Interaction of *IL1B* and *IL1RN* polymorphisms, smoking habit, gender, and ethnicity with aggressive and chronic periodontitis susceptibility

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Abstract

Background: Although the interleukin-1 (IL-1) plays a critical role in the pathogenesis of periodontitis, associations between *IL1* gene cluster polymorphisms and the disease remains unclear. **Aims:** To investigate the importance of *IL1B*-511C>T (rs16944), *IL1B* +3954C>T (rs1143634), and *IL1RN* intron 2 variable number tandem repeat (VNTR) (rs2234663) polymorphisms, individually or in combination, as the risk factors of periodontitis in a Southeastern Brazilian population with a high degree of miscegenation. **Subjects and Methods:** A total of 145 individuals, with aggressive (aggressive periodontitis [AgP], *n* = 43) and chronic (chronic periodontitis [CP], *n* = 52) periodontitis, and controls (*n* = 50) were genotyped by polymerase chain reaction (PCR) (*IL1RN* intron 2 VNTR) or PCR-restriction fragment length polymorphism (PCR-RFLP) (*IL1B*-511 C>T and *IL1B* + 3954C>T) techniques. **Statistical Analysis:** The independent *t*-test, Chi-square, and Fisher's exact tests were used. The SNPStats program was used for haplotype estimation and multiplicative interaction analyses. **Results:** The *IL1B* +3954T allele represented risk for CP (odds ratio [OR] = 2.84), particularly in smokers (OR = 4.43) and females (OR = 6.00). The minor alleles *IL1RN**2 and *3 increased the risk of AgP (OR = 2.18), especially the *IL1RN**2*2 genotype among white Brazilians (OR = 7.80). Individuals with the combinations of the *IL1B* + 3954T and *IL1RN**2 or *3-containing genotypes were at increased risk of developing CP (OR = 4.50). Considering the three polymorphisms (rs16944, rs1143634, and rs2234663), the haplotypes TC2 and CT1 represented risk for AgP (OR = 3.41) and CP (OR = 6.39), respectively. **Conclusions:** Our data suggest that the *IL1B* +3954C>T and *IL1RN* intron 2 VNTR polymorphisms are potential candidates for genetic biomarkers of periodontitis, particularly in specific groups of individuals.

Keywords: Biomarkers, genotype combinations, haplotypes, interleukin-1, periodontitis, polymorphisms

Introduction

Periodontal disease is a chronic inflammatory process of infectious origin that includes gingivitis, an early reversible form of the disease, and periodontitis, which is characterized by loss of connective tissue, supporting alveolar bone, and eventually tooth.[1] The chronic periodontitis (CP) has slow progression and is the most common form of the disease, while the aggressive periodontitis (AgP) is rare, progresses rapidly and affects younger individuals.[2] Although bacterial plaque accumulation is essential, the number and variety of microorganisms are not related to the disease severity.[3] Individuals react differently to bacterial aggression, which reinforces the concept that the host response, rather than bacterial etiology, is the main determinant of disease expression.[4] It is very well known that the host response may be altered by several factors, including smoking and diabetes.[5] In addition, a study in twins showed a possible genetic influence on the development of periodontal disease.[6] It has been suggested that allelic variants of *IL1* interfere with the production and function of interleukin-1 (IL-1), indicating that they might play an important role in the susceptibility and/or severity of periodontitis.[7-9] This is an interesting hypothesis because this family of cytokines is important in the metabolism of collagen in bone destruction and other inflammatory processes.[8,10]

The genes controlling the production of IL-1 are located on human chromosome 2q13. Two of them, named *IL1A* and *IL1B*, encode the proteins IL-1α and IL-1ß, respectively, while a third member, known as *IL1RN*, encodes a protein receptor antagonist (IL-1Ra). Associations between genetic

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polymorphisms of *IL1* family and the severity of CP were initially assessed by Kornman *et al*.[3] The authors observed that the simultaneous occurrence of *IL1A*-889C>T and *IL1B* +3953/4C>T polymorphisms was associated with severe periodontitis in nonsmokers. Since then, polymorphisms in the *IL1* gene cluster have been evaluated in patients with CP and/or AgP, with conflicting data.[11-13] Potential associations between *IL1* polymorphisms and periodontitis remain inconclusive, reinforcing the importance of further studies in populations of different ethnicities.[9]

Brazilian studies have been conducted, but in most of them, periodontitis was not classified into chronic and aggressive forms, or periodontitis was studied in relation to another pathological condition, such as chronic kidney disease[14] and human immunodeficiency virus infection.[15] In addition, several studies involved only the *IL1B* +3954C>T polymorphism or this polymorphism in combination with other polymorphic loci not considered in our work, and in some of them, the control group was not in Hardy–Weinberg equilibrium.[16-18]

Taking all this into consideration, the aim of this study was to investigate possible associations of two single nucleotide polymorphisms (SNPs) in the *IL1B* gene, -511C>T (rs16944) and +3954C>T (rs1143634), and the penta-allelic 86 bp variable number tandem repeat (VNTR) polymorphism in intron 2 (rs2234663) of the *IL1RN* gene, individually or combined, with AgP and severe CP in a Southeastern Brazilian population, which was stratified by smoking status, gender, and skin color/ethnicity.

Subjects and Methods

Study population

One hundred and forty-five individuals living in Rio de Janeiro, aged between 20 and 60, were selected from two Faculties of Dentistry, Estácio de Sá University, and Rio de Janeiro Catholic University. Information of all volunteers included records of medical history, periodontal history, and periapical radiography. All participants were in good general health and signed a consent form before being examined. This study was approved by the Research Ethics Committee of Pedro Ernesto University Hospital, State University of Rio de Janeiro (#1635 CEP/HUPE/UERJ).

A questionnaire containing the following information: gender, age, self-declared skin color/ethnicity (white and nonwhite Brazilians), smoking habit (nonsmoker and smoker/ex-smoker), medications used, systemic diseases, and socioeconomic status was applied to all participants. Periodontal examination included visible plaque index (VPI),[19] gingival bleeding (GBI),[19] probing pocket depth (PD), and clinical attachment loss (CAL). PD and CAL were measured at four sites per tooth (mesial, buccal, distal, palatal, or lingual) using a calibrated periodontal probe (HU-Friedy®, Chicago, IL, USA). Bone loss was measured on periapical radiographs with the aid of a millimeter rule. The distances between cementoenamel junction (CEJ) and the root apex, and the CEJ and the alveolar crest were calculated.[20]

Participants were classified according to clinical and radiographic data. The AgP group included 43 patients (mean age of 33.1 ± 4.8 years) with interproximal CAL and radiographic bone loss ≥50% of the length of the root structure on at least three teeth other than the first molars or incisors. The severe CP group was composed of 52 patients (mean age of 50.6 ± 5.8 years) showing at least five sites with CAL ≥6 mm. The control group included fifty periodontally healthy individuals (mean age of 40.1 ± 7.8 years) without evidence of CAL at interproximal sites and no radiographic signs of bone loss.

Collection of biological material and isolation of genomic DNA

A sample of oral mucosa cells was obtained by scraping the inner cheek with a sterile swab. After being transferred into a 1.5 mL plastic tube containing 1.0 mL of TE buffer (10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid - EDTA, pH 8.0) to release the cells, the collector swab was properly discarded. The samples were stored at −20ºC until DNA extraction, which was performed as previously described.[21] An aliquot of the resulting DNA-containing solution was stored at 4ºC until its use in the molecular assays aiming at *IL1* genotyping, and a second aliquot was kept at −20ºC for long-term storage.

Molecular analysis of *IL1B* and *IL1RN* polymorphisms

IL1B-511C>T and *IL1B* +3954C>T genotyping was performed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique using primers described by Soga *et al*.[22] An aliquot of 5 µL of each amplicon was digested with 5 U of a specific restriction endonuclease for approximately 16 h, and the digestion products were resolved in ethidium bromide-stained polyacrylamide gels (8%).

Amplification of the VNTR of 86 bp in intron 2 of *IL1RN* gene was performed using a pair of primers described by Parkhill *et al*.[12] Electrophoretic analysis of the PCR products in 8% polyacrylamide gels stained with ethidium bromide allowed the identification of the genotypes formed by the combination of the five alleles.

Primer sequences, amplification reaction mixtures (final volume of 25 µL), PCR programs, restriction endonucleases, and PCR or digestion products are presented in Table 1.

Genotype identification was blindly performed by two scientists. Ten percent of the total samples were genotyped twice to validate the technique used.

Table 1: Genotyping conditions for the *IL1B*−511C>T, *IL1B*+3954C>T, *IL1RN* intron 2 variable number tandem repeat polymorphisms

Polymorphism	Primer	PCR reaction mixture	PCR program	Restriction endonuclease	PCR or digestion products
IL1B−511C >T	Forward 5'-TGGCATTGATCTGGTTCATC-3' Reverse 5'-GTTTAGGAATCTTCCCACTT-3'	1×reaction buffer with 2 mM Mg Cl_2 (Biotools) 80 μM dNTPs (Amersham) 5 pmol of each primer (Alpha DNA) 3 U Taq DNA polymerase (Biotools) 100-200 ηg of genomic DNA	95°C/10 min 35 cycles: 95°C/1 min, 53°C/1 min, 74°C/1 min 72°C/10 min	*Ava*I (New England BioLabs)	Digested C allele – 115 and 190 bp Nondigested T allele – 305 bp
IL1B+3954C >T	Forward 5'-GTTGTCATCAGACTTTGACC-3' Reverse 5'-TTCAGTTCATATGGACCAGA-3'	1×reaction buffer with 2 mM Mg Cl_2 (Biotools) 80 μM dNTPs (Amersham) 5 pmol of each primer (Alpha DNA) 3 U Taq DNA polymerase (Biotools) 100-200 ηg of genomic DNA	95°C/5 min 38 cycles: 95°C/45 s, 55°C/45 s, 72°C/45 s 72°C/10 min	*Taq*I (Fermentas)	Digested T allele – 114 and 136 bp Nondigested C allele (250 bp)
IL1RN intron 2 VNTR	Forward 5'-CTCAGCAACACTCCTAT-3' Reverse 5'-TCCTGGTCTGCAGGTAA-3'	1×reaction buffer (Invitrogen) 1.5 mM $MgCl_2$ (Invitrogen) 200 μM of dNTPs (Amersham) 7.5 pmol of each primer (Alpha DNA) 5 U of Taq DNA polymerase – Platinum (Invitrogen) 100-200 ηg of genomic DNA	94°C/4 min 32 cycles: 94°C/1 min, 50°/1 min, 70°C/1 min 72°C/10 min		*IL1RN**1 (four repeats) – 410 bp *IL1RN**2 (two repeats) – 240 bp *IL1RN**3 (three repeats) – 500 bp *IL1RN**4 (five repeats) – 325 bp *IL1RN**5 (six repeats) – 595 bp

VNTR: Variable number tandem repeat; PCR: Polymerase chain reaction

Statistical analysis

The independent *t*-test was used to assess differences between normally distributed values. The control group was tested for deviation from the Hardy–Weinberg equilibrium by the Chi-square test. The Fisher's exact test was used to analyze the differences in allele and genotype frequencies between the study groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the magnitude of association of *IL1* polymorphisms and the susceptibility to AgP or severe CP. The reference categories were defined as the most frequent allele for each polymorphism and the homozygote genotype for this allele. Statistical analyses were performed using the software GraphPad Prism version 5 (GraphPad Software, Inc., San Diego, CA, USA). Haplotype estimation and multiplicative interaction analyses between the *IL1* polymorphisms and the response or other variables were performed using the online SNPStats program (http://bioinfo.iconcologia.net/SNPStats).[23] Statistical significance was set at 0.05.

Results

Demographic and clinical characteristics of AgP, CP, and control groups are presented in Table 2. The values of mean age and frequency of men were higher in the CP group in comparison with the control and AgP groups. Significantly more white Brazilians were observed among controls. Regarding clinical characteristics, the mean number of teeth was higher, whereas the mean % of sites with GBI was significantly lower in controls in comparison with the other two groups. The mean VPI was higher in the CP group than the corresponding values in control and AgP groups. Controls did not present sites with PD ≥6 mm, CAL ≥6 mm, and bone loss ≥50%, and no differences were found between AgP and CP groups with respect to these clinical characteristics.

IL1B and *Il1RN* polymorphisms and the development of periodontitis

Genotype and allele distributions of the *IL1B*-511C>T, *IL1B* +3954C>T, and *IL1RN* intron 2 VNTR polymorphisms in the three study groups are shown in Table 3. The control group is in Hardy–Weinberg equilibrium regarding the three polymorphisms. No differences in *IL1B*-511C>T genotype or allele frequencies were observed between patients with periodontitis, chronic or aggressive, and controls.

For *IL1B* +3954C>T polymorphism, allele and genotype distributions were statistically different between CP and control groups, with higher frequencies of the allele T (OR = 2.84), in homozygosis or heterozygosis (CT + TT) (OR = 2.41), among patients with CP. The prevalence of the allele *IL1B* +3954T was even higher among smokers or ex-smokers (OR = 4.43; 95% CI = 1.29–15.24; P = 0.025), and females (OR = 6.00; 95% CI = 2.13–16.93; P = 0.027) of the CP group in comparison with controls.

Comparative analyses between AgP and control groups showed a significant difference for *IL1RN* intron 2 VNTR,

Table 2: Demographic and clinical characteristics of aggressive periodontitis and chronic periodontitis groups and controls

Characteristic	Group		
	AgP (*n*=43)	CP (*n*=52)	Control (*n*=50)
Demographic			
Male (%)	28	56*	42
Age (years old) (mean±SD)	33.1±4.8	50.6±5.8*	40.1±7.8†
Smoker/ex-smoker (%)	35	42	38
White Brazilian (%)	67	71	88‡
Clinical (mean±SD)			
Number of teeth	26.0±3.8	23.0±11.9	28.7±3.1‡
VPI (%)	42.8±26.2	56.3±24.8*	39.5±26.3
GBI (%)	53.0±26.1	61.0±24.1	20.6±15.6§
PD ≥6 (mm)	16.1±13.6	15.2±11.3	0
CAL ≥6 (mm)	23.0±13.2	22.0±11.6	0
Bone loss ≥50%	14.4±7.2	12.3±6.1	0

*Significantly higher as compared to control and AgP groups; †Significantly higher as compared to AgP group; ‡Significantly higher as compared to AgP and CP groups; §Significantly lower as compared to AgP and CP groups. SD: Standard deviation; VPI: Visible plaque index; GBI: Gingival bleeding index; PD: Probing pocket depth; CAL: Clinical attachment loss; AgP: Aggressive periodontitis; CP: Chronic periodontitis

with the variant alleles 2 (two repeats) and 3 (five repeats) being more prevalent in the AgP group (OR = 2.18). When only white Brazilian patients were considered, just a slightly higher frequency of the alleles *IL-1RN**2 and *3 (OR = 3.08; 95% CI = 1.40–6.77; *P* = 0.006) was observed, but a markedly higher frequency of the *ILRN**2 * 2 genotype (OR = 7.80; 95% CI = 1.34-45.28; *P* = 0.019) in the AgP group was found when the codominant genetic model (*IL1RN**2*2 *vs IL1RN**1*1) was used.

Genotype combination and haplotype regarding the three polymorphisms as risk factor for the development of periodontitis

Genotype combinations of *IL1B*-511C>T with *IL1B* +3954C>T or *IL1RN* intron 2 VNTR polymorphisms did not reveal any difference between the study groups (data not shown). However, the combination of *IL1B* + 3954T allele-containing genotypes and *IL1RN**2 or *3 alleles-carrying genotypes was significantly higher among patients with CP as compared with controls (OR = 4.50) [Table 4].

Regarding the three polymorphisms, *IL1B*-511C>T, *IL1B* +3954C>T, and *IL1RN* intron 2 VNTR, haplotype estimation showed higher frequencies of the haplotypes TC2

Table 3: Genotype and allele distributions with respect to the three polymorphisms, *IL1B*−511C>T, *IL1B*+3954 C>T, and *IL1RN* intron 2 variable number tandem repeat, in aggressive periodontitis and chronic periodontitis groups and controls

Polymorphism	Genotype or allele	AgP, *n* (% or f)	CP, *n* (% or f)	Control#, *n* (% or f)	AgP versus controls		CP versus controls	
					OR	95% CI	OR	95% CI
IL1B − 511C > T	CC	11 (29)	11 (26)	12 (30)	Reference		Reference	
	CT	17 (45)	24 (57)	19 (48)	0.98	0.34-2.78	1.38	0.50-3.80
	TT	10 (26)	7 (17)	9 (22)	1.21	0.36-4.09	0.85	0.24-3.06
	CT + TT	27 (71)	31 (74)	28 (70)	1.05	0.40-2.79	1.21	0.46-3.17
	C	39 (0.51)	46 (0.55)	43 (0.54)	Reference		Reference	
	T	37 (0.49)	38 (0.45)	37 (0.46)	1.10	0.59-2.07	0.96	0.52-1.77
IL1B+3954C > T	CC	23 (59)	22 (47)	34 (68)	Reference		Reference	
	CT	12 (31)	17 (36)	16 (32)	1.11	0.44-2.77	1.64	0.69-3.91
	TT	4 (10)	8 (17)	0	NA		NA	
	CT + TT	16 (41)	25 (53)	16 (32)	1.48	0.62-3.54	**2.41**	**1.06-5.51***
	C	58 (0.74)	61 (0.65)	84 (0.84)	Reference		Reference	
	T	20 (0.26)	33 (0.35)	16 (0.16)	1.81	0.86-3.79	**2.84**	**1.44-5.62**†
IL1RN intron 2 VNTR	11	15 (42)	25 (54)	28 (64)	Reference		Reference	
	12	14 (39)	17 (37)	13 (29)	2.01	0.75-5.36	1.46	0.59-3.61
	22	6 (16)	4 (9)	3 (7)	3.73	0.82-17.09	1.49	0.30-7.33
	13	1 (3)	0	0	NA		NA	
	12+22+13	21 (58)	21 (46)	16 (36)	2.45	0.99-6.05	1.47	0.63-3.42
	1	45 (0.62)	67 (0.73)	69 (0.78)	Reference		Reference	
	2+3	27 (0.38)	25 (0.27)	19 (0.22)	**2.18**	**1.09-4.37**‡	1.35	0.68-2.69

#Hardy-Weinberg equilibrium: (Chi-square test: *IL1B*−511C>T: *P*=0.76, *IL1B*+3954C>T: *P*=0.33; *IL1RN* intron 2 VNTR: *P*=0.38); **P*=0.041; †*P*=0.003; ‡*P*=0.035. Significant results are in bold. AgP: Aggressive periodontitis; CP: Chronic periodontitis; OR: Odds ratio; CI: Confidence interval; f: Frequency; NA: Not applicable; VNTR: Variable number tandem repeat

and CT1 in the AgP (OR = 3.41) and CP (OR = 6.30) groups, respectively, as compared to controls [Table 5].

Discussion

Periodontitis development is related to the disruption of periodontal host-microbe homeostasis, and imbalances in pro- and anti-inflammatory cytokines production upon microbial challenge play an important role in this process.[24] Allelic variations of the *IL1* family genes may affect periodontitis susceptibility,[8] and several studies have been conducted although the association of *IL1* polymorphisms with severe periodontitis, aggressive or chronic remains unclear. In this study, we investigated potential associations of two SNPs in the *IL1B* gene, *IL1B*-511C>T, and *IL1B* +3954C>T, and the VNTR polymorphism in intron 2 of the *IL1RN* gene with periodontal conditions in a Southeastern Brazilian population, which is characterized by a complex background with a high degree of miscegenation.[25]

High levels of IL-1ß in the gingival crevicular fluid are correlated to an increased susceptibility to periodontitis and seem to play a critical role in the pathogenesis of periodontal disease.[26] As the *IL1B*-511C>T polymorphism is located in the promoter region of the gene, it could interfere with the IL-1ß expression.[27] The T allele frequency showed to be high in Japanese,[7] Chinese,[28] Caucasian,[9] and South Indian populations.[27] The frequency of the allele T in our control group was 0.46, and no significant differences were observed between allele and genotype distributions in AgP or CP groups in comparison with controls [Table 2]. Similarly, different studies have failed to establish an association of the *IL1B*-511C>T polymorphism with the development of AgP and/or CP.[3,27,29] However, in a meta-analysis, a weak association between this polymorphism and CP was observed, with the T allele representing a risk for the disease, especially among Asians.[30]

Although the *IL1* +3954C>T polymorphism has been extensively studied, its correlation with high IL-1β levels is still controversial.[11,14,31] Low prevalence of the *IL1B* +3954T allele has been reported in Chinese,[28,32] Thai,[33] and Japanese populations.[7] In contrast, the prevalence of individuals carrying the variant allele is high in Caucasians (31–50%).[9] In our control group, composed of 88% of white Brazilians [Table 1], the prevalence of the *IL1B* + 3954T allele was 0.16 [Table 2], which was similar to the values found in studies conducted in different regions of Brazil.[17,34,35]

No significant differences for *IL1B* +3954C>T genotype or allele distributions were observed between the AgP and control groups. Similar results were obtained by other researchers.[31,36] However, several reported results are not consistent with ours. Quappe *et al*.[37] observed an association

Table 4: Genotype combinations concerning the *IL1B*+3954 C>T and *IL1RN* intron 2 variable number tandem repeat polymorphisms in aggressive periodontitis and chronic periodontitis groups and controls

Genotype combination *IL1B*+3954/*IL1RN* VNTR	AgP, *n* (%)	CP, *n* (%)	Control, *n* (%)	AgP versus controls		CP versus controls	
				OR	95% CI	OR	95% CI
CC/11	7 (20.6)	9 (21.4)	18 (40.9)	Reference		Reference	
CC/12+22+13	13 (38.2)	11 (26.2)	12 (27.3)	2.79	0.86-9.01	1.83	0.58-5.76
CT + TT/11	7 (20.6)	13 (31.0)	10 (22.7)	1.80	0.49-6.62	2.60	0.82-8.20
CT + TT/12+22+13	7 (20.6)	9 (21.4)	4 (9.1)	4.50	1.00-20.32	**4.50**	**1.08-18.70***

**P*=0.046. Significant results are in bold. VNTR: Variable number tandem repeat; OR: Odds ratio; CI: Confidence interval; AgP: Aggressive periodontitis; CP: Chronic periodontitis

Table 5: Haplotype estimation with respect to the three polymorphisms, *IL1B*−511C>T, *IL1B*+3954 C>T, and *IL1RN* intron 2 variable number tandem repeat, in aggressive periodontitis and chronic periodontitis groups and controls

Haplotype (*IL1B*−511/ *IL1B*+3954/*IL1RN* VNTR#)	AgP (f)	CP (f)	Control (f)	AgP versus controls		CP versus controls	
				OR	95% CI	OR	95% CI
CC1	0.2445	0.2133	0.4107	Reference		Reference	
TC1	0.1919	0.2120	0.2385	1.32	0.40-4.38	1.79	0.58-5.54
TC2	0.2218	0.1121	0.1055	**3.41**	**1.10-10.53***	1.10	0.27-4.42
TT1	0.0492	0.1284	0.0918	0.92	0.16-5.41	2.34	0.64-8.61
CC2	0.0841	0.1090	0.0853	1.63	0.36-7.38	3.56	0.74-17.20
CT1	0.1494	0.1747	0.0414	5.26	0.85-32.58	**6.30**	**1.22-32.57†**
TT2	0.0339	0	0.0268	1.71	0.15-19.50	NA	
CT2	0.0252	0.0505	0	NA		NA	

#For haplotype estimation, the only *IL1RN**3 allele, found in the AgP group, and the alleles *IL1RN**2 were grouped together; **P*=0.036; †*P*=0.031. Significant results are in bold. f: Frequency; OR: Odds ratio; CI: Confidence interval; AgP: Aggressive periodontitis; CP: Chronic periodontitis; NA: Not applicable; VNTR: Variable number tandem repeat

between this polymorphism and AgP in a Chilean population, with the C allele showing a protective effect. Contradictory results were reported by Parkhill *et al*.,[12] who observed the higher frequency of the C allele in patients with AgP as compared to controls. Nevertheless, we observed risk effects for the T allele and the T allele-containing genotypes (CT + TT) on the development of CP. Our results are consistent with those obtained in other studies performed in Australia,[38] Chile,[39] Germany,[40] and Brazil,[34] and the results of the different meta-analysis, in which an association between the *IL1B* +3954C>T polymorphism and CP was found.[30,41,42]

Although Il-1RA may play a defense role in periodontitis, its increase is not sufficient to limit the release of IL-1β.[43] The allele *IL1RN**2 has been associated with reduced levels of gene transcripts in patients with periodontitis.[14] In the present work, the minor alleles *IL1RN**2 (two repeats) and *3 (five repeats) were overrepresented in the AgP group, in comparison with controls [Table 3], which suggests their effects on the risk of AgP in our population. The minor alleles *IL1RN**2, *3, *4, *5 also proved to be associated with AgP in Japanese,[7] Turkish,[44] and Iranian individuals,[45] but their presence was found not to be important for the development of AgP in Caucasians[12,36,46] and Chinese populations.[28] On the other hand, the *IL1RN* intron 2 VNTR polymorphism showed no effect on the risk of CP in our population as had already been observed in other studies.[3,45,46] In fact, published data on the association of this polymorphism with periodontal diseases are inconsistent and conflicting, which may be explained by variation in the allele frequencies across different ethnic groups.[46,47] In a recent meta-analysis, for example, the authors suggested that *IL1RN* intron 2 VNTR polymorphisms might contribute to an increased risk of CP in Asians and a decreased risk of AgP in Caucasians.[48]

It is very well known that association between genetic and environmental factors may affect the disease susceptibility, and smoking habit has been considered an important risk factor for the progression and prognosis of periodontal diseases.[4,49] No difference was found between the number of current or former smokers in the AgP, CP, and control groups [Table 1]. However, our data indicated that smoking increased even more (1.56 times) the risk of *IL1B* +3954T allele carriers to develop CP (OR = 4.43). Other authors did not observe this association,[3,11] reinforcing the need of further investigations.

Gender has also been considered as a risk factor for periodontal disease, and it seems that differences in lifestyle rather than in genetic factors are responsible for the higher prevalence of severe periodontitis observed in men as compared to women.[49] Men were overrepresented in the CP groups in comparison with AgP and control groups [Table 1]. Nevertheless, the interaction analysis revealed that being of female sex markedly increased (2.11 times) the risk of *IL1B* +3954T allele-carriers to develop CP (OR = 6.00).

As previously mentioned, studies concerning possible associations of *IL1* polymorphisms and periodontal diseases have been conducted in different ethnic groups, with inconsistent and sometimes contradictory results. In the present work, the skin color/ethnicity modified the risk of developing AgP in association with the *IL1RN* intron 2 VNTR polymorphisms as a higher prevalence of the *IL1RN**2 or *3 alleles was observed in white Brazilian AgP patients in comparison with white Brazilian controls (OR = 3.08). This association was confirmed by using the codominant genetic model, and a markedly higher frequency of the *ILRN**2*2 genotype was found in white Brazilian AgP patients (OR = 7.80).

Analysis of genotype combinations revealed a synergistic effect of both *IL1B* +3954C>T and *IL1RN* VNTR polymorphisms on the risk of developing CP, with individuals carrying composite genotypes that combine minor alleles at each locus (*IL1B* + 3954CT or TT and *IL1RN* VNTR *1*2, *1*3, or *2*2) presenting even higher risk of developing the disease (OR = 4.50) [Table 4]. In one of the few studies considering genotype combinations, the authors also suggested that the presence of those alleles could increase the risk of severe CP in nonsmoking patients.[50]

Finally, the haplotype estimation using the data corresponding to the three polymorphisms, *IL1B*-511C>T, *IL1B* +3954C>T, and *IL1RN* VNTR, allowed the identification of two risk haplotypes, TC2 e CT1, for aggressive [OR = 3.41] and chronic [OR = 6.30] periodontitis, respectively [Table 5]. Few studies regarding the association of *IL1* gene cluster haplotypes with periodontitis have been reported, but the comparison with our results was not possible since these studies included either different genes or different polymorphic loci.[14,51]

Conclusions

In summary, interaction analyses revealed that the association of *IL1B* +3954C>T polymorphism with CP can be modified by smoking habit and gender, while the skin color/ethnicity modified the *IL1RN* VNTR polymorphism-associated risk for AgP. In addition, to our knowledge, this is the first report in a Brazilian population, and one of the few reports in the world, showing that *IL1* haplotypes are associated with the risk of AgP and CP. However, further studies in larger populations, preferably of different ethnicities, might be conducted to validate our findings to use this information not only for developing prevention and diagnosis strategies but also for clarifying the molecular mechanisms of periodontitis.

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Conflicts of interest

There are no conflicts of interest.

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