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Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis

Raquel M. Scarel-Caminaga[1,2], Paula C. Trevilatto[1,3], Ana P. Souza[1,2], Rui B. Brito[1], Luis E. A. Camargo[4] and Sergio R. P. Line[1]

[1]Department of Morphology, Dental School of Piracicaba – FOP/UNICAMP, Piracicaba, SP, Brazil; [2]University of the Sacred Heart, Bauru, SP, Brazil; [3]School of Dentistry, Pontifical Catholic University of Paraná (PUC-PR), Curitiba, PR, Brazil; [4]Laboratory of Molecular Genetics – ESALQ/USP, Piracicaba, SP, Brazil

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Abstract

Background: Chronic periodontitis (CP) is characterized by an inflammation in the supporting tissues of the teeth caused primarily by bacterial infection. Interleukin 10 (IL10) is an anti-inflammatory cytokine whose genetic polymorphisms may influence the expression of the protein.

Objective: In this study we investigated the hypothesis that single-nucleotide polymorphisms (SNPs) in the promoter of *IL10* gene might be related to CP.

Materials and Methods: DNA was obtained from $n = 67$ CP patients and $n = 43$ control subjects. All studied individuals were non-smokers. The –1087 SNP was investigated by DNA sequencing, and the –819 and –592 SNPs by restriction fragment length polymorphism of PCR products.

Results: Frequencies of –819 and –592 SNPs showed differences between the control and CP groups. The ACC haplotype was more prevalent in the control group and the ATA haplotype more prevalent in the CP group. The ATA haplotype seemed to increase susceptibility to CP in women (odds ratio (OR) = 2.57). The heterozygous haplotype GCC/ACC was predominant in the control group (OR = 8.26; $p = 0.001$).

Conclusions: Specific haplotypes and SNPs in *IL10* gene are associated with susceptibility to CP in Brazilian patients.

Key words: chronic periodontitis; haplotypes; interleukin 10; polymorphisms

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Periodontitis is a chronic multifactorial disease which is caused mainly by gram-negative microorganisms present in the plaque adjacent to the gingiva, resulting in stimulation of host cells to produce molecules important in the immunoinflammatory response (Kornman & Newman 2000). There is a complex network of pro- and anti-inflammatory cytokines acting in the inflamed periodontal tissues (Okada & Murakami 1998). An important cytokine recently investigated in chronic periodontitis (CP) is interleukin 10 (IL10). The expression of mRNA *IL10* in periodontitis lesions was significantly higher compared with autologous peripheral blood mononuclear cells (PBMC) (Yamazaki et al. 1997). IL10 is an anti-inflammatory cytokine, produced by T-helper 2 cells (Th2), macrophages and B cells, which inhibits synthesis of pro-inflammatory cytokines such as IL1, IL2, IL6, IL8, tumor necrosis factor-α and interferon-γ. However, IL10 is a B cell stimulator, enhancing B cell proliferation and differentiation. These facts suggest that IL10 can play important roles in the regulation of cellular and humoral immune responses (Hajeer et al. 1998). The expression levels of the IL10 may be modulated by genetic polymorphisms in regulatory regions of the gene, mainly the promoter region localized upstream of the start codon of the gene (Stern 2000). There are three single-nucleotide polymorphisms (SNPs) in the *IL10* gene at positions –1087, –819 and –592 from the transcriptional start site which have been associated with altered synthesis of IL10 in response to inflammatory stimuli (Turner et al. 1997, Berglundh et al. 2003). The –1087 SNP is a G to A substitution and lies within a putative Ets transcription factor binding site (Kube et al. 1995). The –1087G allele is known to be associated with high in vitro IL10 production (Turner et al. 1997). The –819 SNP presents a dimorphic polymorphism, a C to T substitution, and may affect an estrogen responsive element (Lazarus et al. 1997). The –592 SNP is a C to A substitution and lies within a region with a negative regulatory function (Kube et al. 1995). The three dimorphisms exhibit strong linkage disequilibrium and appear in three preference potential haplotypes: GCC (G at position –1087, C at position

–819 and C at position –592), ACC and ATA. The haplotype GCC has been associated with high production of IL10 (Turner et al. 1997).

Given the important role of IL10, it seems possible that genetic polymorphisms in the *IL10* gene may modulate the levels of the protein, and might be associated with CP. In the present study we have tested if the –1087, –819 and –592 SNPs in the *IL10* gene and its haplotypes are associated with CP.

Material and Methods

Selection of subjects

A sample of 110 unrelated, non-smoking subjects >25 years of age (mean 41.2 ± 12.5), were recruited for study from the patient pool at the Dental Clinics of the Faculty of Dentistry of Piracicaba – UNICAMP. The patients are from the South-eastern of Brazil. All subjects signed a consent form that was approved by an Institutional Review Board (85/2000). The baseline clinical parameters for the subject population are presented in Table 1. All individuals involved in this study were in good general health and they did not have any of the exclusion criteria described previously (Scarel-Caminaga et al. 2002a). Diagnosis and classification of disease severity were made on the basis of dental clinical parameters including probing depth, assessment of clinical attachment loss (CAL), tooth mobility and observation of bleeding on probing. Measurements of probing depth and attachment level were recorded at six points around each tooth. Subjects were categorized in two groups:

1. *Control group*: subjects found to exhibit no signs of periodontal disease as determined by the absence of CAL and no sites with probing depth >3 mm, together with less than 10% of sites with gingivitis upon clinical examination ($n = 43$).
2. *CP group*: formed by two subgroups with different levels of the disease. The subgroups were *moderate periodontitis* – patients with teeth exhibiting ⩾3 mm and ⩽6 mm CAL ($n = 31$), and *severe periodontitis* – patients with teeth exhibiting ⩾7 mm CAL ($n = 36$). All patients had at least three teeth exhibiting sites CAL in at least two different quadrants.

The dental clinical categories according to CP generalized severity, which were used above to discriminate the subjects have been published previously (Scarel-Caminaga et al. 2002a, de Souza et al. 2003, Trevilatto et al. 2003).

Analysis of genetic polymorphisms

DNA was extracted from epithelial buccal cells with sequential phenol/chlorophorm solution and precipitated with salt/ethanol solution (Scarel et al. 2000). The *IL10* gene promoter region (GenBank accession number X78437) was amplified by PCR (total volume of 50 μl) in two fragments.

Promoter region of IL10 (fragment 1)

The following primers were used: reverse – 5′ TTC TGT GGC TGG AGT CTA AAG TT 3′ and forward –5′ TTC CTC CCA GTT ACA GTC TA 3′ (1 μM each primer). Amplification reactions were performed with 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 200 μM of each dNTP, 500 ng genomic DNA and 2.5 U *Taq* DNA Polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden). The PCR temperature profile included an initial denaturation step at 95°C for 5 min, followed by 35 cycles each of 95°C (1 min), 59°C (1 min) and 72°C (1 min), with a final extension step of 72°C (5 min).

Promoter region of IL10 (fragment 2)

Amplification reactions were performed utilizing the following primers: reverse – 5′ GGT CTC TGG GCC TTA GTT TCC 3′ and forward – 5′ AAC TTT AGA CTC CAG CCA CAG AA 3′ in the same conditions described above (annealing temperature was 62°C for 1 min).

The amplified fragment 1 (395 bp) of *IL10* promoter region (containing the –1087 SNP) was submitted to purification using a commercial Kit (GFX PCR DNA and Gel Band Purification Kit, Amersham Pharmacia Biotech). Polymorphisms were investigated by sequencing in the ABI 377 DNA Sequencer (Perkin-Elmer Corporation) using 4 μl DYEnamic ET Terminator (Amersham Pharmacia Biotech, Uppsala, Sweden).

In order to investigate the –819 and –592 SNPs, the amplified fragment 2 of *IL10* promoter region (492 bp) was subjected to the RFLP (restriction fragment length polymorphism) method and performed similarly as described previously (Eskdale et al. 1997), but with different band lengths due to the different primers utilized.

Statistical analysis

The significance of the differences in observed frequencies of each polymorphism in control and CP groups was assessed by standard chi-squared (χ^2) tests or using the CLUMP program (http://www.mds.qmw.ac.uk/statgen/dcurtis/software.html) that employs the Monte Carlo simulations (Sham & Curtis 1995). The use of the Monte Carlo method avoids the need for a Bonferroni correction and the difficulty of assessing the significance of rarer alleles. The CLUMP program is designed for use in genetic case–control studies where multiple alleles are being considered and the observed frequencies of some alleles are rare (Hodge et al. 2001). Differences were considered significant when $p < 0.05$, and the risk associated with individual alleles or genotypes was calculated as the odds ratio (OR) with 95% confidence

Table 1. Baseline clinical parameters of the subject population

	Control ($n = 43$)	Chronic periodontitis		Total ($n = 110$)
		Moderate ($n = 31$)	Severe ($n = 36$)	
Age (years)				
Mean (± D)	43 (± 14.0)	37 (± 11)	44 (± 10.5)	41.2 (12.5)
Gender	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Female	28 (65.1)	25 (80.6)	30 (83.4)	83 (75.5)
Male	15 (34.9)	6 (19.4)	6 (16.6)	27 (24.5)
Ethnic group	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Caucasoid	36 (83.7)	24 (77.4)	24 (66.7)	84 (76.3)
Afro-American	3 (7.0)	5 (16.1)	5 (13.9)	13 (11.8)
Mulatto	3 (7.0)	2 (6.5)	7 (19.4)	12 (10.9)
Japanese	1 (2.3)	0.0	0.0	1 (0.9)

intervals (CI) using SAS statistical package (v.6.11). In order to calculate gene heterozygosity, Hardy–Weinberg expectations, linkage disequilibrium and haplotypes, the computer program package ARLEQUIN was used (v. 2.0 – http://anthro.unige.ch/arlequin) (Schneider et al. 2000).

Results

Frequency analysis in the control and CP groups

The subject sample included in this study was composed mainly by female subjects (75.5%) (Table 1). Regarding ethnic status, Caucasians were prevalent (77.2%) compared with Afro-Americans together with Mulatto individuals (22.8%). We had just one subject of Asian origin. We have made statistical analysis considering only the Caucasian individuals (female = 72.6%; male = 27.4%), the results were very similar to the obtained with the total sample (Tables 2–4).

Since the –819 SNP of the *IL10* gene is responsive to estrogen, the allelic and genotypic frequencies were analysed considering the total sample and the female sample (Table 2). This SNP had a significant difference in the genotype frequency ($p = 0.017$) considering the total sample, and the calculation of the OR revealed that individuals with CC genotype seemed to be three times less susceptible to CP (OR = 3.04; 95% CI = 1.34–6.91). When the same analysis was made only in the female subjects, the OR increased to 4.13 (95% CI = 1.55–11.02).

The –592 SNP of *IL10* gene revealed the same tendency observed in the analysis for the –819 locus. Significant differences in genotype frequencies of –592 locus between control and CP groups were confirmed through the OR values, especially when only the female subjects were analysed (OR = 3.38; 95% CI = 1.29–8.82) (Table 1). Regarding the –1087 SNP, no significant differences were found in the total sample and in the female group nor when only Caucasians were analysed.

Haplotype frequencies

The distribution of haplotypes arranged as alleles (Table 3) showed no significant difference in the total sample ($p = 0.179$) nor in Caucasians ($p = 0.267$), between control and CP groups. However, in the female subjects, the *p*-value ($p = 0.061$) suggested a trend with the predominance of haplotype ACC in the control group and the predominance of haplotype ATA in the CP group.

We have found rare haplotypes such as GTA, ATC, GTC and ACA in all subgroups analysed (Table 3). A considerable percentage of the haplotype ATC was observed in the CP groups of

Table 2. Frequencies of *IL10* SNPs in control and chronic periodontitis (CP) individuals

SNP	Total ($n = 110$)			Female subjects ($n = 83$)			Caucasians ($n = 84$)		
	Control *n* (%)	CP *n* (%)	p^a	Control *n* (%)	CP *n* (%)	p^a	Control *n* (%)	CP *n* (%)	p^a
– 1087			0.488			0.748			0.455
Allele	$n = 86$	$n = 134$		$n = 56$	$n = 110$		$n = 72$	$n = 96$	
A	55 (64.0)	93 (69.4)		35 (62.5)	73 (66.4)		47 (65.3)	69 (71.9)	
G	31 (36.0)	41 (30.6)		21 (37.5)	37 (33.6)		25 (34.7)	27 (28.1)	
Genotype	$n = 43$	$n = 67$	0.462*	$n = 28$	$n = 55$	0.844*	$n = 36$	$n = 48$	0.162*
AA	17 (39.5)	34 (50.7)		11 (39.3)	25 (45.5)		14 (38.9)	27 (56.3)	
AG	21 (49.0)	25 (37.3)		13 (46.4)	23 (41.8)		19 (52.8)	15 (31.2)	
GG	5 (11.6)	8 (12.0)		4 (14.3)	7 (12.7)		3 (8.3)	6 (12.5)	
– 819			0.096			0.017			0.031
Allele	$n = 86$	$n = 134$		$n = 56$	$n = 110$		$n = 72$	$n = 96$	
C	58 (67.4)	74 (55.2)		42 (75.0)	60 (54.5)		53 (73.6)	54 (56.3)	
T	28 (32.6)	60 (44.8)		14 (25.0)	50 (45.5)		19 (26.4)	42 (43.7)	
OR		–		OR = 2.5 (95% CI = 1.55–11.02)			OR = 2.2 (95% CI = 1.11–4.2)		
Genotype	$n = 43$	$n = 67$	0.017	$n = 28$	$n = 55$	0.011*	$n = 36$	$n = 48$	0.015*
CC	21 (48.8)	16 (23.9)		15 (53.6)	12 (21.8)		19 (52.8)	11 (22.9)	
CT	16 (37.2)	42 (62.7)		12 (42.8)	36 (65.5)		13 (36.1)	32 (66.7)	
TT	6 (14.0)	9 (13.4)		1 (03.6)	7 (12.7)		4 (11.1)	5 (10.4)	
Genotype			0.013			0.009			0.007
CC	21 (48.8)	16 (23.9)		15 (48.8)	12 (23.9)		19 (52.8)	11 (22.9)	
CT/TT	22 (51.2)	51 (76.1)		13 (51.2)	43 (76.1)		17 (47.2)	37 (77.1)	
OR	OR = 3.04 (95% CI = 1.34–6.91)			OR = 4.13 (95% CI = 1.55–11.02)			OR = 3.78 (95% CI = 1.47–9.61)		
– 592			0.451			0.127			0.068
Allele	$n = 86$	$n = 134$		$n = 56$	$n = 110$		$n = 72$	$n = 96$	
C	59 (68.6)	84 (62.7)		42 (75.0)	68 (61.8)		54 (75.0)	58 (60.4)	
A	27 (31.4)	50 (37.3)		14 (25.0)	42 (38.2)		18 (25.0)	38 (39.6)	
Genotype	$n = 43$	$n = 67$	0.007*	$n = 28$	$n = 55$	0.012*	$n = 36$	$n = 48$	0.007*
CC	21 (48.8)	19 (28.4)		15 (53.6)	14 (25.5)		19 (52.8)	12 (25.0)	
CA	17 (39.5)	46 (68.6)		12 (42.8)	40 (72.7)		14 (38.9)	34 (70.8)	
AA	5 (11.6)	2 (3.0)		1 (3.6)	1 (1.8)		3 (8.3)	2 (4.2)	
Genotype			0.048			0.022			0.017
CC	21 (48.8)	19 (28.4)		15 (48.8)	14 (25.5)		19 (52.8)	12 (25.0)	
CA/AA	22 (51.2)	48 (71.6)		13 (51.2)	41 (74.5)		17 (47.2)	36 (75.0)	
OR	OR = 2.41 (95% CI = 1.08–5.36)			OR = 3.38 (95% CI = 1.29–8.82)			OR = 3.35 (95% CI = 1.33–8.45)		

[a] *p* Values marked by (*) were obtained by the T4 values of computer program CLUMP and *p* values not marked were calculated by χ^2-test.

Table 3. Distribution of *IL10* locus haplotypes found in control and chronic periodontitis (CP) individuals

Haplotypes	Total ($n = 110$)		Female subjects ($n = 83$)		Caucasians ($n = 84$)	
	Control *n* (%)	CP *n* (%)	Control *n* (%)	CP *n* (%)	Control *n* (%)	CP *n* (%)
− 1087 − 819 − 592	$n = 86$	$n = 134$	$n = 56$	$n = 110$	$n = 72$	$n = 96$
G C C	28 (32.5)	37 (27.6)	20 (35.7)	33 (30.0)	25 (34.7)	25 (26.0)
A C C	30 (34.9)	36 (27.0)	22 (39.3)	27 (24.5)	26 (36.1)	28 (29.2)
A T A	24 (27.9)	48 (35.8)	13 (23.2)	41 (37.3)	20 (27.8)	36 (37.5)
G T A	3 (3.5)	1 (0.7)	1 (1.8)	1 (1.0)	0	1 (1.0)
A T C	1 (1.2)	8 (6.0)	0	5 (4.5)	1 (1.4)	4 (4.2)
G T C	0	3 (2.2)	0	3 (2.7)	0	1 (1.0)
A C A	0	1 (0.7)	0	0	0	1 (1.0)
p[a]	0.179*		0.061*		0.267*	
Haplotype						
A C C	30 (34.9)	36 (27.0)	22 (39.3)	27 (24.5)	26 (36.1)	28 (29.2)
A T A	24 (27.9)	48 (35.8)	13 (23.2)	41 (37.3)	20 (27.8)	36 (37.5)
p[a]	0.199		0.043		0.259	
OR	—		2.57 (95% CI = 1.10–5.95)		–	

[a]*p* Values marked by an asterisc (*) were obtained by the T4 values of computer program CLUMP and *p* values not marked were calculated by χ^2-test.

Table 4 Distribution of *IL10* locus haplotypes (arranged as genotypes) found in control and chronic periodontitis (CP) individuals

Genotypes	Total ($n = 110$)		Female subjects ($n = 83$)		Caucasians ($n = 84$)	
	Control *n* (%)	CP *n* (%)	Control *n* (%)	CP *n* (%)	Control *n* (%)	CP *n* (%)
− 1087 –819 –592/ − 1087 –819 –592	$n = 43$	$n = 67$	$n = 28$	$n = 55$	$n = 36$	$n = 48$
G C C/G C C	3 (6.9)	7 (10.4)	3 (10.6)	6 (11.0)	3 (8.3)	5 (10.4)
A C C/A C C	6 (14.0)	5 (7.5)	5 (17.9)	4 (07.3)	5 (13.9)	5 (10.4)
A T A/A T A	4 (9.3)	2 (3.0)	1 (3.6)	1 (1.8)	3 (8.3)	2 (4.2)
G C C/A C C	12 (28.0)	3 (4.5)	7 (25.0)	2 (3.6)	11 (30.6)	1 (2.1)
G C C/A T A	9 (20.9)	19 (28.3)	6 (21.4)	18 (32.7)	8 (22.2)	13 (27.0)
A C C/A T A	6 (14.0)	19 (28.3)	5 (17.9)	16 (29.1)	5 (13.9)	15 (31.2)
G C C/G T A	1 (2.3)	1 (1.5)	1 (3.6)	1 (1.8)	0	1 (2.1)
A T C/A T A	1 (2.3)	4 (6.0)	0	3 (5.5)	1 (2.8)	2 (4.2)
G T A/G T A	1 (2.3)	0	0	0	0	0
A C C/A C A	0	1 (1.5)	0	0	0	1 (2.1)
G T C/A T C	0	1 (1.5)	0	1 (1.8)	0	0
G T C/A T A	0	2 (3.0)	0	2 (3.6)	0	1 (2.1)
A C C/A T C	0	3 (4.5)	0	1 (1.8)	0	2 (4.2)
p[a]	0.011*		0.051*		0.023*	
Genotype						
G C C/A C C	12 (28.0)	3 (4.5)	7 (25.0)	2 (3.6)	11 (30.6)	1 (2.1)
Others	31 (72.0)	64 (95.5)	21(75.0)	53 (96.4)	25 (69.4)	47 (97.9)
p[a]	0.001		0.009		0.007	
OR	8.26 (95% CI = 2.17–31.4)		8.83 (95% CI = 1.69–46.0)		–	

[a]*p* Values marked by an asterisk (*) were obtained by the T4 values of computer program CLUMP and *p* values not marked were calculated by χ^2-test.

the total sample (6.0%) and Caucasians (4.2%).

Considering the three SNPs independently, the genotype distributions were consistent with the assumption of Hardy–Weinberg equilibrium in the control group of the total sample. The data were organized as genotypes (Table 4). There was significant difference in the frequency of the haplotypes in the total sample ($p = 0.011$), in female sample ($p = 0.051$) and in Caucasians ($p = 0.023$). Similar to the total sample, female subjects with the heterozygous haplotype GCC/ACC seemed to be over than eight times less likely to develop CP than individuals with other haplotypes (OR = 8.83; 95% CI = 1.69–46.03).

Discussion

The present study has analysed three SNPs in the promoter region of the *IL10* gene in non-smokers individuals. It is worth mentioning that just three genotyped subjects are former smokers, one in the control group (he had quit smoking 15 years before the present analysis), and two patients in the CP group (they had quit smoking 10 years before the analysis). The results of this study indicate that the –819 and –592 polymorphisms in the *IL10* gene are associated to the susceptibility of the CP, with relevant OR values (Table 2). Two previous studies have failed to show associations between genetic polymorphisms in *IL10* gene and periodontal disease (Kinane et al. 1999, Yamazaki et al. 2001). A possible

reason to these conflicting results is that genotypic differences in cytokine genes are differently inherited to ethnically different populations (Scarel-Caminaga et al. 2002*b*). Although the –1087 *IL10* polymorphism was not associated with CP in the Brazilian population (even when only Caucasians were analysed – Table 2), it has been associated with severe CP in Caucasian subjects of north European origin (Berglundh et al. 2003).

Interestingly, the statistical analysis considering only female subjects revealed a higher positive association of the –819 and –592 SNPs of the *IL10* gene to CP. The –819 SNP lies within a DNA motif forming a putative estrogen responsive element (Lazarus et al. 1997) and the –592 SNP is located between consensus sequences for DNA binding by two types of transcription factors: members of the Ets family and Sp1 (Hobbs et al. 1998). A study involving individuals with systemic lupus erythematosus (SLE) in a male:female ratio of 1:12, showed a strong association between the disease and a cytosine at positions –819 and –592 of *IL10* gene (Lazarus et al. 1997). The authors commented that it may not be coincidental that SLE is almost 10 times more prevalent in women than in men, and that women taking oral contraceptives are more prone to SLE. Despite this, the real role of the estrogen in the regulation of the IL10 production needs more investigation. It is interesting to observe that in both studies (SLE and this study with CP), the patients were predominantly women, and that the genetic status can influence the predisposition of women to disease. However, while the C alleles in the –819 and –592 positions of *IL10* gene promoter seem to be associated with SLE disease, the same C alleles in homozygous fashion seem to ''protect'' individuals against CP. This apparent discrepancy can be explained due to the dual role of IL10: it can act as an anti-inflammatory cytokine but it stimulates B cell proliferation and immunoglobulin secretion. The haplotype GCC has been associated with high production of IL10 (Turner et al. 1997) and individuals with SLE had the production of autoantibodies only in the presence of GCC haplotype. As the SLE is an autoimmune disease whose pathogenesis is attributed to the production of autoantibodies, individuals with the high producer haplotype of IL10 are more prone to develop SLE. Regarding CP, although there was no statistical association between the –1087 SNP and CP, the predominance of CC genotype both in –819 and –592 positions in the control group may indicate a genetic combination that could collaborate to high production of IL10. Individuals who are high producers of IL10 might be more protected against CP due to the anti-inflammatory role of IL10. Therefore, a genetically determined increase of anti-inflammatory IL10 cytokine would downregulate the immune response against periodontopathogenic bacteria.

The analysis of *IL10* haplotypes arranged as alleles (Table 3) and genotypes (Table 4) showed significant results, especially considering the female subjects separately. Among women, the ATA haplotype seemed to be 2.5 times more likely to develop CP than ACC haplotype. It is known that ATA/ATA genotype is associated with lower IL10 production following microbial lipopolysaccharide (LPS) stimulation than other genotypes (Crawley et al. 1999; Yamazaki et al. 2001). Therefore, it is biologically understandable that in the control group there is a predominance of ACC haplotype, which secretes higher levels of anti-inflammatory IL10 compared with the ATA haplotype. Individuals in the diseased group with ATA haplotype would have lower levels of IL10, and it could explain the excess of inflammatory cytokines present in periodontal lesions of the chronic form of the disease. In this study, the most significant haplotypes were the predominance of GCC/ACC compared with other genotypes in the control group. The GCC/ACC haplotype configures a heterozygosity with respect to the –1087 locus (G/A). It is worth mentioning that individuals with –1087G are higher producers of IL10 than those with –1087A (Turner et al. 1997).

The haplotype GTA was found at a low frequency in the south-eastern Brazilian population (Table 3), this haplotype was previously described in a Southern Chinese population (Mok et al. 1998). The haplotypes ATC, GTC and ACA have been considered new, since its presence could not be found in the literature. Despite the fact that the population living in South-eastern of Brazil is predominantly of European ancestry, the presence of new haplotypes (ATC, GTC, ACA) and the rare haplotype GTA in this study, indicate the genetic heterogeneity of the that population, probably due to the high miscegenation of the races (Carvalho-Silva et al. 2001).

We conclude that the –819 and –592 SNPs in the promoter of *IL10* gene and specific haplotypes are associated with CP in Brazilian patients.

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Address:
Sergio R. P. Line
Faculdade de Odontologia de
Piracicaba – UNICAMP
Av. Limeira 901, CEP
13414-018 Piracicaba, SP
Brazil
Fax: +55 019 3412 5218
E-mail: serglin@fop.unicamp.br