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Interleukin-1 receptor gene variants are associated with aggressive periodontitis in the Japanese

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ABSTRACT

Objective: Previous studies have indicated that type-1 and type-2 interleukin-1 (IL-1) receptors (IL-1R1 and IL-1R2) play important roles in periodontitis progression. We investigated the association between periodontitis and polymorphisms in the IL-1R1 and IL-1R2 genes (*IL1R1* and *IL1R2*).

Design: We searched for genetic variants in *IL1R1* and *IL1R2* in 24 Japanese patients with aggressive periodontitis (AgP) and 24 periodontally healthy controls. Thirty-eight single nucleotide polymorphisms (SNPs) were identified within genomic regions containing all exons and relevant exon-intron boundaries in *IL1R1* and *IL1R2*. Possible associations of each gene locus with AgP were investigated in 119 AgP patients and 102 periodontally healthy controls using allelotypes, genotypes, and haplotypes.

Results: Significant differences were noted in the frequencies of 3 SNPs in *IL1R2* (rs3819370, rs3218974 and rs3218977) for AgPs and controls ($p = 0.012$, $p = 0.008$, and $p = 0.038$, respectively), after adjustment for gender and smoking status in the additive model ($p = 0.016$, $p = 0.007$, and $p = 0.027$, respectively) and 2 haplotypes ($p = 0.010$ and $p = 0.011$, respectively) constructed from 2 SNPs (rs3819370 and rs3218974) that showed the lowest *p*-values after adjustment of covariates in additive models.

Conclusion: A genetic susceptibility locus for AgP may lie within or close to the *IL1R2* locus. Further studies in other populations are necessary to confirm these results.

1. Introduction

Periodontitis is an inflammatory disease that causes periodontal tissue destruction; however, its etiology has not yet been well defined. The evidence accumulated thus far indicates that cytokines play a pivotal role in the pathogenesis of periodontal disease and that interleukin-1 (IL-1), in particular, contributes to periodontal destruction.[1] Therefore, understanding the effects of IL-1 on the pathogenesis of

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periodontitis is thought to be an important key for the prevention and treatment of this disease.

The IL-1 family comprises 2 proteins with similar biological activities, IL-1α and IL-1β, as well as the IL-1 receptor antagonist (IL-1Ra), which is a non-signalling ligand. These ligands bind to 2 distinct and separate receptors, the type-1 and type-2 IL-1 receptors (IL-1R1 and IL-1R2, respectively), which are expressed across a variety of cells.[2,3] Both receptors contain 3 Ig-like domains and are therefore classified as members of the Ig superfamily. Binding of IL-1α and IL-β to IL-1R1 leads to cellular signalling and subsequent biological effects.[4] In contrast, because IL-1R2 lacks a signalling domain, binding of IL-1α and IL-1β to IL-1R2 does not lead to cellular and biological effects, and IL-1R2 acts as a decoy receptor for IL-1 family members.[5] Both receptors can be cleaved from the cell surface and circulate as soluble proteins (sIL-1R1 and sIL-1R2) that are still able to bind all members of the IL-1 family.[2,6,7] A study showed a significantly increased number of IL-1R1 molecules in cells from inflamed gingiva compared with cells from healthy gingiva. Expression of IL-1R1 in gingival fibroblasts from patients with periodontitis increases at the protein level.[8] Another study reported that over-expression of IL-1R2 in human gingival fibroblasts down-regulated the expression of IL-1β mRNA in response to IL-1β stimulation.[9] Accordingly, these receptors are considered to play important roles along with their ligands in the progression of periodontal disease.

Studies of twins and families suggest that genetic factors play an important role in determining the susceptibility to periodontal diseases, especially aggressive periodontitis (AgP).[10–12] Many researchers have investigated associations between periodontitis and variants in the *IL-1* gene family.[13–16] Loci for the human IL-1α, IL-1β, IL-1Ra, IL-1R1 and IL-1R2 genes (*IL1A*, *IL1B*, *IL1RA*, *IL1R1*, and *IL1R2*, respectively) are found as a cluster on chromosome 2 from 2q12 to 2q14.[17,18] Furthermore, positive associations between genetic markers in *IL1A*, *IL1B*, and *IL1RA* and periodontitis have been observed.[19,20] Therefore, genetic polymorphisms of the IL-1 gene cluster on chromosome 2 are thought to be associated with increased susceptibility to periodontitis. However, a positive association between the *IL1R1* and *IL1R2* genes and periodontal disease, including AgP, has not yet been reported.

In this study, we considered the *IL1R1* and *IL1R2* loci as candidate regions that may be associated with periodontal disease, and examined 119 Japanese patients with AgP and 102 periodontally healthy control subjects for possible associations.

2. Materials and methods

2.1. Subjects

One hundred nineteen Japanese patients with AgP (AgPs) (mean age at the time of initial examination, 31.4 ± 6.3 years) were recruited at the Department of Periodontology in Aichi Gakuin University from 2002 to 2008. The regionally matched control group consisted of 102 healthy volunteers (mean age, 43.6 ± 7.0 years). The diagnostic criteria of AgP were based on a published study[21] and were defined as follows: (1) age of onset of periodontitis less than 35 years and (2) attachment loss of at least 4 mm on at least four permanent teeth, with at least one first molar affected. Alveolar bone loss of all AgPs was assessed using full-mouth radiographs. In this study, 117 AgPs showed attachment loss of at least 4 mm on at least eight teeth. At the time of their initial visit, 36 AgPs were older than 35 years; however, oral disease history confirmed that the age of onset of periodontitis was less than 35 years. Control subjects were all older than 35 years; otherwise, they may have been too young to have AgP and could have been misclassified. They were chosen so that gender ratio and smoking status became similar to those of AgPs. They showed no attachment loss, although some showed gingival pockets in a few teeth. The systemic health of all participants was confirmed by general blood tests. The extent of periodontitis in all participants was assessed by measuring probing pocket depth (PD), clinical attachment level (CAL), bleeding on probing, and degree of tooth mobility. The clinical characteristics of AgPs and controls are summarized in Table 1.

Genomic DNA was extracted from whole peripheral blood by using the Nucleon Genomic DNA Extraction kit (Tepnel Life Sciences PLC, Manchester, UK).

2.2. Identification of single nucleotide polymorphisms (SNPs)

Sequence information for *IL1R1* and *IL1R2* was obtained from the National Center for Biotechnology Information (NCBI, USA). Accession number NT_022171 corresponds to the DNA

Table 1 – Clinical characteristics of patients with AgP and control subjects.

Characteristics	AgP (n = 119)	Control (n = 102)	p-Value
Gender (female/male)	65/54	45/57	0.14
Age (years)	31.4 ± 6.3	43.6 ± 7.0	<0.001
Current or former smokers (%)	32.8	38.2	0.45
Number of present teeth	27.6 ± 2.8	27.7 ± 2.1	0.55
Mean PD (mm)	4.12 ± 1.41	1.96 ± 0.33	<0.001
Mean CAL (mm)	4.73 ± 1.72	2.15 ± 0.39	<0.001
Teeth with PD ≥ 4 mm (%)	78.2 ± 25.3	9.9 ± 14.7	<0.001
Teeth with CAL ≥ 4 mm (%)	80.1 ± 25.6	18.0 ± 18.4	<0.001
Teeth with bleeding on probing (%)	70.8 ± 27.5	15.9 ± 18.2	<0.001
Teeth with mobility ≥ 2 degrees (%)	13.7 ± 19.6	0 ± 0	<0.001

Values are means ± SD. PD, probing pocket depth; CAL, clinical attachment level.
The χ^2 test was used for analysis of gender and smoker ratios and the Mann–Whitney U test was used for the other analyses.