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The *IL1A* (−889) gene polymorphism is associated with chronic periodontal disease in a sample of Brazilian individuals

P. R. Moreira[1], J. E. Costa[2], R. S. Gomez[2], K. J. Gollob[3], W. O. Dutra[1]

[1]Laboratory of Cell–Cell Interactions, Department of Morphology, [2]Department of Clinical, Pathology and Surgery, School of Dentistry and [3]Laboratory of Lymphocyte Biology, Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

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Background and Objective: It has been proposed that genotypes reflective of polymorphisms in cytokine genes can predispose individuals to disease by enhancing inflammatory processes. The C/T polymorphism at position −889 of the *IL1A* gene influences interleukin-1α expression, with the T allele inducing higher expression. The aim of this study was to evaluate the association of the *IL1A* (−889) gene polymorphism in Brazilian individuals with different clinical forms of periodontitis and severity of disease.

Material and Methods: DNA was obtained from oral swabs of 163 Brazilian individuals and was amplified using the polymerase chain reaction (PCR). Products were submitted to digestion and were analyzed by electrophoresis to distinguish the C and T alleles.

Results: A significant difference in the genotype distribution was observed when comparing the chronic periodontitis group with the control group, evaluating only nonsmokers (chi-squared analysis = 9.91; $p = 0.007$), as well as when smokers were included (chi-squared analysis = 6.36; $p = 0.04$). Moreover, we observed a higher incidence of the T allele in the chronic periodontitis group (37.8%) when compared with the control group (18.4%) in nonsmokers ($p = 0.006$, odds ratio = 2.69, confidence interval = 1.27–5.68) and also when smokers were included ($p = 0.03$, odds ratio = 1.87, confidence interval = 0.98–3.56). No statistical difference was observed when the aggressive periodontitis group was compared with the control group. With regard to severity of disease, no statistical difference was observed.

Conclusion: These data show an association of the *IL1A* (−889) polymorphism with chronic periodontitis in Brazilian individuals.

Walderez O. Dutra, Departmento de Morfologia, ICB- Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos 6627, Pampulha, Caixa Postal 486, CEP 31.270–901, Belo Horizonte, Minas Gerais, Brazil
Tel: +55 31 3499 2809
Fax: +55 31 3499 2655
e-mail: waldutra@mono.icb.ufmg.br

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Inflammatory responses clearly play an important role in the pathogenesis of periodontal disease. While several immune mediators influence the development of tissue inflammatory responses, interleukin-1 is likely to be a major cytokine involved in most inflammatory responses. Interleukin-1 is an inflammatory cytokine that has been implicated in mediating acute and chronic inflammatory diseases (1) and is produced mainly by stimulated monocytes, macrophages, keratinocytes, smooth muscle and endothelial cells (2). Interleukin-1 represents the

primary activator of early cytokines and is responsible for the induction of adhesion molecules on endothelial cells, thereby facilitating the migration of leucocytes from blood vessels into the tissues. Furthermore, interleukin-1 triggers enzymes leading to the production of prostaglandin E2 (PGE2) and is a primary regulator of matrix metalloproteinases and their inhibitors. Importantly for periodontal disease, interleukin-1 also is known to be one of the most active stimulators of osteoclastic activity (3).

The interleukin-1 family consists of three homologous proteins: interleukin-1α and interleukin-1β, which are pro-inflammatory proteins, and interleukin-1ra, an antagonist protein. These proteins are encoded by the genes *IL1A*, *IL1B* and *IL1RN*, respectively (4), which are clustered on chromosome 2q13–21 and are polymorphic in several loci (5).

Single nucleotide polymorphisms (SNPs) in the interleukin-1 locus, their functional consequences and their association with susceptibility to and severity of various chronic inflammatory diseases, have been described in literature (6). It has been proposed that a particular genotype of interleukin-1 could predispose individuals to disease by enhancing inflammation processes (7). The C/T single base variation was described in the *IL1A* promoter (−889), with the allele T (also called allele 2) being associated with a fourfold increase of interleukin-1α expression (8). Additionally, the *IL1A* (−889) CC genotype has been associated with a significantly lower transcriptional activity of the gene and lower levels of interleukin-1α in plasma compared with the TT genotype (7).

Several studies have shown the functional significance of the promoter region in the *IL1A* gene for regulation of expression (7,9). The greatest differences in protein level were observed in individuals with genotypes related to the polymorphism *IL1A* (−889), which is located in the promoter region. The increase in synthesis of interleukin-1α may be the result of a more active promoter, because the polymorphism creates a consensus site for at least one novel transcription factor, Skn-1 (7).

Several studies have evaluated the occurrence of *IL1* gene polymorphisms in individuals with periodontitis. The control allele of the *IL1A* (−889) polymorphism was observed more frequently than the T allele in African–Americans with aggressive periodontitis (10). Additionally, the T allele in Caucasian individuals with severe chronic periodontitis tended to be more frequent than in individuals with mild chronic periodontitis (11). However, contradictory findings reported no evidence for association between the *IL1A* (−889) polymorphism and chronic periodontitis or aggressive periodontitis in Caucasians (12).

Considering that the frequency of many alleles varies between ethnic groups and geographically distinct populations (13), and that studies concerning *IL1A* gene polymorphism has not yet been performed in Brazilian individuals, the aim of the present study was to describe the occurrence of the *IL1A* (−889) gene polymorphism in a sample of Brazilian individuals with different clinical forms of periodontitis and to evaluate the association of this polymorphism with the severity of disease.

Material and methods

Patients

The study employed a cross-sectional design involving individuals from the State of Minas Gerais in the southeastern region of Brazil. A total of 163 patients receiving treatment at the Dentistry School, Federal University of Minas Gerais, were included in this study. The patients were stratified into three groups: subjects with aggressive periodontitis ($n = 55$); subjects with chronic periodontitis ($n = 67$); and healthy volunteers, as the control group ($n = 41$). All patients came from the same geographical area, had a similar socio-economic status, and displayed no significant differences in the ratio of men to women, or age, between the groups. Patients in the aggressive periodontitis group were 15–46-yr old and exhibited highly destructive forms of periodontitis with evidence of early onset; in these patients, the amount of microbial deposits did not justify the severity of periodontal tissue destruction. Moreover, individuals with localized aggressive periodontitis, characterized by periodontal damage localized to permanent first molars and incisors, and individuals with generalized aggressive periodontitis, characterized by interproximal attachment loss affecting at least three permanent teeth other than the permanent first molars and incisors, were included in the aggressive periodontitis group. Patients in the chronic periodontitis group were 25–67-yr old, and exhibited loss of clinical attachment and amount of destruction consistent with the presence of local factors. Individuals with more than three sites with a probing depth of > 5 mm and lesions distributed on more than two teeth in each quadrant, were included in this group. No case that produced doubt in classification was included in the study.

Diagnosis of disease was made considering the patient's medical and dental histories, radiographic findings and observation of clinical signs and parameters, including probing depth, assessment of clinical attachment loss, observation of tooth mobility, bleeding on probing and the presence of plaque/calculus. A clinical diagnosis of periodontitis was based on criteria established in 1999 at the International Workshop for a Classification of Periodontal Diseases and Conditions (14). Measurements of probing depth and clinical attachment loss were assessed at six locations around each tooth. The severity of disease was characterized on the basis of the mean of clinical attachment loss, within each clinical form. Patients exhibiting a clinical attachment loss of ≥ 5 mm were considered to have severe periodontitis, and those exhibiting clinical attachment loss of 3–5 mm were considered to have moderate periodontitis, as previously determined (14). Control healthy individuals included in the study were 20–70 yr of age and did not have, at the time of sample collection, periodontal disease, as determined by the absence of clinical attachment loss and no sites with a probing depth of > 3 mm. Moreover, upon question-

naire and clinical evaluation, control individuals did not have a history of periodontal disease.

A questionnaire was applied to all individuals enrolled in this study, in order to obtain information regarding dental history, family history of periodontal disease, smoking habit and general health concerns, as well as to evaluate demographic characteristics of individuals, such as education, employment status, family monthly income and type of residence. Exclusion criteria were: use of orthodontic appliances; chronic usage of anti-inflammatory drugs; a history of diabetes, hepatitis or human immunodeficiency virus infection; immunosuppressive chemotherapy; bleeding disorders; severely compromised immune function; and/or pregnancy or lactation. Except for the presence of periodontitis, the patients included in this study were systemically healthy. Because tobacco smoking is an important risk factor for periodontitis, we also analyzed our data taking the habit of smoking into consideration. 'Smokers' were defined as current smokers/former smokers (more than 10 cigarettes per day) and 'nonsmokers' included individuals who had never smoked. Table 1 summarizes the patient data, as well as their classification into different groups.

This study was approved by the Universidade Federal de Minas Gerais's Ethics Committee (no. 003/03 and no. 132/00) and signed informed consent was obtained from all participants.

Table 1. Characteristics of the study groups

	Clinical forms		
	Aggressive periodontitis	Chronic periodontitis	Healthy control
Number of individuals (*n*)	55	67	41
Age range (yr)	15–46	25–67	20–77
Gender			
Male (%)	20 (36.4)	21 (31.3)	17 (41.5)
Female (%)	35 (63.6)	46 (68.7)	24 (58 .5)
CAL (mm) mean (± standard deviation)	6.17 ± 1.57	5.68 ± 1.19	–
Smoking status			
Nonsmokers (%)	36 (65.5)	37 (55.2)	38 (92.7)
Smokers (%)	19 (34.5)	30 (44.8)	3 (7.3)

CAL, clinical attachment loss.

Sample collection and DNA extraction

Epithelial cells were obtained through an oral swab performed with a sterile plastic spatula. After gently scraping the oral mucosa, the tip of the spatula was immediately immersed in 2-ml sterile microtubes containing 1500 µl of Krebs buffer (7.25 gIL NaCl, 0.30 gIL KCl, 2% $CaCl_2$, 2% H_2O, 0.29 gIL $MgSO_4$, 5.95 gIL KH_2PO_4, 1.80 gIL $C_6H_{12}O_6$). DNA extraction was performed as described by Boom *et al.* (15) and modified as follows. A pellet of epithelial cells was obtained by centrifugation at 200 ***g*** for 5 min. The supernatant was removed and 20 µl of silica (SiO_2; Sigma, St Louis, MO, USA) and 450 µl of lysis buffer (6.0 M GuSCN, 65 mM Tris-HCl, pH 6.4, 25 mM EDTA and 1.5% Triton X-100) were added to the microtubes. Samples were homogenized using a Vortex and incubated for 30 min at 56°C. After this incubation, samples were submitted to another centrifugation and the supernatant was discarded. The pellet obtained (with DNA adsorbed on the silica) was washed twice with 450 µl of washing buffer (6.0 M GuSCN, 65 mM Tris-HCl, pH 6.4), twice with 450 µl of 70% ethanol, once with 450 µl of acetone and dried at 56°C for 20 min. Finally, 100 µl of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) was added and incubated at 56°C for 12 h to release the DNA. After incubation, the solution was homogenized, centrifuged and the supernatant containing DNA was transferred to a new tube.

Polymerase chain reaction and restriction endonuclease digestion

The *IL1A* (−889) polymorphism was assessed after polymerase chain reaction (PCR) amplification and digestion. The sequences of PCR primers were 5′-aagcttgttctaccacctgaactaggc-3′ and 5′-ttacatatgagccttccatg-3′ with an expected PCR product size of 99 bp, as described previously (6). PCR was carried out in a total volume of 50 µl, containing 10 µl of solution DNA, Premix buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.4, 0.1% Triton X-100, 1.5 mM $MgCl_2$, deoxynucleoside triphosphates, *Taq* DNA polymerase) and primers (20 pmol per reaction). The amplification conditions consisted of 94°C for 3 min, followed by 45 cycles of 94°C for 30 s, 56°C for 35 s and 72°C for 30 s. The run was terminated by a final elongation at 72°C for 5 min. The products were digested with 5 U of *Nco*I at 37°C for 4 h and formed 83- and 16-bp DNA products for allele C and 99-bp DNA products for allele T. The visualization was performed following polyacrylamide gel electrophoresis in a 10% gel.

Statistical analysis

Statistical analysis of data was performed using the JMP statistical software (SAS, Cary, NC, USA). The chi-squared test was used to compare the genotype distributions between control and chronic periodontitis groups, between control and aggressive periodontitis groups, and between chronic periodontitis and aggressive periodontitis groups [3×2 contingency table, degrees of freedom (d.f.) = 2]. The C/T allele and T^+/T^- genotype distribution between control and chronic periodontitis groups, between control and aggressive periodontitis groups, and between chronic periodontitis and aggressive periodontitis groups were assessed in 2×2 contingency table (d.f. = 1), as well as in regard to severity. Analysis of the 2×2 contingency table (d.f. = 1) was performed using Fisher's exact test.

The study groups were tested for Hardy–Weinberg equilibrium comparing the expected with the observed genotype frequencies. To exclude the possible confounding effect of smoking, in a second analysis we excluded smokers from all the different clinical groups. A p-value of < 0.05 was considered significant.

Results

The *IL1A* (−889) polymorphism is associated with chronic periodontal disease in Brazilian individuals

The genotypic and allele distributions of the *IL1A* (−889) polymorphism are shown in Tables 2 and 3, respectively. No difference was found between observed and expected distributions of genotypes for the control group and therefore it was considered to be in Hardy–Weinberg equilibrium. Evaluation of the genotype and the allele distributions was performed comparing between individuals with the different clinical forms of periodontal disease and between each clinical form and individuals without disease, considering or not the habit of smoking. There was a significant difference in the genotype distribution when comparing the chronic periodontitis group with the control group, considering only nonsmokers (chi-squared analysis = 9.91, $p = 0.007$), as well as when smokers were included in the analysis (chi-squared analysis = 6.36, $p = 0.04$) (Table 2). No statistical difference was observed when comparing the control and aggressive periodontitis groups, whether or not the habit of smoking was considered. The data indicate that the frequencies of CT and TT genotypes may be related to chronic periodontitis, with or without the habit of smoking. With regard to allele distribution, statistical difference was also observed among groups (Table 3). A higher incidence of the T allele in the chronic periodontitis group (37.8%) was observed when compared with the control group (18.4%) in nonsmokers ($p = 0.006$, odds ratio = 2.69, confidence interval = 1.27–5.68), and also when smokers were included in the analysis ($p = 0.03$, odds ratio = 1.87, confidence interval = 0.98–3.56). These data indicate that the individuals carrying the T allele have an increased risk of displaying chronic periodontitis. There were no differences between the aggressive periodontitis group and any other of the groups (Table 3). The analysis of the presence of the interleukin-1α high-producer allele in the population, as evaluated by the frequency of T^+ individuals, was significantly different when comparing the control and chronic periodontitis groups and analyzing only nonsmokers ($p = 0.01$, odds ratio = 0.31, confidence interval = 0.12–0.81). When smokers were included, it was observed that the chronic periodontitis group displayed a suggestive bias for the presence of the T^+ genotype as compared with the control group ($p = 0.06$, odds ratio = 2.09, confidence interval = 0.94–4.61). These data are shown in Fig. 1 and demonstrate the association of the T^+ genotype with the chronic periodontitis group.

Table 2. Distribution of the IL1A (−889) genotypes (CC, CT and TT) in the study groups

	Healthy control*	Aggressive periodontitis	Chronic periodontitis*
Nonsmokers			
CC (%)	24 (63.2)	19 (52.8)	13 (35.1)
CT (%)	14 (36.8)	15 (41 7)	20 (54.1)
TT (%)	–	2 (5,5)	4 (10.8)
Nonsmokers + smokers			
CC (%)	24 (58.5)	28 (50.9)	27 (40.3)
CT (%)	17 (41.5)	22 (40)	36 (53.7)
TT (%)	–	5 (9.1)	4 (6)

*Statistical difference (3 × 2 contingency table): nonsmokers: chi-square = 9.91, $p = 0.007$ (control vs. chronic periodontitis); nonsmokers + smokers: chi-square = 6.36, $p = 0.04$ (control vs. chronic periodontitis).

Table 3. Distribution of the IL1A (−889) alleles (C and T) in the study groups

	Healthy control*	Aggressive periodontitis	Chronic periodontitis*
Nonsmokers			
C (%)	62 (81.6)	53 (73.6)	46 (62.2)
T (%)	14 (18.4)	19 (26.4)	28 (37.8)
Nonsmokers + smokers			
C (%)	65 (79.3)	78 (70.9)	90 (67.2)
T (%)	17 (20.7)	32 (29.1)	44 (32.8)

*Statistical difference (2 × 2 contingency table): nonsmokers: $p = 0.006$, odds ratio = 2.69, confidence interval = 1.27–5.68 (control vs. chronic periodontitis); nonsmokers + smokers: $p = 0.03$, odds ratio = 1.87, confidence interval = 0.98–3.56 (control vs. chronic periodontitis).

The *IL1A* (−889) polymorphism is not associated with severity of periodontal disease in Brazilian individuals

When we evaluated the association of genotypes with severity of periodontitis by stratifying the groups according to clinical attachment loss, no statistical difference was observed between the aggressive periodontitis and chronic periodontitis groups (Table 4).Finally, there were no statistical differences when comparing genotype distribution between severe and moderate disease within each clinical form (Table 4).

Discussion

Periodontitis is an inflammatory disease characterized by the loss of connective tissue and alveolar bone. Although the causative agent in periodontitis is the pathogenic bacterial plaque, the progression of disease depends on the production of host mediators in response to bacteria and/or metabolic products (16). Several mediators have been proposed to induce

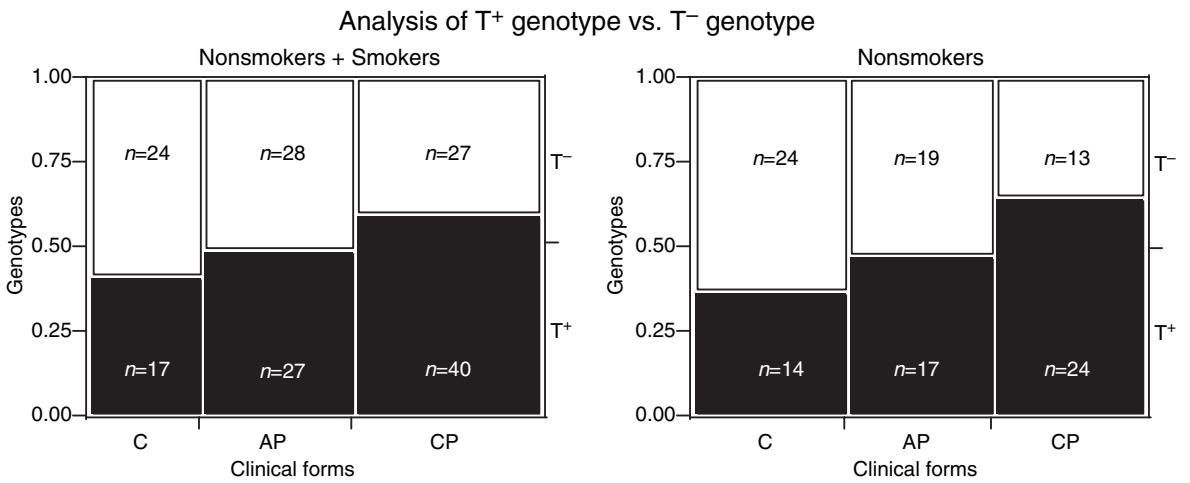

Fig. 1. Analysis of T^+ and T^- genotype segregation amongst individuals with different clinical forms of periodontitis. AP, aggressive periodontitis; C, control; CP, chronic periodontitis. White boxes are representative of the negative T genotype and black boxes are representative of the positive T genotype. Statistical analysis (2×2 contingency table): nonsmokers, $p = 0.01$, odds ratio = 0.32, confidence interval = 0.12–0.82 (control vs. chronic periodontitis); nonsmokers + smokers, $p = 0.05$, odds ratio = 2.09, confidence interval = 0.95–4.61 (control vs. chronic periodontitis).

Table 4. Distribution of the IL1A (−889) genotypes (CC, CT and TT) in the study groups, considering the severity of disease

	Nonsmokers + smokers		Nonsmokers	
	Aggressive periodontitis	Chronic periodontitis	Aggressive periodontitis	Chronic periodontitis
Severe				
CC (%)	25 (53.2)	19 (37.2)	16 (53.3)	9 (33.3)
CT (%)	17 (36.2)	29 (56.9)	12 (40)	15 (55.6)
TT (%)	5 (10.6)	3 (5.9)	2 (6.7)	3 (11.1)
Moderate				
CC (%)	3 (37.5)	8 (50)	3 (50)	4 (40)
CT (%)	5 (62.5)	7 (43.8)	3 (50)	5 (50)
TT (%)	–	1 (6.2)	–	1 (10)

Measurements of clinical attachment loss were assessed at six locations around each tooth and the mean clinical attachment loss for each individual was obtained considering the multiple sites. Patients exhibiting a mean clinical attachment loss of ≥ 5 mm were considered to have severe periodontitis, and those exhibiting clinical attachment loss of 3–5 mm were considered to have moderate periodontitis. No statistical difference was observed among the groups.

periodontal disease. Cytokines play a central role in the immune response by the production of 'appropriate' cytokines, resulting in the development of protective immunity, and by the production of 'inappropriate' cytokines, leading to tissue destruction and disease progression (17).

There are several lines of evidence that implicate the pro-inflammatory cytokine, interleukin-1, in the pathogenesis of periodontal disease. Interleukin-1 is produced in several types of host cells found in the periodontal environment in response to periodontal pathogens (18) and has multiple biological activities, including stimulation of osteoclast recruitment and activation (19). Recent findings indicate that specifically blocking both interleukin-1 and tumor necrosis factor-α (TNF-α) significantly reduce periodontal bone destruction (20). Moreover, interleukin-1α and -β have been found at elevated concentrations in the gingival crevicular fluid of patients with periodontitis, although these concentrations may vary widely between different subjects (21).

Previous studies have revealed associations between polymorphisms in *IL1* genes and periodontitis (6,22,23). Recently, a genetic susceptibility test (Periodontal Susceptibility Test; Interleukin Genetics Inc., Waltham, MA, USA) for severe chronic periodontitis became commercially available based on an assessment for the simultaneous presence of specific polymorphisms, namely allele 2 at *IL1A* (−889) and allele 2 at *IL1B* (+3954) (13). Individuals bearing a combination of these genetic polymorphisms were referred to, by Kornmam *et al.* (6), as being genotype positive. This genotype-positive classification has been associated with the detection of higher concentrations of interleukin-1 in both the gingival tissues and the inflammatory exudates originating from the marginal periodontium (24). However, the prevalence of genotype-positive individuals in different ethnic groups, and their correlation to clinical manifestations of disease, has displayed contradictory results (13).

Previous studies performed by our group suggested that the polymorphism in the locus +3954 of the *IL1B* gene could be a risk factor for chronic periodontitis in Brazilian individuals (25). However, to our knowledge, studies evaluating the polymorphism in locus −889 of the *IL1A* gene in Brazilian individuals with periodontitis have not yet been performed. Thus, analysis of the *IL1A* gene polymorphism

in a sample of the Brazilian individuals represents an important advance in the study of periodontal disease in Brazil, particularly considering the importance of this cytokine in inflammatory responses. In the present study, organization of the sample of Brazilian individuals into ethnic groups was not performed as a result of the strong miscegenation among Brazilians. A recent publication, concerning genetic analysis of Brazilian individuals, does not recommended grouping Brazilians into ethnic groups based on color, race and geographical origin (26). This study has shown that Brazilian individuals, classified as 'white' or 'black', have significantly overlapping genotypes, probably as a result of miscegenation. In our study, all the patients and controls were selected from the same geographical area and were at the same socio-economic level, as evaluated for the demographic characteristics of individuals, such as education, employment status, family monthly income and type of residence. Moreover, the individuals analyzed in our study, from Minas Gerais State, are highly representative of the Brazilian population, as shown by Parra *et al*. (26).

In the present study, we evaluated the polymorphism in the locus −889 (C/T) of the *IL1A* gene in a sample of the Brazilian population, who were suffering or not from periodontal disease, and found an association between the occurrence of this polymorphism and chronic periodontitis. It was observed that the chronic periodontitis group displayed higher frequencies of T^+ genotypes compared with the control group. This finding is in accordance with Kornmam *et al*. (6), who observed that the carriage rate of the T allele in Caucasians by the severe chronic periodontitis group tended to be higher than for the mild group, defined as individuals with probing depths of no greater than 3 mm. Studying the Greek population, Sakellari *et al*. (27) also observed that the T^+ genotype was more common in the chronic periodontitis group than in the control group, although this difference did not reach statistical significance. However, Rogers *et al*. (12) reported no association of this *IL1A* −889 (C/T) polymorphism with chronic periodontitis in Caucasians.

In our study, we observed that the genetic association with chronic periodontitis was more evident when smokers were excluded from the study group, confirming the importance of this risk factor and suggesting that its effect is strong, even in subjects who are not genetically susceptible to the disease. This data suggests that the smoking-related risk may obscure the polymorphism-related risk, as described by Kornman *et al*. (6). Moreover, a previous study reported a reduced interleukin-1α concentration in the gingival crevicular fluid in smokers (8). Possible explanations for this observation included a general reduction in inflammation in smokers, reduced neutrophil numbers in gingival crevicular fluid resulting in decreased production of interleukin-1α, or a direct suppression of interleukin-1α production as a result of smoking (21).

With regard to aggressive periodontitis, no association with the *IL1A* polymorphism was observed in our study, regardless of smoking habit. Our results are in accordance with other studies that observed no significant differences in the frequencies of the alleles between aggressive periodontitis and control in Caucasians, African–Americans, Central American individuals and a Thai ethnic group (12,28–30).

We observed that the frequency of the T allele in controls from the present study was 41.5%, while the frequency of the same allele reported in the literature was 45% in Caucasians (12), 57.1% in European Caucasians (31) and 7.1% in Koreans (32). These data show that the allelic distribution may vary among ethnic groups and emphasize the importance of investigation of polymorphisms in different populations.

When we evaluated the severity of periodontal disease, stratifying the groups according to mean clinical attachment loss, no difference was observed among the groups. To our knowledge, no association between the *IL1A* (−889) polymorphism alone with severity of periodontitis has been found. Previous investigations have associated the severity of periodontitis in nonsmoker Caucasians with the carriage of a composite genotype that included the presence of the polymorphic *IL1A* (−889) allele and the polymorphic *IL1B* (+3954) allele (6). However, other studies have generated conflicting results regarding the importance of this positive genotype in different populations (33). These findings may reflect differences in the prevalence of this genotype in different racial groups. It is clear therefore that genetic tests, based upon these specific genes, do not appear to be useful markers applicable to all patients, independently of the population analyzed.

In the Brazilian population, we previously observed that the *IL1B* (+3954) polymorphism is associated with chronic periodontitis (25), and in the present study we also verified an association of the *IL1A* (−889) polymorphism with chronic periodontitis. There is evidence that these two polymorphisms may be in linkage disequilibrium (34) and that the TT genotype of the *IL1A* (−889) polymorphism influences interleukin-1β levels in whole blood (9). The occurrence of these two functional polymorphisms results in an increased production of interleukin-1α and interleukin-1β cytokines, which plays an important role in the up-regulation of a host response, resulting in tissue destruction (18). It is speculated that over-expression of interleukin-1α and interleukin-1β in response to organisms in subgingival plaque may increase gingival inflammation, gingival crevicular fluid flow and subgingival species (35). The initiation of an inflammatory process through the induction of interleukin-1 results in stimulation of the production of secondary mediators, which would amplify the degree of inflammation (18). Thus, the polymorphisms referent to *IL1A* (−889) and *IL1B* (+3954) genes would directly influence chronic periodontal disease pathogenesis via an effect on cytokine synthesis.

In our study, the subjects involved were matched for gender and had the same socio-economic status. Although they were not matched for age, the age

ranges were extremely close: 15–46 yr (mean 29.8 yr) in the aggressive periodontitis group; 25–67 yr (mean 46.4 yr) in the chronic periodontitis group; and 20–70 yr (mean 30 yr) in the control group. The age-dependent nature of periodontal disease was recently questioned in criteria established in 1999 at the International Workshop for a Classification of Periodontal Diseases and Conditions (14). Difficulties in identifying a control group have been reported as a result of the continuous variability of periodontitis and the different age of onset of disease between individuals (33). Finally, in many periodontal studies, age-matched studies between patients and controls have not been considered necessary because the genetic patterns do not change with age (36,37).

In conclusion, the present study shows that the polymorphism in locus −889 (C/T) of the *IL1A* gene could be a risk factor for chronic periodontitis in the Brazilian population. We believe that the identification of genetic markers for susceptibility to periodontitis will permit the early identification of individuals with high risk for periodontitis and could eventually help through individualized forms of therapy.

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