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Association between interleukin-6 polymorphisms and periodontitis in Indian non-smokers

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Abstract

Aim: Genetic polymorphisms (SNPs) in the interleukin-6 (*IL-6)* gene have been associated with the presence of periodontitis. The aim of this study was to investigate the association between five SNPs in the *IL-6* promoter region and the periodontal status of a rural Indian population.

Materials and Methods: Two hundred and fifty-one systemically healthy volunteers were clinically assessed by a single calibrated examiner and divided into: healthy individuals and periodontitis patients based on the European Workshop on Periodontitis definitions and on a recently suggested definition, which takes into account age and clinical attachment levels. Their genomic DNA was analysed blindly using real-time polymerase chain reaction to study *IL-6* variants. The association between genetic factors and the presence of periodontitis was assessed by logistic regression.

Results: The *IL-6* − 174 GG genotype was associated with periodontitis in non-smokers and older subjects (>45 years old). No statistically significant associations were detected between *IL-6* haplotypes and periodontal status, after adjusting for confounders.

Conclusions: The *IL-6* − 174 polymorphism showed some evidence of an association with the periodontal status in non-smokers and older subjects in this rural Indian population. This association might be mediated by the effect of *IL-6* on inflammatory responses.

Key words: diagnosis; genetic; interleukin; periodontitis

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Periodontitis is an infectious/inflammatory disease characterized by an amplifying cascade of biochemical and cellular events that ultimately lead to the destruction of the periodontal ligament and resorption of the surrounding bone. Although the primary role of the inflammatory cascade is to protect the host, when the immune response is not enough to eliminate the invading microorganisms, the persistence of excessive inflammatory mediators may lead to the destruction of the tooth-supporting tissues with irreversible pathological changes affecting the dental attachment apparatus (Page et al. 1997).

Some studies suggest that high levels of inflammatory mediators such as interleukin-1 (*IL-1*) and tumour-necrosis factor (TNF)-α are correlated with periodontal destruction (Ebersole et al. 1993), and are able to aggravate the inflammatory response. High levels of interleukin-6 (*IL-6*) in biological fluids and blood have been described in infections and chronic inflammatory diseases including early-onset and severe chronic periodontitis (Shapira et al. 1994, Nakajima et al. 2009). *IL-6* is strongly related to bone resorption and remodelling (Roodman et al. 1992). Therefore, the individual ability to produce higher levels of *IL-6* at the periodontal level may be

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considered as predisposing to the inflammatory tissue damage characteristic of severe periodontitis. It has previously been suggested that this pattern of response to environmental stimuli might be genetically determined and may predispose to some chronic inflammatory disorders (Fishman et al. 1998).

The complexity of multifactorial diseases such as periodontitis means that a combination of a number of different functional genetic variants as well as environmental factors could contribute to the clinical phenotype (Page et al. 1997). For example, the G to C substitution found at position −174 in the promoter of the *IL*-6 gene located close to the transcription start site was shown to alter the *IL-6* gene transcription response to stimuli such as LPS and *IL-1* (Fishman et al. 1998).

The aim of the present cross-sectional study was to investigate the association between five *IL-6* SNPs in the promoter region of the gene and their combinations (haplotypes) with the severity of periodontal disease in a rural Indian population. A secondary aim of the analysis was to test the use of different case definitions for use in cross-sectional studies with genetic outcomes.

Materials and Methods

Subject selection

The study was carried out under the auspices of the Institute for Rural Health Studies (IRHS; Hyderabad, Andhra Pradesh, India), and took place in the village of Dokur, 120 km south of the state capital, where a rural clinic is run by IRHS. All adult inhabitants (predominantly from a ''backward caste'', Deb et al. 2002) between the ages of 18 and 70 were invited to attend a screening visit by means of local advertising within the village. A group of 500 villagers attended and were initially screened based on the following exclusion criteria:

- a medical history that included any serious systemic conditions (e.g. malaria, tuberculosis or cardiovascular disease);
- presence of infectious or parasitic diseases;
- current pregnancy or breastfeeding;
- antibiotic or anti-inflammatory medications within the last 3 months;
- fewer than 16 standing teeth.

This screening identified 251 suitable subjects (10% of the total village population), who were selected and invited to take part in the study, and none of whom refused the invitation. Part of this population had been described elsewhere (Madden et al. 2000). Among the 251 subjects, there was no report of consanguinity.

Ethical considerations

Ethical approval for the study was obtained from the Medical Board of IRHS and conformed to the Indian Council of Medical Research Guidelines on Medical Ethics 2000 (Indian Council of Medical Research, Ansari Nagar, New Delhi 110 029, http://icmr.nic.in/vsicmr/ethicalidx.htm). All subjects were native to the village, and voluntary informed consent to participation in the study was obtained from each subject by means of a written consent form, translated into Telugu and also read aloud to each subject by a fluent Telugu speaker. Demographic data and self-reported data on education, social status, type of toothbrushing, alcohol consumption and smoking habit were recorded.

Clinical data collection

Full-mouth periodontal examination of probing pocket depth (PPD) and clinical attachment level (CAL) was carried out for each subject with an EN-15 probe on six sites per tooth. Dental plaque samples were taken from each subject, but the results are not described in this paper. The clinical examination was performed by one single operator (I. M.), previously calibrated to an exact κ level of 0.85 for PPD and bleeding on probing. Patients were divided into two groups based on their periodontal status. The two suggested European Workshop on Periodontitis (EWP) definitions were used (Tonetti & Claffey 2005):

1. presence of proximal attachment loss of $\geqslant$3 mm in $\geqslant$2 non-adjacent teeth (EWP1);
2. presence of proximal attachment loss of $\geqslant$5 mm in $\geqslant$30% of teeth present (EWP2).

Furthermore, to account for the wide age spectrum between subjects and the non-matched study design, a definition based on an age-related reference curve (ARC), using the 50th percentile to discriminate between periodontitis and non-periodontitis, was also used (Meisel & Kocher 2009).

Genetic analysis

Samples of buccal mucosal cells were taken bilaterally (one from each side) from all subjects using wire cytology brushes (Medical Wire and Equipment Company, Corsham, Wiltshire, UK). The material from the buccal mucosa was immediately transferred from the brush heads into 1 ml screw-top cryophials (Invitrogen BV, Groningen, the Netherlands) containing 0.15 ml of 10 mM Tris-HCl/EDTA, pH 7.6 (TE buffer). After inserting the samples and thorough mixing from the brush, 0.15 ml of 0.5 M NaOH was added to preserve the samples. The cryophials were then sealed with parafilm and all samples were stored at ambient temperatures. All samples were processed in the laboratory up to 4 weeks after collection. Two microlitres of Proteinase-K (Invitrogen BV) was added to both samples, which were then incubated at 56°C for 1 h. 31 μl sodium acetate and 610 μl cold absolute ethanol were added to each sample and the solution was centrifuged for 15 min. at 12,000 rpm. The supernatant was removed and the pellet was evaporated under vacuum (Savant automatic environmental speedvac, Savant Instruments, Holbrook, NY, USA) for 30 min. Fifty microlitres of filtered distilled water was added to each sample and the samples were stored at −20°C. After extraction of the DNA from the swabs, vials were labelled and coded. Samples were evaluated in a double-blind fashion. An Applied Biosystems 7300 real-time PCR System was used to perform the Allelic discrimination assays at the Eastman Dental Institute (London, UK), as described previously. Five polymorphisms in the *IL-6* gene promoter region were selected for genotyping, including SNPs at positions −174 (CCTTTAGCAT[C-G]GCAAGAC, rs 1800795), −572 (CAACAGC C[C-G] CTCACAG, rs 1800796), −1363 (CACTGTTTTATC[G-T]GATCTTG, rs 2069827) and −6106 (TCTCTACA[A-T]TAAGAAATAC), and a base-pair deletion at position −1480 (ACCGTCTCT [C-G]TGTTTAG) (Nibali et al. 2008). A total of 55 genotypes (55/1255 = 4%) among all subjects for all SNPs were not scored because they remained unclear after repeated analysis. The distribution of genetic polymorphisms for the *IL-6* −572, −1480, −6106 and −174 satis-

fied the χ^2 analysis for Hardy–Weinberg equilibrium. *IL-6* −1363 satisfies Hardy–Weinberg equilibrium for the whole sample, but not for the no-periodontitis group due to the presence of a very rare homozygote; we therefore have no difficulty in concluding that the genotyping is correct for this sample.

Statistical analysis

Comparisons of continuous and categorical data between groups (periodontitis and non-periodontitis) were performed with ANOVA and the χ^2 test, respectively. The alpha value was set at 0.05.

The association between each SNP and periodontal disease was evaluated using the SPSS 12.0 package. A binary independent variable (periodontitis or non-periodontitis) was used to ascertain the association between each SNP and periodontal diagnosis. Multiple logistic regression analysis adjusting for confounders (age, smoking, gender, type of toothbrushing, previous dental care) was performed to investigate the association between genotypes and diagnosis. Analyses investigating two different types of genetic models (presence of one copy or two copies of the suspected predisposing allele) were performed. Because of the known relationship between age and smoking and the presence of periodontitis, with the subsequent risk of finding spurious associations (Pritchard & Rosenberg 1999), separate analyses were performed in non-smoking subjects (Haber et al. 1993), and in all subjects divided by three age groups (<35, 35–45 and >45 years old) (Neely et al. 2001).

The second analytic approach consisted of analysis of linkage disequilibrium between SNPs located in the same genes, and an association study between haplotypes and periodontitis. These aimed at exploring possible interactions between polymorphisms located in the same gene in determining disease susceptibility. The GC utilities package (Curtis et al. 2006) was used for linkage disequilibrium (LD) analysis. The LD pairs programme allowed investigation of linkage disequilibrium between SNPs. Haplotype associations were analysed using the WHAP package (Sham et al. 2004) as described previously (Nibali et al. 2008). The RunGC programme was used for a separate analysis of haplotype frequencies, and to test for sig-

Table 1. Demographic characteristics of the participants, as divided by their periodontal status (EWP classification 1, EWP classification 2 or age-related curve (ARC) as suggested by Meisel & Kocher 2009)

		All subjects ($n = 251$)	EWP1		EWP2		ARC	
			periodontitis ($n = 105$)	non-periodontitis ($n = 146$)	periodontitis ($n = 47$)	non-periodontitis ($n = 204$)	periodontitis ($n = 92$)	Non-periodontitis ($n = 159$)
Age			45.8 ± 10.5***	37.5 ± 11.1***	49.6 ± 10.3***	39.0 ± 10.9***	43.5 ± 9.5*	39.3 ± 12.4*
Gender (female)		162	62	100	28	134	56	106
		(64.5%)	(59.0%)	(68.5%)	(59.6%)	(65.7%)	(60.9%)	(66.7%)
Smoking	Current	44	26*	18*	33*	174*	23*	21*
		(17.5%)	(24.8%)	(12.3%)	(70.2%)	(85.3%)	(25.0%)	(13.2%)
	Never	207	79*	128*	14*	30*	69*	138*
		(82.5%)	(75.2%)	(87.7%)	(29.8%)	(14.7%)	(75.0%)	(86.8%)
Clinical data	Number of sites PPD 4–6 mm	16.1 ± 22.1	38.5 ± 17.4***	0.1 ± 0.4***	45.5 ± 15.9***	8.7 ± 15.6***	41.0 ± 5.3***	1.8 ± 7.1***
	Number of sites PPD >6 mm	3.3 ± 8.1	7.9 ± 11.1***	0.0 ± 0.2***	15.1 ± 13.1***	0.6 ± 1.8***	7.9 ± 10.9***	0.7 ± 4.1***
	% of sites PPD 4–6 mm	13.0 ± 14.0	24.7 ± 10.8***	0.0 ± 0.2***	31.7 ± 9.2***	5.4 ± 9.7***	26.0 ± 10.2***	1.3 ± 5.0***
	% of sites PPD >6 mm	2.2 ± 5.7	5.3 ± 7.8***	0.0 ± 0.2***	10.2 ± 9.5***	0.4 ± 1.2***	5.2 ± 7.6***	0.5 ± 3.1***
	Number of teeth with PPD >6 mm	1.9 ± 3.8	4.6 ± 4.8***	0.0 ± 0.1***	8.3 ± 4.8***	0.5 ± 1.3***	4.6 ± 4.7***	0.4 ± 2.1***
	Number of sites CAL >4 mm	16.6 ± 22.1	39.5 ± 16.3***	0.1 ± 0.6***	47.5 ± 15.5***	9.5 ± 16.6***	42.2 ± 4.8***	1.8 ± 6.9***
	Number of sites CAL >6 mm	6.1 ± 14.0	14.5 ± 18.5***	0.0 ± 0.4***	27.5 ± 21.1***	1.1 ± 2.7***	13.8 ± 7.8***	1.7 ± 8.4***
	%CAL>4 mm	10.6 ± 13.9	25.3 ± 9.8***	0.1 ± 0.3***	3.4 ± 0.7***	0.8 ± 1.2***	26.7 ± 9.0***	1.3 ± 4.9***
	%CAL>6 mm	4.1 ± 10.2	10.0 ± 14.0***	0.0 ± 0.3***	2.2 ± 1.3***	0.2 ± 0.5***	9.1 ± 12.3***	1.3 ± 7.6***

Statistical analysis for differences between the groups was presented for each classification group. Comparisons of continuous and categorical data between groups were analysed with ANOVA and the χ^2 test, respectively.
*$p < 0.05$ and > 0.001; ***$p < 0.00.1$.

nificant differences using a likelihood-ratio test (LRT).

Results

A total of 251 subjects took part in the study (Table 1). Eighty-nine of these subjects (35.5%) were males, while 162 (64.5%) were females. Their age ranged from 18 to 65 years (mean age 41 years). Tobacco use was reported by 17.5% of all subjects, while alcohol consumption was more frequent (56.6%) in this population. 215 subjects (85.7%) reported not having ever received previous dental care. The average number of teeth present was 26.4 (range 16–29). Clinically, the average number of sites with PPD 4–6 mm was 16.1% (SD = 22.1), while the average sites with PPD > 6 mm was 3.3% (SD = 8.1) per patient. Participants had on average 6.1% (SD = 13.9) sites with CAL > 6 mm. A total of 95 subjects (38%) had at least one site with CAL > 6 mm. Applying the two EWP guideline definitions, 42% and 19% of the subjects were diagnosed with periodontitis, according to the first and the second definition, respectively (Tonetti & Claffey 2005). According to the ARC (Meisel & Kocher 2009), 92 subjects (36.7%) belonged to the periodontitis group, while 159 (63.3%) belonged to the non-periodontitis group. Site-based data for the periodontitis and non-periodontitis groups were found to be similar for subjects divided by EWP1 and ARC definitions.

The following factors were associated with the presence of periodontitis (unadjusted analyses): age ($p < 0.001$ for EWP1 and EWP2, $p = 0.06$ for ARC), smoking ($p = 0.012$, 0.019 and 0.025, respectively, for EWP1, EWP2 and ARC diagnoses), alcohol consumption ($p < 0.001$, $p = 0.012$ and $p < 0.001$, respectively, for EWP1, EWP2 and ARC diagnosis) and type of tooth brushing were also associated with the presence of periodontitis ($p = 0.020$ for EWP1 and $p = 0.32$ for ARC diagnoses, respectively).

Linkage disequilibrium

Significant LD was found between all the studied *IL-6* polymorphisms (*IL-6* − 174, − 1480, − 1363, − 572), except for the one at position –6106. This polymorphism was in LD only with the 572 SNP (Fig. 1).

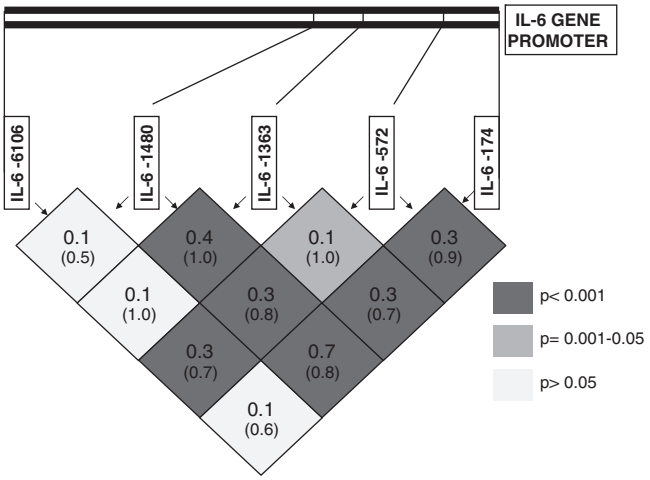

Fig. 1. Schematic representation of LD between the five analysed *IL-6* polymorphisms. R^2 for LD between each marker are reported. D′ values between each marker are reported in parenthesis. The different colour shades represent *p*-values (see legend).

Comparison between groups (periodontitis and non-periodontitis)

The clinical parameters in subjects divided by age groups are reported in Table 2. The genotype data gathered from the five markers are shown in Table 3. Allelic distributions are shown in Table 4. *IL-6* − 572 and − 6106 allelic distribution was associated with the EWP2 classification, while − 572 was also associated with ARC classification. The distribution of the different *IL-6* genetic polymorphisms in the whole population in relation to the predetermined diagnoses of periodontal diseases showed no statistically significant difference for any of the studied SNPs.

The division of subjects by age groups (described in the statistical analysis paragraph) identified three roughly equal groups of patients: 94 (37.5%) were < 35 years old, 77 (30.7%) were 36–45 years old and 80 (31.9%) were > 45 years old. Sub-analysis of the relationships between the presence of periodontitis and genotypes revealed an association between *IL-6* − 174 GG and periodontitis limited to subjects older than 45 years [$p = 0.042$, odds ratio (OR) = 3.21, 95% confidence interval (CI) = 1.04–9.89, and $p = 0.025$, OR = 4.85, 95% CI = 1.39–16.95, respectively, for EWP1 and age-related diagnosis, at logistic regression analysis adjusted for confounders).

Analysis in the non-smoking population

A further analysis in subjects who had never smoked showed a moderate association between *IL-6* − 174 and the presence of periodontitis, statistically significant only for the age-related definition ($p = 0.019$, OR = 3.6, 95% CI = 1.19–6.96 adjusted for confounders) (Table 5 and Fig. 2).

Haplotypes

WHAP haplotype analysis revealed no statistically significant associations with the disease trait, once the analysis was adjusted for known confounders.

Discussion

Most genetic epidemiological studies in the periodontal literature have been performed in populations exposed to dental prevention and care (Kinane et al. 2005). Owing to the role of *IL-6* as an inflammatory mediator, it is thought that individual variability in the ability to synthesize and release *IL-6* may modulate the predisposition to a number of inflammatory diseases such as atherosclerosis and rheumatoid arthritis (Fishman et al. 1998, Fife et al. 2005). In the present cross-sectional study, the relative frequency of different genotypes of polymorphisms on the *IL-6* gene promoter (− 174, − 572, − 1363, − 1480, − 6106) and their combinations (haplotype) were investigated in smokers and non-smokers in a rural Indian popula-

Table 2. Clinical parameters in subjects divided by age groups (according to EWP1 definition)

	Age group <35 ($n = 94$, periodontitis = 24, non-perio = 70)	Age group 35–45 ($n = 77$, periodontitis = 30, non-periodontitis = 47)	Age group >45 ($n = 80$, periodontitis = 51, non-periodontitis = 29)
Clinical data			
Number of PPD 4–6 mm	8.7 ± 16.0	15.7 ± 23.6	25.3 ± 24.1
Number of PPD > 6 mm	1.3 ± 4.5	2.2 ± 4.7	6.8 ± 12.1
Number of teeth with PPD > 6 mm	0.8 ± 2.3	1. 6 ± 3.0	3.7 ± 5.2
Number of CAL > 4 mm	9.1 ± 1.2	16.7 ± 23.5	25.4 ± 23.3
Number of CAL > 6 mm	1.9 ± 6.6	3.6 ± 7.1	13.4 ± 20.8

Average and standard deviations are reported.

Table 3. Distribution of all the genotypes for the studied polymorphisms in all subjects ($n = 251$) for the three definitions adopted: European Workshop on Periodontology (EWP) 1 and 2, and age-related curve (ARC as described by Meisel & Kocher 2009)

	EWP1		EWP2		ARC	
	periodontitis ($n = 105$)	non-periodontitis ($n = 146$)	periodontitis ($n = 47$)	non-periodontitis ($n = 204$)	periodontitis ($n = 92$)	non-periodontitis ($n = 159$)
IL-6 − 174						
CC	6 (5.8%)	7 (5.0%)	4 (8.5%)	9 (4.6%)	3 (3.3%)	10 (6.5%)
CG	18 (17.5%)	28 (19.9%)	9 (19.1%)	37 (18.8%)	16 (17.8%)	30 (19.5%)
GG	79 (76.7%)	106 (75.2%)	34 (72.3%)	151 (76.6%)	71 (78.9%)	114 (74.0%)
IL-6 − 572						
CC	33 (31.4%)	53 (40.8%)	14 (29.8%)	72 (38.3%)	58 (40.6%)	28 (30.4%)
CG	50 (47.6%)	56 (43.1%)	19 (40.4%)	87 (46.3%)	61 (42.7%)	45 (48.9%)
GG	22 (21.0%)	21 (16.2%)	14 (29.8%)	29 (15.4%)	24 (16.8%)	19 (20.7%)
IL-6 − 1363						
AA	1 (1.0%)	0 (0%)	0 (0%)	1 (0.5%)	1 (1.1%)	0 (0%)
AC	6 (5.8%)	6 (4.4%)	3 (6.4%)	9 (4.7%)	5 (5.5%)	7 (4.7%)
CC	96 (93.2%)	130 (95.6%)	44 (93.6%)	182 (94.8%)	85 (93.4%)	141 (95.3%)
IL-6 − 1480						
CC	78 (74.3%)	96 (68.1%)	33 (70.2%)	141 (70.9%)	70 (76.1%)	104 (67.5%)
CG	26 (24.8%)	40 (28.4%)	14 (29.8%)	52 (26.1%)	21 (22.8%)	45 (29.2%)
GG	1 (1.0%)	5 (3.5%)	0 (0%)	6 (3.0%)	1 (1.1%)	5 (3.2%)
IL-6 − 6106						
AA	77 (74.8%)	88 (65.7%)	39 (23.0%)	126 (66.3%)	67 (73.6%)	98 (67.1%)
AT	24 (23.3%)	40 (29.9%)	7 (14.9%)	57 (30.0%)	22 (24.2%)	42 (28.8%)
TT	2 (1.9%)	6 (4.5%)	1 (2.1%)	7 (3.7%)	2 (2.2%)	6 (4.1%)

tion with limited access to dental care and periodontal phenotypes ranging from healthy to severe periodontitis. Limitations of cross-sectional studies of this nature include difficulties in case definition. Therefore, the other objective of the analysis was to test and compare the applicability of different disease definitions to the sample. In order to distinguish periodontitis and non-periodontitis subjects in this study, we applied the two definitions suggested by the European Workshop on Periodontology (EWP) (Tonetti & Claffey 2005). According to these, 42% and 19% of the subjects, respectively, were diagnosed with periodontitis. However, because of the effect of age on periodontal disease presentation, we also applied a definition based on an ARC recently suggested by Meisel and Kocher, using data from another larger cross-sectional study (Hensel et al. 2003). This overcomes, to some extent, the drawbacks of the cross-sectional nature of the study in terms of lack of a match between groups. Using the 50th percentile as a cut-off, 37% of the subjects were diagnosed with periodontitis. Interestingly, EWP1 and ARC case definitions identified similar groups of subjects. As observed in Tables 1 and 3, the clinical features (PPD and CAL) and genotype data were almost identical. In fact, all but 14 out of 105 subjects defined as having periodontitis by the EWP definition (based on attachment loss of $\geqslant 3$ mm in $\geqslant 2$ non-adjacent teeth) were also defined as having periodontitis according to the ARC definition (which takes into account $\geqslant 4$ mm CAL in relation to age). One single subject defined as having disease by the ARC definition was considered as having ''non-periodontitis'' in the EWP1. Age, smoking and type of toothbrushing were associated with periodontal status. In particular, older subjects, smokers, alcohol users and subjects using neem stick or a toothbrush rather than a finger to clean their teeth had a higher prevalence of periodontitis. The association between toothbrushing technique and the presence of periodontitis is probably due to interactive effects with smoking (a higher percentage of smokers brushed with a neem stick rather than a finger, data not presented). The associations between smoking and periodontitis and between age and periodontitis have been well documented in previous studies (Haber et al. 1993, Neely et al. 2001). Owing to these associations and the potential for residual confounding effects (especially due to the wide age range in the study), we performed

Table 4. Allele distribution in periodontitis and non-periodontitis subjects for all the polymorphisms studied

Allele frequency		EWP1		EWP2		ARC	
		periodontitis ($n = 105$)	non-periodontitis ($n = 146$)	periodontitis ($n = 47$)	non-periodontitis ($n = 204$)	periodontitis ($n = 92$)	non-periodontitis ($n = 159$)
IL-6 −174	C	30 (14.5%)	42 (14.9%)	17 (18.1%)	55 (13.9%)	22 (12.2%)	50 (16.2%)
	G	176 (85.5%)	240 (85.1%)	77 (81.8%)	339 (86.1%)	158 (87.8%)	258 (83.8%)
IL-6 −572	C	116 (55.2%)	162 (62.3%)	47 (50.0%)*	231 (61.4%)*	177 (61.9%)***	101 (52.1%)***
	G	94 (44.8%)	98 (37.7%)	47 (50.0%)*	145 (38.6%)*	109 (38.1%)***	93 (47.9%)***
IL-6 −1363	A	8 (3.9%)	6 (2.2%)	3 (3.1%)	11 (2.9%)	7 (3.8%)	7 (2.4%)
	C	198 (96.1%)	266 (97.8%)	91 (96.9%)	373 (97.1%)	175 (86.2%)	289 (97.6%)
IL-6 −1480	C	182 (86.7%)	232 (82.2%)	80 (85.1%)	334 (83.9%)	161 (87.5%)	253 (82.1%)
	G	28 (13.3%)	50 (17.8%)	14 (14.9%)	64 (16.1%)	23 (12.5%)	55 (17.9%)
IL-6 −6106	A	178 (86.4%)	216 (80.6%)	85 (90.4%)**	309 (81.3%)**	156 (85.7%)	238 (81.5%)
	T	28 (13.6%)	52 (19.4%)	9 (9.6%)**	71 (8.7%)**	26 (14.3%)	54 (18.5%)

Two-tailed Fisher's exact test p values are reported in the last column.
*$p = 0.043$; **$p = 0.035$; ***$p = 0.032$.

Table 5. Results of logistic regression analyses for the association between *IL-6* −174 GG and different diagnosis of periodontitis in non-smokers ($n = 207$) for the three definitions adopted: European Workshop on Periodontology (EWP) 1 and 2, and age-related curve (ARC; as described by Meisel & Kocher 2009)

Definitions of periodontitis	EWP1			EWP2			ARC		
Logistic regression analyses (adjusted for gender, age, tooth brushing and previous dental care)	$p =$	OR	95% CI	$p =$	OR	95% CI	$p =$	OR	95% CI
All subjects ($n = 251$)	0.150	1.7	0.8–3.3	0.318	0.6	0.3–1.5	0.084	1.8	0.9–3.6
Non-smokers ($n = 207$)	0.062	2.3	0.9–5.4	0.333	1.7	0.6–5.2	0.019	2.9	1.2–7.0

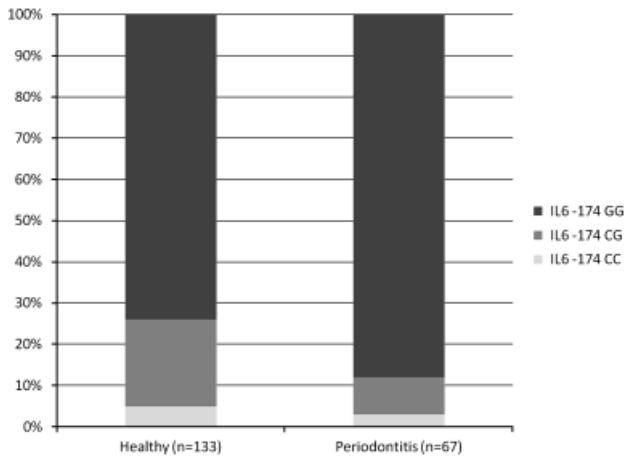

Fig. 2. Representation of the *IL-6* −174 genotype distribution in non-smokers with and without periodontitis. Logistic regression analysis for *IL-6* −174 GG *versus* CG/CC $p = 0.0017$, odds ratio = 3.6, 95% confidence interval = 1.26–10.43.

further analyses in non-smokers and in subjects divided by age groups. It was observed that among individuals who had never smoked, subjects homozygous for *IL-6* –174 GG had a higher incidence of periodontitis. This was statistically significant only when the age-adjusted definition was considered (Meisel & Kocher 2009). No statistically significant association was found for the other markers (−572, −1363, −1480, −6106), although −572 and −6106 allelic distributions showed moderate associations with periodontitis. The fact that a genetic association with periodontitis was evident only in non-smokers confirms the importance of smoking as a periodontal disease modifier and as a confounder in periodontal epidemiological studies (Korn-

man et al. 1997). The *IL-6* −174 GG genotype was also associated with periodontitis in older subjects (>45 years) irrespective of smoking status (for both classifications), suggesting that a cumulative/chronic effect of this genotype over time may increase the susceptibility to periodontitis.

Some studies support the presence of genetic factors in determining the predisposition to and progression of periodontitis (Kinane et al. 2005). In this study, an increased prevalence of the GG genotype was observed for the *IL-6* –174 polymorphism in non-smoking subjects with periodontitis, suggesting that subjects harbouring the GG genotype may be more prone to developing periodontitis. This is consistent with other studies showing increased *IL-6* −174 GG prevalence in Caucasian subjects with aggressive periodontitis (Nibali et al. 2008) and in Brazilian individuals with chronic periodontitis (Trevilatto et al. 2003), and with another study showing association between the same genotype and extent of periodontitis (Tervonen et al. 2007). These results may be explained by the supposedly more pronounced inflammatory response associated with the *IL-6* −174 G allele (Fishman et al. 1998, Bennermo et al. 2004), also in periodontitis patients (Raunio et al. 2007). It remains to be explained why the same effect was not found when smokers were included in the analysis.

In the present investigation, no association could be found between *IL-6* haplotypes with supposed clinical relevance (Terry et al. 2000, Fife et al. 2005, Nibali et al. 2008) and periodontal disease. This is consistent with another study on chronic periodontitis (Nibali et al. 2009) and may be due to the different ethnicities of the populations studied or due to the different disease phenotypes (chronic or aggressive periodontitis) included.

One of the limitations of the study might be the criteria selected to define the presence of periodontitis (Preshaw 2009). This study presents a first application of the suggested ARC for cross-sectional studies on periodontitis (Meisel & Kocher 2009), although clear differences exist in ethnicity and socioeconomic factors between subjects who took part in the SHIP study in Pomerania (Hensel et al. 2003), where these data are derived from, and the Indian villagers who took part in this study. The association between genetic factors and periodontitis in the older age group was observed for both EWP1 and age-related classifications. However, only the latter showed a statistically significant association with periodontitis in non-smokers. We cannot exclude the possibility of a type I error linked to the cross-sectional nature of the study, although the results of this study point towards the same direction as those obtained in independent populations (Tervonen et al. 2007, Nibali et al. 2009).

Within the limitations of this study, the results support the hypothesis of a possible association between *IL-6* polymorphisms (in particular at position −174) and periodontal disease in non-smoking subjects living in a rural Indian village. Further studies in other populations and with larger sample sizes and haplotype analyses are required to confirm these findings. This study also highlights the importance of a case definition in cross-sectional studies and supports a role for ARC in the diagnosis of periodontitis in research settings.

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Clinical Relevance

Scientific rationale for study: Genetic factors are thought to affect the onset and progression of periodontitis.

Principal findings: Interleukin-6 genotypes were associated with periodontitis in non-smokers and older subjects living in a rural village in India.

Practical implications: Interleukin-6 genetic factors may predispose to periodontitis in some subjects not exposed to routine dental care.