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Single-nucleotide polymorphisms in the IL-4 and IL-13 promoter region in aggressive periodontitis

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Abstract

Introduction: IL-4 and IL-13 polymorphisms have been shown to influence the susceptibility to systemic diseases. In this study, possible associations between the IL-4 − 590 C → T, IL-4 − 34 C → T, IL-13 − 1112 C → T and IL-13 − 1512 A → C promoter polymorphisms were investigated in subjects with generalized aggressive periodontitis (AgP) compared with healthy individuals.

Material and Methods: Fifty-eight patients with diagnosis of generalized AgP and 51 matched healthy controls participated in the study. Blood samples were collected and DNA isolated. Molecular analyses were performed by PCR-RFLP in a blind fashion. Genotype and allele frequencies among study groups were compared using Fisher's exact test (α value: 0.05). Pearson's χ^2 test was used for analysis of Hardy–Weinberg equilibrium.

Results: The frequency of the IL-4 − 590 T/T and IL-4 − 34 T/T genotypes differed significantly between groups ($p = 0.05$, 0.02, respectively), although the allele frequencies were similar. There was a higher frequency of the IL-4 − 590 T/T and IL-4 − 34 T/T genotypes in patients with AgP compared with controls. The genotype and allele frequencies of the IL-13 polymorphisms did not differ between groups.

Conclusions: This study demonstrated an association between the IL-4 − 590 T/T and IL-4 − 34 T/T genotypes and AgP. Further research is necessary to prove if there is an association of these polymorphisms with AgP, and if the polymorphisms have a functional effect.

Key words: aggressive periodontitis; genetic polymorphisms; interleukin-4; interleukin-13; risk factors

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Periodontitis is an inflammatory disease characterized by immune cell infiltration into the gingival tissues, leading to connective tissue destruction, attachment loss and alveolar bone resorption (Page & Kornman 1997). Although periodontal bacteria are the causative agents in periodontitis, subsequent progression and disease severity are determined by the host immune response in which many cell types, notably polymorphonuclear leucocytes, macrophages, lymphocytes and fibroblasts, are involved (Meyle 1993, Offenbacher 1996). Functional defects or deficient numbers of PMN have profound effects on the host's susceptibility to periodontitis (Page & Beck 1997, Oyaizu et al. 2003).

A significant number of T-cells producing a range of cytokines are found in local lesions and in peripheral blood of periodontitis patients (Kobayashi et al. 2000, Yamazaki et al. 2000, Petit et al. 2001, Nakajima et al. 2005). Dichotomy in the pattern of this cytokine production and hence the balance between T-helper type 1 (Th-1) and Th-2 cytokines has led to the formulation of several hypotheses as to which T-cell subsets are associated with periodontitis (Shapira et al. 1992, Seymour et al. 1993, Ebersole & Taubman 1994, Dennison & Van-Dyke 1997). Seymour et al. (1993) suggested that nonsusceptibility to periodontal breakdown may involve a predominantly Th-1-like response, resulting in T-cell activation, cell-mediated

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immunity and interferon-γ (IFN-γ) enhancement of innate immunity. In susceptible individuals, disease progression may involve a predominantly Th-2-like response in which T-cells produce the cytokines required for B-cell proliferation and differentiation, leading to polyclonal B-cell activation, and the continued production of B-cell IL-1 (Seymour et al. 1993).

It has been shown that interleukin (IL)-4 inhibits the persistence of macrophages in periodontitis lesions and downregulates CD14, one of the major receptors for the periodontopathogen *Porphyromonas gingivalis* LPS, which could lead to decreased tissue destruction (Lauener et al. 1990, Yamamoto et al. 1996). Thus, it was proposed that a lack of downregulation of monocytes by IL-4 leads to tissue destruction in periodontitis (Shapira et al. 1992). However, IL-13 shares most of its activities on human monocytes with IL-4, and IL-13 expression has also been demonstrated in periodontitis lesions (de Waal et al. 1993, Fujihashi et al. 1996, Yamazaki et al. 1997, Nakajima et al. 1999). Moreover, IL-13 may act directly or indirectly on fibroblasts by activating transforming growth factor-β (TGF-β) production in macrophages (Wynn 2003). Other target cells that regulate a variety of IL-13-associated effector functions are B cells, smooth muscle, endothelium and epithelium.

Recent studies have demonstrated that susceptibility to chronic and aggressive periodontitis has a genetic component (Hart & Kornman 1997, Michalowicz et al. 2000, Kubota et al. 2001, Diehl et al. 2003, Li et al. 2004, Brett et al. 2005). Consequently, various immunological risk factors involved in the pathogenesis of periodontitis have been analysed at the molecular level, showing different results (Hart & Kornman 1997).

Promoter regions influence transcription and possess a number of sequence-dependent characteristics, which make them distinct from the rest of the genome. With regard to IL-4, two common promoter polymorphisms consisting of a C–T exchange at positions -590 and -34 have been identified and associated with asthma, atopy, Crohn's disease and rheumatoid arthritis (Rosenwasser et al. 1995, Takabayashi et al. 1999, Aithal et al. 2001, Beghe et al. 2003, Pawlik et al. 2005, Moreno et al. 2006). These SNP have been shown to increase the promoter activity of IL-4 in a luciferase reporter–gene construct, suggesting that this mutation increases the expression of IL-4 in humans (Rosenwasser et al. 1995). The -590 C $\rightarrow$ T polymorphism has been analysed in patients with aggressive periodontitis in different populations (Michel et al. 2001, Gonzales et al. 2004, Pontes et al. 2004). Michel et al. (2001) reported a higher frequency of this polymorphism in Northern European AgP patients compared with healthy subjects ($p<0.05$). However, this has not been confirmed in two recent studies in other populations (Gonzales et al. 2004, Pontes et al. 2004).

The known polymorphisms in the promoter region of the IL-13 gene include two C–T exchanges at positions -1055 and -1112 and an A–C exchange at position -1512 (van der Pouw Kraan et al. 1999, Graves et al. 2000). The IL-13 -1055 C $\rightarrow$ T and -1112 C $\rightarrow$ T polymorphisms have been recently shown to be associated with allergic asthma and Graves' disease (van der Pouw Kraan et al. 1999, Hiromatsu et al. 2005).

Given the proposed role of the IL-4/IL-13 pathway in the T-cell-mediated immune response in periodontitis lesions and the previously reported associations of IL-4 and IL-13 promoter polymorphisms with systemic diseases, this explorative study compared the frequency of the IL-4 -590 C $\rightarrow$ T and -34 C $\rightarrow$ T polymorphisms, the IL-13 -1112 C $\rightarrow$ T and -1512 A $\rightarrow$ C polymorphisms, and the distribution of the genotypes among patients with aggressive periodontal disease (AgP) and healthy controls (HC).

Material and Methods

Study subjects

The study was a single-blind case–control trial, which conformed to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of the Justus-Liebig University of Giessen, Germany (No. 113/05). A total of 58 patients presenting at the Department of Periodontology were included. Except for the presence of periodontitis, patients were systemically healthy and did not present any signs of disease at the time of inclusion. Additionally, a total of 51 unrelated, age and ethnically matched healthy individuals were included in the control group. Before inclusion in the study, written, informed consent was obtained from all subjects.

Inclusion criteria for AgP patients

All patients were Caucasians and presented with the diagnosis of generalized aggressive periodontitis. This diagnosis was made based on the clinical and radiographic criteria described on the 1999 Consensus Classification of Periodontal Diseases (Armitage 1999). The patients had to be healthy except for the presence of periodontitis and be younger than 35 years. If patients were older than this age at the initial examination, they were still diagnosed as having AgP if there were clear clinical signs of AgP and the onset of disease occurred before this age. This was proved by radiographs obtained when the patient was younger than 35 years. As smoking can be a confounding factor in genetic analyses in periodontal diseases, patients had to be nonsmokers or light smokers (<10 cigarette/day).

The following clinical parameters were registered at six sites/tooth by a single experienced clinician (J. R. G.): probing pocket depth (PPD) and clinical attachment level (CAL, as the distance from the cement–enamel junction to the base of the periodontal pocket) measured with a PCP-UNC15 probe; bleeding on probing (BOP) registered as present or absent as a percentage of the total number of sites. Modified plaque (PLI) and papillary bleeding indexes (PBI) were assessed at four sites/tooth (O'Leary et al. 1972, Saxer & Mühlemann 1975).

For the diagnosis of generalized AgP, they had to present with a total of at least 20 teeth, with interproximal PPD and CAL of $\geqslant 5$ mm present on a minimum of eight teeth, three of which were other than first molars and incisors.

Panoramic radiographs of diagnostic quality were taken and evaluated for interproximal bone loss measurements from the cement–enamel junction of the tooth to the bone crest, expressed as a percentage of the total root length. All radiographs of the AgP patients showed at least three permanent teeth other than first molars and incisors with interproximal sites with $\geqslant 50\%$ bone loss. In addition, patients were not included if it was assumed that periodontal destruction was caused by local risk factors like poor restorations and poor oral hygiene.

Inclusion criteria for controls

The control subjects were students or staff of the University who voluntarily enrolled in the present study. The age and ethnicity of the controls were matched with that of the patients. However, no individual under the age of 21 years was recruited as one needs to be old enough to have developed any signs of disease (Albandar et al. 1997). The same clinical assessments as for the patients (PPD, CAL, BOP, PLI, and PBI) were performed by the same experienced investigator. To assure periodontal health, the controls must have presented with a minimum of 26 teeth, with $\geqslant 95\%$ of the tooth sites with <4 mm PPD without BOP, no PPD $\geqslant 5$ mm and no loss of CAL (Gonzales et al. 2003). Under these conditions, no radiographic examination was considered necessary in this group. Also controls had to be nonsmokers or light smokers (<10 cigarettes/day).

Blood samples and DNA isolation

Peripheral venous blood samples of 10 ml were obtained by standard venipuncture using tubes containing EDTA (1 mg/ml) (Sarstedt, Nümbrecht, Germany). Genomic DNA was isolated according to the instructions of the manufacturer (InstaGene Whole Blood Kit, Bio-Rad Laboratories GmbH, Munich, Germany).

Genotyping of cytokine polymorphisms

Genotyping was performed by polymerase chain reaction (PCR) (Eppendorf Mastercycler-Gradient, Eppendorf/Netheler/Hinz, Hamburg, Germany) and detection of polymorphisms by restriction fragment length polymorphism (RFLP) using restriction enzymes. PCR reactions were carried out in a total volume of 100 μl containing 50 ng of genomic DNA. HotStarTaq® was used under standard buffer conditions (Qiagen, Hilden, Germany). After PCR and before the digestion with restriction enzymes, nucleic acid products were purified using silica-gel membranes (QIAquick PCR Purification Kit, Quiagen, Hilden, Germany). The products were analysed directly by electrophoresis on 2% agarose gels stained with ethidium bromide.

For analysis of the IL-4 -590 C $\rightarrow$ T polymorphism, the following primers were used 5′-ACTAGGCCT CACCTGATACG-3′ and 5′-GTTGT AATGCAGTCCTCCTG-3′ (Walley & Cookson 1996). The cycle conditions were 95°C for 15 min., 94°C for 1 min., followed by 40 cycles of 57°C for 1 min., 72°C for 1 min., and 72°C for 10 min. This resulted in a PCR product of 252 bp spanning positions -522 to -774 in the IL-4 promoter region, verified by using one-fifth of the volume of the PCR-reaction on a 2% agarose gel after electrophoresis and ethidium bromide staining. The remaining PCR product was digested with 6 U *BsmF*I (New England BioLabs, Schwalbach, Taunus) in 1 × NEB-buffer 4 in a total volume of 40 μl for 6 h at 65°C. Cleavage by *BsmF*I results in two fragments of 192 and 60 bp, whereas the -590 C–T polymorphism abolishes this site.

The following primers were used for IL-4 -34 C $\rightarrow$ T genotypes: 5′-CTCATTTTCCGTCGGTTTCAGC-3′ and 5′-GAAGCAGTTGGGACGTGA GA-3′. PCR conditions were an initial denaturation phase at 94°C for 5 min., followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 15 s (Takabayashi et al. 1999). Samples were digested with *Mnl*I enzyme at 37°C for 6 h (NEB, Schwalbach, Taunus). This yielded fragments of 150 and 38 bp for allele C and a 190 bp for allele T.

For IL-13 -1112 C $\rightarrow$ T the following primers were used: 5′-GGAATCCA GCATGCCTTGTGAGG-3′ and 5′-GT CGCCTTTTCCTGCTCTTCCCGC-3′. The primers for IL-13 -1512 A $\rightarrow$ C genotypes were 5′-CAACCGCCGCGC CAGCGCCTTCTC-3′ and 5′-CCGCTA CTTGGCCGTGTGACCGC-3′. The PCR conditions for both IL-13 genotypes were similar. Samples were denatured at 94°C for 2 min. followed by 33 cycles of 94°C for 40 s, 54°C for 40 s and 72°C for 50 s and then a final extension for 10 min. at 72°C. Restriction digestion was accomplished with *BstU*I (NEB, Schwalbach, Taunus) at 60°C for 6 h. For IL-13 -1112 C $\rightarrow$ T genotype, a 224 bp PCR fragment was generated for allele C and a 247 bp fragment for allele T (Graves et al. 2000). For IL-13 -1512 A $\rightarrow$ C genotypes a 214 bp PCR fragment was generated for allele A and a 192 bp fragment for allele C (Graves et al. 2000).

Examination of the agarose gels clearly showed the previously described fragments, so that only one RFLP procedure was necessary. Nevertheless, initial analysis was confirmed by reanalysis of randomly selected samples.

Statistical analysis

The distribution of the five clinical parameters (PPD, CAL, BOP, PLI, PBI) was described for patients and controls by median and the inter-quartile range. The Fisher exact test was used to compare the frequencies for the wild-type and polymorphic alleles among periodontitis patients and the representative control group, and for analysis of the distribution of the different genotypes of the IL-4 -590 C $\rightarrow$ T and -34 C $\rightarrow$ T, IL-13 -1112 C $\rightarrow$ T and -1512 A $\rightarrow$ C polymorphisms. Deviation from Hardy–Weinberg equilibrium (HWE) was assessed by Pearson's goodness-of-fit between the observed and expected numbers using χ^2 test with 1 degree of freedom. All statistical procedures were performed at a level of significance of 5% ($p<0.05$).

Results

Demographical data and clinical parameters

A total of 58 generalized AgP patients and 51 healthy controls took part in the study. Clinical characteristics, smoking, age and gender distribution are summarized in Table 1. As expected, the values of the clinical parameters (PPD, CAL, BOP, PLI, and PBI) were higher in the patient group. The differences between patients and controls are in accordance with the inclusion criteria.

Distribution of genotypes

The distribution of genotypes in periodontitis patients and the healthy control group, as well as the results of Fisher exact test are shown in Table 2. The distribution of the genotypes for the studied genetic polymorphisms was consistent with the literature data (Walley & Cookson 1996, Takabayashi et al. 1999, Graves et al. 2000).

Homozygosity for the IL-4 T allele at position -590 was found in 25.9% of patients and in 11.8% of controls. A total of 24.1% individuals with AgP and 43.1% of controls were heterozygous (C/T). Homozygosity for the C allele was found in 50% of patients and in 45.1% of controls. The distribution of the genotypes between patients and controls was different ($p = 0.05$).

Table 1. Clinical characteristics, age and gender distribution among periodontitis and control groups

Characteristics	Periodontitis ($n = 58$)	Controls ($n = 51$)
Age (years)	34 (29–38)	30 (27–32)
Male/female (%)	43.5/56.5	38.3/61.7
Smokers (< 10 cigarettes/day) (%)	12	17.6
PPD (mm)	3.9 (3.3–4.4)	1.8 (1.6–1.9)
CAL (mm)	4.5 (3.9–5.3)	1.8 (1.6–1.9)
BOP (%)	53 (28–73)	5.5 (2.4–11.8)
PLI (%)	47 (26–66)	39 (24–53)
PBI (%)	19 (4–42)	5 (0–8.8)

Values represent median and interquartiles (in parentheses).
PPD, probing pocket depth; CAL, clinical attachment level; BOP, bleeding on probing; PLI, plaque index; PBI, papillary bleeding index.

Table 2. Distribution of the genotypes of the IL-4 C(−590)T, C(−34)T, IL-13 C(−1112)T and A(−1512)C polymorphisms in the periodontitis and healthy control groups

Genotype frequency	AgP ($n = 58$)	Controls ($n = 51$)	p-value
IL-4 −590 C → T			0.05
C/C	29 (50) [37.4–63]	23 (45.1) [32.2–58.6]	
C/T	14 (24.1) [15–37]	22 (43.1) [30.4–56.8]	
T/T	15 (25.9) [16.3–38.4]	6 (11.8) [5.6–23.4]	
IL-4 −34 C → T			0.02
C/C	32 (55.1) [42.4–67.2]	24 (47) [34–60.5]	
C/T	11 (19) [11–31]	21 (41.2) [29–55]	
T/T	15 (25.9) [16.4–38.4]	6 (11.8) [5.6–23.4]	
IL-13 −1112 C → T			0.51
C/C	19 (32.8) [22.1–46]	17 (33.3) [22–47.1]	
C/T	29 (50) [37.4–63]	29 (56.9) [43.2–69.5]	
T/T	10 (17.2) [10–29]	5 (9.8) [4.4–21]	
IL-13 −1512 A → C			0.33
A/A	31 (53.4) [41–66]	20 (39.2) [27–53]	
A/C	24 (41.4) [30–54.2]	28 (55) [41.3–68]	
C/C	3 (5.2) [2–14.1]	3 (5.8) [2.1–16]	

Values represent the number of subjects in genotype frequency (percentages in parentheses).
Confidence intervals of proportions in square brackets.
AgP, aggressive periodontitis

The prevalence of the T allele was 38% in patients (44/116) and 33% (34/102) in the healthy control group. The frequency of the IL-4 −590T allele was not significantly different between the groups ($p = 0.89$).

Homozygosity for the IL-4 −34T allele was found in 25.9% and 11.8% of the periodontitis and control subjects, respectively. A total of 19% of the individuals with AgP and 41.2% of the control subjects were heterozygous (C/T). The distribution of the genotypes within both groups was significantly different ($p = 0.02$). The prevalence for the T allele was 35% in patients (41/116) and 32% (33/102) in the healthy control group. The frequency of the IL-4 −34T allele was not significantly different between both groups ($p = 0.78$). The distribution of IL-4 genotypes at positions −590 and −34 in the AgP patients differed from HWE. The distribution of the two IL-4 genotypes in the control group was in HWE.

There was a strong association between the IL-4 alleles −34C/−590C and −34T/−590T. On the basis of this association, individuals were grouped into simultaneous carriages of two alleles at the two loci. The frequency of the haplotypes between the groups was different ($p = 0.06$). There was a higher frequency of the IL-4 −34T/−590T alleles in aggressive periodontitis patients than in controls.

Homozygosity for the IL-13 −1112T allele was found in 17.2% of patients and in 9.8% of healthy controls. A total of 50% of the patients and 57% of the controls were heterozygous (C/T). The distribution of the genotypes within both groups was not significantly different ($p = 0.51$). The prevalence of the T allele was 42% in patients (49/116) and 38% in the healthy control group (39/102). The frequency of the IL-13 −1112T allele was not significantly different between the two groups ($p = 0.67$).

Homozygosity for the IL-13 −1512 C allele was found in 5.2% of patients with aggressive periodontitis and in 5.8% of controls. A total of 41.4% of the patients and 55% of the controls were heterozygous (A/C). The distribution of the genotypes within both groups was not significantly different ($p = 0.33$). The prevalence of the C allele was 26% in patients with periodontitis (30/116) and 33% in the control group (34/102). The frequency of the IL-13 −1512 C allele was not significantly different between the two groups ($p = 0.29$).

The distribution of IL-13 genotypes at positions −1112 and −1512 in patients and controls did not differ from HWE.

Discussion

By comparing a North European sample of 58 patients with generalized aggressive periodontitis and 51 controls with healthy periodontium, an association of the IL-4 −590 T/T and IL-4 −34 T/T genotypes with aggressive periodontitis was identified.

To our knowledge, this is the first study investigating the IL-4 −34 C → T, IL-13 −1112 C → T and IL-13 −1512 A → C polymorphisms in patients with periodontitis. The results of this study confirm the data of a previous study conducted in our laboratory, in which a higher frequency of the IL-4 −590 C → T polymorphism and a repeat in intron 2 was found in Northern European AgP patients compared with healthy subjects (Michel et al. 2001).

The hypothesis that these polymorphisms constitute a putative common risk factor for periodontitis may warrant testing in larger groups of patients. Th-1 cells produce IL-2 and IFN-γ, while Th-2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 (Romagnani 1997). IL-4 is a key cytokine that controls differentiation to the Th-2 effector cells and shares biological features with IL-13 (de Vries et al. 1993). Recently, it has been proposed that due to the shift in lymphocyte populations in the inflammatory infiltrate from predominantly T-cells in gingivitis to an increased proportion of B-cells in periodontitis, susceptibility to periodontal disease

progression may involve a predominantly Th-2-like response (Seymour et al. 1993, Gemmell et al. 2002b). This means that Th-2-cells produce the cytokines required for B-cell proliferation and differentiation, leading to polyclonal B-cell activation, the production of elevated levels of nonprotective antibodies and the production of high levels of B-cell IL-1.

To test this hypothesis, studies investigating Th-1/Th-2 profiles in periodontitis have been conducted, showing conflicting results (Fujihashi et al. 1996, Salvi et al. 1998, Sigusch et al. 1998, Bartova et al. 2000, Gemmell et al. 2002a). Thus, it appears that the relative dominance of B cells and plasma cells in periodontitis lesions cannot entirely be explained by enhanced Th-2 functions, but it may be due to an imbalance between Th-1 and Th-2 cells (Berglundh et al. 2002, Berglundh & Donati 2005). However, several studies support the hypothesis that Th-1 cells are associated with the stable lesion and a Th-2 response with the progression of periodontitis (Yamazaki et al. 1994, Aoyagi et al. 1995, Yamamoto et al. 1997, Bartova et al. 2000, Gemmell et al. 2002b, Yun et al. 2003).

Owing to reports that demonstrated an absence of IL-4-producing T cells in periodontal lesions, it has been suggested that a lack of downregulation of monocytes by IL-4 leads to tissue destruction (Shapira et al. 1992). However, the lack of macrophages in the advanced lesions is consistent with the presence of IL-4 and/or IL-13 and thus of a Th-2 response (Chapple et al. 1998, Gemmell & Seymour 1998).

The promoter of the IL-4 gene largely contributes to the control of T-cell differentiation, as a key binding site for specific Th-2 transcription factors (Li-Weber et al. 1997a, b). IL-4 and IL-13 are functionally related and recent data indicate that only the combined analyses of genetic alterations in the IL-4/IL-13 pathway reveal its actual significance in the development of these diseases (Kabesch et al. 2006). Thus, in view of the proposed model of the immunopathogenesis of periodontitis, the genetically determined IL-4 -590 and -34 C $\rightarrow$ T, IL-13 -1112 C $\rightarrow$ T and IL-13 -1512 A $\rightarrow$ C promoter polymorphisms may contribute to interindividual differences in susceptibility to aggressive periodontitis. These promoter polymorphisms have been mainly implicated in the regulation of total IgE production (de Vries et al. 1993, Graves et al. 2000, Laundy et al. 2000).

Linkage disequilibrium between the IL-4 -34 and -590 loci has been reported in Japanese and Caucasian populations (Takabayashi et al. 1999, Beghe et al. 2003). In this study, we found a strong association between the IL-4 alleles -34C/-590C and -34T/-590T, respectively. The frequency of the simultaneous carriage of the IL-4 -34T/-590T alleles was higher in aggressive periodontitis patients than in controls.

The distribution of the IL-4 genotypes differed from HWE in the patient group. There are many causes for deviation from this equilibrium, including genotyping error, chance, non-random mating, differential survival of marker carriers, genetic drifting, population stratification or combinations of these reasons (Trikalinos et al. 2006). In this, RFLP results were confirmed by reanalysis of randomly selected samples of patients and controls. This might be due to the selection criteria of patients with AgP.

The role of IL-13 in periodontitis is still unclear. In a recent study, messenger RNA for IFN-γ and IL-13 was upregulated whereas IL-4 and -10 were downregulated following stimulation of peripheral blood mononuclear cells with *Porphyromonas gingivalis* (Nakajima et al. 1999). Recently, mRNA expression of IL-13 was determined in T cells extracted from gingival biopsies from patients with aggressive periodontitis, gingivitis and healthy controls. IL-13 was only detected in the gingivitis and healthy groups (Suarez et al. 2004).

There was no significant difference in the prevalence of the IL-13 -1112 C $\rightarrow$ T and -1512 A $\rightarrow$ C SNP among patients and controls in this study. However, there is still insufficient evidence to support this conclusion. A limitation of this study is the small sample size, as one must have larger samples to avoid type II errors. In addition, periodontitis is a multifactorial disease with a complex polygenic background and the relative risk for developing aggressive periodontitis might involve particular haplotypes in the IL-13 genes that were not investigated in this study.

Overall conclusions

Taken together and within the limitations of this study, the significant association between IL-4 -34 T/T and IL-4 -590 T/T genotypes with aggressive periodontitis and also the higher frequency in the simultaneous carriage of the IL-4 -34T/-590T mutant alleles in these patients support the previous hypothesis that IL-4 plays a regulatory role in the immune response of periodontitis. There is a need for further investigations of these polymorphisms in a larger sample, combined with functional studies of gene and protein function, in order to support this conclusion.

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Clinical Relevance

Scientific rationale for the study: Polymorphisms in the genes coding for the T-helper (Th)-2 cytokines IL-4 and IL-13 have been associated with systemic inflammatory diseases. It has been proposed that a Th-2, rather than a Th-1 response, might play the dominant role in progressive periodontal lesions.

Principal findings: The basic finding of this study was the association between the IL-4 −590 T/T and −34 T/T genotypes and AgP in Caucasians as compared with healthy controls.

Practical implications: The knowledge of genetic factors in association with aggressive periodontitis provides future tools for diagnostic and preventive strategies, especially if the IL-4 promoter polymorphisms have a functional effect.