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Interleukin-2 −330 and 166 gene polymorphisms in relation to aggressive or chronic periodontitis and the presence of periodontopathic bacteria

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Background and Objective: As a pro-inflammatory cytokine, interleukin-2 mediates the activation, growth and differentiation of T and B lymphocytes and natural killer cells. Promoter polymorphisms of the interleukin-2 gene have been associated with altered interleukin-2 production or identified as prognostic markers for various infectious diseases. Therefore, the aim of this study was to evaluate two polymorphisms at positions −330 T/G and 166 G/T in patients with generalized chronic periodontitis ($n = 58$) or generalized aggressive periodontitis ($n = 73$) in comparison with periodontitis-free controls ($n = 69$).

Material and Methods: Both interleukin-2 polymorphisms were analyzed using the polymerase chain reaction with sequence-specific primers. Distributions of single alleles, genotypes and haplotypes were calculated using the chi-square test. Risk factor analyses were carried out by logistic regression with respect to established cofactors for periodontitis. The presence of subgingival bacteria in an individual were analyzed using a molecular biological method (the micro-Ident® test).

Results: The interleukin-2 genotype −330 TG occurred less frequently in patients with chronic periodontitis (25.9% vs. 49.3%). Moreover, this genotype decreased the adjusted odds ratio for chronic periodontitis (odds ratio = 0.394), whereas the interleukin-2 genotype 166 TT and the haplotype combination interleukin-2 −330,166 TT : TT were associated with an increased adjusted odds ratio (odds ratio = 2.82 or 2.97). For the latter interleukin-2 combination, a positive association for the subgingival presence of *Porphyromonas gingivalis* (81.3% vs. 59.5%) and bacteria of the 'red complex' (78.1% vs. 56.0%) was shown.

Conclusion: The interleukin-2 genotypes −330 TG and 166 TT, as well as the combination genotype interleukin-2 TT : TT, could be putative prognostic factors for chronic periodontitis.

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Periodontitis is a chronic infectious disease of the supporting tissues of the teeth (1). However, most of the bacterial species isolated from subgingival plaque are indigenous to the oral cavity (2). Moreover, the risk for periodontal diseases is not uniform for all individuals (3). For instance, only 10–15% of the general United States population develops severe, destructive forms of periodontal diseases (4). Beside environmental effects and behavioural patterns such as dental care, socioeconomic status and tobacco consumption, it is assumed that approximately 50% of the population variance for periodontitis may be attributed to genetic factors (5). Several cytokine polymorphisms have been identified as disease-modifying genetic variants (6). On the basis of these polymorphisms there are striking differences between individuals in their production of cytokines and, consequently, in their response to periodontopathic antigens, as well as in their individual ability to affect both the growth and the virulence of various bacterial species (7–11).

Interleukin-2 is a 15 kD glycoprotein that is mainly produced by $CD4^+$ T cells. However, mouse splenic B cells and B-cell tumor lines may also produce interleukin-2 when activated with anti-immunoglobulin (12). Interleukin-2 plays a central role in the initiation and development of an immune response to antigen through its direct effect, via specific receptors, on the activation, growth and differentiation of T and B lymphocytes and natural killer cells (13–15).

There is evidence that interleukin-2 may be a relevant factor in the pathway of periodontal disease. For instance, in comparison with control subjects, the concentrations of interleukin-2 and of the soluble interleukin-2 receptor were found to be elevated in the sera of patients with juvenile or severe generalized periodontitis (16). Moreover, the expression rate of the interleukin-2 receptor on T-helper cells and B cells was highest in gingival crevicular flow, lower in gingival tissue and lowest in peripheral blood (17). Furthermore, interleukin-2 upregulates the expression of intercellular adhesion molecule-1 on the surface of human gingival fibroblasts and consequently the adhesion of neutrophils is also increased (18). In animal *in vitro* experiments it was shown that the release of interleukin-2 by mononuclear cells could be activated by a fimbrial protein of *Porphyromonas gingivalis* (19). Moreover, interleukin-2 induced an elevation in the production of osteoclastic acid, corresponding to an increased resorption of bone (20).

Various polymorphisms in the genes encoding cytokines were found to be functionally relevant and to cause differences in the immunoregulatory activity of their cytokine molecules (6). Two interleukin-2 gene polymorphisms at positions −330 (T → G) and 166 (G → T) were identified by John *et al.* 1998 (21). Anti-CD3/CD28-stimulated peripheral lymphocytes of individuals who were homozygous for the interleukin-2 −330 GG gene polymorphism showed an early and sustained *in vitro* production of interleukin-2 (22). Moreover, the interleukin-2 −330 polymorphism was indicative for infectious diseases such as Brucellosis (23) and gastric atrophy caused by *Heliobacter pylori* (24), as well as diseases with an autoimmune background such as Takayasu's arteritis (25) or multiple sclerosis (26). Furthermore, the presence of at least one interleukin-2 −330 G allele was associated with an increased risk of acute graft-vs.-host disease after allogeneic bone marrow transplantation (27).

To date, only one study has been carried out (in an ethnically mixed Brazilian cohort of patients) to investigate the −330 T → G polymorphism in patients with chronic periodontitis (28). The authors revealed a lower frequency of both the T allele and the genotype TT in patients with severe chronic periodontitis. The polymorphism at position 166 and patients with aggressive periodontitis were not investigated. Therefore, the first purpose of the present study was to investigate putative associations of the interleukin-2 gene polymorphisms at positions −330 and 166 in German Caucasoid subjects suffering from aggressive or chronic periodontitis in comparison with periodontitis-free controls. Allele, genotype and haplotype frequencies were considered. Multivariate analyses, including established confounders for periodontitis, were performed to generate adjusted odds ratios for a certain cytokine polymorphism. The second aim of the study was to examine whether polymorphisms of interleukin-2 were associated with the detection of *Aggregatibacter actinomycetemcomitans*, *P. gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis* and *Treponema denticola*.

Material and methods

Study population and clinical investigations

The study was approved by the local ethics committee and was carried out in accordance with the ethical guidelines of the 'Declaration of Helsinki' and its amendment in 'Tokyo and Venice'. The study was performed at the Department of Operative Dentistry and Periodontology of the Martin-Luther-University Halle-Wittenberg from March 2005 to February 2008. Seventy-three patients with generalized aggressive periodontitis, 58 patients with generalized severe chronic periodontitis and 69 periodontitis-free controls were included. All individuals were unrelated Germans of Caucasian descent. Exclusion criteria for all participants were pregnancy, the use of antibiotics during the last 6 mo or the chronic usage of anti-inflammatory drugs. Furthermore, persons with drug-induced gingival hyperplasia, a history of human immunodeficiency virus infection or acute intra-oral infection (e.g. herpetic gingivostomatitis) were excluded. Other criteria for exclusion were: oral pemphigus or pemphigoid; Beçet disease; type I or type II diabetes mellitus; coronary heart disease; rheumatic diseases; lupus erythematodes; and Crohn's disease.

During the anamnesis, the participants were questioned regarding the occurrence of other general diseases, drug consumption and their smoking status. Each person who smoked at least one cigarette per day was considered a smoker. In order to assess a

putative family aggregation of periodontitis, all study participants were asked whether their parents, siblings or children lost their teeth early as a consequence of periodontitis. Moreover, all periodontitis patients were asked whether symptoms of periodontitis, such as bleeding, swelling of the gingiva, pocket formation, increased tooth mobility, etc., occurred before they reached 35 years of age. The clinical assessment included determination of the approximal plaque index (29), bleeding on probing, pocket depth, and clinical attachment loss.

For determination of the approximal plaque index, all teeth were stained using a plaque relevator (Mira-2-Ton; Hager & Werken, Duisburg, Germany). After the patient had rinsed their mouth with water, the presence of plaque in the approximal spaces was registered from the oral aspect of the first and third quadrants and from the buccal aspect of the second and fourth quadrants. The number of positive findings was divided by the number of measurements to yield a per cent plaque index.

For each tooth, both the maximal clinical pocket depth (the distance between the gingival margin and the bottom of the pocket) and the maximal clinical attachment loss (the distance between the cemento–enamel junction and the bottom of the pocket) were derived by measuring six sites around each tooth and recording the maximum values. In order to determine the extent of periodontitis, in one person the percentage of teeth with clinical attachment loss values of 4–6 mm and > 6 mm were recorded.

As previously described (30) all patients and controls were assessed in accordance with the new classification system for periodontal diseases (31). In particular, patients with generalized chronic periodontitis were selected if they showed an attachment loss in at least 30% of the teeth with a minimum pocket depth of 4 mm. The amount of attachment loss was consistent with the presence of mineralized plaque. More horizontal than vertical approximal bone loss was visible in X-rays. Patients with generalized aggressive periodontitis were included only when evidence was available (from dental history and/or radiographs) that the onset of the disease occurred before the age of 35 years, who had attachment loss of ≥ 4 mm in at least 30% of their teeth and (in order to exclude localized aggressive periodontitis) in whom at least three teeth were affected in addition to the first molars or incisors. Unlike chronic periodontitis, the severity of attachment loss was inconsistent with the amount of mineralized plaque, and more vertical than horizontal approximal bone loss was visible in the X-rays. Periodontitis-free individuals were included if they were at least 30 years old and had no attachment loss (probing depth ≤ 3.5 mm and no gingival recession as a result of periodontitis). Clinical attachment loss of > 3.5 mm as a consequence of traumatic toothbrushing or extensive overhanging subgingival dental fillings was not considered to be a case of periodontitis.

Molecular assessment of periodontopathic bacteria

To avoid any reduction in the bacterial count and, consequently, false-negative results, microbial samples were taken before subgingival scaling was performed. The microbial samples were taken from the deepest pocket of each quadrant by insertion of a sterile paper point for 20 s. For each subject the mean pocket depth of these test sites (pocket $\text{depth}_{\text{Bacteria}}$) was recorded. All bacterial plaque samples of each individual were pooled in one tube. Preparation of bacterial DNA was carried out using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's manual. For specific amplification of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythensis* and *T. denticola*, the micro-Ident® test (Hain Lifescience, Nehren, Germany), which is based on an alkaline phosphatase-mediated staining reaction, was used. Polymerase chain reaction (PCR) amplification was performed (5 min at 95°C; 10 cycles of 30 s at 95°C, 2 min at 58°C, 40 s at 70°C; 20 cycles of 25 s at 95°C, 40 s at 53°C, 40 s at 70°C; and a final incubation of 8 min at 70°C) in a personal cycler (Biometra, Göttingen, Germany). The PCR products were hybridized to a strip containing DNA sequences of each bacterium as well as positive controls for amplification and hybridization. The presence of bacteria was determined visually by means of colored bands.

Genetic assessment of the polymorphisms interleukin-2 −330 T/G and interleukin-2 166 G/T

Preparation of genomic DNA from fresh human venous EDTA-anticoagulated blood was carried out using the QIAamp® blood extraction kit (Qiagen), according to the manufacturer's instructions. Both polymorphisms were detected using the CYTOKINE Genotyping array PCR-SSP Tray kit of the Collaborative Transplant Study (University of Heidelberg, Germany). For each PCR, an 89-bp fragment of the β-globin gene was co-amplified as a positive control. The PCR amplifications were performed using sequence-specific primers to detect possible haplotypes, prepipetted and lyophilized in thin-walled plastic 96-well PCR trays. For each PCR, 10 μL of a Mastermix containing 1 U of Taq-Polymerase (Invitek, Berlin, Germany), 100 ng of genomic DNA, 5% glycerol and PCR reaction buffer was added. PCR was performed on a Mastercycler Gradient (Eppendorf, Hamburg, Germany). The following cycling conditions were used: an initial denaturation of 2 min at 94°C; 10 cycles of 15 s at 94°C, 1 min at 64°C, 30 s at 72°C; and 20 cycles of 15 s at 94°C, 50 s at 61°C and 30 s at 72°C. After separation of the PCR products by electrophoresis though a 2% agarose gel containing ethidium bromide (0.5 μg/mL), the haplotype-specific pattern of bands became visible on a cross-linker (Roth, Karlsruhe, Germany) when viewed under ultraviolet light (312 nm). The 562-bp fragment corresponded to the haplotype interleukin-2 TG (T at position −330, G at position 166). The 564-bp fragment corresponded to the haplotype interleukin-2 GG (G at position −330, G at position 166). The haplotypes interleukin-2 GT (G at position −330, T at

position 166) and TT (T at position −330, T at position 166) corresponded to a band of 569 bp.

Statistical analysis

The SPSS 15.0 package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Clinical variables were described in terms of means ± standard deviation. The equality of variances was checked using Levenes test, and *t*-tests were performed for bivariate comparisons.

The genotype distributions of the investigated polymorphisms were tested according to the Hardy–Weinberg equilibrium. Genotype and allele frequencies were calculated by direct counting and were then divided by the number of subjects to produce genotype frequency, or were divided by the number of chromosomes to produce allele frequency. Differences between patients and controls were checked using the chi-square test and Yates' continuity correction (p_Y). If $n < 5$, Fisher's exact test was performed (p_F). For multiple comparisons, significant deviations were additionally tested by Bonferroni correction (p_B). Stepwise binary logistic regression (backwards elimination analysis) with respect of the cofactors bleeding on probing, gender, age and smoking was used in order to determine adjusted odds ratios of interleukin-2 genotypes, or combinations, for the presence of periodontitis. A variable was excluded if the significance of the chance from the previous step exceeded $p = 0.10$. In general, *p*-values of ≤ 0.05 were accepted as being statistically significant.

Results

Clinical data

Patients with aggressive periodontitis (Table 1) were significantly younger than the periodontitis-free controls ($p = 0.001$). There were no significant differences between patients and controls regarding the mean percentage of current smokers and the percentage of women. Among their relatives, both groups of patients reported significantly more often early tooth loss as a consequence of periodontitis ($p < 0.001$). Moreover, in the two periodontitis study groups, all clinical conditions were significantly more pronounced than in the control group ($p < 0.001$). However, there was no significant difference between patients with aggressive periodontitis and controls with respect to the approximal plaque index. Additionally, *A. actinomycetemcomitans* was not significantly more prevalent among patients with chronic periodontitis in comparison with the control group. In aggressive periodontitis, as in chronic periodontitis, the mean percentage of teeth with clinical attachment loss of ≥ 6 mm was about 30%. The mean values for both pocket depth and clinical attachment loss were > 5 mm. These results indicate a generalized severe attachment loss for both patient groups.

Interleukin-2 −330 T/G polymorphism

The distribution of the interleukin-2 genotypes −330 TT, TG and GG fulfilled the criteria of the Hardy–Weinberg disequilibrium among the patients with aggressive periodontitis and the group of healthy controls. However, the distribution of these

Table 1. Clinical characteristics of the groups of patients (aggressive periodontitis and chronic periodontitis) and the healthy control group (no periodontitis)

Variable	Aggressive periodontitis ($n = 73$)	Chronic periodontitis ($n = 58$)	Healthy controls ($n = 69$)
Clinical parameters			
Age ± SD (years)	40.9 ± 9.8*	49.1 ± 9.6	46.7 ± 10.7
Women, *n* (%)	46 (63.0)	38 (65.5)	38 (55.1)
Current smokers, *n* (%)	24 (32.9)	12 (20.7)	15 (21.7)
Early tooth loss as a result of periodontitis within the family, *n* (%)	35 (47.9)*	22 (37.9)*	6 (8.7)
Approximal plaque index, mean % ± SD	53.4 ± 28.9	60.9 ± 26.6*	45.9 ± 20.7
Bleeding on probing, mean % ± SD	78.3 ± 23.2*	69.4 ± 25.1*	44.7 ± 24.4
Probing depth (mm), mean ± SD	6.4 ± 5.6*	5.3 ± 1.1*	2.6 ± 0.8
Clinical attachment loss (mm), mean ± SD	6.6 ± 1.5*	6.0 ± 1.4*	2.9 ± 0.9
Teeth with CAL 4–6 mm, mean % ± SD	40.3 ± 21.3*	46.8 ± 21.0*	15.0 ± 17.4
Teeth with CAL >6 mm (%), mean % ± SD	47.6 ± 26.1*	38.8 ± 26.5*	0.1 ± 0.9
Missing teeth, mean ± SD	4.0 ± 3.9*	4.2 ± 3.3*	2.6 ± 3.0
Individual occurrence of periodontal bacteria in subgingival pockets			
Aggregatibacter actinomycetemcomitans, *n* (%)	29 (39.7)*	19 (32.8)	15 (21.7)
Porphyromonas gingivalis, *n* (%)	58 (79.5)*	51 (87.9)*	17 (24.6)
Prevotella intermedia, *n* (%)	47 (64.4)*	36 (62.1)*	21 (30.4)
Tannerella forsythensis, *n* (%)	65 (89.0)*	56 (96.6)*	45 (65.2)
Treponema denticola, *n* (%)	64 (87.8)*	57 (98.3)*	42 (60.9)
Porphyromonas gingivalis. + *Tannerella forsythensis* + *Treponema* denticola, *n* (%)	54 (74.0)*	48 (82.8)*	17 (24.6)

CAL, clinical attachment loss; SD standard deviation.
*$p \leq 0.05$ in comparison with the control group.

Table 2. Allele and genotype frequencies of the interleukin-2 polymorphism T → G at position −330 among patients with chronic periodontitis (CP) or aggressive periodontitis (AP) compared with healthy controls

Interleukin-2 −330 T/G	Aggressive periodontitis *n* (%)	Chronic periodontitis *n* (%)	AP + CP *n* (%)	Healthy controls *n* (%)	AP vs. controls p_c	CP vs. controls p_c	AP + CP vs. controls p_c
Allele	*n* = 146	*n* = 116	*n* = 262	*n* = 138			
T	94 (64.4)	85 (73.3)	179 (68.3)	96 (69.6)	NS	NS	NS
G	52 (35.6)	31 (26.7)	83 (31.7)	42 (30.4)	NS	NS	NS
Genotype	*n* = 73	*n* = 58	*n* = 131	*n* = 69			
TT	29 (39.7)	35 (60.3)	64 (48.9)	31 (44.9)	NS	NS	NS
TG	36 (49.3)	15 (25.9)	51 (38.9)	34 (49.3)	NS	0.012	NS
GG	8 (11.0)	8 (13.8)	16 (12.2)	4 (5.8)	NS	NS	NS

p_c, corrected (Yates or Fisher); NS, not significant ($p_c > 0.05$).

genotypes within the group of patients with chronic periodontitis did deviate significantly from the Hardy–Weinberg equilibrium.

Compared with the periodontitis-free controls, the interleukin-2 −330 TG genotype (Table 2) was less common in patients with chronic periodontitis. This result remained significant after Bonferroni correction ($p_B = 0.036$).

Interleukin-2 166 G/T polymorphism

The distribution of interleukin-2 GG, GT and TT fulfilled the criteria of the Hardy–Weinberg disequilibrium among all cohorts investigated. Moreover, there was a trend towards an increased frequency of IL-2 166 TT among patients with chronic periodontitis. This result did not remain significant after Yates' or Bonferroni correction (Table 3).

Interleukin-2 haplotypes and haplotype combinations

The frequencies of the four investigated interleukin-2 haplotypes −330,166 TG, GG, GT and TT were not significantly different among the groups of patients in comparison with the healthy controls. The haplotype combination interleukin-2 −330,166 TT : TT was increased among patients with chronic periodontitis (Table 4), but was not significant after Bonferroni correction ($p_B = 0.234$). Moreover, there was a trend towards a higher occurrence of interleukin-2 −330,166 GG : GG in all patient groups and a trend towards a lower frequency of interleukin-2 −330,166 GG : TT among patients with chronic periodontitis. The rare combinations interleukin-2 −330,166 GG : GT, GG : TG, GT : TT and TT : TG were found only once among periodontitis-free controls.

Backwards binary logistic regression analysis

With respect to the cofactors age, gender, smoking and bleeding on probing, the occurrence of the interleukin-2 genotype 166 TT or the expression of the combination interleukin-2 −330, 166 TT : TT in an individual increased the adjusted odds ratio for chronic periodontitis, while carriers of interleukin-2 −330 TG had a decreased adjusted odds ratio for chronic periodontitis. Besides certain interleukin-2 genotypes or combinations, bleeding on probing increased slightly the odds ratio for chronic periodontitis (Table 5). None of the interleukin-2 genotypes could be identified as a risk or resistance indicator for aggressive periodontitis or periodontitis in general (aggressive periodontitis + chronic periodontitis group).

Interleukin-2 genotypes and combinations in relation to periodontopathic bacteria

Among the total study group, *P. gingivalis* (Table 6) and bacteria of the 'red-complex', namely *P. gingivalis*, *T. forsythensis* and *T. denticola*, were found to occur at a significantly higher frequency in interleukin-2 −330,166 TT:TT-positive individuals. Moreover, there was a trend that subjects who expressed interleukin-2 166 TT were more often infected with *P. gingivalis*

Table 3. Allele and genotype frequencies of the interleukin-2 polymorphism G → T at position 166 among patients with chronic periodontitis (CP) or aggressive periodontitis (AP) compared with healthy controls

Interleukin-2 166 G/T	Aggressive periodontitis *n* (%)	Chronic periodontitis *n* (%)	AP + CP *n* (%)	Healthy controls *n* (%)	AP vs. controls p_c	CP vs. controls p_c	AP + CP vs. controls p_c
Allele	*n* = 146	*n* = 116	*n* = 261	*n* = 138			
G	101 (69.2)	65 (56.0)	166 (63.4)	87 (63.0)	NS	NS	NS
T	45 (30.8)	51 (44.0)	96 (36.6)	51 (37.0)	NS	NS	NS
Genotype	*n* = 73	*n* = 58	*n* = 131	*n* = 69			
GG	36 (49.3)	23 (39.7)	59 (45.0)	27 (39.1)	NS	NS	NS
GT	29 (39.7)	19 (32.8)	48 (36.6)	33 (47.8)	NS	NS	NS
TT	8 (11.0)	16 (27.6)	24 (18.3)	9 (13.0)	NS	NS	NS

p_c, corrected (Yates or Fisher); NS, not significant ($p_c > 0.05$).

Table 4. Distribution of interleukin-2 haplotype combinations, arranged as genotype frequencies, found among patients with chronic periodontitis (CP) or aggressive periodontitis (AP) compared with healthy controls

Interleukin-2 −330,166: −330,166	Aggressive periodontitis *n* (%)	Chronic periodontitis *n* (%)	AP + CP *n* (%)	Healthy controls *n* (%)	AP vs. controls p_c	CP vs. controls p_c	AP + CP vs. controls p_c
Combinations	*n* = 73	*n* = 58	*n* = 131	*n* = 69			
GG : GG	8 (11.0)	8 (13.8)	16 (12.2)	3 (4.3)	NS	NS	NS
GG : TT	18 (24.7)	7 (12.1)	25 (19.1)	16 (23.2)	NS	NS	NS
TG : GG	18 (24.7)	8 (13.8)	26 (19.8)	16 (23.2)	NS	NS	NS
TG : TG	10 (13.7)	7 (12.1)	17 (13.0)	7 (10.1)	NS	NS	NS
TG : TT	11 (15.1)	12 (20.7)	23 (17.6)	15 (21.7)	NS	NS	NS
TT : TT	8 (11.0)	16 (27.6)	24 (18.3)	8 (11.0)	NS	0.039	NS
GG : GT	0	0	0	1 (1.4)	NS	NS	NS
GG : TG	0	0	0	1 (1.4)	NS	NS	NS
GT : TT	0	0	0	1 (1.4)	NS	NS	NS
TT : TG	0	0	0	1 (1.4)	NS	NS	NS

p_c, corrected (Yates or Fisher); NS, not significant ($p_c > 0.05$).

Table 5. Backward stepwise binary logistic regression carried out to determine the genetic impact of the genotypes interleukin-2 −330 TG (model 1), interleukin-2 166 TT, and the combination interleukin-2 −330,166 TT : TT, for chronic periodontitis with respect to the cofactors age, gender, smoking and bleeding on probing (only variables with significant odds ratios are shown)

Interleukin-2 genotype/combination vs. Others	Regression coefficient	SE	*p*-values	Odds ratio	95% CI lower	upper
Model 1						
Interleukin-2 −330 TG	−0.932	0.432	0.031	0.394	0.169	0.918
Bleeding on probing	0.038	0.009	< 0.0001	1.039	1.022	1.057
Model 2						
Interleukin-2 166 TT	1.037	0.542	0.048	2.820	1.010	7.875
Bleeding on probing	0.039	0.008	< 0.0001	1.040	1.023	1.057
Model 3						
Interleukin-2 TT:TT	1.091	0.532	0.040	2.977	1.040	8.540
Bleeding on probing	0.039	0.008	< 0.0001	1.040	1.023	1.057

CI, confidence interval; IL, interleukin; SE, standard error.

Table 6. Detection frequencies of *Porphyromonas gingivalis* and bacteria of the 'red complex' (*P. gingivalis* + *Tannerella forsythensis* + *Treponema denticola*) in dependence of the expression of certain interleukin-2 genotypes and haplotype combinations

Genotype/combination	*P. gingivalis* *n* (%)	Red complex *n* (%)
Interleukin-2 −330 TG vs.	49 (57.6)	44 (51.8)
Interleukin-2 −330 TT or GG	77 (67.0)	75 (65.2)
p_c	NS	NS
Interleukin-2 166 TT vs.	26 (78.8)	25 (75.8)
Interleukin-2 166 GT or GG	100 (59.5)	94 (56.3)
p_c	NS	NS
Interleukin-2 TT:TT vs.	26 (81.3)	25 (78.1)
other combinations	100 (59.5)	94 (56.0)
p_c	0.033	0.032

p_c, corrected (Yates or Fisher); NS, not significant ($p_c > 0.05$).

and bacteria of the 'red complex'. Additionally, there was a tendency for a decreased occurrence of *P. gingivalis* and bacteria of the 'red complex' in interleukin-2 −330 TG-positive subjects.

Discussion

Interleukin-2 has been identified as an important cytokine in the immune response against periodontopathogens (17,19). Moreover, the promoter polymorphism −330 T → G of the interleukin-2 gene was found to be associated with the amount of cytokine produced. In *in vitro* experiments, T lymphocytes from −330 GG homozygous subjects were able to produce higher amounts of interleukin-2 than T lymphocytes from −330 TG heterozygous or −330 TT homozygous individuals (32). For the interleukin-2 166 G → T polymorphism, no association with the amount of cytokine produced was described. However, this single nucleotide polymorphism was associated with susceptibility to subacute sclerosing panencephalitis and multiple myeloma (33,34).

This study was designed to investigate whether the polymorphisms interleukin-2 −330 T → G and 166 G → T could be putative risk or resistance indicators for aggressive or chronic periodontitis. In addition, a

further aim of the study was to show whether the expression of a certain interleukin-2 genotype was associated with an infection with key periodontopathogens.

In the bivariate analyses we obtained a tendency of an increased frequency of the genotypes interleukin-2 −330 GG, interleukin-2 −330 TT and interleukin-2 166 TT among patients with chronic periodontitis, whereas the interleukin-2 −330 TG genotype occurred significantly less frequently. In the multivariate analyses the associations of interleukin-2 −330 TG, interleukin-2 166 TT and interleukin-2 −330 166 TT : TT were statistically significant after adjustment for established cofactors for periodontitis, such as age (35,36), gender (36,37), bleeding on probing (38–40) and smoking (41–43). Thus, the interleukin-2 −330 TG genotype could be a putative indicator for a lower susceptibility to chronic periodontitis, whereas interleukin-2 166 TT and the combination interleukin-2 −330 166 TT : TT could be associated with a higher risk for the disease. The associations revealed suggest that polymorphisms in the interleukin-2 gene influence cytokine release and, in consequence, the immune response against periodontopathogens. In this context it is noteworthy that the combination interleukin-2 −330 166 TT : TT was also significantly associated with the occurrence of *P. gingivalis* or bacteria of the 'red complex' in individuals. Moreover, another study revealed that the genotype interleukin-2 −330 TT was associated with gastric atrophy as a result of *H. pylori* infection (24). Apparently, subjects who are carriers of 'low producer' genotypes, such as interleukin-2 −330 TT, have a higher risk of infection with certain bacteria. This knowledge could be important for the identification of at-risk patients and the employment of new therapeutic approaches. For instance, recombinant interleukin-2 administrated to patients with lepromatous leprosy (44) achieved clinically successful results.

To date, no functional relevance for the interleukin-2 166 GT polymorphism has been shown. However, a recent study reports that the interleukin-2 −330 + 160 TG haplotype is more frequent in patients with subacute sclerosing panencephalitis, which is caused by mutated measles viruses variants (33). Furthermore, the interleukin-2 166 genotype GT was found to be associated with multiple myeloma (34). Nevertheless, the reasons for the association of the interleukin-2 166 GT polymorphism with chronic periodontitis remain unclear. It can be speculated that this polymorphism is in linkage disequilibrium with unknown susceptibility genes for periodontitis or other infectious diseases.

The results yielded in the present study were not supported by those of the study of Scarel-Caminaga *et al.* 2002 (28), and this may be explained by differences in the study design. For instance, Scarel-Caminaga *et al.* compared patients with severe chronic periodontitis to a mixed group of patients with moderate periodontitis and with periodontitis-free controls. Moreover, in all investigated groups the number of women was at least double that of the number of men. In addition, the groups were inhomogeneous regarding their ethnic origin.

Besides the investigated interleukin-2 polymorphisms, gingivitis assessed as bleeding on probing increased slightly the odds ratio for chronic periodontitis. This result is supported by a longitudinal study which shows that an increased gingivitis index is a value predictor for further attachment loss (40).

In summary, the results of the present study revealed an association of both interleukin-2 polymorphisms investigated with the occurrence of chronic periodontitis. The results were adjusted for established cofactors for periodontitis, such as age, gender, smoking and bleeding on probing. Moreover, the haplotype combination interleukin-2 −330 166 TT : TT was associated with infection with *P. gingivalis* and bacteria of the 'red complex'.

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