J Clin Periodontol 2006; 33: 704–709 doi: 10.1111/j.1600-051X.2006.00978.x

Journal of Clinical Periodontology

Interleukin-6 receptor gene polymorphisms and periodontitis in a non-smoking Japanese population

Johnah C. Galicia, Hideaki Tai, Yasutaka Komatsu, Yasuko Shimada, Ikuyo Ikezawa and Hiromasa Yoshie

Division of Periodontology, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Galicia JC, Tai H, Komatsu Y, Shimada Y, Ikezawa I, Yoshie H. Interleukin-6 receptor gene polymorphisms and periodontitis in a non-smoking Japanese population. J Clin Periodontol 2006; 33: 704–709. doi: 10.1111/j.1600-051X.2006.00978.x.

Abstract

Aim: The indispensable role of interleukin-6 receptor (IL-6R) in regulating IL-6 responses has been clearly established. We have previously reported that *IL6R* polymorphisms strongly influenced the serum levels of soluble IL-6R. In this study, we investigated the association between these genetic variations and periodontitis.

Material and Methods: Among the seven novel *IL6R* single-nucleotide polymorphisms (SNPs) reported, we genotyped two important sites: the +48892 A/C in exon 9 and the −183 G/A in the promoter region. The SNP in exon 9 results in Asp → Ala substitution in the proteolytic cleavage site of IL-6Rα. In total, 212 periodontitis cases and 210 healthy controls were genotyped using polymerase chain reaction, restriction fragment length polymorphisms and direct sequencing methods.

Results: Analysis of the genotype distribution of the +48892 A/C SNP in periodontitis patients and in controls revealed a suggestive association with aggressive ($p = 0.04$) and chronic periodontitis ($p = 0.04$). In addition, the carriage rate for the A allele was significantly higher in chronic periodontitis patients [$p = 0.02$, odds ratio (OR) = 2.25]. No association was found in the −183 G/A SNP. The two markers were in linkage disequilibrium (LD) ($|D'| = 0.53$).

Conclusion: The *IL6R* +48892 A/C polymorphism could act as a risk factor for periodontitis; however, further association and biological studies are needed.

Key words: case–control; IL-6 receptor; Japanese; periodontitis; SNP

Accepted of publication 14 June 2006

The important biological activities of interleukin-6 (IL-6) have been proven over the past decades (Kishimoto 2005). IL-6 exerts its biological effects via specific receptors expressed on the surface of target cells (Kishimoto 1992). Initially, IL-6 binds to an 80 kDa glycoprotein, the membrane-bound IL-6 receptor (IL-6Rα), forming an IL-6/IL-6Rα complex. This complex associates with a signal transducer, a 130 kDa glycoprotein called gp130. This high-affinity binding results in cellular activation. The main signalling pathway is JAK/STAT (Heinrich et al. 1998). Polymorphisms in *IL6* served as protective or susceptibility factors for periodontitis and were associated with serum levels of IL-6 (Trevilatto et al. 2003, D'Aiuto et al. 2004, Holla et al. 2004, Brett et al. 2005, Komatsu et al. 2005).

Genes that encode IL-6Rα were located on chromosomes 1q21 and 9, although the gene on chromosome 9 was found out to be a pseudogene (Szpirer et al. 1991, Snyder et al. 1995). While gp130 is widely expressed in almost all organs of the body, the cellular distribution of IL-6Rα is largely limited to hepatocytes and leucocyte subsets (Jones et al. 2001, Jones & Rose-John 2002). For this reason, cells that do not express the membrane-bound form of IL-6R depend on the soluble form of IL-6 receptor (sIL-6R) to elicit IL-6 responses in cells (Romano et al. 1997).

Two mechanisms are involved in the generation of sIL-6R. The first mechanism consists of proteolytic cleavage or shedding of the membrane-bound form, and the second mechanism, which represents a small fraction of the total sIL-6R present in the human serum, consists of alternative mRNA splicing, resulting in a transcript encoding a soluble receptor (Lust et al. 1992, Müllberg et al. 1993, Horiuchi et al. 1998). The cleavage site was determined at one major location (Gln357/Asp358) close to the transmembrane region of the protein (Müllberg et al. 1994). Shedding

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

may be enhanced by bacterial pore-forming toxins, phorbol ester, formyl peptide and others (Müllberg et al. 1993, Walev et al. 1996, Modur et al. 1997). On the other hand, high-level production of an alternatively spliced soluble interleukin-6 receptor was detected in the serum of patients with adult T cell leukaemia/HTLV-I-associated myelopathy (Horiuchi et al. 1998).

Membrane-bound IL-6Rά from lymphocyte infiltration into periodontal tissues were thought to be the source of large amounts of sIL-6R in inflamed gingival tissue from chronic periodontitis patients (Naruishi et al. 2001, Takashiba et al. 2003). The sIL-6R was found to be agonistic to IL-6 responses by cells, resulting in impaired gingival fibroblast adherence (Naruishi et al. 2001). It also triggered osteoclast formation by IL-6 and induced bone resorption (Ishimi et al. 1990, Tamura et al. 1993). However, studies have shown that the combination of sIL-6R and soluble gp130 (sgp130) led to a drastic reduction of the IL-6 responses, confirming the antagonistic activity of sIL-6R in the presence of the ubiquitous sgp130 (Müller-Newen et al. 1998).

Recently, we reported that the single-nucleotide polymorphisms (SNPs) in *IL6* were associated with increased serum levels of sIL-6R (Galicia et al. 2004). As with the previous study, two of the seven polymorphisms described by Kim et al. (2003) in the Korean population were also genotyped this time: the −183 G/A in the promoter region and the +48,892 A/C in exon 9. The second locus corresponds to the proteolytic cleavage site of IL-6Rα and was linked to obesity and type 2 diabetes mellitus (Wolford et al. 2003, Hamid et al. 2004, Wang et al. 2005). In this paper, we studied the role of IL-6R gene polymorphisms in both aggressive (AgP) and chronic periodontitis (CP) in a Japanese population.

Material and Methods

Study population

Potential participants who were referred for treatment or routine check-up to the Periodontology Department of the Niigata University Medical and Dental Hospital were recruited and then interviewed. Those who fell under any one of the following criteria were excluded: (1) systemic illness, pregnancy or recent medication; (2) previous history of periodontal surgery or periodontal therapy in the past 6 months; (3) less than 20 natural teeth present; (4) a history of smoking; and (5) non-Japanese descent. A total of 422 subjects aged 22–77 years were finally selected to participate in this study. A description of the subjects is presented in Table 1. All procedures were performed in accordance with the Helsinki protocol and with the guidelines set by the Niigata University Ethical Committee. The nature and objectives of the study were carefully explained to each participant and an informed consent was obtained.

Table 1. Characteristics of periodontitis patients and control groups studied

Characteristics	CP ($n = 169$)	Healthy controls for CP ($n = 70$)	AgP ($n = 43$)	Healthy controls for AgP ($n = 140$)
Age (years)	55 ± 10.0 (37–76)	54 ± 9.9 (34–77)	32 ± 3.0 (25–35)	25 ± 3.0 (22–34)
Gender (male in %)	41	26	44	47
mPPD (mm)*	3.0 ± 1.2	2.0 ± 0.4	4.0 ± 1.1	1.9 ± 0.31
mPAL (mm)*	4.0 ± 1.5	2.2 ± 0.4	5.0 ± 1.3	1.9 ± 0.31
mBL (%)*,†	32 ± 13.1	11.6 ± 2.1	43 ± 12.8	–
%BL ⩾50*,†	18 ± 19.9	0.0 ± 0.28	40 ± 22.7	–

*Significantly different compared with controls ($p > 0.001$, Mann–Whitney *U*-test).
†Not measured in AgP controls.
Values represent the mean ± standard deviation.
mPPD, mean probing pocket depth; mPAL, mean probing attachment level; mBL, mean bone loss; %BL ⩾50, percentage of sites with bone loss equal to or more than 50%.

Clinical assessments

All subjects were evaluated clinically and radiographically at a first visit to assess the following periodontal parameters: number of teeth, probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), alveolar bone loss (BL), plaque index (PlI) and gingival index (GI). Six sites were examined on each tooth: mesio-buccal, buccal, disto-buccal, disto-lingual, lingual and mesio-lingual. Several calibrated examiners took the measurements and recorded the results. Full-mouth radiographs were also evaluated to examine alveolar bone levels (BL) on the mesial and distal aspect of the tooth. BL was measured from the cemento-enamel junction to the root apex at an arbitrary point on a radiograph, and expressed as a percentage of the total root length (Schei et al. 1959).

Selection of periodontitis cases was carried out according to the criteria set by the European Federation of Periodontology (Tonetti & Claffey 2005). In brief, periodontitis patients were grouped according to the two proposed threshold levels: patients with proximal attachment loss of ⩾3 mm in ⩾2 non-adjacent teeth (inclusive of incipient cases), and patients with proximal attachment loss of ⩾5 mm in ⩾30% of teeth present (with substantial extent and severity). Controls were healthy, age-matched individuals who did not conform to the criteria mentioned. Aggressive periodontitis patients were identified based on the clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions (Armitage 1999), using five clinical parameters and full-mouth or panoramic radiographs of diagnostic quality. Familial aggregation of the disease could not be confirmed; however, all patients presented the generalized form of AgP, with interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors and a CAL of ⩾5 mm at more than one site on ⩾8 teeth. In this paper, the term ''periodontitis'' means the sum of the AgP and CP patients included in the study.

DNA isolation and genotyping

The method described by Galicia et al. (2004) was used for DNA isolation and for genotyping of the two *IL6R* SNPs, namely the −183 G/A (rs4845617) in the promoter region and the +48892 A/C (rs8192284) in exon 9.

Joint analysis with IL6 gene polymorphisms for periodontitis risk

Scientific evidence clearly shows that IL-6 and IL-6R are functionally related in the same biological pathway (Kishi-

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Table 2. Distribution of the IL6R −183 G/A and +48,892 A/C allele and genotype in Japanese periodontitis patients and controls

		−183 G/A (rs4845617)			+48892 A/C (rs8192284)	
		Healthy controls*, $n = 210$ (%)	Periodontitis†, $n = 211$ (%)		Healthy controls*, *n = 210 (%)*	Periodontitis†, *n = 212 (%)*
Genotype frequency	*A/A*	47 (22.4)	49 (23.2)	*A/A*	76 (36.2)	70 (33)
	A/G	106 (50.5)	109 (51.7)	*A/C*	93 (44.3)	116 (54.7)
	G/G	57 (27.1)	53 (25.1)	*C/C*	41 (19.5)	26 (12.3)
p value		$p = 0.89$			$p = 0.05$	
Allele frequency	*A*	200 (47.6)	207 (49.1)	*A*	245 (58.3)	256 (60.4)
	G	220 (52.4)	215 (50.9)	*C*	175 (41.7)	168 (39.6)
p value		$p = 0.68$			$p = 0.54$	
Carriage rate	*A (+)*	153 (72.9)	158 (74.9)	*A (+)*	169 (80.5)	186 (87.7)
	A (−)	57 (27.1)	53 (25.1)	*A (−)*	41 (19.5)	26 (12.3)
	G (+)	163 (77.6)	162 (76.8)	*C (+)*	134 (63.8)	142 (67)
	G (−)	47 (33.4)	49 (23.2)	*C (−)*	76 (36.2)	70 (33)
p value (OR; 95% CI)		A: $p = 0.74$			A: $p = 0.04$ (1.73; 0.99-3.06)	
		G: $p = 0.84$			C: $p = 0.49$	

*Sum of the AgP and CP controls.
†Sum of the AgP and CP patients.
OR, odds ratio; CI, confidence interval.

moto 1992, Tamura et al. 1993, Yawata et al. 1993, Romano et al. 1997, Heinrich et al. 1998, Naruishi et al. 1999, 2001, Jones & Rose-John 2002). Hence, we hypothesized that genetic variations in *IL6R* and *IL6* could interact synergistically to predispose an individual to periodontitis. It was possible for us to carry out this joint analysis because the raw data from our previous report on *IL6* SNPs and periodontitis were still available (Komatsu et al. 2005). The same periodontitis cases and healthy controls were genotyped for both *IL6* and *IL6R* genes, although six healthy controls and nine periodontitis cases had to be disregarded because their DNA samples were no longer available during the genotyping of *IL6R*.

Table 3. Distribution of the IL6R −183 G/A allele and genotype in Japanese periodontitis patients and controls

	Healthy controls for AgP, $n = 140$ (%)	AgP, $n = 42$ (%)	Healthy controls for CP, $n = 70$ (%)	CP, $n = 169$ (%)
Genotype frequency				
A/A	28 (20)	10 (23.8)	19 (27.1)	39 (23.1)
A/G	72 (51.4)	23 (54.8)	34 (48.6)	86 (50.9)
G/G	40 (28.6)	9 (21.4)	17 (24.3)	44 (26)
p value	$= 0.64$		$= 0.80$	
Allele frequency				
A	128 (45.7)	43 (51.2)	72 (51.4)	164 (48.5)
G	152 (54.3)	41 (48.8)	68 (46.6)	174 (51.5)
p value	$= 0.38$		$= 0.56$	
Carriage rate				
A(+)	100 (71.4)	33 (78.6)	53 (75.7)	125 (74)
A(−)	40 (28.6)	9 (21.4)	17 (24.3)	44 (26)
G(+)	112 (80)	32 (76.2)	51 (72.9)	130 (76.9)
G(−)	28 (20)	10 (23.8)	19 (27.1)	39 (23.1)
p value	A: $p = 0.36$; G: $p = 0.59$		A: $p = 0.78$; G: $p = 0.50$	

Statistical analysis

A genetic statistical software (SNPalyze™ Pro ver. 3.2.3, Dynacom, Yokohama, Japan) was used to test for deviation of genotype frequencies from Hardy–Weinberg expectations (HWE) and for LD. Differences in the clinical parameter values between the *IL6R* −183 A/G and +48,892 A/C alleles were compared using the non-parametric Mann–Whitney *U*-test. The association of genotype frequency, allele frequency and high-risk and low-risk *IL6-IL6R* genotypes between cases and controls was determined by χ^2 analysis. Statistical analyses were performed by using the standard statistical software (StatView J-4.5 application program, SAS Institute Inc., Cary, NC, USA). Significance was set at 5% ($p < 0.05$). For results that generated a significant *p* value, the odds ratios (OR) were calculated with 95% confidence intervals (CIs).

Results

Genotype distributions of the *IL6R* −183 G/A SNP in cases and controls did not deviate from (HWE; $p > 0.05$). In the +48892 A/C SNP, all the control groups and the CP group were in HWE, but the total periodontitis and the AgP cases deviated from HWE ($p = 0.04$ and 0.005, respectively). The two sites appeared to be in LD ($|D'| = 0.53$, $\chi^2 = 175.2$, $p < 0.0001$), as also reported in previous studies (Kim et al. 2003, Galicia et al. 2004). Out of the 422 subjects enrolled and genotyped, 169 and 70 were classified as CP and non-CP, while 43 and 140 were categorized as AgP patients and their controls, respectively. As expected, clinical variables displayed significant differences between CP and AgP patients and their respective controls. The characteristics of the patient and control groups studied are shown in Table 1.

Prevalence of IL6R genotypes and alleles in the promoter region

Analysis of the genotype distribution of the *IL6R* −183 G/A (rs4845617) in periodontitis, AgP, CP and their respective controls did not reveal any signifi-

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Table 4. Distribution of the IL6R +48892 A/C allele and genotype in Japanese periodontitis patients and controls

	Healthy controls for AgP, $n = 140$ (%)	AgP, $n = 43$ (%)	Healthy controls for CP, $n = 70$ (%)	CP, $n = 169$ (%)
Genotype frequency				
A/A	52 (37.1)	12 (27.9)	24 (34.3)	58 (34.3)
A/C	66 (47.1)	29 (67.4)	27 (38.6)	87 (51.5)
C/C	22 (15.8)	2 (4.7)	19 (27.1)	24 (14.2)
p value	$= 0.04$		$= 0.04$	
Allele frequency				
A	170 (60.7)	53 (61.6)	75 (53.8)	203 (60.1)
C	110 (39.3)	33 (38.4)	65 (46.4)	135 (39.9)
p value	$= 0.80$		$= 0.19$	
Carriage rate				
A(+)	118 (84.3)	41 (95.3)	51 (72.9)	145 (85.8)
A(−)	22 (15.7)	2 (4.7)	19 (27.1)	24 (14.2)
C(+)	88 (62.9)	31 (72.1)	46 (65.7)	111 (65.7)
C(−)	52 (37.1)	12 (27.9)	24(34.3)	58 (34.3)
p value (OR; 95% CI)	A: $p = 0.06$; C: $p = 0.27$		A: $p = 0.02$ (2.25; 1.08–2.25) C: $p = 0.99$	

OR, odds ratio; CI, confidence interval.

cant results (Tables 2 and 3). The same outcome was observed in the analysis between healthy controls and periodontitis cases with substantial extent and severity as described by Tonetti and Claffey (2005) (data not shown).

Prevalence of IL6R genotypes and alleles in exon 9

Analysis of the *IL6R* +48892 A/C (rs8192284) genotype and allele distribution revealed a modest difference in the genotype frequency and in the carriage rate for the A allele between periodontitis patients and controls ($p = 0.05$ and $p = 0.04$, respectively; Table 2). The same observation was also noted in the genotype frequency ($p = 0.04$) and carriage rate for the A allele ($p = 0.02$) between CP and healthy controls (Table 4). In AgP, only the genotype frequency between the patients and controls was significant ($p = 0.04$; Table 4). The carriage rate for the A allele was significantly higher in the periodontitis and CP groups than in the controls ($p = 0.04$ and 0.02, respectively). However, the results indicated did not turn out to be statistically significant after Bonferroni's adjustment. But as this marker is in LD ($|D'| = 0.53$) with the −183 marker and the latter is not associated with periodontitis, a Bonferroni-type correction would be too conservative and it is best to simply report the disease-marker association results without correction (Nyholt 2001). No significant association was found between healthy controls and periodontitis cases with substantial extent and severity as described by Tonetti and Claffey (2005) (data not shown).

Joint IL6–IL6R analysis

The results indicate that the two genes do not interact to increase an individual's risk for periodontitis (data not shown).

Discussion

Among the seven polymorphisms described in the Korean population, we only genotyped two SNPs based on the importance of their locations and the degree of LD as described by Kim et al. (2003). Selecting only the more important loci within a group exhibiting strong LD is encouraged as it could reduce the cost of genotyping with little loss of information (Wang et al. 2005). Moreover, the *IL6R* +48892 A/C SNP described in this study is also represented in other ethnicities, promising a global applicability of this locus in terms of disease association (Wolford et al. 2003, Hamid et al. 2004, Wang et al. 2005).

Reports have clearly shown the important biological roles that both the IL-6Rα and sIL-6R play in regulating the immune response, including the IL-6 responses of cells related to the periodontium (Tamura et al. 1993, Müller-Newen et al. 1998, Naruishi et al. 1999, 2001). However, in vivo studies have demonstrated that instead of being an agonist, sIL-6R of the human plasma should be regarded as a protein that enables sgp130 to trap IL-6 efficiently, decreasing the bioavailability of the latter (Müller-Newen et al. 1998, Jostock et al. 2001).

In our recently published report, we have found that the *IL6R*+ 48892 A allele-negative subjects (C/C genotypes) had significantly higher serum levels of sIL-6R (Galicia et al. 2004). However, although the subjects with the C/C genotype have a higher soluble IL-6 receptor, this does not necessarily mean that their receptors containing the substituted amino acid could aid cells that do not express IL-6Rα trigger the IL-6-signalling cascade. Site-directed mutagenesis of *IL6R* barely affected IL-6 binding but it did abolish the IL-6-signalling capability of sIL-6R (Yawata et al. 1993). This could mean that the site-directed mutant receptors could only bind to IL-6, and not to the IL-6 signal-transducing protein, gp130, inhibiting cells to respond to IL-6.

Putting together our present case–control study results and the results of our previous study regarding the effect of *IL6R* polymorphisms on sIL-6R serum levels, the following statements could be hypothesized to suggest further studies in the future: (i) the presence of significantly higher A allele carriers in periodontitis and CP subjects could mean that this allele, comprising the normal amino acid codon for IL-6R, produces biologically active soluble and membrane-bound IL-6 receptors that could confer reactivity of cells to the pro-inflammatory action of IL-6; (ii) the non-A allele carriers, which are higher in the healthy controls than in the diseased subjects (27.1% *versus* 14.2% in CP and 15.7% *versus* 4.7% in AgP, respectively – Table 4), produce significantly higher amounts of mutant sIL-6R receptors that, based on studies, could potentiate the antagonistic capacity of sgp130 to IL-6 (Müller-Newen et al. 1998). Regulation of IL-6 in the periodontal tissue is one of the prospects in controlling biological events involved in the pathogenesis of periodontal disease (Takashiba et al. 2003).

In addition to the case–control analysis performed in the present study, a joint *IL6*/*IL6R* analysis was also carried out. However, the detailed method used in the joint analysis was not included. This is because we wanted to limit this paper to a case–control study and at the same time, explore the possibility that

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

an interaction could occur between *IL6* and *IL6R*. The results obtained suggest the possible role that the *IL6R* polymorphisms play in the susceptibility to periodontitis regardless of the disease classification and independent of *IL6*. However, the polymorphisms could not be associated with the disease severity.

Although only modest differences in the genotype frequencies and carriage rate between the cases and the controls were obtained, the importance of the biological effects arising from the polymorphisms studied substantiates the importance of our findings. Our present report is in accordance with studies of other IL-6-mediated conditions like type 2 diabetes mellitus and obesity (Wolford et al. 2003, Hamid et al. 2004). We strongly encourage further association and biological studies to investigate the effect of the *IL6R* polymorphisms on the activities of IL-6R and IL-6, as these could have consequences not only on the general but also on the periodontal immune responses.

Acknowledgements

This study was supported by Grant-in-Aids for Scientific Research (16592065, 16390612 and 17791553) from the Ministry of Education, Science, Sports and Culture of Japan, and by Tanaka Industries Co. Ltd. (Niigata, Japan).

References

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

Brett, P. M., Zygogianni, P., Griffiths, G.S, Tomaz, M., Parkar, M., D'Aiuto, F. & Tonetti, M. (2005) Functional gene polymorphisms in aggressive and chronic periodontitis. *Journal of Dental Research* **84**, 1149–1153.

D'Aiuto, F., Parkar, M., Brett, P. M., Ready, D. & Tonetti, M. S. (2004) Gene polymorphisms in pro-inflammatory cytokines are associated with systemic inflammation in patients with severe periodontal infections. *Cytokine* **28**, 29–34.

Galicia, J. C., Tai, H., Komatsu, Y., Shimada, Y., Akazawa, K. & Yoshie, H. (2004) Polymorphisms in the IL-6 receptor (IL-6R) gene: strong evidence that serum levels of soluble IL-6R are genetically influenced. *Genes and Immunity* **5**, 513–516.

Hamid, Y. H., Urhammer, S. A., Jensen, D. P., Glumer, C., Borch-Johnsen, K., Jorgensen, T., Hansen, T. & Pedersen, O. (2004) Variation in the interleukin-6 receptor gene associates with type 2 diabetes in Danish whites. *Diabetes* **53**, 3342–3345.

Heinrich, P. C., Behrmann, I., Müller-Newen, G., Schaper, F. & Graeve, L. (1998) Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochemical Journal* **334**, 297–314.

Holla, L. I., Fassmann, A., Stejskalova, A., Znojil, V., Vanek, J. & Vacha, J. (2004) Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients with chronic periodontitis. *Journal of Periodontology* **75**, 30–36.

Horiuchi, S., Ampofo, W., Koyanagi, Y., Yamashita, A., Waki, M., Matsumoto, A., Yamamoto, M. & Yamamoto , N. (1998) High-level production of alternatively spliced soluble interleukin-6 receptor in serum of patients with adult *T*-cell leukaemia/HTLV-I-associated myelopathy. *Immunology* **95**, 360–369.

Ishimi, Y., Miyaura, C., Jin, C. H., Akatsu, T., Abe, E., Nakamura, Y., Yamaguchi, A., Yoshiki, S., Matsuda, T. & Hirano, T. (1990) IL-6 is produced by osteoblasts and induces bone resorption. *Journal of Immunology* **145**, 3297–3303.

Jones, S. A., Horiuchi, S., Topley, N., Yamamoto, N. & Fuller, G. M. (2001) The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB Journal* **15**, 43–58.

Jones, S. A. & Rose-John, S. (2002) Soluble receptors in cytokine biology: the agonistic properties of the sIL-6R/IL-6 complex. *Biochimica et Biophysica Acta* **1592**, 251–263.

Jostock, T., Mullberg, J., Ozbek, S., Atreya, R., Blinn, G., Voltz, N., Fischer, M., Neurath, M. F. & Rose-John, S. (2001) Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *European Journal of Biochemistry* **268**, 160–167.

Kim, L. H., Lee, H. S., Kim, Y. J., Jung, J. H., Kim, J. Y., Park, B. L. & Shin, H. D. (2003) Identification of novel SNPs in the interleukin 6 receptor gene (IL6R). *Human Mutation* **21**, 450–451.

Kishimoto, T. (1992) Interleukin-6 and its receptor in autoimmunity. *Journal of Autoimmunity* **5**, 123–132.

Kishimoto, T. (2005) Interleukin-6: from basic science to medicine – 40 years in immunology. *Annual Review of Immunology* **23**, 1–21.

Komatsu, Y., Tai, H., Galicia, J. C., Shimada, Y., Endo, M., Akazawa, K., Yamazaki, K. & Yoshie, H. (2005) Interleukin-6 (IL-6)–373 A9T11 allele is associated with reduced susceptibility to chronic periodontitis in Japanese subjects and decreased serum IL-6 level. *Tissue Antigens* **65**, 110–114.

Lust, J., Donovan, K., Kline, M., Greipp, P., Kyle, R. & Maihle, N. (1992) Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. *Cytokine* **4**, 96–100.

Müllberg, J., Oberthür, W., Lottspeich, F., Mehl , E, Dottrich , E, Graeve, L., Heinrich, P. C. & Rose-John, S. (1994) The soluble human IL-6 receptor–mutational characterization of the proteolytic cleavage site. *Journal of Immunology* **15**, 4958–4968.

Müllberg, J., Schooltnik, H., Stoyan, T., Gunther, M., Graeve, L., Buse, G., Mackiewicz, A., Heinrich, P. C. & Rose-John, S. (1993) The soluble human interleukin-6 receptor is generated by shedding. *European Journal of Immunology* **23**, 473–480.

Müller-Newen, G., Kuster, A., Hemmann, U., Keul, R., Horsten, U., Martens, A., Graeve, L., Wijdenes, J. & Heinrich, P. C. (1998) Soluble IL-6 receptor potentiates the antagonistic activity of soluble gp130 on IL-6 responses. *Journal of Immunology* **161**, 6347–6355.

Modur, V., Li, Y., Zimmerman, G. A., Prescott, S. M. & McIntyre, T. M. (1997) Retrograde inflammatory signaling from neutrophils to endothelial cells by soluble interleukin-6 alpha. *Journal of Clinical Investigation* **100**, 2752–2756.

Naruishi, K., Takashiba, S., Chou, H. H., Arai, H., Nishimura, F. & Murayama, Y. (1999) Role of soluble interleukin-6 receptor in inflamed gingiva for binding of interleukin-6 to gingival fibroblasts. *Journal of Periodontal Research* **34**, 296–300.

Naruishi, K., Takashiba, S., Nishimura, F., Chou, H. H., Arai, H., Yamada, H. & Murayama, Y. (2001) Impairment of gingival fibroblast adherence by IL-6/sIL-6R. *Journal of Dental Research* **80**, 1421–1424.

Nyholt, D. R. (2001) Genetic case–control association studies – correcting for multiple testing. *Human Genetics* **109**, 564–565.

Romano, M., Sironi, M., Toniatti, C., Polentarutti, N., Fruscella, P., Ghezzi, P., Faggioni, R., Luini, W., van Hinsbergh, V., Sozzani, S., Bussolino, F., Poli, V., Ciliberto, G. & Mantovani, A. (1997) Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* **6**, 315–325.

Schei, O., Waerhaug, J., Lovdal, A. & Arno, A. (1959) Alveolar bone loss related to oral hygiene and age. *Journal of Periodontology* **30**, 7–16.

Snyder, L., Renauld, J. C., Szpirer, C. & Content, J. (1995) Cloning and chromosal localization of a pseudogene corresponding to an mRNA for a soluble IL-6R. *DNA Sequence* **5**, 311–314.

Szpirer, J., Szpirer, C., Riviere, M., Houart, C., Baumann, M., Fey, G. H., Poli, V., Cortese, R., Islam, M. Q. & Levan, G. (1991) The interleukin-6-dependent DNA-binding protein gene (transcription factor 5: TCF5) maps to human chromosome 20 and rat chromosome 3, the IL-6 receptor locus (IL-6R) to human chromosome 1 and the rat chromosome 2, and the rat IL-6 gene to rat chromosome 4. *Genomics* **10**, 539–546.

Takashiba, S., Naruishi, K. & Murayama, Y. (2003) Perspective of cytokine regulation for periodontal treatment: fibroblast biology. *Journal of Periodontology* **74**, 103–110.

Tamura, T., Udagawa, N., Takahashi, N., Miyaura, C., Tanaka, S., Yamada, Y., Koishihara, Y., Ohsugi, Y., Kumaki, K., Taga, T., Kishimoto, T. & Suda, T. (1993) Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. *Proceedings of the National Academy of Science USA* **90**, 11924–11928.

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Tonetti, M. S. & Claffey, N.European Workshop in Periodontology group C (2005) Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. *Journal Of Clinical Periodontology* **32** (Suppl. 6), 210–213.

Trevilatto, P. C., Scarel-Caminaga, R. M., de Brito, R. B. Jr, de Souza, A. P. & Line, S. R. (2003) Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. *Journal of Clinical Periodontology* **30**, 438–442.

Walev, I., Vollmer, P., Palmer, M., Bhakdi, S. & Rose-John, S. (1996) Pore-forming toxins trigger shedding of receptors for interleukin 6 and lipopolysaccharide. *Proceedings of the National Academy of Science USA* **93**, 7882–7887.

Wang, W. Y., Barratt, B. J., Clayton, D. G. & Todd, J. A. (2005) Genome-wide association studies: theoretical and practical concerns. *Nature Review Genetics* **6**, 109–118.

Wang, H., Zhang, Z., Chu, W., Hale, T., Cooper, J. J. & Elbein, S. C. (2005) Molecular screening and association analyses of the interleukin 6 receptor gene variants with type 2 diabetes, diabetic nephropathy, and insulin sensitivity. *Clinical Endocrinology and Metabolism* **90**, 1123–1129.

Wolford, J. K., Colligan, P. B., Gruber, J. D. & Bogardus, C. (2003) Variants in the interleukin 6 receptor gene are associated with obesity in Pima Indians. *Molecular Genetics and Metabolism* **80**, 338–343.

Yawata, H., Yasukawa, K., Natsuka, S., Murakami, M., Yamasaki, K., Hibi, M., Taga, T. & Kishimoto, T. (1993) Structure-function analysis of human IL-6 receptor: dissociation of amino acid residues required for IL-6-binding and for IL-6 signal transduction through gp130. *EMBO Journal* **12**, 1705–1712.

Address:
Hiromasa Yoshie
Division of Periodontology
Department of Oral Biological Science
Niigata University Graduate School of Medical and Dental Sciences
5274, Gakkocho-Dori 2-ban-cho
Niigata 951-8514
Japan
E-mail: yoshie@dent.niigata-u.ac.jp

Clinical Relevance

Scientific rationale: Polymorphisms in various candidate genes have been associated with periodontitis. Recently, type 2 diabetes mellitus and obesity have been linked to *IL6R* polymorphisms. Like periodontitis, diabetes and obesity are also complex diseases.

Principal findings: The results of this study are in accordance with the findings in diabetes and obesity. Although the +48892 A/C SNP in *IL6R* displayed a modest association with periodontitis, the biological effects of the *IL6R* polymorphism mentioned substantiate the importance of this finding.

Practical implications: The *IL6R* +48892 A/C polymorphism could be included as one of the candidate markers of periodontitis risk.

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.