**IL-1RN** gene polymorphism is associated with peri-implantitis

**Marja L. Laine**  
**Asa Leonhardt**  
**Ann-Marie Roos-Jansäter**  
**A. Salvador Peña**  
**Arie Jan van Winkelhoff**  
**Edwin G. Winkel**  
**Stefan Renvert**

**Authors’ affiliations:**  
Marja L. Laine, Arie Jan van Winkelhoff,  
Department of Oral Microbiology, ACTA  
Amsterdam, Amsterdam, The Netherlands  
Asa Leonhardt, Department of Periodontology,  
Specialist Dental Clinic, Mölndal Hospital,  
Mölnsdal, Sweden  
Asa Leonhardt, Department of Oral Microbiology,  
Faculty of Odontology, Sahlgrenska Academy of  
Göteborg University, Göteborg, Sweden  
Ann-Marie Roos-Jansäter, Stefan Renvert,  
Department of Health Sciences, Kristianstad  
University, Kristianstad, Sweden  
A. Salvador Pena, Laboratory of Immunogenetics,  
VU Medical Centre, Amsterdam, The Netherlands  
A. Salvador Peña, Department of Gastroenterology,  
VU Medical Centre, Amsterdam, The Netherlands  
Edwin G. Winkel, Clinic of Periodontology  
Amsterdam, The Netherlands  
Edwin G. Winkel, University Medical Center  
Groningen, Groningen, The Netherlands

**Correspondence to:**  
Prof. Dr. S. Renvert  
Department of Health Sciences  
Kristianstad University  
S 291 88 Kristianstad  
Sweden  
Tel.: +46 44 204090  
Fax: +46 44 204053  
e-mail: stefan.renvert@hv.hkr.se

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**Abstract**

**Objectives:** Interleukin (IL)-1α, IL-1β and their natural specific inhibitor IL-1 receptor antagonist (IL-1ra) play a key role in the regulation of the inflammatory response in periodontal tissues. Polymorphisms in the IL-1 gene cluster have been associated with severe adult periodontitis. We aimed to investigate the IL-1 gene cluster polymorphisms in patients with peri-implantitis.

**Material and methods:** The study included 120 North Caucasian individuals. A total of 71 patients (mean age 68 years, 76% smokers) demonstrating peri-implantitis at one or more implants as evidenced by bleeding and/or pus on probing and bone loss amounting to $\geq 3$ threads on Brånemark implants and 49 controls (mean age 66 years, 45% smokers) with clinical healthy mucosa and no bone loss around the implants were recruited for the study. The titanium implants, *ad modum* Brånemark, had been in function for at least 2 years. Mouthwash samples were collected and used for genotyping of the bi-allelic polymorphisms IL-1A/*C*889, IL-1B/*C*3953, IL-1B/*C*511 and a variable number of tandem repeat IL-1RN gene polymorphisms using PCR technique.

**Results:** Significant differences were found in the carriage rate of allele 2 in the IL-1RN gene between peri-implantitis patients and controls (56.5% vs. 33.3%, respectively; odds ratios (OR) 2.6; 95% confidence interval (CI) 1.2–5.6; *P* = 0.015). Logistic regression analysis taking smoking, gender and age into account confirmed the association between the IL-1RN allele 2 carriers and peri-implantitis (OR 3; 95% CI 1.2–7.6; *P* = 0.02).

**Conclusions:** Our results provide evidence that IL-1RN gene polymorphism is associated with peri-implantitis and may represent a risk factor for this disease.

Bacteria in the subgingival plaque on implant surfaces can trigger a host response that may lead to peri-implantitis [Lindhe et al. 1992, Schou et al. 1993, Lang et al. 1994]. Bacteria are most likely an essential factor for the onset and progression of peri-implantitis [Mombelli 1997, Klokkevold & Newman 2000]. However, the disease is probably the result of several factors that may influence the host inflammatory response, including smoking, stress and genetic variation in relevant genes (polymorphism) [Genco & Loe 1993].

Although osseointegrated implants have been reported to have high survival and success rates, infections do occur and can ultimately lead to the loss of implants [Adell et al. 1990; Buser et al. 1997]. Smoking is an important risk factor for peri-implantitis, but non-smoking patients also may develop peri-implantitis [Lindquist et al. 1997; Esposito et al. 1998].

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Previous studies have indicated that peri-implantitis and implant failures appear to cluster in subsets of individuals, and that a patient who has lost one implant is at elevated risk of experiencing other implant losses [Weyant & Burt 1993; Hutton et al. 1995]. These observations have led to the question whether there is a common denominator for susceptibility to develop peri-implantitis.

The host immune system reacts to a microbial challenge by production of inflammatory mediators. The pro-inflammatory cytokine, interleukin-1 (IL-1) is considered a major mediator of chronic inflammatory diseases, such as periodontitis [Dinarello 1994]. Previous reports have indicated elevated levels of IL-1 in gingival crevicular fluid (GCF) in patients with advanced peri-implant infections when compared with healthy peri-implant control sites [Kao et al. 1995; Panagakos et al. 1996; Salcetti et al. 1997]. IL-1 plays a central role in the process of bone resorption and destruction of the extracellular matrix by up-regulating matrix metalloproteinase (MMP) production [Stashenkov et al. 1987; Birkedal-Hansen 1993]. The production of the pro-inflammatory cytokine IL-1 by monocytes and macrophages is stimulated by lipopolysaccharides of Gram-negative bacteria. IL-1 receptor antagonist (IL-1ra) is an anti-inflammatory cytokine, which binds to the host cell surface using the same receptor as the pro-inflammatory IL-1, thereby inhibiting the signal transduction by blocking the receptor.

The role of IL-1 in the initiation and progression of periodontitis has been established [Gemmell et al. 1997]. Recently, polymorphisms of the IL-1 gene cluster were associated with periodontitis [Kornman et al. 1997; Laine et al. 2001]. Kornman and co-workers [Kornman et al. 1997; Laine et al. 2001] reported on an association between an IL-1A*2 (carrier of allele 2) and an IL-1B*2 genotype and periodontitis in non-smoking patients, whereas Laine and co-workers [Kornman et al. 1997; Laine et al. 2001] reported on an association between an extended IL-1A*2, IL-1B*2 and IL-1RN*2 genotype and periodontitis, taking into account smoking and the prevalence of the two major periodontal pathogens, Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans.

A retrospective analysis of changes in bleeding on probing (BOP) during periodontal maintenance has indicated that patients with an IL-1A*2 and an IL-1B*2 genotype have significantly higher BOP percentages [Lang et al. 2000]. Furthermore, it has been shown that patients positive for an IL-1A*2 and an IL-1B*2 genotype have a higher risk for tooth loss [2.7 times] and that smoking in combination with IL-1A*2 and IL-1B*2 genotypes resulted in a 7.7 times greater likelihood for tooth loss after periodontal therapy [McGuire & Nunn 1999]. An association between the IL-1A and IL-1B genotype and increased levels of IL-1 produced by monocytes has been shown in vitro [Pociot et al. 1992; Dominici et al. 2002] and in GCF in vivo [Engelbreton et al. 1999]. Carriage of allele 2 of the IL-1RN gene has been associated with decreased levels of the IL-1 receptor antagonist in vitro [Andus et al. 1997].

We hypothesize that individuals carrying allele 2 in the IL-1 gene cluster are susceptible to develop peri-implantitis through altered IL-1 and IL-1ra production. To our knowledge there is no report on the polymorphism in IL-1RN gene, encoding for the anti-inflammatory IL-1ra, and peri-implantitis. We aimed to investigate if IL-1A^{-889}, IL-1B^{3953}, IL-1B^{-511} and a variable number of tandem repeat IL-1RN gene polymorphisms and their combinations are associated with peri-implantitis taking into account smoking status, gender and age.

Material and methods

Subjects
One hundred and twenty systemically healthy, unrelated Northern Caucasian individuals (38 edentulous, 62 dentate) aged 32–88 years (mean age 67 years), with 1–12 implants (median 6 implants) ad modum Bränemark [Nobel Biocare AB, Göteborg, Sweden] were included and gave informed consent to participate in the present study. The study protocol was approved by the ethical committee of the Lund University, Sweden. Smoking was defined as follows: (i) patients who were current smokers or had stopped smoking were considered smokers and (ii) patients who had never smoked were considered as non-smokers. The implants were required to be in place and functional in the oral cavity for at least 2 years. A total of 71 patients demonstrated peri-implantitis defined as bone loss >3 threads on Bränemark implants and evidence of bleeding and/or pus on probing at one or more implants. Forty-nine patients with Bränemark implants without clinical or radiographic signs of peri-implant disease served as controls. Any periodontal disease was adequately treated before installation of the implants. At the clinical examination, the patients were asked why they had lost their teeth.

DNA isolation from mouthwash samples
DNA from all subjects was isolated according to the method of de Vries et al. [1996] as modified and validated for the study of cytokine gene polymorphisms [Laine et al. 2000]. In short, each individual rinsed out his/her mouth with 10 ml of 0.9% saline for 60 s. Cells were centrifuged at 300 × g for 10 min. The pellet was washed twice in 0.9% saline, re-suspended in 100 µl of 50 mM NaOH and boiled for 10 min. Samples were neutralized with 14 µl of 1 M Tris [pH 7.5] and centrifuged at 14,000 × g for 3 min. Supernatants were collected and stored at 4°C until analysis.

Analysis of polymorphisms in genes of the IL-1 family
The bi-allelic polymorphisms at position –889 within the promoter region of the IL-1A gene [McDowell et al. 1995], at position +3954 [Taql restriction fragment length polymorphism] within exon 5 [Bioquee et al. 1995] and at position –511 within the promoter region of the IL-1B gene [di Giovine et al. 1992], and the penta-allelic variable number of the tandem repeat polymorphism within the second intron of the IL-1RN gene were determined according to previously described methods [Mansfield et al. 1994].

Statistical methods

The IL-1A^{-889}, IL-1B^{3954}, IL-1B^{-511} and IL-1RN variable number tandem repeat (VNTR) genotypes, allele 2 and carrier of allele 2 frequencies and their combinations were compared between the controls and cases by the χ²-test and Fisher’s two-tailed exact test, and odds ratios [ORs] with 95% confidence intervals [CIs] were deter-
minded. *IL-1B* VNTR alleles other than 2 were combined for data analysis. Subsequently, differences in the carriage of allele 2 in *IL-1* cluster in patients and controls (as binary variables) were analyzed with logistic regression models. Age, gender and smoking status were entered in the models, the significant determinants were determined and adjusted OR and CI were calculated. Differences in genotype frequencies between control and case subgroups were explored. A *P*-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 11.0 for windows (SPSS Inc., Chicago, IL, USA).

**Results**

Demographic and clinical data of the study groups are presented in Table 1. Information of the number of implants was available from 57 peri-implantitis patients and 44 controls. A total of 365 implants (mean 6.4, range 2–12) was presented in patients with and 236 implants (mean 5.4, range 1–12) in patients without peri-implantitis. The majority of the patients in both the peri-implantitis group (56%) and in the control group (82%) claimed they had lost their teeth because of periodontal disease, whereas 13% in the peri-implantitis group and 11% in the control group said it was not because of periodontal disease. A big portion of the peri-implantitis group (31%) did not know why they had lost their teeth.

The *IL-1* cluster composite genotypes consisting of the allelic variants (i) *IL-1A*/*IL-1B* VNTR alleles 1 and 2 and (ii) *IL-1A*/*IL-1B* VNTR alleles 1 and 2 genotypes were at least one copy of allele 2 frequencies were similar in the peri-implantitis patients and controls. An association was found between the *IL-1RN* genotype and peri-implantitis. Differences in the carriage of allele 2 frequencies, and peri-implantitis patients and controls (mean 6.8 and 7.6) (Table 3). Also, smoking represented a significant risk factor for peri-implantitis.

**Discussion**

The present study is to our knowledge the first report that shows an association between the carrier state of allele 2 of the *IL-1RN* gene and peri-implantitis. The carriers of these *IL-1RN* genotypes were at higher risk for peri-implantitis with an OR of 3, taking in to account smoking status, gender and age of the patients. The carriage rate of *IL-1RN* allele 2 was 13% in healthy controls, which is in accordance with previous reports for healthy populations of

### Table 1. Demographic and clinical data of the peri-implantitis cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (N = 71)</th>
<th>Controls (N = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>68</td>
<td>66</td>
</tr>
<tr>
<td>Range (years)</td>
<td>51–84</td>
<td>32–88</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>47/24</td>
<td>43/26</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>76</td>
<td>49</td>
</tr>
<tr>
<td>Loss of teeth because of periodontitis (%)</td>
<td>56.3</td>
<td>75.5</td>
</tr>
<tr>
<td>Loss of teeth because of unknown reason (%)</td>
<td>29.6</td>
<td>16.3</td>
</tr>
<tr>
<td>Dentate (%)</td>
<td>57.7</td>
<td>42.9</td>
</tr>
</tbody>
</table>

### Table 2. The different *IL-1* cluster composite, *IL-1A*/*IL-1B* VNTR genotypes, allele 2 and carriage of at least one copy of allele 2 frequencies in peri-implantitis cases and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>IL-1A</em>/<em>IL-1B</em> VNTR *2</td>
<td>26.2</td>
<td>8.3</td>
</tr>
<tr>
<td><em>IL-1A</em>/<em>IL-1B</em> VNTR *2/2</td>
<td>47.8</td>
<td>44.9</td>
</tr>
<tr>
<td><em>IL-1A</em>/<em>IL-1B</em> VNTR *2/2</td>
<td>33.8</td>
<td>38.8</td>
</tr>
<tr>
<td>Allele 2 frequency</td>
<td>63.2</td>
<td>55.1</td>
</tr>
<tr>
<td>Allele 2 carriers</td>
<td>3/</td>
<td>6.1</td>
</tr>
<tr>
<td>Allele 2 frequency</td>
<td>34.6</td>
<td>33.7</td>
</tr>
<tr>
<td>Allele 2 carriers</td>
<td>66.2</td>
<td>61.2</td>
</tr>
<tr>
<td><em>IL-1B</em> VNTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL-1B</em> VNTR</td>
<td>49.3</td>
<td>49</td>
</tr>
<tr>
<td>Allele 2 frequency</td>
<td>44.9</td>
<td>40.8</td>
</tr>
<tr>
<td>Allele 2 carriers</td>
<td>5.8</td>
<td>10.2</td>
</tr>
<tr>
<td>Allele 2 frequency</td>
<td>28.3</td>
<td>30.6</td>
</tr>
<tr>
<td>Allele 2 carriers</td>
<td>50.7</td>
<td>51</td>
</tr>
<tr>
<td><em>IL-1B</em> VNTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other than 2/other than 2</td>
<td>43.5</td>
<td>66.7</td>
</tr>
<tr>
<td>Allele 2 frequency</td>
<td>43.5</td>
<td>22.9</td>
</tr>
<tr>
<td>Allele 2 carriers</td>
<td>13</td>
<td>10.4</td>
</tr>
<tr>
<td>Allele 2 frequency</td>
<td>34.8</td>
<td>21.9</td>
</tr>
<tr>
<td>Allele 2 carriers</td>
<td>56.5</td>
<td>33.3</td>
</tr>
</tbody>
</table>

*VNTR, variable number tandem repeat.*
Caucasian origin [Garcia-Gonzalez et al. 2001; Laine et al. 2001; Meisel et al. 2002]. In comparison with the carriage rate of IL-1RN allele 2 in adult Caucasian periodontitis patients (36-46%) [Laine et al. 2001; Meisel et al. 2002] peri-implantitis patients carry more often IL-1RN allele 2 (56.5%). Smoking, irrespective of the IL-1 cluster genotype, is a risk factor for peri-implant bone loss and an elevated rate of implant failures has been associated with heavy smoking [Bain & Moy 1993; Wilson & Nunn 1999]. The present study confirms an association between smoking and peri-implantitis.

Other authors have investigated the relationship between gene polymorphisms in the pro-inflammatory cytokine genes IL-1A and IL-1B and peri-implantitis, and found no association [Feloutzis et al. 2003; Gruica et al. 2004]. In these papers gene polymorphism in the anti-inflammatory cytokine IL-10 gene was not studied. However, using subgroup analysis Feloutzis et al. (2003) and Gruica et al. (2004) reported that IL-1A*2 and IL-1B*2 composite genotype-positive heavy smokers were at high risk for the development of inflammatory complications and increased peri-implant marginal bone loss in comparison to non-smokers with the same genotype. In the present study, no association was found between the IL-1A and IL-1B genotypes separately, or in combination, and peri-implantitis.

Wilson & Nunn (1999) did not find any association between the IL-1 genotype and implant loss. However, they did not report which IL-1 gene polymorphisms they studied. Also, in their study, more than 50% of the failures (implant losses) occurred during the first year after placement, which may present a different disease entity than the one analyzed in the present study.

Table 3. Logistic regression analysis of patients with peri-implantitis and controls

<table>
<thead>
<tr>
<th></th>
<th>P-value</th>
<th>Odds ratio (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>IL-1A 889G&gt;932†</td>
<td>0.42</td>
<td>1.6 (0.5-5.1)</td>
</tr>
<tr>
<td>IL-1B 3954A&gt;2</td>
<td>0.86</td>
<td>0.9 (0.3-2.8)</td>
</tr>
<tr>
<td>IL-1B 5112G&gt;2</td>
<td>0.12</td>
<td>0.46 (0.2-1.2)</td>
</tr>
<tr>
<td>IL-1RN/IN/32</td>
<td>0.02</td>
<td>3.1 (0.7-14.8)</td>
</tr>
<tr>
<td>Age</td>
<td>0.2</td>
<td>0.97 (0.9-1)</td>
</tr>
<tr>
<td>Gender</td>
<td>0.15</td>
<td>1.9 (0.8-4.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.004</td>
<td>3.6 (1.5-8.8)</td>
</tr>
</tbody>
</table>

†2, carrier of allele 2.
CI, confidence interval.

In conclusion, our results provide evidence that IL-1RN gene polymorphism is associated with peri-implantitis and may represent a risk factor for peri-implantitis.

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Shasheen, P., Dewhirst, F.E., Peros, W.J., Kent, R.L. & Ago, J.M. (1987) Synergistic interactions between interleukin 1, tumor necrosis factor, and


