker M, Weizenegger M, Bartel J. Reverse hybridization assay for rapid identification of periodontitis-associated interleukin-1 alleles. *Clin Lab* 1999;45:499-505.
 21. Cullinan MP, Westerman B, Hamlet SM. A longitudinal study of interleukin-1 gene polymorphisms and periodontal disease in a general adult population. *J Clin Periodontol* 2001;28:1137-1144.
 22. Engebretson SP, Lamster IB, Herrera-Abreu M, Celenti RS, Timms JM, Chaudhary AG, di Giovine FS, Kornman KS. The influence of interleukin gene polymorphism on expression of interleukin-1beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. *J Periodontol* 1999;70:567-573.
 23. Shirodaria S, Smith J, McKay IJ, Kennett CN, Hughes FJ. Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease. *J Dent Res* 2000;79:1864-1869.
 24. Mark LL, Haffajee AD, Socransky SS, Kent RL Jr, Guerrero D, Kornman K, Newman M, Stashenko P. Effect of the interleukin-1 genotype on monocyte IL-1 beta expression in subjects with adult periodontitis. *J Periodontol* 2000;35:172-177.
 25. McDevitt MJ, Wang HY, Knobelmann C, Newman MG, di Giovine FS, Timms H, Duff GW, Kornman KS. Interleukin-1 genetic association with periodontitis in clinical practice. *J Periodontol* 2000;71:156-163.
 26. Diehl SR, Wang YF, Brooks CN. Linkage disequilibrium of interleukin-1 genetic polymorphisms with early onset periodontitis. *J Periodontol* 1999;70:418-430.
 27. Ehmke B, Kress W, Karch H, Grimm T, Klaiber B, Flemming TF. Interleukin-1 haplotype and periodontal disease progression following therapy. *J Clin Periodontol* 1999;26:810-813.
 28. Gore EA, Sanders JJ, Pandey JP, Palesch Y,

- Galbraith GM. Interleukin-1 beta+3953 allele 2: association with disease status in adult periodontitis. *J Clin Periodontol* 1998;25:781-785.
29. Parkhill JM, Henning BJW, Chapple ILC, Heasman PA, Taylor JJ. Association of interleukin-1 gene polymorphisms with early onset periodontitis. *J Clin Periodontol* 2000;27:682-689.
 30. Rider LG, Artlett CM, Foster CB, Ahmed A, Neeman T, Chanock SJ, Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A, Duff GW. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Human Genetics* 1993;91:403-404.
 31. Shin SY, Kim KH, Park OJ, Kim KK, Ku Y, Yoshie H, Chung CP. Genetic polymorphisms of the periodontally healthy Korean population. *J KAP* 2003;33:739-745.
 32. Caffesse RG, De LaRosa MR, De LaRosa MG, Mota LF. Prevalence of interleukin-1 periodontal genotype in a Hispanic dental population. *Quintessence Int* 2002;33:190-194.
 33. Tseng LH, Chen PJ, Lin MT, Shau WY, Chung SM, Martin PJ, Hansen JA. Single nucleotide polymorphisms in intron 2 of the human interleukin -1 receptor antagonist (IL-Ra1) gene: Fruther definition of the IL-1 beta and IL-1Ra polymorphisms in North American Caucasians and Taiwanese Chinese. *Tissue Antigens* 2001;57:318-324.
 34. Anusaksathien O, Sukboon A, Sitthiphong P, Teanpaisan R. Distribution of Interleukin-1 β +3954 and IL-1 α -889 Genetic Variations in a Thai Population Group. *J Periodontol* 2003;74:1796-1802.
 35. Guzman S, Karima M, Wang HY, Van Dyke TE. Association Between Interleukin-1 Genotype and Periodontal Disease in a Diabetic Population. *J Periodontol* 2003;74:1183-1190.
 36. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma level are coordinately regulated by both IL-1Ra and IL-1 β genes. *Eur J Immunol* 1998; 28: 2598-2602.
 37. Rawlinson A, Dalati MHN, Rahman S, Walsh TF, Fairclough AL. Interleukin-1 and IL-1 receptor antagonist in gingival crevicular fluid. *J Clin Periodontol* 2000;27:738-743.
 38. Ishihara Y, Nishihara T, Kuroyanagi T, Shirozu N, Yamagishi E, Ohguchi M, Koide M, Ueda N, Amano K, Noguchi T. Gingival crevicular interleukin-1 and interleukin-1 receptor antagonist levels in periodontally healthy and diseased sites. *J Periodontal Res* 1997;32:524-529.
 39. Perrier S, Coussediere C, Dubost JJ, Albuisson E, Sauvezie B. IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjögren's syndrome and rheumatoid arthritis. *Clin Immunol Immunopathol* 1998;87: 309-313.

한국인 치주염 환자에서의 IL-1 유전자 다변성 연구

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중증 만성 치주염과 IL-1B+3954 및 IL-1A+4845 유전자의 대립유전자 2 보유 유전자 다변성이 관련된다고 보고되었다. 그러나 이러한 IL-1 복합유전자 다변성과 만성 치주염 및 급진성 치주염과의 관련성에 대해서는 상반되게 보고되고 있는데 이는 인종적 배경과 질환특성의 차이에 기인한 것으로 보인다. 이 연구는 한국인에서 경도, 중등도와 중증의 만성 치주염 그리고 급진성 치주염 환자를 대상으로 하여 IL-1A+4845, IL-1B+3954, IL-1B-511, IL-1 RN intron 2 (VNTR) 유전자 다변성의 분포를 평가하고, 치주질환의 심도와 유형에 관련되는지 알아보도록 시행되었다.

전남대학교 병원 치주과에서 검진과 치료를 받은 100명의 치주질환자를 대상으로 하였고 질환군은 치주낭 깊이, 부착 소실, 골 소실을 기준으로 하여 경도, 중등도, 중증의 만성 치주염, 급진성 치주염군으로 분류하였다. 대조군으로는 전남대학교 병원 소아치과에 내원한 전신적으로 건강한 92명의 아동을 포함하였다. 각 대상 환자에서 채취된 협점막상피에서 genomic DNA를 얻어 IL-1A+4845, IL-1B+3954, IL-1B-511 genotype은 중합효소 연쇄반응을 시행한 후 제한 효소분해과정을 거쳐 전기영동 후 분리한 결과를 해석하였으며 IL-1 RN (VNTR) 유전형은 중합효소연쇄반응 후 분리한 결과를 해석하여 다음의 결과를 얻었다.

대립유전자 2 보유자 비율은 치주질환자에서 IL-1A+4845, IL-1B+3954, IL-1B-511, IL-1 RN이 각각 61%, 13%, 76.6%, 34%였으며 대조군에서는 76.9%, 7.7%, 62.2%, 19.1%였다. IL-1B+3954과 IL-1A+4845 대립유전자 2 보유자인 양성유전자형 비율은 경도, 중등도, 중증의 만성치주염, 급진성 치주염환자에서 각각 10%, 7.9%, 22.2%, 12%였으며 치주질환자의 13%, 대조군의 7.7%에서 양성 복합유전자형(positive genotype)을 보였다. IL-1B-511 유전자 다변성은 치주질환자에서 대조군에 비하여 높았으며 급진성 치주염환자에서 대립유전자 2 보유자율이 유의하게 높았다($p < 0.01$). IL-1 RN intron 2 유전자 다변성은 중등도 및 중증 만성 치주염환자에서 대립유전자 2 보유자율이 유의하게 증가하였다.

이러한 결과는 IL-1 gene cluster의 유전형이 한국인에서도 치주염의 유형과 질환 심도에 관련될 수 있음을 시사하였다.

주요어: IL-1 유전자, 유전자 다변성, 대립유전자, 복합유전자형, 한국인 치주염 환자.