Acta Odontologica Scandinavica, 2008; 66: 105–112

informa
healthcare

ORIGINAL ARTICLE

Association of interleukin-6 (IL-6) haplotypes with plaque-induced gingivitis in children

LYDIE IZAKOVICOVA HOLLA[2,4], KRISTINA MUSILOVA[2], JAN VOKURKA[1], LUCIE KLAPUSOVÁ[2], PAVLA PANTUCKOVA[1], MARTINA KUKLETOVA[1], LUBOMIR KUKLA[3] & VLADIMIR ZNOJIL[4]

[1]*Faculty of Medicine, Masaryk University Brno, Czech Republic,* [2]*Clinics of Stomatology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University Brno, Czech Republic,* [3]*Department of Social Medicine and Health Care Administration, Masaryk University Brno, Czech Republic, and* [4]*Department of Pathophysiology, Faculty of Medicine, Masaryk University Brno, Czech Republic*

Abstract

Objectives. The proinflammatory cytokine interleukin-6 (IL-6) is a key regulator of the host response to microbial infection and major modulator of extracellular matrix catabolism and bone resorption. The aim of this case-control study was to test differences between children with and without gingivitis in the distribution of IL-6 alleles at positions −174, −572, and −597 and their haplotypes. ***Material and methods.*** A total of 455 Caucasian children, aged 11 to 13 years, were enrolled in this study. According to gingival bleeding on probing indices, 183 were classified as healthy subjects and 272 as children with plaque-induced gingivitis. DNA for genetic analysis was obtained from buccal epithelial cells and PCR-RFLP methods were used for genotyping three selected IL-6 promoter polymorphisms. ***Results.*** Complex analysis revealed significant differences in haplotype frequencies between patients and healthy subjects ($p < 0.01$). The CGA haplotype was significantly more frequent in children with gingivitis than in healthy subjects (41.5% versus 34.1%). In subanalyses, we found that IL-6 −174C allele was more frequent in patients (44.3%) than in healthy children (36.1%, $p = 0.016$, $P_{corr} < 0.05$). Multivariate logistic regression analysis showed that allele C remained a risk factor for gingivitis in children ($p = 0.03$) regardless of plaque or gender. However, the proportions of the IL-6 −597 and −572 genotypes were comparable between the two groups. ***Conclusions.*** Our results indicate that the three promoter polymorphisms in the IL-6 gene act in a cooperative fashion and suggest that IL-6 haplotypes could play a role in the pathogenesis of gingivitis in Caucasian children.

Key Words: *Cytokine, gene, gingivitis, PCR, polymorphism*

Introduction

Periodontal diseases (gingivitis and periodontitis) are inflammatory processes of the gingiva and supporting structures of teeth induced by a microbial biofilm, but individual differences in the host immune response to infection may affect susceptibility and severity of the disease.

According to a new classification [1], gingival diseases can be divided into four major groups: (a) dental plaque-induced gingival diseases, (b) gingival diseases modified by systemic factors, (c) gingival diseases modified by medications, and (d) gingival diseases modified by malnutrition. Plaque-induced gingivitis is the most common form of the periodontal diseases, affecting a significant proportion of the population of susceptible individuals. Although plaque and calculus are the major developing factors in this form of gingival disease, the host response that involves complex interactions between cells, the extracellular matrix, and circulating cytokines also plays an important role [2]. Allelic variations in cytokine genes and factors regulating their expression result in phenotypic differences in cytokine responses between individuals [3]. Increased expression of interleukin-6 (IL-6) mRNA and crevicular levels of IL-6 have been found in patients with gingivitis and periodontitis, suggesting a potential role of the IL-6 gene for susceptibility to periodontal diseases [4,5].

IL-6 is a pleiotropic cytokine with a broad range of effects, including differentiation and/or activation of

Correspondence: Lydie Izakovicova Holla, Department of Pathophysiology, Medical Faculty Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. Tel: +420 549494229. Fax: +420 549494340 (2330). E-mail: holla@med.muni.cz

(Received 16 August 2007; accepted 21 February 2008)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350802004664

macrophages and T cells, growth and differentiation of B cells, stimulation of hematopoiesis, osteoclast differentiation, and bone resorption [6]. IL-6 is produced by a variety of different cells, such as gingival fibroblasts, endothelial cells, monocytes, and T lymphocytes, playing a crucial role at the interface of adoptive and innate immunity. It is not constitutively expressed, but is highly inducible and is produced in response to inflammatory stimuli such as tumor necrosis factor α (TNFα), interleukin-1, and bacterial endotoxin. The human IL-6 gene has been assigned to chromosome 7p21 and comprises five exons and four introns [7]. The IL-6 5′ region contains numerous polymorphisms that directly influence the expression of the protein. It has been shown that the polymorphism at position −174 (G/C) affects IL-6 transcription [8]. Furthermore, two other functional SNPs, at positions −572 (G/C) and −597 (A/G), have been reported to have an effect on IL-6 secretion capacity [9]. Moreover, functional analyses indicate a cooperative influence of these polymorphisms on the IL-6 transcriptional regulation, suggesting that the SNPs at the three sites do not act independently of one another, and implying the investigation of IL-6 −597/−572/−174 haplotypes [10,11].

There are several studies of polymorphisms of the IL-6 gene in chronic and/or aggressive periodontitis [5,12–17]. To our knowledge, however, only one study on the potential association between IL-6 gene polymorphisms and risk of gingivitis has been published so far [18]. With respect to the key role of interleukins in inflammatory response in gingivitis, the IL-6 gene is an obvious functional candidate for this disorder. This study was undertaken to compare the distributions of the IL-6 −174G/C, −572G/C, and −597A/G alleles, genotypes, and haplotypes in healthy Czech children aged 11 to 13 years and those with plaque-induced gingivitis.

Material and methods

Study subjects

In this case-control study, a total of 455 Caucasian children (249 boys and 206 girls) of exclusively Czech nationality, aged 11 to 13 years, randomly selected from the ELSPAC Brno study (European Longitudinal Study of Pregnancy and Childhood), which comprises over 5000 children and their families, were examined to assess gingival health. The ELSPAC is a prospective study in several European countries where the chosen group of children and their families are examined from pregnancy of the mother, birth of the child, up to 18 years of age [19]. Pediatric, anthropological, and psychological examinations have already been performed at the 8th, 11th, 13th year of age of subjects, and currently the examination at the 15th year is in progress. These age phases were chosen in order to record health and developmental changes associated with the prepubertal, pubertal, and postpubertal phases of child development. Children participating in this study also underwent a dental examination at the Clinics of Stomatology, St. Anne's University Hospital and the Faculty of Medicine, Masaryk University. Inclusion criteria therefore consist of a simple informed consent of the respective children and their families and of their willingness to participation. Thus, the randomness of the set is ensured; although a slight drift towards families with mothers with higher education can be expected. The general health and oral hygiene of the study set is varied as a result of the subject set randomness.

The study group ($n=272$; 173 boys and 99 girls) comprised children with clinical evidence of plaque-induced gingivitis. The healthy group ($n=183$; 76 boys and 107 girls) included children who had healthy gingiva with no clinical signs of inflammation.

Clinical examination

The clinical assessment was carried out by one investigator (LK) using the following clinical parameters: gingival index (GI), plaque index (PI), and calculus index (CSI). Gingivitis was measured using the modified Löe-Silness GI index [20] on teeth 16, 12, 24, 32, 36, 44. This index uses a 0 to 3 scale to assess gingivitis on or adjacent to 4 sites (mid-buccal, mesio-buccal, disto-buccal, and lingual) of the individual tooth according to the following criteria: the complete absence of visual signs of inflammation scored 0; a slight change in color, slight edema, and no bleeding on probing scored 1; visual inflammation, redness, edema, glazing, and bleeding on pressure scored 2; finally, severe inflammation, marked redness, edema, ulceration, and a tendency to spontaneous bleeding scored 3. The scores from the four areas of the tooth were added and divided by 4 to give the GI for the tooth. The GI for the patient was then obtained by adding the indices for the teeth and dividing by 6 (number of teeth examined). Therefore, the index for the patient was an average score for the areas examined. Mean and SD, median GI scores, and quartile intervals (QI) were calculated from all individual scores. Patients were classified as having gingivitis if the total sum of the ordinal values of classifications of localities with inflammation was at least 4 (of the total possible value 72, i.e. 6 examined teeth $\times 4$ localities $\times$ score 3), whereas control children were those who had healthy gingiva (i.e. zero value in all 24 examined localities). According to these findings, gingival inflammation was present in 272 children, whereas 183 had healthy gingiva. Children with sum of the ordinal values between 1 and 3 were not included in this study ($n=28$). The presence of

plaque and calculus was recorded according to Silness-Löe (PI) and calculus surface index (CSI), respectively [21,22] without any disclosing agents. A World Health Organization (WHO) probe was used for the assessment. In the PI, cervical plaque was scored on the same selected teeth as in GI on three tooth surfaces (mesial interdental area superior to the tip of the papilla and the facial and oral surfaces). When no plaque was visible adjacent to the marginal or papillary gingiva and also scraping with explorer revealed no plaque, the score was 0. A very thin microbial deposit near the marginal or papillary gingiva was scored 1. Any soft deposits present adjacent to the marginal gingival and in the interdental area were scored 2. A clearly visible, thick deposit adjacent to the gingival margin and often extending far up the crown was scored 3. A mean PI score was derived for each subject at each examination by summing the individual PI scores and dividing the sum by the number of sites graded for the subject. Mean and SD, median PI scores and quartile intervals (QI) were calculated from all individual scores. The CSI used is a yes-or-no index that determines whether or not calculus (supra- and subgingival) is present on the 16 surfaces of the 4 mandibular incisors. The CSI for each subject may thus be a maximum of $4 \times 4 = 16$; mean and SD, median CSI scores, and quartile intervals (QI) were calculated from all individual scores. The radiograph examination was not performed because it was not part of routine care for these children and would therefore have been deemed unethical. Phenotype status was assigned without knowledge of genotypes by two independent investigators.

The study was performed with the approval of the Committee for Ethics of the Medical Faculty, Masaryk University Brno, and informed consent was obtained from all parents (in the case of children), in line with the Helsinki declaration, before inclusion in the study.

DNA isolation

Genomic DNA for PCR was isolated from buccal epithelial cells by the conventional method using proteinase K digestion of cells.

IL-6 genotyping

The IL-6 −174G/C polymorphism was genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) as described previously [8]. The −597G/A and −572G/C variant genotypes were determined using primers (5′-AAACAGTGGTGAAGAGACTCAGTG-3′ and 5′-AAAGAGTAAAGCTGAAGTCATGCA-3′) to generate a PCR fragment of 543 bp. The reaction volume was 25 μl with final reaction component concentrations of 200 $\mu mol \cdot l^{-1}$ of each dNTP, 1.5 $mmol \cdot l^{-1}$ $MgCl_2$, 10 $mmol \cdot l^{-1}$ Tris base (pH 8.4), 50 $mmol \cdot l^{-1}$ KCl, and 0.15 U Taq polymerase. PCR was performed with an initial denaturation temperature of 95°C for 2 min, followed by 40 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with the final extension lasting for 10 min at 72°C. Twelve microliters of the PCR product was digested with FokI (−597G/A) for 2 h at 37°C and 12 μl was digested with BsrbI (−572G/C) for 4 h at 37°C. The digested products were electrophoresed on 2% agarose gel and visualized by ethidium bromide staining.

All samples were successfully genotyped, and to verify and confirm the results genotyping was repeated on 10% randomly selected DNA samples for each of the methods.

Statistical analysis

The computer package EpiInfo6, available from the Centers for Disease Control and Prevention, USA (http://www.cdc.gov/epo/epi/epiinfo.htm), was used to estimate the statistical power to detect significant differences between the study groups. The power calculation performed for this study demonstrated that the sample size required to ascertain the significance of association of gingivitis with the studied genetic polymorphisms with an alpha value of 0.05 and OR = 1.5 80% power was 175 for healthy children and 261 for cases.

Comparisons were made between allele, genotype, and haplotype frequencies in the patients and healthy populations. The allele frequencies were calculated from the observed numbers of genotypes. The significance of differences in the allele frequencies between each of the two groups was determined using Fisher's exact test. The Pearson chi-squared test was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium and for comparison of differences in genotype combinations among groups. Testing of allele or genotype frequencies for several polymorphisms among the groups involves multiple comparisons; therefore necessary correction had to be applied. Where appropriate, Bonferroni correction was used to adjust the α level according to the number of independent comparisons to an overall value of 0.05. Adjusted p-values for particular analyses are denoted as P_{corr}. Comparison of estimated haplotype frequencies was performed as described previously [23]. Briefly, haplotype frequencies were calculated from the observed genotype frequencies assuming that during zygote formation haplotypes combine at random depending on their frequencies in the set (the assumption being analogous to Hardy-Weinberg equilibrium). This assumption was verified by a reversed calculation of the expected genotype frequencies from the calculated haplotype frequencies. Differences between both groups (patients and healthy subjects) were tested by a simulation using

Table I. PCR–RFLP conditions for analysis of the IL-6 gene polymorphisms.

Polymorphism	Primers (5′– 3′)	PCR product (bp) / enzyme		Digested fragments / alleles
−174 G/C	5′- TGACTTCAGCTTTACTCTTTGT-3′(sense)	198	*Nla III*	C: 122+45+31
	5′- CTGATTGGAAACCTTATTAAG-3′ (antisense)			G: 167+31
−572 C/G	5′- AAACAGTGGTGAAGAGACTCAGTG-3′ (sense)	543	*Bsrb I*	C: 531
	5′- AAAGAGTAAAGCTGAAGTCATGCA-3′ (antisense)			G: 263+268
−597 A/G				A: 250+281
			Fok I	G: 531

the Monte Carlo method. In this test, 10,000 random simulations were performed to generate tables having the same marginal totals as the observed data, and the number of occurrences of a chi-squared value greater than or equal to the chi-squared value associated with the real table was counted.

Clinical parameters of subjects distributed by three possible genotypes were compared using the analysis of variance in relation to the amount of plaque. The relationship between genotypes and disease status was analyzed by multiple logistic regression analysis while adjusting for gender and plaque level.

Contingency table analysis, odds ratio (OR), 95% confidence intervals, and significance values were estimated with the use of the statistical program package Statistica v. 6.0 (Statsoft Inc., Tulsa, Okla., USA).

Results

Gingivitis was observed in 272 (59.8%) of the children examined (i.e. cases); the rest of the group were considered healthy. Children with gingivitis had mean (SD) GI score 0.45 (0.37), [0.31 (median), 0.17–0.51 (25% quartile to 75% quartile)] and the number of positive sites per subject was 12.7 ± 8.14. However, in healthy subjects, all parameters had 0 values. In gingivitis, mean (SD) and medians (quartiles) of PI and CSI indexes were 1.14 (1.19) [1.00 (0.00–2.00)] and 0.31 (1.90) [0.00 (0.00–0.00)], in contrast to the group of healthy gingiva where the values were 0.33 (0.69) [0.00 (0.00–0.00)] for PI and 0.59 (2.60) [0.00 (0.00–0.00)] for CSI. The distributions of genotypes and alleles of the IL-6 in children with and without gingivitis are given in Table II. All groups were in Hardy-Weinberg equilibrium with non-significant chi-squared values comparing the observed and expected genotype frequencies of each of the tested polymorphisms. The IL-6 −174C, −572G, and −597A alleles were found in 36.1%, 94.5%, and 37.4% of the healthy children, and in 44.3%, 93.9%, and 42.6% of the patients with gingivitis, respectively. Differences in allele frequencies were not significant for IL-6 −572 and −597 variants, but patients with gingivitis had significantly increased frequency of the IL-6 −174 C allele ($p = 0.016$, $P_{corr} < 0.05$, OR = 1.41; 95% CI = 1.07–1.85). Analysis by logistic regression considering gender and plaque in addition to the IL-6 −174 genotypes as independent variables showed significant association between −174G/C IL-6 genotypes and gingivitis in children ($p = 0.03$, OR = 1.41, 95% CI: 1.07–1.85) regardless of plaque ($p = 0.000001$, OR = 4.50, 95% CI: 2.97–6.84) or gender ($p = 0.00037$, OR = 2.46, 95% CI: 1.68–3.61). The children at highest risk of having gingivitis were −174 allele C-positive boys who had abundant plaque.

When the clinical parameters (severity of gingival inflammation expressed first by including all localities with inflammation, i.e. values of ordinal scale of GI 1-3, second by the percentage of sites having 2 or 3 GI scores out of the total number of sites studied, extent, and further the amount of plaque and calculus) were distributed by the three genotypes of IL-6 −174 variant, statistically significant associations were observed between plaque levels (PI) and gingival inflammation. However, we observed less significant association between the amount of plaque and gingival inflammation in children with IL-6 −174 CC genotype (according to our results "possible risk genotype"); these children showed more pronounced gingival inflammation with lower values of plaque index than children with "non-risk genotypes" (IL-6 −174 C/G and GG; see Table III).

When we combined multiple SNP (single nucleotide polymorphism) sites and analyzed haplotypes, we observed only five haplotypes with frequency more than 1%, because all three SNPs in the IL-6 promoter are in linkage disequilibrium with each

Table II. Genotype distributions of the IL-6 in children with and without gingivitis.

Locus	Allele	Genotype	Healthy (%)	Gingivitis (%)	*P*-value*
SNP1		GG	72 (39.3)	88 (32.4)	
−174	G/C	GC	90 (49.2)	127 (46.7)	0.016
		CC	21 (11.5)	57 (20.9)	$P_{corr} < 0.05$
SNP2		GG	163 (89.1)	240 (88.2)	
−572	G/C	GC	20 (10.9)	31 (11.4)	0.820
		CC	0 (0.0)	1 (0.4)	
SNP3		AA	20 (10.9)	52 (19.1)	
−597	A/G	AG	97 (53.0)	128 (47.1)	0.133
		GG	66 (36.1)	92 (33.8)	

*Comparisons performed by means of the Fisher exact two-sided test for allele frequencies.
Bonferroni corrected *P*-value (P_{corr}) for single test (3 independent comparisons).

Table III. Clinical parameters (mean and standard deviation) distributed by plaque levels in subjects with "risk genotype (IL-6 −174/CC)" versus "nonrisk genotype (IL-6 −174CG and GG) groups".

Plaque level (PI)	*n*	Sex (boys/girls)	No. of positive sites, mean (SD)	Severity of inflammation, mean (SD)	Severity of inflammation* (%)	CSI mean (SD)
IL-6 −174 CC genotype						
0	37	23/14	5.84 (8.02)	0.19 (0.30)	2.85	0.32 (1.97)
1	21	11/10	10.00 (8.09)	0.35 (0.36)	7.07	0.00 (0.00)
2	5	4/1	15.00 (10.12)	0.58 (0.53)	16.7	2.40 (5.37)
3	14	9/5	16.64 (9.39)	0.56 (0.36)	9.77	0.00 (0.00)
P level		0.687	0.0002	0.0002	0.034	0.078
IL-6 −174 CG genotype						
0	121	57/64	5.24 (7.04)	0.17 (0.25)	2.02	0.69 (2.81)
1	51	22/29	7.82 (8.37)	0.24 (0.27)	1.96	0.00 (0.00)
2	9	9/0	14.33 (10.37)	0.45 (0.32)	5.23	1.33 (4.00)
3	23	23/10	12.00 (10.16)	0.44 (0.43)	9.29	0.36 (2.09)
P level		0.002	<0.0001	<0.0001	<0.0001	0.246
IL-6 −174 GG genotype						
0	90	44/46	4.11 (7.15)	0.15 (0.30)	2.85	0.40 (2.17)
1	37	19/18	7.81 (10.40)	0.31 (0.51)	7.96	0.00 (0.00)
2	8	6/2	13.50 (11.36)	0.45 (0.38)	7.30	1.50 (4.24)
3	24	21/3	14.46 (7.68)	0.58 (0.44)	12.34	0.50 (2.45)
P level		0.005	<0.0001	<0.0001	<0.0001	0.312

*It is expressed as the percentage of sites having 2 or 3 GI scores out of the total number of sites studied.

other to various degrees. The frequencies of the IL-6 haplotypes are summarized in Table IV. There was a significant difference ($p < 0.01$) between cases and healthy children in the frequency of haplotypes. The risk of having gingivitis was noted to increase 1.4-fold in children positive for CGA (OR = 1.37, 95% CI: 1.12–1.68) and to decrease 0.8-fold in children with GGG (OR = 0.80, 95% CI: 0.66–0.97) haplotypes.

Discussion

Chronic gingivitis is the most common periodontal infection among children and adolescents. It is characterized by inflammation of the marginal gingiva without detectable loss of bone or connective tissue attachment. It seems that gingivitis, like several other forms of periodontal disease, is a result of the interaction between microbiological components of the plaque together with the genes or, more precisely, of the genetic variability in these loci [24].

Table IV. The frequencies of the IL-6 haplotypes in both groups.

Haplotypes			Healthy subjects	Gingivitis patients
−174G/C	−572G/C	−597A/G		
C	G	A	0.341	0.415
G	G	A	0.034	0.009
G	C	G	0.055	0.050
C	G	G	0.020	0.021
G	G	G	0.551	0.496
3 others*			0.000	0.009
		$p < 0.01$		

*Three pooled remaining haplotypes with very low frequencies of occurrence.

Today, the role of environmental factors is clearer than the role of genes; their influence is much harder to measure. Our study represents an investigation of the role of the IL-6 gene in gingivitis in the Czech population. Frequency of gingivitis in approximately 60% of children in our study is consistent with prevalence of gingivitis among school children in the United States that ranged from 40% to 60% [25].

In this study, three SNPs at positions −174, −572, and −597, which form IL-6 haplotypes, were analyzed. Power calculation indicates that our sample size was large enough to detect association with an acceptable level of significance No previous study has investigated the role of the above-mentioned IL-6 haplotype in gingivitis, and only a few articles on the polymorphisms in this gene in periodontitis patients have been published so far. First, Trevillato et al. [12] suggested that polymorphism at position −174 could be associated with susceptibility to chronic periodontitis (CP), and the −572G/C variant in this gene was related to decreased susceptibility to CP in our previous study [13]. In contrast, Jansson et al. [16] and Wohlfahrt et al. [15] did not find any significant difference in IL-6 −174G/C genotypes between chronic periodontitis patients (CP) and healthy controls. However, most later studies have confirmed an association of the −174 G/C polymorphism in the IL-6 gene with susceptibility to CP [5,14,17,26]. Moreira et al. [5] suggested that the GG genotype may represent a protective role in severity of periodontitis. In contrast, Babel et al. [17] described significantly higher frequency of the CC genotype in the group of patients with CP. However, Brett et al. [14] found that carriage of the GC genotype was associated with an increased risk of chronic disease, while Tervonen et al.

[26] described an evident association between the GG genotype and the extent of periodontal disease. These discrepancies can be explained by different microbial composition of plaque, because 5 variants (including −174G/C) in the IL-6 gene were associated with increased odds of detecting periodontal pathogens, such as *A. actinomycetemcomitans*, *P. gingivalis* or *T. forsythensis* [27]. Our current results of an association of IL-6 −174 polymorphism with susceptibility to gingivitis are in accordance with these previous findings, but in contrast to a very recent study by Scapoli et al. [18]. These authors examined the role of IL-6, TNFα and LTα variants in modulation of the clinical expression of plaque-induced gingivitis and found no effect of selected polymorphisms in relatively small investigated groups of subjects.

The functional significance of the IL-6 polymorphisms has been debated and the expression of IL-6 mRNA involves synergistic interaction of a number of transcription factors with the IL-6 promoter, including NF-κB and NF-IL6. Binding sites for these factors occur in close proximity to nucleotide −174 [10]. Polymorphisms such as the 174G/C SNP, which is located adjacent to the negative regulatory domain (nucleotides −173 to −151 relative to the transcription start site) and in close proximity to an NF-IL-6 binding motif, seem to result in interindividual variation in IL-6 transcription and expression. Indeed, a frequent G/C polymorphism at nucleotide position −174 and other, less frequent, variants in the promoter region have been discussed as possibly influencing serum IL-6 [8,9]. However, the effect of interleukin-6 variants on the concentration of IL-6 in plasma is still controversial. There is disagreement between data concerning genotypes associated with higher IL-6 plasma concentrations. In some studies, the homozygous GG genotype has been associated with increased serum IL-6, whereas the authors of other studies have reported that the homozygous CC genotype might be associated with higher systemic IL-6 expression [reviewed in 28]. However, individual SNPs do not exist in isolation, but are genetically linked, forming distinct haplotypes. A previous, very recent, study has suggested that although basal IL-6 mRNA expression in peripheral blood leucocytes did not differ between subjects with different −597/−572/−174 haplo- or genotypes, individuals with the −597/−572/−174 GGG haplotype showed a marginally significant decrease in IL-6 secretion upon LPS stimulation ($p = 0.058$) [11]. In addition, homozygous carriers of the GGG/GGG −597/−572/−174 genotype had significantly lower IL-6 concentrations versus all other genotypes [11]. An even stronger link to low IL-6 secretion was detected for both the −597 and −174 GG genotype. In our study, children without gingivitis had an increased frequency of this low-secretion haplotype, in contrast to children with gingivitis with a presence of "opposite" (probably more active) −174C and −597A alleles that were associated with increased IL-6 values. These increased IL-6 levels were found in patients with periodontal diseases [4,5]. Therefore, our data suggest that there can be a component of gingivitis attributable to the genotype.

Unlike a number of studies analyzing the role of gene polymorphisms in periodontitis, only a few studies investigating the role of genetic factors at the gingivitis origin or development have been published to date [29–32]. However, during experimental gingivitis studies, it was noted that subjects varied in their rates of gingival inflammation development, and the differences in gingivitis susceptibility were often independent of quantitative plaque accumulation differences or qualitative differences in plaque [33]. The reported significant differences in gingival inflammatory response under quantitatively and/or qualitatively almost identical plaque accumulation [33] suggest that the level of the gingival tissue response to plaque accumulation may be an individual trait [34], dependent on host-related factors, possibly genetic in origin. The results of one of the latest studies suggest the association between IL-1 receptor antagonist polymorphism and subject-based clinical behavior of the gingiva in response to *de novo* plaque accumulation, as well as a possible association between IL-1 beta −511 polymorphism and gingivitis susceptibility [32]. Besides IL-1 (a key proinflammatory mediator), anti-inflammatory cytokines such as IL-10 play a role in controlling the inflammatory response. IL-10 is therefore considered a candidate gene that encodes susceptibility to gingivitis. In 2005, Dashash et al. [30] found that the −1082 A allele could be a risk factor for gingivitis in children regardless of plaque or age. The same authors later approved a role of the IL-10 gene in gingivitis and found that haplotype combination, which is associated with increased production of IL-10 (and thus with stronger anti-inflammatory activity) was more frequent in controls than in children with gingivitis [31].

In our study, IL-6 −174 C allele could be a risk factor for gingivitis regardless of plaque and sex. In addition, children with −174 CC genotype (possible "risk genotype") had more intensive gingival inflammation even with lower values of plaque index than children with "non-risk genotypes" (IL-6 −174 C/G and GG).

In addition, preliminary genetic evidence supports the possibility that there are inter-individual differences in the ability to develop gingival inflammation and shows that specific genetic characteristics may contribute to exacerbated gingival inflammation in response to plaque accumulation. Gingivitis susceptibility may probably influence periodontitis susceptibility [35], but the state of current knowledge does not permit us to say which way the relationship goes. In conclusion, our present findings indicate that the

three promoter polymorphisms in the IL-6 gene act in cooperative fashion and suggest that IL-6 haplotypes could play a role in the pathogenesis of plaque-induced gingivitis in Caucasian children regardless of plaque or gender. However, as differences in the genetic background greatly influence allele frequencies of a disease-associated gene, further studies with a larger sample size would be helpful to ascertain the genetic association between IL-6 haplotypes and gingivitis in Caucasians and children from different ethnic groups.

Acknowledgments

The study was supported by grant IGA: NR8394-3/05 and partially by grant IGA: NR9129-3/06 and project 1M0528.

References

[1] Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4:1–6.

[2] Genco RJ. Host responses in periodontal diseases: current concepts. J Periodontol 1999;63:338–55.

[3] D'Aiuto F, Parkar M, Brett PM, Ready D, Tonetti MS. Gene polymorphisms in pro-inflammatory cytokines are associated with systemic inflammation in patients with severe periodontal infections. Cytokine 2004;28:29–34.

[4] Hirose M, Ishihara K, Saito A, Nakagawa T, Yamada S, Okuda K. Expression of cytokines and inducible nitric oxide synthase in inflamed gingival tissue. J Periodontol 2001;72: 590–7.

[5] Moreira PR, Lima PM, Sathler KO, Imanishi SA, Costa JE, Gomes RS, et al. Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. Clin Exp Immunol 2007;148:119–26.

[6] Barton BE. The biological effects of interleukin 6. Med Res Rev 1996;16:87–109.

[7] Bawcock AM, Kidd JR, Lathrop GM, Daneshvar L, May LT, Ray A, et al. The human "interferon-beta 2/hepatocyte stimulating factor/interleukin-6" gene: DNA polymorphism studies and localization to chromosome 7p21. Genomics 1988;3:8–16.

[8] Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369–76.

[9] Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab 2003;88: 255–9.

[10] Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 2000;275:18138–44.

[11] Müller-Steinhardt M, Ebel B, Härtel C. The impact of interleukin-6 promoter $^{-597/-572/-174}$ genotype on interleukin-6 production after lipopolysaccharide stimulation. Clin Exp Immunol 2007;147:339–45.

[12] Trevilatto PC, Scarel-Caminaga RM, de Brito RB Jr, de Souza AP, Line SR. Polymorphism at position −174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. J Clin Periodontol 2003;30:438–42.

[13] Holla LI, Fassmann A, Stejskalova A, Znojil V, Vanek J, Vacha J. Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients with chronic periodontitis. J Periodontol 2004;75:30–6.

[14] Brett PM, Zygogianni P, Griffiths GS, Tomaz M, Parkar M, D'Aiuto F, et al. Functional gene polymorphisms in aggressive and chronic periodontitis. J Dent Res 2005;84:1149–53.

[15] Wohlfahrt JC, Wu T, Hodges JS, Hinrichs JE, Michalowicz BS. No association between selected candidate gene polymorphisms and severe chronic periodontitis. J Periodontol 2006;77:426–36.

[16] Jansson H, Lyssenko V, Gustavsson A, Hamberg K, Söderfeldt B, Groop L, et al. Analysis of the interleukin-1 and interleukin-6 polymorphisms in patients with chronic periodontitis. A pilot study. Swed Dent J 2006;30:17–23.

[17] Babel N, Cherepnev G, Babel D, Tropmann A, Hammer M, Volk HD, et al. Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. J Periodontol 2006;77:1978–83.

[18] Scapoli C, Mamolini E, Trombelli L. Role of IL-6, TNF-A and LT-A variants in the modulation of the clinical expression of plaque-induced gingivitis. J Clin Periodontol 2007; 34:1031–8.

[19] [No authors listed] European longitudinal study of pregnancy and childhood (ELSPAC). Paediatr Perinat Epidemiol 1989;3:460–9.

[20] Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol 1967;38:610–6.

[21] Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:112–35.

[22] Ennever J, Sturzenberger OP, Radike AV. The calculus surface index method for scoring clinical calculus studies. J Periodontol 1961;32:54–7.

[23] Holla LI, Fassmann A, Benes P, Halabala T, Znojil V. 5 polymorphisms in the transforming growth factor-beta 1 gene (TGF-beta 1) in adult periodontitis. J Clin Periodontol 2002;29:336–41.

[24] Loos BG, John RP, Laine ML. Identification of genetic risk factors for periodontitis and possible mechanisms of action. J Clin Periodontol 2005;32:159–79.

[25] Bhat M. Periodontal health of 14–17-year-old US schoolchildren. J Public Health Dent 1991;51:5–11.

[26] Tervonen T, Raunio T, Knuuttila M, Karttunen R. Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. J Clin Periodontol 2007;34:377–83.

[27] Nibali L, Ready DR, Parkar M, Brett PM, Wilson M, Tonetti MS, et al. Gene polymorphisms and the prevalence of key periodontal pathogens. J Dent Res 2007;86:416–20.

[28] Endler G, Marsik C, Joukhadar Ch, Marculescu R, Mayr F, Mannhalter Ch, et al. The interleukin-6 G(–174)C promoter polymorphism does not determine plasma interleukin-6 concentrations in experimental endotoxemia in humans. Clin Chem 2004;50:195–200.

[29] Dashash M, Blinkhorn AS, Hutchinson IV, Pravica V, Drucker DB. The relationship between interleukin-10 gene polymorphism at position −1082 and susceptibility to gingivitis in children. J Periodontol 2005;76:1455–62.

[30] Dashash M, Drucker DB, Blinkhorn AS. Interleukin-10 haplotype frequencies in children with gingivitis. J Periodontol 2006;77:1503–9.

[31] Jepsen S, Eberhard J, Fricke D, Hedderich J, Siebert R, Acil Y. Interleukin-1 gene polymorphisms and experimental gingivitis. J Clin Periodontol 2003;30:102–6.

[32] Scapoli C, Tatakis DN, Mamolini E, Trombelli L. Modulation of clinical expression of plaque-induced gingivitis: interleukin-1 gene cluster polymorphisms. J Periodontol 2005;76:49–56.

[33] Trombelli L, Tatakis DN, Scapoli C, Bottega S, Orlandini E, Tosi M. Modulation of clinical expression of plaque-induced gingivitis. II. Identification of "high-responder" and "low-responder" subjects. J Clin Periodontol 2004;31:239–52.

[34] Trombelli L, Scapoli C, Calura G, Tatakis DN. Time as a factor in the identification of subjects with different susceptibility to plaque induced gingivitis. J Clin Periodontol 2006; 33:324–8.

[35] Shapira L, Wilensky A, Kinane DF. Effect of genetic variability on the inflammatory response to periodontal infection. J Clin Periodontol 2005;32:72–86.