Analysis of polymorphisms in *Interleukin 10*, *NOS2A*, and *ESR2* genes in chronic and aggressive periodontitis

Virginia Régia Souza SILVEIRA[(a)]
Suzane Cristina PIGOSSI[(b)]
Raquel Mantuaneli SCAREL-CAMINAGA[(b)]
Joni Augusto CIRELLI[(c)]
Rodrigo RÊGO[(a)]
Nádia Accioly Pinto NOGUEIRA[(d)]

[(a)]Universidade Federal do Ceará – UFC, *Campus* Sobral, School of Dentistry, Department of Dentistry, Sobral, CE, Brazil.

[(b)]Universidade Estadual Paulista Júlio de Mesquita Filho – UNESP, School of Dentistry at Araraquara, Department of Morphology, Araraquara, SP, Brazil.

[(c)]Universidade Estadual Paulista Júlio de Mesquita Filho – UNESP, School of Dentistry at Araraquara, Department of Oral Diagnosis and Surgery, Araraquara, SP, Brazil.

[(d)]Universidade Federal do Ceará – UFC, Faculty of Pharmacy, Dentistry and Nursing, Department of Clinical and Toxicological Analyses, Fortaleza, CE, Brazil.

Corresponding Author:
Virginia Régia Souza da Silveira
E-mail: virrginia50@hotmail.com

Abstract: The objective of this study was to investigate the association between single nucleotide polymorphisms (SNPs) in the IL10, NOS2A, and ESR2 genes and chronic periodontitis (CP) and aggressive periodontitis (AgP). Three groups of patients underwent periodontal and radiographic evaluations: CP (n = 61), AgP (n = 50), and periodontally healthy (control group=61). Genomic DNA was extracted from oral epithelial cells and used for genotyping by real-time polymerase chain reaction using TaqMan® probes. The investigated SNPs were: -1087G > A, -819C > T and -592C > A in the IL10; +2087G > A in the NOS2A, and +1730G > A in the ESR2 gene. Differences in genotype and allele frequencies of each polymorphism and some individual characteristics were analyzed using the chi-square test and multivariate logistic regression analysis. Analysis of SNPs and haplotypes in the IL10 and SNP in the ESR2 gene did not present any significant association with AgP or CP. The +2087G allele of the NOS2A gene tended to be significantly associated with periodontal disease. Patients carrying the genotype +2087GG in the NOS2A gene were genetically protected against the development of CP (p = 0.05; OR = 0.44; 95%CI = 0.20–0.95). This result showed greater significance when patients with AgP and CP were combined (total PD) (p = 0.03; OR = 0.46; 95%CI = 0.23–0.92). In conclusion, the studied Brazilian population had a significantly higher frequency of the GG genotype for the +2087 SNP in the NOS2A gene in individuals without periodontitis, although statistical significance was not maintained after multiple logistic regression.

Keywords: Aggressive Periodontitis; Interleukin-10; Nitric Oxide Synthase; Estrogen Receptor Beta; Single Nucleotide Polymorphism.

Introduction

Periodontitis is a chronic infectious disease of the supporting tissues of the teeth. Due to bacterial infection, periodontal tissues become inflamed and are slowly destroyed by the action of the inflammatory process, which could lead to tooth loss.[1] The two main forms of periodontitis are chronic periodontitis (CP) and aggressive periodontitis (AgP).[2] CP is the most common form of destructive periodontal disease in adults.[3] AgP is a rapidly progressing form of periodontal disease that occurs in otherwise clinically healthy individuals[4] and is characterized by familial aggregation.[5]

Periodontitis is considered a complex disease,[6] since the pathophysiology presents various biological pathways. Complex diseases are associated with variations in multiple genes, each of which has a small overall contribution and relative risk for disease process.[1] In periodontal disease, the phenotype is determined by both the genetic makeup and the environmental influences on the affected individual.[6] Most of the genetic research into periodontitis has focused on gene polymorphisms that play a role in immunoregulation or metabolism.[6]

Interleukin-10 is an anti-inflammatory cytokine that plays a role in controlling the inflammatory response.[1,6] Periodontitis lesions presented a significantly higher expression for IL-10 than that in autologous peripheral blood mononuclear cells.[7] Therefore, IL-10 has been considered a candidate susceptibility gene in periodontitis.[1]

Three promoter single nucleotide polymorphisms (SNPs) have been described in the *IL10* gene, namely (-1087)G > A; (-819)C > T; and (-592)C > A, and have been associated with altered IL-10 synthesis in response to inflammatory stimuli.[8] The SNPs -819 and -592 in the *IL10* gene and specific haplotypes were associated with CP in Brazilian patients.[9] In the *IL10* gene, the A allele of the –592 SNP may contribute to a relative increase in the risk for CP, while for –819 gene polymorphism, the T allele may result in a slight increase in CP susceptibility in Caucasians.[10,11] The ATA/ATA haplotype was associated with CP[9] and AgP[12] and with low levels of the IL-10 protein.[8]

Nitric oxide (NO) is a short-lived molecule that plays a key role in many physiological and pathological processes.[13] Nitric oxide is synthesized from the amino acid L-arginine by NO synthase (NOS) which exists in three distinct isoforms: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS).[14] iNOS is expressed almost exclusively under inflammatory conditions and is produced by immunocompetent cells, such as macrophages and neutrophils.[15] Several SNPs within the *NOS2A* gene could influence its production or activity.[14,16] Increases in iNOS activity have been reported in periodontal disease, suggesting the production and participation of NO in the disease process.[17]

Estrogens act by binding to and activating two estrogen receptors (ERs), ERα and ERβ,[18] encoded respectively by two separate genes, *ESR1* and *ESR2*.[19] There is evidence in the literature to suggest an association between estrogen and periodontal disease, since ERs were detected in gingival tissues,[19] periodontal ligament cells, and jaw bone.[20] Also, various effects of estrogen on periodontal tissues were found in humans and animals, such as enhancement of cellular proliferation in blood vessels, inhibition of neutrophilic chemotaxis, stimulation of fibroblast proliferation, and change in collagen metabolism.[19,20] ERβ is the most predominant ER in healthy and diseased human gingivae, implying that the effects of estrogen on both health and diseased gingival tissues are mediated by this receptor.[19] Polymorphisms in both ER genes may modify the effects of endogenous estrogen and have been associated with several pathological conditions, such as cardiovascular disease,[21] breast cancer,[22] and CP.[23]
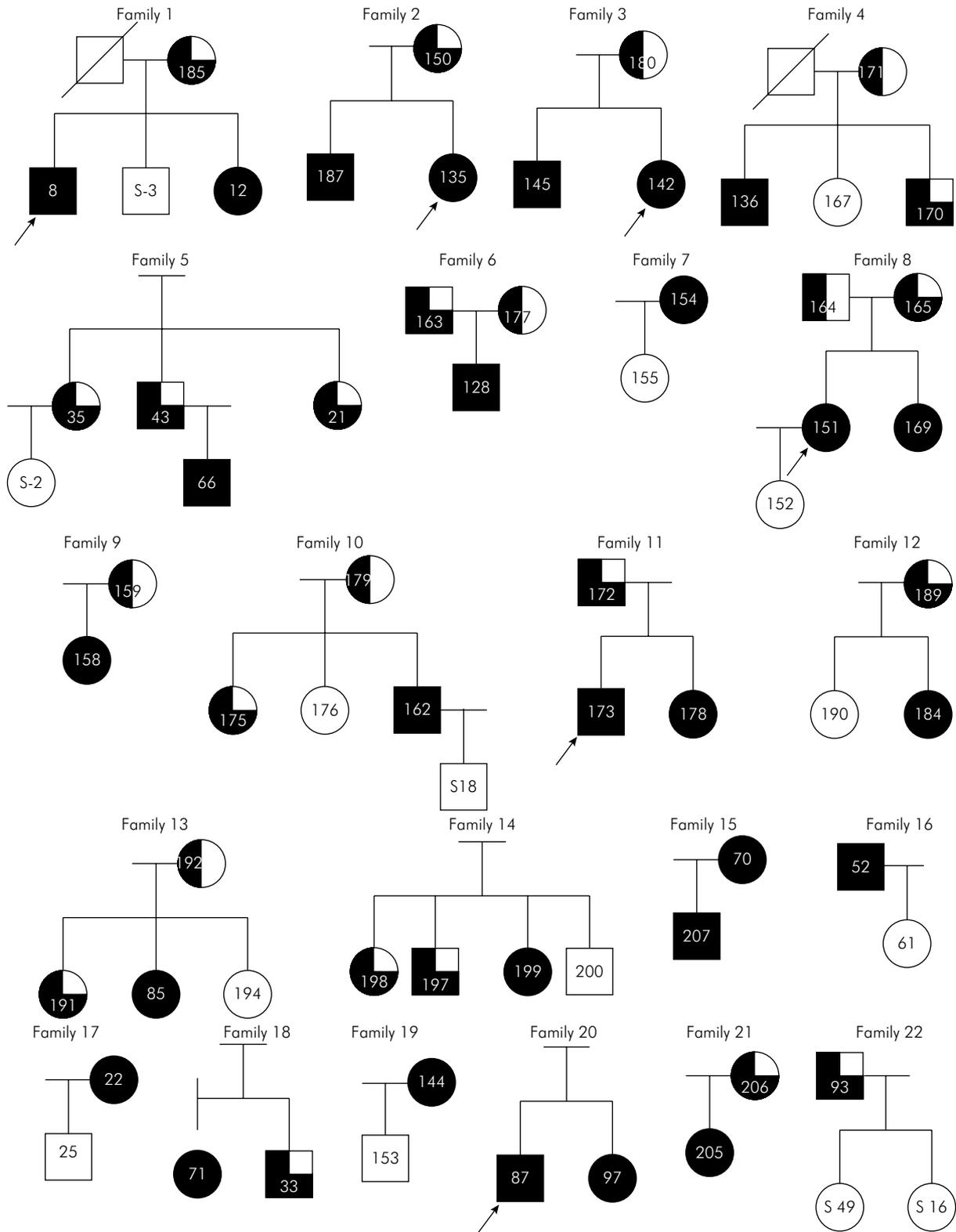
There are currently few data on the association of polymorphisms in the *NOS2A* and *ESR2* genes with periodontitis, as well as SNPs in the *IL10* gene with AgP. Therefore, in the present study, we investigated the association of SNPs in the promoter region of the *IL10* gene and its haplotypes, in addition to SNPs in the *NOS2A* and *ESR2* genes, with CP and AgP in Brazilian patients.

Methodology

This cross-sectional study was conducted at the Periodontology Clinic, in the in the School of Pharmacy, Dentistry, and Nursing, of the Federal University of Ceará, Brazil, between June 2011 and June 2014. The study protocol was approved by the Ethics Committee 098/11. All participants or guardians were informed about the purpose of the study and provided written consent.

Study population

This study enrolled 42 AgP, 42 CP and 49 periodontally healthy patients. In addition, 39 family members (parents and/or siblings) of 22 AgP patients were also enrolled (Figure 1). The AgP relatives were included and allocated to the study groups according to their periodontal condition. Six relatives were completely edentulous, with history of tooth loss in adulthood

Figure 1. Family tree of families affected by AgP. Squares designate males and circles designate females (numbers and letters refer to the patients' codes). The proband is indicated with an arrow in families that have more than one member with AgP. Family members with gingivitis were designated as periodontally healthy.

due to periodontal disease, and were included in the CP group. Thus, a total of 172 subjects were recruited and placed in three study groups as follows: 50 patients with AgP, 61 patients with CP, and 61 periodontally healthy patients (control group). All of the referent subjects were systemically healthy and non-smokers.

AgP patients were classified according to the clinical criteria proposed by the American Academy of Periodontology (AAP).[5] CP patients were also classified according to the same criteria.[3,24,25] In addition to the AAP[5] criteria, patients with AgP should present age $\geq$ 12 and $\leq$ 40 years; history of familial aggregation; presence of at least 20 teeth, excluding third molars and including at least three first molars and five incisors; presence of at least three sites on different teeth (other than central incisors or first molars) with probing depth (PD) and clinical attachment loss (CAL) $\geq$ 5 mm, bleeding on probing (BOP), and radiographic evidence of moderate to severe bone loss.

The control group comprised subjects with at least 24 teeth in the oral cavity, excluding third molars, absence of sites presenting PD and CAL > 3 mm, and presenting gingival index[26] < 10%; tooth mobility was not found.

The exclusion criteria were as follows: periodontal treatment within the previous 6 months, antibiotic therapy within the previous 3 months, ongoing orthodontic therapy and systemic alterations that could have interfered with periodontal conditions.

Periodontal examination

All completely erupted permanent teeth, excluding third molars, were submitted to a clinical periodontal examination with the North Carolina manual probe (Trinity®, São Paulo, Brazil). The following parameters were evaluated: plaque index (PI),[26] gingival index (GI),[26] probing depth (PD), clinical attachment level (CAL), and dichotomous bleeding on probing (BOP) score. PD and CAL were taken at six sites per tooth. A single examiner evaluated all the clinical parameters, and measurement reproducibility was calculated using the intraclass correlation coefficient (ICC) for PD and CAL. The agreement between replicate measurements was high (ICC > 0.80).

DNA collection and genetic polymorphism analysis

DNA was obtained from buccal epithelial cells[27] and extracted with ammonium acetate.[28] DNA concentration was determined with the aid of a spectrophotometer - Nanodrop 2000 (Thermo Scientific, DE, USA), with purity estimated by OD 260/280 ratio and diluted to 10ng/µl.

The analysis of genetic polymorphisms was performed using quantitative real-time polymerase chain reaction (qPCR). The SNPs in the *IL10* gene -1082 (G > A) (rs1800896), -819 (C >T) (rs1800871) and -592 (C > A) (rs1800872); in the *ESR2* gene +1730 (G > A) (rs4986938); and in the *NOS2A* gene +2087 (G > A) (rs2297518) were genotyped using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA).

DNA amplification was carried out in a 13-µl volume containing 1µL of genomic DNA (10ng/µl), 6.25µL of Master Mix 2x (Applied Biosystems), 0.63µL 20x TaqMan Assay Mix (Applied Biosystems), and 4.63µL of RNA/DNase free water (Applied Biosystems). The amplifications were made in a StepOne Real-Time PCR System (Applied Biosystems) in 96-well plates. At the end of the reaction, the result was evaluated by observing the groups or clusters of samples that carried each genotype. The distinction between the different genotypes was determined by their particular fluorescence. Each point was determined using the StepOne Real-Time PCR System. The analysis of polymorphisms was performed in the Molecular Genetics Laboratory at the School of Dentistry at Araraquara, UNESP-São Paulo State University.

Data analysis

Differences in the allele and genotype frequencies of gene polymorphisms were analyzed by the chi-square test and odds ratio using the GraphPad Instat software – version 3.05 (Graph Pad Software Inc. San Diego, CA-USA). A multivariate logistic regression was performed to estimate the relationship between the investigated SNPs and susceptibility to periodontal disease and other covariates (age, gender, CAL, BOP), using the R statistical package (Development Core Team, Vienna, Austria). The distribution of each polymorphism in each group was tested for

the Hardy-Weinberg equilibrium by means of the ARLEQUIN software,[29] which was also used to investigate linkage disequilibrium between the loci and the estimated frequency of the possible *IL10* haplotypes. The frequency of *IL10* haplotypes in different groups was confirmed by manual counting and assessed through the CLUMP software.[30] All tests were performed at a significance level of 5%.

Results

The clinical data are summarized in Table 1. The population under investigation was composed mainly of female subjects (65.7%). All AgP patients showed the generalized form of the disease. The mean age was 28.0±10.0 years for the control group, 28.2±6.5 years for AgP patients, and 44.9±11.3 years for CP patients, which was statistically different from the other groups (p<0.05). It is important to take into consideration that the present study included direct family members of 22 AgP families. Our findings showed that in most of those families, periodontal disease was present in more than one individual, as either AgP or CP (Figure 1). Unfortunately, the number of families affected by AgP enrolled in this study was insufficient to apply an AgP family-based association analysis.

Table 1. Demographic and clinical periodontal parameters.

Parameters	Control group	AgP	CP
	n = 61	n = 50	n = 61
Age (years; mean ± SD)	28.0 ± 10.0	28.1 ± 6.5	44.9 ± 11.3*
Male/ Female	18/43	15/35	26/35
Number of teeth (mean ± SD)	26.8 ± 3.0	25.8 ± 2.0	22.2 ± 5.2*
PI (% sites; mean ± SD)	ne	33.4 ± 17.3	37.8 ± 21.1
GI (% sites; mean ± SD)	ne	13.7 ± 19.2	17.2 ± 19.9
PD (mm; mean ± SD)	ne	3.3 ± 0.7**	2.8 ± 0.7
CAL (mm; mean ± SD)	ne	3.8 ± 0.9**	3.5 ± 1.0
BOP (% sites; mean ± SD)	ne	43.1 ± 17.4**	32.0 ± 0.2

AgP: aggressive periodontitis; CP: chronic periodontitis; PI: plaque index; GI: gingival index; PD: probing depth; CAL: clinical attachment level; BOP: bleeding on probing; ne: not estimated. *p < 0.05 = difference between CP and the other groups; **p < 0.05 = difference between AgP and CP.

Regarding clinical periodontal aspects, the control group had an average of 26.8 (±3.0) remaining teeth compared with 25.8 (±2.0) in the AgP group, and 22.2 (±5.2) in the CP group. There was a statistically significant difference between the number of teeth in all groups (p < 0.05). The mean number of teeth with PD and CAL > 3 mm was, respectively, 19.1 (±4.4) and 20.4 (±3.9) for the AgP group, 12.4 (±5.3) and 15.5 (±4.9) for the CP group, and zero for the control group. AgP patients presented signs of severe periodontal destruction and inflammation, as observed from the significantly higher mean of PD, CAL and BOP compared with the CP groups (Table 1).

Allele and genotype frequencies were not significantly different between control and disease groups regarding any of the three SNPs in the *IL10* and *ESR2* genes (Table 2). The genotype distributions of each SNP were consistent with the assumption of the Hardy-Weinberg equilibrium for each group. Considering the SNP +2087 in the *NOS2A* gene, the G allele showed a trend towards a significant association with CP (p = 0.07; OR = 0.52; 95%CI = 0.26–1.01). Patients carrying the genotype GG were genetically protected against the development of CP (p = 0.05; OR = 0.44; 95%CI = 0.20–0.95). This result was considered significant when AgP and CP patients were combined (total PD) (p = 0.03; OR = 0.46; 95%CI = 0.23–0.92) (Table 2). However, the multiple logistic regression analysis demonstrated that the genotypes of any of these genes were not associated with AgP or CP after adjusting for common covariates, including age, gender, CAL, and BOP (Table 3). The distribution of haplotypes in the *IL10* gene, arranged as genotypes, showed no significant differences between the control and periodontitis groups (Table 4).

Haplotypes as genotypes consisted of -1082 -819 -592/-1082 -819 -592. Control: healthy patients; AgP: aggressive periodontitis; OR (95%CI): odds ratio values with the respective 95% confidence intervals; CP: chronic periodontitis; p-value obtained from the Clump program. Total PD = AgP + CP groups.

By utilizing the G*Power 3 software, the calculations showed that a sample size of 121 individuals demonstrated a power of 77% for the *NOS2A* (+2087) genotype analysis between CP (n = 60) and Control (n = 61), with OR = 0.44. When Total PD (AgP+CP n=110)

Table 2. Allele and genotype frequencies of the SNPs in the *IL10*, *NOS2A*, and *ESR2* genes.

Gene (SNP)	CONTROL (n[%])	AgP (n[%])	p	CP (n[%])	p	Total PD (AgP+CP) (n[%])	p
IL 10 (-1082) alleles	n = 82	n = 100		n = 120		n = 220	
A	60 (73.2)	65 (65.0)	0.26	79 (64.8)	0.28	144 (64.9)	0.21
G	22 (26.8)	35 (35.0)	0.26	41 (33.6)	0.28	76 (34.2)	0.21
IL 10 (-1082) genotypes	n = 41	n = 50	-	n = 60	-	n = 110	-
AA	23 (56.1)	20 (40.0)	0.14	27 (44.3)	0.31	47 (42.3)	0.19
AG	14 (34.2)	25 (50.0)	0.14	25 (41.0)	0.54	50 (45.0)	0.45
GG	4 (9.7)	5 (10.0)	1.00	8 (13.1)	0.75	13 (11.7)	1.00
H-W disequilibrium	p = 0.41	p = 0.48	-	p = 0.56	-	p = 0.95	-
IL 10 (-819) alleles	n = 122	n = 100	-	n = 122	-	n = 222	-
C	41 (33.6)	37 (37.0)	0.67	46 (37.7)	0.59	83 (37.4)	0.56
T	81 (66.4)	63 (63.0)	0.67	76 (62.3)	0.59	139 (62.6)	0.56
IL 10 (-819) genotypes	n = 61	n = 50	-	n = 61	-	n = 111	-
CC	28 (45.9)	21 (42.0)	0.71	22 (36.1)	0.36	43 (38.7)	0.25
CT	25 (41.0)	21 (42.0)	1.00	32 (52.5)	0.28	53 (47.7)	0.75
TT	8 (13.1)	8 (16.0)	0.79	7 (11.5)	1.00	15 (13.5)	1.00
H-W disequilibrium	p = 0.52	p = 0.48	-	p = 0.36	-	p = 0.83	-
IL 10 (-592) alleles	n = 122	n = 100	-	n = 122	-	n = 222	-
C	80 (65.6)	61 (61.0)	0.48	76 (62.3)	0.68	137 (61.7)	0.48
A	42 (34.4)	39 (39.0)	0.48	46 (37.7)	0.68	85 (38.3)	0.48
IL 10 (-592) genotypes	n = 61	n = 50	-	n = 61	-	n = 111	-
CC	27 (44.3)	20 (40.0)	0.70	22 (36.1)	0.46	42 (37.8)	0.25
CA	26 (42.6)	21 (42.0)	1.00	32 (52.5)	0.36	53 (47.7)	0.87
AA	8 (13.1)	9 (18.0)	0.59	7 (11.5)	1.00	16 (14.4)	1.00
H-W disequilibrium	p = 0.66	p = 0.41	-	p = 0.36	-	p = 0.91	-
NOS2A (+2087) alleles	n = 122	n = 100	-	n = 122	-	n = 222	-
A	17 (13.9)	14 (14.1)	1.00	29 (23.8)	0.07	43 (19.4)	0.24
G	105 (86.1)	86 (86.0)	1.00	93 (76.2)	0.07	179 (80.6)	0.24
NOS2A (+2087) genotypes	n = 61	n = 50	-	n = 60	-	n = 110	-
AA	2 (3.3)	0 (0.0)	0.50	3 (4.9)	1.00	3 (2.7)	1.00
AG	13 (21.3)	14 (28.0)	0.51	23 (37.7)	0.07	37 (33.3)	0.15
GG	**46 (75.4)**	36 (72.0)	0.83	**35 (57.4)**	**0.05**	**71 (64.0)**	**0.03**
	-	-	-	OR = 0.44 (95%C I= 0.20–0.95)	-	OR = 0,46 (95%CI = 0.23–0.92)	-
H-W disequilibrium	p = 0.38	p = 0.24	-	p = 0.75	-	p = 0.47	-
ESR2 (+1730) alleles	n = 122	n = 100	-	n = 122	-	n = 222	-
G	88 (72.1)	75 (75.0)	0.65	83 (68.0)	0.57	158 (71.2)	0.9
A	34 (27.9)	25 (25.0)	0.65	39 (32.0)	0.57	64 (28.8)	0.9
ESR2 (+1730) genotypes	n = 61	n = 50	-	n = 61	-	n = 111	-
GG	30 (49.2)	26 (52.0)	0.85	30 (49.2)	1.14	56 (50.5)	1.00
GA	28 (45.9)	23 (46.0)	1.00	23 (37.7)	0.46	46 (41.4)	0.33
AA	3 (4.9)	1 (2.0)	0.63	8 (13.1)	0.20	9 (8.1)	0.75
H-W disequilibrium	p = 0.26	p = 0.11	-	p = 0.29	-	p = 0.91	-

The control group was compared to each AgP, CP, as well as AgP + CP groups Total PD; p values were calculated by the chi-square test; Bold: significant result; OR: odds ratio; CI: confidence interval.

Table 3. Multiple logistic regression results for the covariates analyzed.

Patient characteristics	Control n = 61	AgP n = 50	CP n = 61	p	OR (95%CI)
Age (mean)	28.0	28.1	44.8	1.0	1.17 (0.8–1.72)
Gender [n (%)]					
Female	43 (70.5)	35 (70)	36 (59)		Reference
Male	18 (29.5)	15 (30)	25 (41)	0.88	1 1.19 (0.09–15.26)
BOP%	NA	43.12	31.97	0.78	1.01 (0.91–1.12)
CAL (mean)	NA	3.8	3.4	1.0	0.46 (0.05–4.03)
IL10 gene polymorphism [n (%)]					
-1082					
AA	23 (56.1)	20 (40)	27 (44.3)		Reference
AG	14 (34.2)	25 (50)	25 (41)	1.0	1.6 (0.09–29.53)
GG	4 (9.7)	5 (10)	8 (13.1)	0.57	2.59 (0.02–277.99)
-819					
CC	28 (45.9)	21 (42)	22 (36)		Reference
TC	25 (41)	21 (42)	32 (52.5)	0.89	1.57 (0.1–24.22)
TT	8 (13.1)	8 (16)	7 (11.5)	1.0	1.39 (0.03–73.96)
-592					
CC	27 (44.3)	20 (40)	22 (36)		Reference
AC	26 (42.6)	21 (42)	32 (52.5)	1.0	1.68 (0.11–26.38)
AA	8 (13.1)	9 (18)	7 (11.5)	1.0	1.64 (0.05–51.74)
NOS2A gene polymorphism [n (%)]					
2087					
GG	46 (75.4)	36 (72)	35 (57.4)		Reference
GA	13 (21.3)	14 (28)	23 (37.7)	1.0	1.36 (0.1–18.73)
AA	2 (3.3)	0	3 (4.9)	1.0	268.3 (0–8.81)
ESR2 gene polymorphism [n (%)]					
1730					
GG	30 (49.2)	26 (52)	30 (49.2)		Reference
GA	28 (45.9)	23 (46)	23 (37.7)	1.0	0.94 (0.08–10.6)
AA	3 (4.9)	1 (2)	8 (13.1)	1.0	4.55 (0–48.2)

Control: healthy patients; AgP: aggressive periodontitis; CP: chronic periodontitis; BOP: bleeding on probing; CAL: clinical attachment level; OR (95%CI): dds ratio values with the respective 95% confidence intervals.

Table 4. Distribution of *IL10* haplotypes (arranged as genotypes) found in the studied groups.

Genotypes	Control (n[%])	AgP (n[%])	p	OR (95% IC)	CP (n[%])	p	OR (95% IC)	Total PD (AgP+CP) (n[%])	p	OR (95% IC)
	n = 61	n = 50	-	-	n = 61	-	-	n = 111	-	-
GCC/ATA	13 (21.3)	16 (32.0)	0.28	1.74 (0.74–4.08)	15 (24.6)	0.83	1.20 (0.51–2.80)	31 (27.9)	0.37	1.43 (0.68–2.99)
ATA/ACC	11 (18.0)	5 (10.0)	0.28	0.50 (0.16–1.56)	16 (26.2)	0.38	1.61 (0.68–3.84)	21 (18.9)	1.00	1.06 (0.47–2.38)
ACC/GCC	12 (9.7)	7 (14.0)	0.46	0.66 (0.24–1.84)	9 (14.8)	0.63	0.70 (0.27–1.82)	16 (14.4)	0.39	0.69 (0.30–1.57)
ACC/ACC	11 (18.0)	8 (16.0)	0.81	0.86 (0.32–2.35)	4 (6.6)	0.09	0.32 (0.09–1.06)	12 (10.8)	0.24	0.55 (0.23–1.33)
ATA/ATA	8 (13.1)	7 (14.0)	1.00	1.08 (0.36–3.21)	7 (11.5)	1.00	0.86 (0.29–2.54)	14 (12.6)	1.00	0.95 (0.37–2.43)
GCC/GCC	4 (6.6)	5 (10.0)	0.73	1.58 (0.40–6.24)	9 (14.8)	0.24	2.47 (0.72–8.49)	14 (12.6)	0.30	2.06 (0.65–6.55)
ACC/ACA	-	-	-	-	1 (1.6)	1.00	3.05 (0.12–76.4)	1 (0.9)	1.00	1.67 (0.07–41.6)
ATA/GTA	-	1 (2.0)	0.45	3.73 (0.15–93.6)	-	-	-	1 (0.9)	1.00	1.67 (0.07–41.6)
ATA/GCA	1 (1.6)	-	1.00	0.40 (0.02–10.0)	-	1.00	0.40 (0.02–10.0)	-	0.35	0.18 (0.01–4.51)
GCC/ACA	1 (1.6)	-	1.00	0.40 (0.02–10.0)	-	1.00	0.33 (0.01–8.21)	-	0.35	0.18 (0.01–4.51)
ACA/GCA	-	1 (2.0)	0.45	3.73 (0.15–93.6)	-	-	-	1 (0.9)	1.00	1.67 (0.07–41.6)

and Control (n= 61) were compared, with OR=0.46, the sample size of 171 individuals demonstrated a power of 89%. Therefore, the number of subjects enrolled in this study was large enough to detect associations with acceptable level of confidence.

Discussion

To our knowledge, this is the first study on the +2087 SNP of the *NOS2A* gene in periodontitis. This study showed a significant association between this gene variant and periodontal disease in a Brazilian population. The *NOS2A* gene is up-regulated in inflammatory processes, leading to an increased production of NO.[14] A study showed a higher expression of iNOS in the gingiva of patients with periodontitis than in healthy tissue samples.[17] High concentrations of NO were associated with severe periodontitis.[13] The GA genotype of the same SNP in the *NOS2A* gene investigated here (rs2297518) demonstrated, in a previous study, a tendency towards higher *NOS2A* activity than the GG genotype.[14] An increased frequency was found for the A allele in this +2087 SNP in patients with migraines with aura and was consistent with increased iNOS activity in such patients.[16] This genetic polymorphism leads to an amino acid substitution of serine with leucine in exon 16, which apparently increases iNOS activity and possibly promotes excessive NO formation and inflammation in the presence of the A allele.[16] In the present study, the GG genotype in the +2087 SNP of the *NOS2A* gene was significantly associated with periodontitis (AgP + CP). While the presence of the G allele seems to indicate a tendency towards protection against the development of CP, patients carrying the GG genotype were, in terms of genetic carriage, significantly better protected against the development of periodontal disease. We suggest that the presence of the G allele in homozygosity could influence the intensity of the host response under bacterial challenge, leading to decreased NO formation and lower inflammation effects in the periodontal tissue. However, considering that CP is a complex disease, only the carriage of this genotype does not necessarily imply that these patients will be protected against the development of periodontitis.

The three SNPs in the promoter region of the *IL10* gene mostly investigated in the literature are -592(C > A),

–819(C > T), and –1082(G > A).[10,11] Two meta-analyses, evaluating case-control studies, concluded that *IL10* -819 and -592 gene polymorphisms were associated with CP, especially among Caucasians.[10,11] The present study showed no evidence of such association, and this result is similar to other cross-sectional studies.[31,32] Probably, the lack of association between *IL10* gene polymorphism and periodontal diseases found in these studies was due to the small number of patients, while in meta-analysis studies, statistics are based on the combination of all the patients included in all studies being evaluated, which results in a larger population sample. In a longitudinal study[33] with a 5-year follow-up, the *IL10* genotype contributed to the progression of periodontal disease. This may reflect the fine balance between IL-10 and disease expression and progression, and the need for prospective longitudinal studies to elucidate the relative contribution of various factors in multifactorial diseases.[33]

This study did not find a significant association between *ESR2* +1730 (G/A) gene polymorphism and periodontitis. Only few studies have investigated the association of ESR gene polymorphisms and periodontal disease.[23,34,35] *ERa* gene polymorphisms were associated with the number of remaining teeth in postmenopausal Japanese women[34] and CP in female Han Chinese.[23]

Even though patients' relatives were included in this investigation, it was not possible to perform a family-based association analysis of AgP. However, the inclusion of affected family members was a positive aspect in the present study. Most of the 22 families investigated here had more than one member affected by periodontal disease (AgP or CP, Figure 1), corroborating the findings of other studies.[36,37] Meng et al.[38] reviewed genetic studies of families affected by AgP. Their findings showed that the familial aggregation of AgP is often very high among certain families, with the percentage of affected siblings and affected pedigree members reaching 40-50%. This finding suggests that genetic factors may be important in disease susceptibility. Also, those authors suggested that family screening could be used as a preventive diagnostic tool for AgP.[38]

Clinical diagnosis is the primary method by which AgP is recognized, but it may be supplemented with

microbiological and family segregation analysis. However, judging from the amount of periodontal tissue loss that is often present in virtually all of these subjects, periodontal destruction may have initiated earlier, sometimes several years before the diagnosis of the disease.[39] Future advancement in the understanding of the genetic predisposition to AgP may allow for the inclusion of specific genetic profiles in future case definitions.[39]

Because of the variable characteristics among the groups, mainly age, multiple logistic regression was used to evaluate the influence of these covariates in the association of each SNP in the disease. Considering common periodontal disease covariates in this analysis, the +2087 NOS2A SNP had lost the significance observed with the chi-square test in Table 2. This result could have been different if a larger number of subjects or different ethnicities had been included.

Another positive aspect of the present study is the analysis of SNPs in the *IL10* gene as haplotypes. Haplotypes are more powerful in detecting susceptibility alleles than individual polymorphisms, and they may provide more information about the onset of the disease.[40] Therefore, this suggests that the lack of association between *IL10* haplotypes and periodontitis in the investigated population (Table 4) does not seem to be an occasional finding.

Further studies with larger sample populations and with different ethnicities are needed in order to better evaluate the potential association between periodontal disease and polymorphisms in the *IL10, ESR2,* and *NOS2A* genes. We can speculate that, in the future, when the role of these genetic factors is finally elucidated, it will be possible to apply this information for the identification of patients at risk, especially for aggressive periodontitis. This will allow for early treatment, thus reducing the functional and esthetic sequelae associated with the disease.[20]

Conclusion

We conclude that, in the studied Brazilian population, there was a significantly higher frequency of the GG genotype for the +2087 SNP in the *NOS2A* gene in individuals without periodontitis, although statistical significance was not maintained after multiple logistic regression.

Acknowledgments

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