

Genetic polymorphisms of the IL-1 β genes in periodontally healthy Korean population

Seung-Yun Shin¹, Kyoung-Hwa Kim¹, Ok-Jin Park², Kak-Kyun Kim²,
Young Ku¹, Hiromasa Yoshie³, Chong-Pyoung Chung¹

¹Department of Periodontology, College of Dentistry, Seoul National University

²Department of Oromaxillofacial Infection and Immunity, Seoul National University

³Division of Periodontology, Department of Oral Biological Science, Niigata University, Japan

I. INTRODUCTION

Inflammatory cytokines have been studied in periodontal diseases.¹⁾ Interleukin 1(IL-1) a multifunctional proinflammatory cytokine, makes inflammatory cells migrate to the infection, activates fibroblasts and other nucleated cell to produce PGE₂ and MMPs and promotes bone resorption. Consequently IL-1 induce the inflammatory cascade of the microbial immune response.²⁾ An increased IL-1 β level has been detected in the gingival crevicular fluid and periodontal tissues of periodontitis patients³⁻⁵⁾ IL-1 receptor antagonist belongs to the same family as IL-1. IL-1 receptors antagonist binds to IL-1 receptor and blocks IL-1 and thereby preventing activation of the target cells.⁶⁾

Three genes that regulate the production of IL-1 were known (IL-1A, IL-1B, IL-1RN).⁷⁾ These genes

are located close to each other on chromosome 2q13. Gene IL-1A and IL-1B control production of proinflammatory proteins, IL-1 α and IL-1 β , respectively. IL-1RN controls the synthesis of an antagonist protein (IL-1ra) that impede IL-1 α and IL-1 β

Kornman and coworkers reported the presence of specific alleles at two IL-1 genetic polymorphisms was associated with increased severity of periodontal disease in adult non-smokers.⁸⁾ Since Kornman's study, many researchers reported that the genetic polymorphism of inflammatory cytokines such as IL-1, TNF- α , Fc γ R can change the susceptibility to the periodontitis.⁹⁻²⁰⁾

However these researches were done in Northern European caucasian heritage,^{8-11,15,21)} in Hispanics^{22,23)} and in African-American.^{10,12)} Armitage and his colleagues reported that the prevalences of both IL-1A and IL-1B polymorphisms are dramatically lower in

* This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea. (02-PJ1-PG10-20507-0008)

Corresponding author : Chong-Pyoung Chung, Department of Periodontology, College of Dentistry, Seoul National University, 28, Yongon-Dong, Chongno-Gu, 110-749, Seoul

Chinese than those reported in Europeans. Only of the 300 subjects(2,3%) carried the composite IL-1 genotype consisting of allele 2 of both IL-1A +4845 and IL-1B +3954.²⁴⁾ Low frequencies of IL-1B +3954 polymorphisms were also reported in Japanese. Only 9.3%(9/97) of the healthy control group carried at least one copy of polymorphic alleles of IL-1 β +3854.

The aim of this study is to survey of the prevalence of the single nucleotide polymorphisms (SNPs)(IL-1 β +3954, IL-1 β -511, IL-1RN) in periodontally healthy Korean population.

II. MATERIALS AND METHODS

1. Subjects and Clinical assessments

Sixty five systemically healthy subjects were included in this study. Subjects(49 males and 16 females; age 19-39 years (mean age: 24.9 \pm 3.0 years), who showed neither attachment loss nor probing depth greater than 4mm at more than one sites. All subjects were Korean and none of them had a history or current manifestation of systemic disease. The study population consists of dentists and dental assistants who works at the Seoul National University Dental Hospital and senior students of College of Dentistry, Seoul National University. The study was approved by the Institution Review Board at Seoul National University Hospital and written informed consent was obtained from all subjects.

Clinical parameters including probing depth, clinical attachment level, bleeding on probing, gingival index were assessed. Probing depth and clinical attachment level was recorded using the Florida Probe[®] (Florida Probe Co., Gainesville, FL, USA) in 6 sites of the tooth.

2. Isolation of genomic DNA

Genomic DNA was obtained from peripheral blood by using a DNA extraction kit(Puregen, Gentra System, Minneapolis, MN) according to the manufacture's instructions.

1) IL-1 β -551 genotyping

Genotypes of the IL-1 β -551 were determined by polymerase chain reaction(PCR) as previously described²⁰⁾ using PCR premixture (Bioneer, Koera) and the following primers: sense 5'-TGG CAT TGA TCT GGT TCA TC-3'; anti-sense 5'-GTT TAG GAA TCT TCC CAC TT-3'. The PCR condition was first denatured for 10 min at 95 $^{\circ}$ C, followed by 35 cycles of a 1-min denaturing at 95 $^{\circ}$ C, a 1-min annealing at 53 $^{\circ}$ C, and a 1-min extension at 74 $^{\circ}$ C. The PCR products were digested with 3 unit of Ava I (New England BioLabs) at overnight. The PCR products were determined by electrophoresis on a 3% agarose gel stained with ethidium bromide. The resulting products of 190 + 114bp (allele 1) and 304bp (allele 2) are diagnostic.

2) IL-1 β +3954 genotyping

Genotypes of the IL-1 β +3954 were also determined by PCR as previously described by Tai et al.²⁰⁾ using PCR premixture(Bioneer, Korea) and the following primers: sense 5'-CTC AGG TGT CCT CGA AGA AAT CAA A-3'; anti-sense 5'-GCT TTT TTG CTG TGA GTC CCG-3'. The PCR condition was first denatured for 10 min at 95 $^{\circ}$ C, followed by 35 cycles of a 30-s denaturing at 94 $^{\circ}$ C, a 30-s annealing at 60 $^{\circ}$ C, and a 30-s extension at 74 $^{\circ}$ C. The products were digested with 3 units of Taq I (New England BioLabs) at 65 $^{\circ}$ C overnight. The PCR products were determined by electrophoresis on a acrylamide gel stained with ethidium bromide. Allele 1 gave products of 12bp, 85bp and 97bp, while allele2

gave products of 12bp and 182bp.

3) IL-1RN genotyping

The IL-1RN intron 2 contains a variable number of tandem repeat(VNTR) of an 86bp length of DNA. IL-1RN genotypings were determined by PCR using the following primers: sense 5'-CTC AGC AAC ACT CCT AT-3', anti-sense 5'-TCC TGG TCT GCA GGT AA-3' The PCR condition was first denatured for 10 min at 95°C, followed by 35 cycles of a 1-min denaturing at 94°C, a 1-min annealing at 60°C and a 1-min extension at 70°C. The PCR products were determined by electrophoresis on 3% agarose gel stained with ethidium bromide. Allele 1 (4 repeats) was 410 bp in size, allele 2(2 repeats) was 240bp, allele 3(3 repeats) was 500bp, allele 4 (3 repeats) was 325 bp, and allele 5(6 repeats) was 595 bp.

III. RESULTS

All healthy subject's clinical evaluation data was shown in Table 1. Mean probing depth was 2,04mm (SD : 0,21) and mean clinical attachment loss was 2,08mm (SD:0,23). The percentage sites of bleeding on probing was 7.53 ± 5.49 and gingival index was 0.16 ± 0.11 .

The genotype frequencies of IL-1 β -551, +3954, IL-1RN were shown in Table 2. In the genotype frequency of IL-1 β -551, almost half of subjects were heterozygous of allele 1 and allele 2. Allele 1

homozygous were 15 subjects(23,1%) and allele 2 homozygous were 17 subjects(26,2%). In the genotype frequency of IL-1 β +3954, most subjects were allele 1 homozygous(89,2%). All of the people who carried the IL-1B polymorphism were heterozygous(10,8%). In IL-1RN polymorphism, all the people have allele 1 genotype whether they were homozygous or heterozygous. Most people were allele 1 homozygous(86,3%), only 9 out of 65 subjects have heterozygous genotypes. Eight subjects have allele 2 genotype and only one subject carried allele 1/allele 4 heterozygous genotypes.

The allele frequency of IL-1 β -551, +3954, IL-1RN was shown in Table 3.

Table 1. Clinical characteristic of subjects (mean \pm SD)

Clinical Parameters	n = 65
Age (years)	24,9 \pm 3,0
Male/Female	49/16
Probing depth mm (mean \pm SD)	2,04 \pm 0,21
Clinical attachment loss mm (mean \pm SD)	2,08 \pm 0,23
% sites with BOP	7,53 \pm 5,49
Gingival index	0,16 \pm 0,11

Table 2. Distribution of genotype frequency of IL-1 β - 511, +3954 and IL-1RN

	n=65	%
IL-1B-551		
allele 11	15	23,1
allele 12	32	49,2
allele 22	17	26,2
IL-1B +3954		
allele 11	58	89,2
allele 12	7	10,8
allele 22	0	0
IL-1RN		
allele 11	56	86,2
allele 12	8	12,3
allele 14	1	1,5

Table 3. Distribution of allelic frequency of IL-1 β - 511, +3954 and IL-1RN

Genes	Allele	%
IL-1B-551	allele 1	47,7
	allele 2	52,3
IL-1B +3954	allele 1	94,6
	allele 2	5,4
IL-1RN	allele 1	93,1
	allele 2	6,1
	allele 4	0,8

In allele frequency of IL-1 β -551, allele 1 was found in 47.7% and allele 2 was found in 52.3%. In IL-1 β +3954, allele 1 was found in 94.6% and allele 2 was found in only 5.4%. In IL-1RN, most allele were allele 1. Allele 1 was found in 93.1%. Allele 2 was found in 6.1%. Allele 4 was found in only 0.8%(1 subject, heterozygous) and allele 3, 5 were not found at all.

IV. DISCUSSION

Many researches related to the genetic polymorphism have been performed. They have been struggling to find relationship between periodontal disease and genetic polymorphism of various gene. Many various genes such as IL-1, TNF- α , Fc γ receptor, TGF- β promoter, N-formyl peptide receptor, IL-6,10, Vitamin D receptor gene are researched.^{8-11,20,21,25-29} Of these many genes, IL-1 genetic polymorphism were studied a lot. It comes from that IL-1 has been known as a proinflammatory cytokine that makes inflammatory cells migrate to the infection, activates fibroblasts and other nucleated cell to produce PGE₂ and MMPs and promotes bone resorption.² And an increased IL-1 β level has been detected in the gingival crevicular fluid and periodontal tissues of periodontitis patients.³⁻⁵

Many researches were done in Northern European caucasian heritage.^{8-11,15,21} In these studies, the presence of allele 2 of the IL-1A and IL-1B polymorphism which was considered as genotype-positive. In Northern European Caucasian heritage, the genotype-positive subjects were large enough to study the relationship between periodontal disease and genetic polymorphisms (28.0-38.0%).^{8,9,21} In African-American 14.5%(N=104) of non-disease individuals and 8% (N=34) of patients with the localized form of aggressive periodontitis were genotype-positive.^{10,12} However, the prevalences of both

IL-1A and IL-1B polymorphisms are dramatically lower in Chinese than those reported for Europeans. Allele 2 of the IL-1B +3954 polymorphism was only 3.3% (10/300) of the study population.²⁴ The investigators concluded that it was not possible to characterize the relationship between genotype and periodontitis susceptibility, because too few subjects were genotype-positive.

In this study, IL-1 β +3954 polymorphism was also found in 7 out of 65 subjects(10.8%). All of them were heterozygous and none was homozygous. It is larger percentage than in chinese. But it is not enough to find any relationship between the susceptibility of the periodontitis and genetic polymorphism.

Tai and his coworkers studied IL-1RN polymorphism in Japanese population. In his study the frequency of IL-1RN polymorphic alleles was found to be significantly increased in generalized from of aggressive periodontitis patients. The frequency of IL-1RN polymorphic genotypes was 8.2% in healthy control group and 25.5% in generalized from of aggressive periodontitis patients. In our study IL-1RN polymorphic alleles was 13.8%.

In IL-1 β -511 polymorphism, the frequency of allele 1 was lower than allele 2 (47.7% vs 52.3%). In others study, the frequencies of allele 1 and allele 2 was similar and even the reversal of the frequency of allele 1 and allele 2 could be seen.^{20,28,30}

V. CONCLUSION

Ethnic and racial differences in periodontal disease-susceptibility gene polymorphisms have been reported. The prevalences of IL-1B +3954 polymorphisms was also lower in Korean than those reported for Europeans. The absence of genotype 22 in the IL-1 β +3954 and IL-1RN polymorphisms was found in the Korean population. It would appear that knowledge of this IL-1 β +3954 polymorphism

would provide little diagnostic or predictive information in Korean population. Further research with more sample size could be necessary.

VI. REFERENCES

1. Salvi GE, Brown CE, Fujihashi K, Kiyono H, Smith FW, Beck JD, Offenbacher S. Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. *J Periodont Res* 1998;33:212-225.
2. Offenbacher S. Periodontal disease: Pathogenesis. *Ann Periodontol* 1996;1:821-878.
3. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of Interleukin-1 α and I- β in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. *J Periodont Res* 1990;25:156-163.
4. Stashenko P, Fujiyoshi P, Obernesser MS, Probst L, Haffajee AD, Socransky SS. Levels of interleukin-1 β in tissue from sites of active periodontal disease. *J Clin Periodontol* 1991;18:548-554.
5. DuFour A, Baran C, Langkamp HL, Piesco NP, Agarwal S. Regulation of differentiation of gingival fibroblasts and periodontal ligament cells by rhIL-1 beta and rhTNF-alpha. *J Periodontal Res*. 1993 Nov;28:566-568.
6. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood*. 1996 Mar 15;87:2095-147.
7. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. *Genomics*. 1994 Jan 15;19:382-384.
8. Kornman KS, 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.
9. Gore EA, Sanders JJ, Pandey JP, Palesch Y, Galbraith GM. Interleukin-1beta+3953 allele 2: association with disease status in adult periodontitis. *J Clin Periodontol*. 1998;25:781-785.
10. Diehl SR, Wang Y, Brooks CN, Burmeister JA, Califano JV, Wang S, Schenkein HA. Linkage disequilibrium of interleukin-1 genetic polymorphisms with early-onset periodontitis. *J Periodontol*. 1999;70:418-430.
11. Galbraith GM, Hendley TM, Sanders JJ, Palesch Y, Pandey JP. Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. *J Clin Periodontol*. 1999;26:705-709.
12. Walker SJ, Van Dyke TE, Rich S, Komman KS, di Giovine FS, Hart TC. Genetic polymorphisms of the IL-1alpha and IL-1beta genes in African-American LJP patients and an African-American control population. *J Periodontol*. 2000;71(5):723-728.
13. Kobayashi T, Westerdaal NA, Miyazaki A, van der Pol WL, Suzuki T, Yoshie H, van de Winkel JG, Hara K. Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. *Infect Immun*. 1997;65:3556-3560.
14. Sugita N, Yamamoto K, Kobayashi T, Van Der Pol W, Horigome T, Yoshie H, Van De Winkel JG, Hara K. Relevance of Fc gamma RIIIa-158V-F polymorphism to recurrence of adult periodontitis in Japanese patients. *Clin Exp Immunol*. 1999;117:350-354.
15. Parkhill JM, Hennig BJ, Chapple IL, Heasman PA, Taylor JJ. Association of interleukin-1 gene polymorphisms with early-onset periodontitis. *J Clin Periodontol*. 2000;27:682-689.
16. Soga Y, Nishimura F, Ohyama H, Maeda H, Takashiba S, Murayama Y. Tumor necrosis factor-alpha gene (TNF-alpha) -1031/-863, -857 sin-

- gle-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese. *J Clin Periodontol*. 2003;30:524-531.
17. Christgau M, Aslanidis C, Felden A, Hiller KA, Schmitz G, Schmalz G. Influence of interleukin-1 gene polymorphism on periodontal regeneration in intrabony defects. *J Periodontol Res*. 2003;38:20-27.
 18. 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.
 19. Endo M, Tai H, Tabeta K, Kobayashi T, Yamazaki K, Yoshie H. Analysis of single nucleotide polymorphisms in the 5'-flanking region of tumor necrosis factor-alpha gene in Japanese patients with early-onset periodontitis. *J Periodontol*. 2001;72:1554-1559.
 20. Tai H, Endo M, Shimada Y, Gou E, Oriina 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.
 21. Lang NP, Tonetti MS, Suter J, Sorrell J, Duff GW, Kornman KS. Effect of interleukin-1 gene polymorphisms on gingival inflammation assessed by bleeding on probing in a periodontal maintenance population. *J Periodontol Res*. 2000;35:102-107.
 22. Caffesse RG, De La Rosa RM, De La Rosa GM, Weltman R. Effect of interleukin-1 gene polymorphism in a periodontally healthy Hispanic population treated with mucogingival surgery. *J Clin Periodontol*. 2002;29:177-181.
 23. Arregui I, Guisasola C, Menendez M, Martin-Villa L, Blanco-Moreno J, Tejerina JM, Sicilia A. IL-1 genotype distribution in a periodontally diseased population in Spain (abstract). *J Clin Periodontol* 2000; 27:86.
 24. 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.
 25. Tachi Y, Shimpuku H, Nosaka Y, Kawamura T, Shinohara M, Ueda M, Imai H, Ohura K. Vitamin D receptor gene polymorphism is associated with chronic periodontitis. *Life Sci*. 2003;73:3313-3321.
 26. de Souza AP, Trevilatto PC, Scarel-Caminaga RM, de Brito RB, Line SR. Analysis of the TGF-beta1 promoter polymorphism (C-509T) in patients with chronic periodontitis. *J Clin Periodontol*. 2003;30(6):519-523.
 27. Gwinn MR, Sharma A, De Nardin E. Single nucleotide polymorphisms of the N-formyl peptide receptor in localized juvenile periodontitis. *J Periodontol*. 1999;70:1194-1201.
 28. Trevilatto PC, Scarel-Caminaga RM, de Brito RB Jr, de Souza AP, Line SR. Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. *J Clin Periodontol*. 2003;30:438-442.
 29. Yamazaki K, Tabeta K, Nakajima T, Ohsawa Y, Ueki K, Itoh H, Yoshie H. Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. *J Clin Periodontol*. 2001;28:828-832.
 30. 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.

치주적으로 건강한 한국인에서 IL-1 β 유전자의 유전자 다형성 발생빈도에 관한 연구

신승윤¹, 김경화¹, 박옥진², 김각균², 구 영¹, Hiromasa Yoshie³, 정종평¹

¹서울대학교 치과대학 치주과학교실

²서울대학교 치과대학 구강악안면 감염 및 면역학교실

³Division of Periodontology, Department of Oral Biological Science, Niigata University, Japan

Interleukine-1(IL-1)은 여러가지 기능을 가진 싸이토카인으로써 미생물에 대한 면역반응을 일으킨다. IL-1의 유전자 다형성과 치주질환과의 관련성에 대한 많은 연구가 있어왔지만, 대부분이 백인을 대상으로 한 연구였다. 이후 중국인과 일본인을 대상으로 한 연구에서 IL-1의 유전자 다형성의 분포가 인종간에 차이를 보인다는 점이 발견되었다. 이번 연구에서는 치주적으로 건강한 한국인에서 IL-1 β -511, IL-1 β +3954, IL-1RN에 대한 유전자형의 분포를 조사하고자 하였다.

서울대학교 치과병원에 근무하는 치과 의사, 치과위생사, 간호조무사 및 서울대학교 치과대학 4학년 학생 중 치주낭 깊이와 부착소실이 4mm 이하인 치주적으로 건강한 한국인 65명을 대상으로 하였다. IL-1 β -511, IL-1 β +3954, IL-1RN의 유전자 다형성은 분리한 DNA에 각 대립유전자에 특이성을 지닌 primer를 넣고 PCR(Polymerase Chain Reaction)법을 이용하여 증폭시킨후 전기영동법을 이용하여 각 대립유전자의 존재를 확인함으로써 결정하였다.

IL-1 β -511 대립유전자 11, 대립유전자 12, 대립유전자 22의 유전자형에 대하여 각각 23.1%, 49.2%, 26.2%의 분포를 보였다. IL-1 β +3954의 유전자 다형성은 대립유전자 11, 대립유전자 12의 유전자형에 대하여 각각 89.2%, 10.8%의 분포를 보였으며, 대립유전자 22의 유전자형을 갖는 사람은 한명도 발견되지 않았다. IL-1RN의 유전자형은 5가지의 대립유전자 중에서 대립유전자 1, 대립유전자 2, 대립유전자 4만이 발견되었으며, 대립유전자 11, 대립유전자 12, 대립유전자 14의 유전자형이 86.2%, 12.3%, 1.5%로 분포하였다.

이를 바탕으로 각 대립유전자의 발생빈도 계산한 결과 IL-1 β -511에서는 대립유전자 1과 2의 비율의 거의 유사하였으나 (47.7%, 52.3%), IL-1 β +3954, IL-1RN에서는 대립유전자 1이 90%이상 발견되었으며, 또한 대립유전자 1외의 다른 대립유전자가 발견된 경우, 모두 이형접합체였다.

이 연구는 IL-1 β -511, IL-1 β +3954, IL-1RN에 대한 유전자형의 분포를 조사한 것으로 한국인에서 이들 유전자의 유전자형의 분포는 백인에서의 분포와 차이를 보이고 있었다. 이후 치주질환자의 유전자형 분포와의 비교로 치주질환과 IL-1 β -511, IL-1 β +3954, IL-1RN의 유전자다형성과의 관련성에 관한 추가적인 연구가 필요할 것으로 여겨진다.

주요어 : IL-1 유전자, 유전자 다형성, 대립유전자