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# Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese

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***Abstract***

**Background/Aims:** Early onset periodontitis (EOP), newly 'aggressive periodontitis', is considered to have genetic basis, which have not been clearly defined. The interleukin-1 (IL-1) gene cluster polymorphism as one of genetic factors may influence the expression of several chronic inflammatory diseases. The aim of this study is to investigate the frequency of single nucleotide polymorphisms (SNPs) in the genes encoding IL-1$\alpha$, IL-1$\beta$ and a variable number of tandem repeat (VNTR) polymorphisms in the IL-1 receptor antagonist gene (IL-1RN) in 47 generalized EOP (G-EOP) patients and 97 periodontally healthy controls.

**Material and methods:** All subjects were of Japanese descent and systemically healthy. They were identified according to established clinical criteria. SNPs in the IL-1$\alpha$ (+4845) and IL-1$\beta$ (−511, +3954) genes were analyzed by amplifying the polymorphic region using polymerase chain reaction (PCR), followed by restriction-enzyme digestion and agarose gel electrophoresis. IL-1RN (VNTR) polymorphisms were then detected by PCR amplification and fragment size analysis.

**Results:** There was no significant difference in the IL-$\alpha$ (+4845) and IL-1$\beta$ (−511, +3954) genotypes and allele frequencies between G-EOP patients and healthy controls. However, the frequency of IL-1RN (VNTR) polymorphic alleles was found to be significantly increased in G-EOP patients ($\chi^2$ test, $P = 0.007$; odds ratio = 3.40). Additionally, the carriage rate of IL-1RN (VNTR) polymorphisms was significantly higher in G-EOP patients than in healthy controls ($\chi^2$ test, $P = 0.005$; odds ratio = 3.81).

**Conclusion:** These findings suggest that IL-1RN (VNTR) polymorphisms are associated with G-EOP in Japanese.

Key words: early onset periodontitis; gene polymorphism; interleukin-1 receptor antagonist; interleukin-1

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Early onset periodontitis (EOP), currently proposed to be replaced with the term 'aggressive periodontitis' (Armitage et al. 1999), represents an inflammatory disease with pathogenic features, such as onset in the juvenile or early adult years and an aggressive clinical course characterized by localized or generalized loss of alveolar bone (Ranney 1992).

The results of population and family studies indicate that genetic factors seem to have a strong influence on susceptibility to EOP (Schenkein 1994, Hart 1996). The genetic influence on chronic adult periodontitis is less apparent, since the prevalence of this disorder is strongly affected by environmental variables such as oral hygiene and smoking. It has also been known that EOP shares many pathogenic features with the more common chronic adult periodontitis in immunopathological studies. Therefore, although EOP is comparatively rare in the general population, studies of this disorder may reveal genetic variants which may also influence chronic inflammatory diseases such as periodontitis (Van Dyke & Schenkein 1996).

Many studies to date have focused on the potential role of pro-inflammatory cytokines, including IL-$\alpha$, IL-$\beta$ and tumor necrosis factor alpha (TNF-$\alpha$). These pro-inflammatory cytokines stimulate many cells to produce matrix metalloproteinases, prostaglandins and pro-inflammatory cytokines, as well as affecting bone metabolism, all of which in turn contribute to the pathogenesis of periodontal disease (Landi et al. 1997). IL-1 receptor antagonist (IL-1ra) belongs to the same family as IL-1 and its only known function is to bind to IL-1 receptors, blocking IL-1 and thereby preventing activation of the target cells (Dinarello 1996). Furthermore, several studies have reported that increased levels of both IL-1$\alpha$ and IL-1$\beta$ in gingival crevicular fluid correlate with the severity of periodontal disease (Wilton et al. 1992, Hou et al. 1995) and a model for severe periodontal disease, emphasizing the importance of individual differences in inflammatory mediators, have been proposed (Offenbacher et al. 1993).

Recent reports have indicated that allelic variation in cytokine genes and factors regulating their expression may influence the clinical outcome, susceptibility and progression of periodontal disease (Kobayashi et al. 1997, Kornman et al. 1997, Gore et al. 1998, Sugita et al. 1999). Dysregulation of cytokine gene expression may be responsible for the repeated cycles of tissue inflammation observed in these disorders (Duff 1998). Thus the genetic basis for many common diseases with multifactorial pathogenesis such as periodontal disease may be influenced by subtle phenotypic variations caused by common alleles of a number of disease-associated polymorphic genes (Hart & Kornman 1997).

The genes for IL-1$\alpha$, IL-1$\beta$ and IL-(1RNa) are all located on the long arm of chromosome 2. Two biallelic base change polymorphisms in the IL-1$\beta$ have been reported to influence the protein production (Pociot et al. 1992, Santtila et al. 1998, Hulkkonen et al. 2000). One is in the promoter region at position $-511$, and the other is in exon 5 at position $+3954$. A polymorphism in the IL-1$\alpha$ promoter region at $-889$, which is $>99\%$ concordant with IL-1$\alpha$ $+4845$ (Cox et al. 1998), and a variable number of tandem repeat (VNTR) polymorphisms in the second intron of the IL-1RN have been also associated with several inflammatory diseases (Bidwell et al. 1999).

In the present study, therefore, we investigated the frequency of single nucleotide polymorphisms (SNPs) in the IL-1$\alpha$, IL-1$\beta$ and IL-1RN (VNTR) polymorphisms in generalized EOP (G-EOP) patients and periodontally healthy controls in Japanese. We then analyzed whether these cytokine gene polymorphisms may be responsible in part for genetic susceptibility to G-EOP.

## Material and methods

### Subjects and clinical assessments

Forty-seven G-EOP patients and 97 healthy control subjects were included in this study. G-EOP patients (19 males and 28 females; age 23–35 years, mean age 31.4 years) referred to the Periodontal Clinic of Niigata University Dental Hospital. According to Diehl et al. (1999), the diagnostic criteria for G-EOP were defined by the following clinical conditions: $\geq 5$ mm attachment loss at more than one site on $\geq 8$ teeth, at least three of which were not first molars and incisors. The onset of the disease was before the age of 35 years. The healthy control subjects (53 males and 44 females; age 22–35 years, mean age 28.6 years), who showed neither attachment loss nor probing depth greater than 3 mm at more than one site, were matched by age with G-EOP patients. According to Kobayashi et al. (2000), G-EOP patients were evaluated clinically at first visit by several periodontists for the following items representing periodontal condition: number of missing teeth (mean $\pm$ standard error; $2.4 \pm 0.4$ teeth), probing pocket depth ($4.3 \pm 0.1$ mm), probing attachment level ($4.9 \pm 0.2$ mm), supragingival plaque accumulation ($54.4 \pm 22.6\%$), bleeding on probing ($49.2 \pm 5.0\%$) and alveolar bone loss ($42.5 \pm 1.9\%$). Incidental attachment loss or bone loss associated with root fractures or endodontic infection was not observed in the G-EOP patients.

All subjects were Japanese and none of them had a history or current manifestation of systemic disease. The study was approved by the Institution Review Board at Niigata University Faculty of Dentistry, and written informed consent was obtained from all patients and healthy subjects, in line with the Helsinki Declaration before inclusion in the study.

### Extraction of DNA

Genomic DNA was obtained from peripheral blood by using a DNA extraction kit (Wako Pure Chemical Industries. Inc., Osaka, Japan) according to the manufacturer's instructions.

*Analysis of the IL-1α (+4845), IL-1β (−511), IL-1β (+3954) SNPs*

Sample DNA 10–35 ng was amplified in 25 $\mu$L volume of the reaction mixture containing $10\times$ reaction buffer, 1.5–2.5 mM $MgCl_2$, 0.2 mM dNTP, a pair of 0.75 M of each primer, and 1.25 U Taq polymerase (AmpliTaq Gold ™, Perkin Elmer, NJ, USA). The PCR conditions in detail are as described below.

*IL-1α (+4845):* The following primers were used: 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′ (Walker et al. 2000) with 2.5 mM $MgCl_2$. The PCR condition was first denatured for 10 min at 95 °C, followed by 35 cycles of a 30-s denaturing at 95 °C, a 30-s annealing at 56 °C, and a 30-s extension at 72 °C, and 1 cycle 72 °C 5 min The PCR products were checked by 2% agarose gel electrophoresis and then, digested with 5 units of Fnu 4H1 (New England BioLabs, Hitchin, UK) at 37 °C overnight. The restriction fragments were determined on 3% agarose gel electrophoresis, stained with ethidium bromide. The resulting products of 124 + 29 bp (allele 1) and 153 bp (allele 2) are diagnostic. IL-1$\beta$ ($-511$): The primers used were as follows: sense 5′-TGG CAT TGA TCT GGT TCA TC-3′; antisense 5′-GTT TAG GAA TCT TCC CAC TT-3′ (Kornman et al. 1997) with 2.5 mM $MgCl_2$. The PCR condition was first denatured for 10 min at 95 °C, followed by 35 cycles of a 1-min denaturing at 95 °C, a 1-min annealing at 53 °C, and a 1-min extension at 74 °C. The PCR products were digested with 3 units of Ava I (New England BioLabs) at 37 °C overnight. The resulting products of 190 + 114 bp (allele 1) and 304 bp (allele 2) are diagnostic.

*IL-1β (+3954):* The primers used were as follows: sense 5′-CTC AGG TGT CCT CGA AGA AAT CAA A-3′; antisense 5′-GCT TTT TTG CTG TGA GTC CCG-3′ (Kornman et al. 1997) with 1.5 mM $MgCl_2$. The PCR condition was first denatured for 10 min at 95 °C, followed by 35 cycles of a 30-s denaturing at 94 °C, a 30-s annealing at 60 °C, and a 30-s extension at 74 °C. The

products were digested with 3 units of Taq I (Gibco BRL, Life Technologies, Inc., Rockville, MD, USA) at 65°C overnight. Allele 1 gave products of 12 bp, 85 bp and 97 bp, while allele 2 gave products of 12 bp and 182 bp.

*Analysis of the IL-1RN (VNTR).* The IL-1RN 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′ (Tarlow et al. 1993). The reaction conditions used were as described above. 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 with1.5 mM $MgCl_2$. The PCR products were determined by electrophoresis on a 3% agarose gel stained with ethidium bromide. Allele 1 (4 repeats) was 410 bp in size, allele 2 (2 repeats) was 240 bp, allele 3 (5 repeats) was 500 bp, allele 4 (3 repeats) was 325 bp, and allele 5 (6 repeats) was 595 bp.

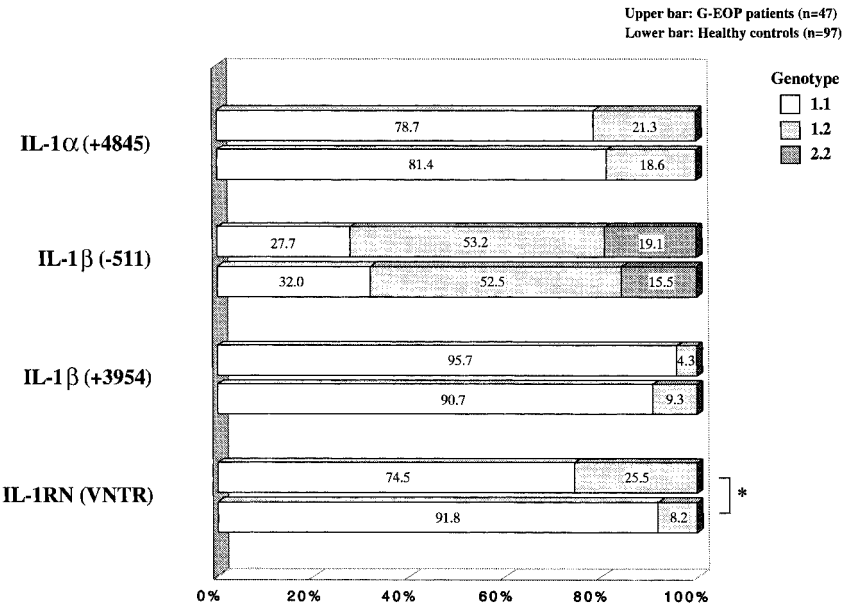


*Fig. 1.* The genotype frequency of IL-1$\alpha$ (+4845), IL-1$\beta$ (−511) (+3954) SNPs, and IL-1RN (VNTR) polymorphisms in G-EOP patients and healthy controls. We described polymorphic alleles as allele 2 in the IL-1RN (VNTR) polymorphisms. A significant difference was observed in the IL-1RN genotype distribution between G-EOP patients and healthy controls ($2 \times 2$ contingency table; $\chi^2 = 7.91$, $P = 0.005$).

### Statistical analysis

The associations of the genotype and allele frequencies between G-EOP patients and healthy controls were determined by Chi square or Fisher's exact test, using the standard statistical software (Stat View J-4.5 application program, SAS Institute Inc., NC, USA). Chi-square analysis was used to test for deviation of genotype frequencies from Hardy–Weinberg expectations. Significance was set at 5% ($P < 0.05$).

## Results

The genotype frequency of IL-1$\alpha$, IL-1$\beta$ SNPs, and IL-1RN (VNTR) polymorphisms in G-EOP patients and healthy controls is shown in Fig. 1. As the IL-1RN (VNTR) alleles 3, 4 and 5 were infrequent in all populations in this study, we described polymorphic alleles as allele 2 in Fig. 1. A significant difference was observed in the IL-1RN genotype distribution between G-EOP patients and healthy controls ($2 \times 2$ contingency table; $\chi^2 = 7.91$, $P = 0.005$), but not in IL-1$\alpha$, IL-1$\beta$ genotype distribution. Separating the IL-1RN (VNTR) genotypes (1.1, 1.2, 1.3, 1.4, or 1.5), the distribution also differed in G-EOP patients and healthy controls ($2 \times 5$ contingency table; $\chi^2 = 10.49$, $P = 0.03$). Interestingly, the absence of 2.2 genotype in the IL-1$\alpha$ (+4845) (+3954) SNPs, and IL-1RN (VNTR) polymorphisms was found in the Japanese populations.

As shown in Fig. 1, genotype frequencies of each variant in healthy controls did not deviate from those predicted by the Hardy–Weinberg law ($\chi^2 = 1.01$, 0.64, 0.24, 0.18, respectively, $P > 0.05$).

We next assessed allele frequencies of each variant. The frequency of IL-1RN (VNTR) polymorphic alleles (alleles 2, 3, 4 and 5) was only found to be significantly increased in G-EOP patients (12.8% in G-EOP patients vs. 4.1% in healthy controls, $\chi^2 = 7.32$, $P = 0.007$; Fisher's exact = 0.009; odds ratio = 3.40, 95% CI = 1.24–9.52). Additionally, the carriage rate (% of individuals who carry at least one copy of polymorphic alleles) of IL-1RN (VNTR) polymorphic alleles was significantly higher in G-EOP patients than in healthy controls (25.5% in G-EOP patients vs. 8.2% in healthy controls, $\chi^2 = 7.91$, $P = 0.005$; Fisher's exact = 0.009; odds ratio = 3.81, 95% CI = 1.31–11.31) (see Fig. 2). However, no significant associations were found in the IL-1$\alpha$ (+4845) and IL-1$\beta$ (−511, +3954) SNPs.

Smoking has been established as a major risk factor in the development of periodontal disease (Grossi et al. 1995) and should be considered when conducting genetic studies of periodontitis. We therefore evaluated the frequency of alleles on IL-1$\alpha$, IL-1$\beta$ SNPs, and IL-1RN (VNTR) polymorphisms in non-smokers as well. In the present study, 37 G-EOP patients and 97 healthy controls were non-smokers. The frequency of IL-1RN (VNTR) polymorphic alleles (alleles 2, 3, 4 and 5) was also found to be significantly increased in non-smoker G-EOP patients (13.5% in G-EOP patients vs. 4.1% in non-smoker healthy controls, $\chi^2 = 7.54$, $P = 0.006$; Fisher's exact = 0.009; odds ratio = 3.63, 95% CI = 1.26–10.63). Additionally, the carriage rate of IL-1RN VNTR polymorphisms was significantly higher in non-smoker G-EOP patients than in non-smoker healthy controls (27.0% in G-EOP patients vs. 8.2% in healthy controls, $\chi^2 = 8.12$, $P = 0.004$; Fisher's exact = 0.007; odds ratio = 4.12, 95% CI = 1.33–12.94). However, no significant associations were found in IL-1$\alpha$ (+4845) and IL-1$\beta$ (−511, +3954) SNPs.

## Discussion

The present study revealed the different frequency of IL-1$\alpha$, IL-1$\beta$ SNPs, and IL-1RN (VNTR) polymorphisms in Caucasian, African-American and Japanese healthy controls. Allele 2 car-

riage rates were 37.5% for the IL-1$\alpha$ (+4845), 59.4% for the IL-1$\beta$ (−511), 61.1% for the IL-1$\beta$ (+3954) SNPs, and 48.6% for IL-1RN (VNTR) polymorphisms in Caucasians (Gore et al. 1998, Parkhill et al. 2000). Moreover, in African-Americans, carriage rates were 26.9% for the IL-1$\alpha$ (+4845), 27.0% for the IL-1$\beta$ (+3954), and 20.4% for the IL-1RN (VNTR) (Walker et al. 2000, Rider et al. 2000). In contrast, our results indicated that allele 2 carriage rates were 18.6%, 68.0%, 9.3%, and 8.2%, respectively, in Japanese. In particular, the rate of IL-1$\alpha$ (+4845), IL-1$\beta$ (+3954), and IL-1RN (VNTR) tended to be lower in Japanese than Caucasians, whereas the rate in African-Americans was between the Caucasian and the Japanese data. There appears, from these findings, to be a great difference in the frequency of IL-1$\alpha$ (+4845) and IL-1$\beta$ (+3954) SNPs, and IL-1RN (VNTR) polymorphisms among Caucasians, African-Americans, and Japanese. In a Chinese population, Armitage et al. (2000) have reported allele 2 carriage rates of 17.0% for the IL-1$\alpha$ (+4845) and 3.3% for IL-1$\beta$ (+3954) SNPs, suggesting comparative similarities in the frequency of IL-1$\alpha$, and IL-1$\beta$ SNPs between Chinese and Japanese. Interestingly, we also revealed that the absence of 2.2 genotype in the IL-1$\alpha$ (+4845), IL-1$\beta$ (+3954) SNPs and IL-1RN (VNTR) polymorphisms was found in the Japanese populations (see Fig. 1). The frequency of 2.2 genotypes in each variant was 12.5%, 4.2%, and 12.5% in Caucasian and 1.9%, 1.0%, and 3.4% in African-American controls, respectively (Gore et al. 1998, Parkhill et al. 2000, Rider et al. 2000, Walker et al. 2000). These findings suggest that the IL-1 genotype distributions in the Japanese population may prevent any meaningful analysis of genotype associations with disease. Moreover, ethnic and racial differences in disease-susceptibility gene polymorphisms have been reported by others. For example, in Caucasians the IL-1RN allele 2 has been associated with the susceptibility to systemic lupus erythematosus (SLE), where this gene was present in 24.1% of controls and 32.7% of patients with SLE ($P<0.05$) (Blakemore et al. 1994). In contrast, only 4.1% of Japanese controls carried this polymorphism compared to 9.7% of the SLE patients ($P<0.05$). The prevalence of the marker gene was clearly lower in Japanese compared to Caucasians (Suzuki et al. 1997).

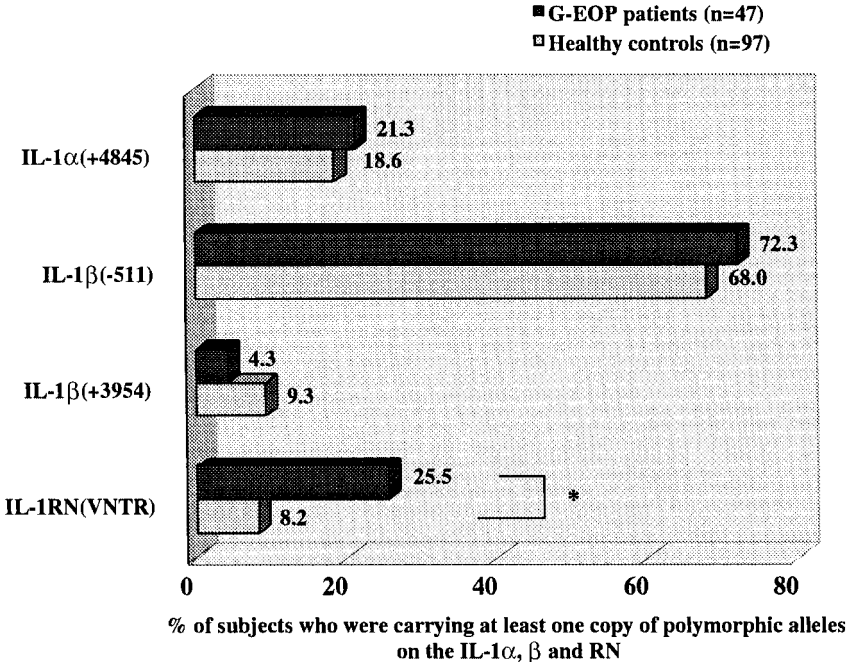


*Fig. 2.* The carriage rate (% of individuals who carry at least one copy of polymorphic alleles) of IL-1$\alpha$ (+4845), IL-1$\beta$ (−511) (+3954) SNPs, and IL-1RN (VNTR) polymorphisms in G-EOP patients and healthy controls. A significant difference was observed in the carriage rate of IL-1RN VNTR polymorphisms when comparing G-EOP patients with healthy controls ($\chi^2=7.91$, $P=0.005$, odds ratio = 3.81, 95% CI = 1.31–11.31).

Several reports revealed that allele 2 of the IL-1$\beta$ (+3954; previously described +3953) SNP, either alone or in combination with the IL-1$\alpha$ (+4845; −889) SNP, is associated with certain groups of patients with severe periodontitis (Kornman et al. 1997, Gore et al. 1998). Current studies have indicated that those individuals homozygous for allele 1 of the IL-1$\beta$ (+3954) SNP have an increased susceptibility to EOP and localized EOP in Caucasians (Diehl et al. 1999, Parkhill et al. 2000). In African-Americans, Walker et al. (2000) have reported that allele 1 of the IL-1$\beta$ (+3953) SNP is carried by >99% of controls and localized EOP patients, and the allele 2 carriage rate of the composite genotypes for the IL-1$\alpha$ (+4845) and IL-1$\beta$ (+3953) SNPs is 14% in controls and 8% in localized EOP patients. Our data revealed that the frequency of homozygous for the IL-1$\beta$ (+3954) allele 1 tended to be higher in G-EOP patients (95.7%) than in healthy controls (90.7%) in Japanese, but this difference was not statistically significant ($P=0.29$). These findings may reflect differences in the role of IL-1$\beta$, especially IL-1$\beta$ (+3954) SNP, in the pathogenesis of EOP and chronic adult periodontitis, or ethnic and racial differences in disease-susceptibility gene polymorphisms between Caucasians and Japanese.

Several studies have shown a significant increase in both the frequency and carriage rate of the IL-1RN allele 2 in chronic inflammatory and autoimmune diseases including SLE, Sjögren's syndrome, and gastric cancer (Suzuki et al. 1997, Perrier et al. 1998, El-Omar et al. 2000). With regard to periodontal diseases in Caucasians, the carriage rate of the IL-1RN allele 2 was not associated with severity of periodontitis (59.2% in the mild group vs. 48.8% in the severe group, Kornman et al. 1997). Moreover, no significant association was found in the distribution of IL-1RN (VNTR) genotypes between EOP patients and healthy controls (allele 2 carriage rate of 31.4% in G-EOP patients vs. 48.6% in healthy controls, Parkhill et al. 2000). However, there have been no previous reports in ethnic populations such as Chinese and Japanese. In this study, we found that the frequency of IL-1RN (VNTR) polymorphic alleles (alleles 2, 3, 4 and 5) was solely found to be significantly increased in G-EOP patients (12.8% in G-EOP patients vs. 4.1% in healthy controls, $\chi^2=7.32$, $P=0.007$; odds ratio = 3.40, 95% CI =

1.24–9.52). Additionally, the carriage rate of IL-1RN (VNTR) polymorphic alleles was significantly higher in G-EOP patients than in healthy controls (25.5% in G-EOP patients vs. 8.2% in healthy controls, $\chi^2 = 7.91$, $P = 0.005$; odds ratio = 3.81, 95% CI = 1.31–11.31). These data may reflect ethnic and racial differences in disease-susceptibility gene polymorphisms between Caucasians and Japanese.

The IL-1RN allele 2 is associated with enhanced IL-1$\beta$ production *in vitro*, and IL-1$\beta$ levels may be influenced by polymorphism outside the IL-1$\beta$ gene rather than by the IL-1$\beta$ (+3954) SNP itself (Hurme & Santtila 1998, Santtila et al. 1998). Clay et al. (1996) have identified five single base change polymorphisms in exons, all of which are in linkage disequilibrium with the intron 2 VNTR polymorphisms of the IL-1RN. However, these novel polymorphisms and the disease-associated VNTR polymorphisms do not alter levels of steady state mRNA for IL-1ra in cultured keratinocytes. Currently, El-Omar et al. (2000) have found that allele 2 of the IL-1$\beta$ (−31) SNP and IL-1RN (VNTR) polymorphisms is associated with an increased risk of both hypochlorhydria induced by *H. pylori* and gastric cancer. These polymorphisms are in near-complete linkage disequilibrium and the IL-1$\beta$ (−31) SNP is a TATA-box polymorphism that markedly affects an increased DNA-binding activity *in vitro*. These findings suggest that increased IL-1$\beta$ production, influenced by the IL-1$\beta$ (−31) SNP and IL-1RN (VNTR) polymorphisms, could facilitate the onset and development of periodontal diseases including EOP, characterized by an increase in severity of inflammation.

On the other hand, the IL-1RN allele 2 was associated with increased IL-1ra production both *in vitro* and *in vivo* (Danis et al. 1995, Hurme et al. 1998, Wilkinson et al. 1999). Few studies have investigated the relationship between IL-1ra production and periodontal disease. Rawlinson et al. (2000) revealed the relationship between the severity of periodontitis and the increasing GCF levels of IL-1$\beta$ and decreasing levels of IL-1ra. Ishihara et al. (1997) reported that total amount of IL-1 (IL-1$\alpha$ + IL-1$\beta$)/IL-1ra ratio was correlated with the severity of periodontitis, but IL-1ra itself was not significant. Recently, Perrier et al. (1998) found that the IL-1RN allele 2 was associated with Sjögren's syndrome. Patients with IL-1RN allele 2 generally had lower salivary IL-1ra levels and higher serum levels than patients without the allele. This discrepancy between local and systemic IL-1ra production levels may result from different effects in different cells, since 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 unclear. Further studies are needed to clarify the relationship between IL-1$\beta$ SNPs, IL-1RN (VNTR) polymorphisms and their production levels in periodontitis patients.

In conclusion, our study has revealed that the frequency of IL-1RN (VNTR) polymorphic alleles is significantly increased in G-EOP. In addition, the carriage rate of IL-1RN (VNTR) polymorphic alleles is significantly higher in G-EOP patients than in healthy controls. These findings suggest that IL-1RN (VNTR) polymorphisms are associated with G-EOP in Japanese, but they are not adequately represented in the Japanese population to draw strong conclusions because of the absence of 2.2 genotype.

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## Zusammenfassung

*Assoziation des Interleukin-1-Rezeptorantagonisten-Genpolymorphismus zu früh beginnender Parodontitis bei Japanern*

**Hintergrund/Zielsetzung:** Man nimmt an, dass früh beginnende Parodontitis (EOP), neuerdings 'aggressive Parodontitis' genannt, einen genetischen Hintergrund hat, der aber nicht klar definiert ist. Der Interleukin-1 (IL-1) Gencluster-Polymorphismus könnte einer der genetischen Faktoren sein, die die Ausbildung verschiedener chronischer Entzündungskrankheiten beeinflusst. Das Ziel dieser Studie war es, die Häufigkeit von Einzelnukleotid-Polymorphismen (SNP) der Gene für IL-1$\alpha$, IL-1$\beta$ und eine variable Zahl von Tandemwiederholungspolymorphismen (VNTR) im IL-1-Rezeptorantagonisten-Gen (IL-1RN) bei 47 Patienten mit generalisierter EOP (G-EOP) und 97 parodontal gesunden Kontrollprobanden zu vergleichen.

**Material und Methoden:** Alle Studienteilnehmer waren von Japanischer Herkunft und allgemein gesund. Die Zuordnung zu Test- (G-EOP) und Kontollgruppe erfolgte nach den bekannten klinischen Kriterien. SNPs im IL-1$\alpha$- (+4845) und IL-1$\beta$-Gen (−511, +3954) wurden durch Vervielfältigung der polymorphen Region mittels Polymerasekettenreaktion (PCR) gefolgt von Zerteilung durch Restriktionsenzyme und Agarosegelelektrophorese nachgewiesen. Die IL-1RN-Polymorphismen (VNTR) wurden durch PCR und Fragmentgrößenanalyse bestimmt.

**Ergebnisse:** Es bestand kein signifikanter Unterschied zwischen den IL-1$\alpha$- (+4845) und IL-1$\beta$-Genotypen (−511, +3954) und Allelfrequenzen zwischen den G-EOP-Patienten und den gesunden Kontrollprobanden. Allerdings wurde das polymorphe Allel für IL-1RN (VNTR) bei G-EOP-Patienten signifikant häufiger gefunden ($\chi^2$-Test, $P$=0,007; Odds ratio=3,40). Zusätzlich war die Trägerrate des IL-1RN-Polymorphismus (VNTR) bei G-EOP-Patienten gegenüber den gesunden Kontrollprobanden signifikant erhöht ($\chi^2$-Test, $P$=0,005; Odds ratio=3,81).

**Schlussfolgerung:** Die Ergebnisse legen den Schluss nahe, dass IL-1RN-Polymorphismen (VNTR) bei Japanern mit G-EOP assoziiert sind.

## Résumé

*Association entre les polymorphismes du gène du récepteur antagoniste de l'interleukine-1 avec la parodontite d'apparition précoce dans une population japonaise*

**Buts:** La parodontite d'apparition précoce (EOP), désormais appelée parodontite agressive, est considérée avoir des bases génétiques, ce qui n'a pas été clairement établi. Le polymorphisme des gènes de l'interleukine-1 (IL-1) en tant que l'un de ces facteurs génétiques pourrait influencer l'expression de plusieurs maladies chroniques inflammatoires. Le but de cette étude était de rechercher la fréquence des polymorphismes d'un unique nucléotide (SNPs) sur les gènes codant pour IL-1$\alpha$, IL-1$\beta$ et un nombre variable de polymorphismes de répétition de tandem (VNTR) sur le gène du récepteur antagoniste de l'IL-1 (IL-1RN) chez 47 patients atteints de EOP généralisées (G-EOP) et 97 contrôles aux parodontes sains.

**Matériel et méthodes:** Tous les sujets étaient japonais d'origine et en bonne santé. Ils furent identifiés en fonction de critères cliniques établis. Les SNPs IL-1$\alpha$ (+4845) et IL-1$\beta$ (−511, +3954) furent analysées par amplification de la région polymorphique par réaction de polymérase en chaîne (PCR), suivi par une digestion par enzyme de restriction et électrophorèse sur gel d'agarose. Les polymorphismes IL-1RN (VNTR) furent détectés par amplification PCR et analyse de la taille des fragments.

**Resultats:** Il n'y avait pas de différences significatives pour les génotypes IL-1$\alpha$ (+4845) et

IL-$\beta$ (−511, +3954) et les fréequences d'allèle entre les patients G-EOP et les contrôles sains. Cependant, la fréquence des allèles polymorphiques IL-1RN (VNTR) fut trouvée augmentée significativement chez les patients G-EOP ($\chi^2$ test, $P$=0.007; odds ratio=3.40). De plus, le taux de portage des polymorphismes IL-1RN (VNTR) étaient significativement plus grand chez les patients G-EOP que chez les contrôles sains ($\chi^2$ test, $P$=0.005; odds ratio=3.81).

**Conclusion:** Ces résultats suggèrent que les polymorphismes IL-1RN (VNTR) sont associés avec la chez les japonais.

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