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Effect of ancestry on *interleukin-10* haplotypes in chronic periodontitis

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1. ABSTRACT

Chronic periodontitis is caused by an inflammatory reaction of the periodontal tissues and alveolar bone. This inflammation is caused by periodontopathic bacteria located in the subgingival biofilm, resulting in inflammatory reactions that may lead to loss of attachment. This tissue destruction is a consequence of host immune and inflammatory responses to specific periodontal pathogens and their metabolic products. Cytokines modulate the immune response, altering its efficiency in the competition against pathogens and increasing periodontal susceptibility. This study investigated genetic polymorphisms in Interleukin 10 (A-1082G, C-819T and C-592A) in 205 individuals from an admixed Brazilian population. A significantly increased risk of developing chronic periodontitis was observed in individuals with low IL-10 production and Amerindian ancestry. These results suggest that the polymorphisms A-1082G, C-819T, and C-592A, which are associated with ancestry, are involved in the susceptibility to the development of chronic periodontitis in an admixed northern Brazilian population.

2. INTRODUCTION

Chronic periodontitis (CP) is complex disease with a pathology that includes microbiological and inflammatory responses and genetic, epigenetic and environmental factors (1, 2). Pathogenic microorganisms colonize and proliferate in the gingival crevice, leading to local infection and periodontal pockets in a susceptible host. These factors cause the destruction of the supporting tissues of the teeth, progressive loss of attachment, and bone loss. CP is characterized by periodontal pocket formation and/or recession of the gingiva (3). The progression of this destructive disease seems to depend on an abnormal host response to pathogenic microorganisms within plaque biofilm (4). The host immune system releases inflammatory cytokines and enzymes, which may act in the degradation of periodontal tissues and lead to the clinical signs of the disease (4, 5).

Chronic inflammation and cytokine levels play a pivotal role in the destructive processes occurring in periodontitis (6). Several inflammatory mediators,

such as pro-inflammatory and anti-inflammatory interleukins, are involved in periodontal diseases (7, 8). Interleukin-10 (IL-10) is an anti-inflammatory cytokine and is a key moderator of inflammation. IL-10 stimulates the production of protective antibodies and down-regulates pro-inflammatory cytokines; therefore, a decrease in the normal levels of IL-10 or its receptor results in inflammatory disorders in mouse and humans (9,10).

Polymorphisms of the IL-10 gene that may reduce IL-10 production are significantly associated with reduced bone mineral density in women who are prone to osteoporosis (11, 12). Low levels of IL-10 may result in an insufficient inhibition of pro-inflammatory cytokines and collagenase, which may impact bone loss diseases, such as osteoporosis and periodontitis (13). However, IL-10 is an important regulator of alveolar bone homeostasis during development (13).

Polymorphisms in cytokine genes often affect cytokine expression profiles and may play an important role in resistance or susceptibility to CP (8, 14). Specifically regarding the IL-10 gene, single nucleotide polymorphisms (SNPs) (A-1082G (rs1800896), C-819T (rs1800871) and C-592A (rs1800872)) are associated with diseases with different pathologies, such as leprosy and type 2 diabetes mellitus (15, 16). The literature reveals a strong correlation of the allele IL-10*$^{-1082}$G with the overexpression of IL-10; in contrast, the allele IL-10*$^{-1082}$A is associated with the low expression of IL-10. For example, the genotype IL-10*$^{-1082}$GG confers overexpression of IL-10, whereas the -IL-10*$^{-1082}$GA and IL-10*$^{-1082}$AA genotypes confer intermediate and low expression of IL-10, respectively (17, 18, 19). IL-10 modulates the immune response for antibody production; thus, reduced IL-10 production could be a risk factor for the development of CP (7, 20).

In addition, these three SNPs can generate haplotypes that also change the level of IL-10 production (9, 17, 18, 19, 21). The modulation of serum IL-10 levels from an individual's haplotype depends on polymorphism A-1082G; ergo, genotypes with two IL-10*$^{-1082}$G alleles (GCC/GCC and other combinations) exhibit increased IL-10 production, genotypes with one IL-10*$^{-1082}$G allele (GCC/ATA and other combinations) exhibit average IL-10 production, and genotypes without an IL-10*$^{-1082}$G allele (ATA/ATA and other combinations) exhibit low IL-10 production (9, 17, 18, 19,21).

Brazil is one of the most genetically heterogeneous populations in the world, with influential genetic contributions from three main continental groups: Europeans, Africans and Amerindians (22). The relative proportions of these three ancestral roots within the Brazilian population have changed considerably over time (23). Therefore, the modern Brazilian population is genetically diverse and considered very heterogeneous (22). Consequently, the analysis of genetic markers in complex diseases within this population may result in misinterpretation due to the existence of population substructure (24). Furthermore, it is important to assess the control of genomic ancestry in association studies using Ancestry Informative Markers (AIMs), especially in populations with a high degree of interethnic admixture (25).

Within this context, we investigated the haplotypes formed by three SNPs within the IL-10 gene (A-1082G (rs1800896), C-819T (rs1800871) and C-592A (rs1800872)) to better understand how genetic mechanisms of a host can affect how factors of susceptibility to chronic periodontitis in an admixed Brazilian population.

3. MATERIALS AND METHODS

3.1. Cases and controls

A total of 205 individuals were included in this study: 55 with chronic periodontitis (CP) who attended dental clinics in Belém (PA, Brazil) and 150 controls subjects (HC) without CP or any other chronic disease were selected from the same geographic area. The diagnosis diagnosed and classified based on dental exams with the following clinical description: probing depth, bleeding upon probing, gingival inflammation, tooth mobility and clinically detected attachment loss >5 mm in at least ≥30% of total sites. Measurements of probing depth and attachment level were recorded at six points around each tooth. The HC group included subjects presenting healthy gingival tissues without evidence of attachment loss (clinical attachment loss ≤1 mm) at more than one site, a probing depth <3 mm and no history of previous periodontal disease. Individuals meeting these criteria were defined as periodontally HC subjects.

3.2. Ethical aspects

All patients were informed about the research and signed informed consent forms. This study was approved by the Ethics Committee in Research (ECR) of the Instituto de Ciências da Saúde (ICS) of the Universidade Federal do Pará, protocol No. 186/97 CEP-ICS/UFPA.

3.3. Sample collection and DNA extraction

Buccal mucosa samples were collected with a cotton swab and stored in a microtube with buffer at -20°C until analysis. The genetic material was extracted from the cell fraction using phenol-chloroform and precipitated using ethanol (26).

3.4. Analysis of polymorphisms

The IL-10 SNPs were investigated using the TaqMan genotyping assay with TaqMan® probes conducted in a 7500 Real-Time PCR System (Life Technologies, CA, USA). PCR genotyping of all three polymorphisms (A-1082G (rs1800896), C-819T (rs1800871) and C-592A (rs1800872)) was performed with 3.5 µL of master mix, 0.17 µL of probe TaqMan 40X, 3.32 µL of water and 1.0 µL of DNA. The final mix was amplified using the following cycling conditions: 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 92°C and 1 minute at 60°C.

3.5. Analysis of genetic ancestry

Population substructure was investigated using 48 AIMs as previously described (25). Three multiplex PCR reactions were performed, each with 16 markers, followed by electrophoresis on an ABI-PRISM 3130 (Life Technologies, CA, USA) sequencer and analysis using GeneMapper ID version 3.2 (Life Technologies, CA, USA).

3.6. Statistical analysis

Allele frequencies were estimated by gene counting, and deviation from Hardy-Weinberg equilibrium was tested by Chi-square with Benjamini and Hochberg False Discovery Rate (FDR) correction. Haplotype frequencies were obtained using PHASE 2.1. software (27, 28, 29). The p-value calculated from a permutation test of the null hypothesis (that the case and control haplotypes were a random sample from a single set of haplotype frequencies) was also obtained using this software.

Haploview version 4.2. (https://www.broadinstitute.org/haploview/haploview) was used to analyze the linkage disequilibrium (LD) structure among polymorphisms (30). Pairwise LD between SNPs was estimated using Lewontin's standardized coefficient D' and LD coefficient r2 (31), and haplotype blocks were defined according to the method of Gabriel *et al.*, 2002 using Haploview 4.2. with default settings. Haplotypes were used to estimate the maximization algorithm (32).

Differences in the demographic, clinical and biochemical characteristics between CP and HC samples were compared by Student's *t*-test for quantitative variables, Fisher's exact test for qualitative variables and the Mann–Whitney test for estimates of differences in genetic ancestry. Multiple comparison corrections and adjusted ORs (and 95% CI) for the association between genetic markers were obtained by logistic regression. These tests were performed with PASW Statistic (formerly SPSS) version 18.0. Statistical significance was accepted if the *p*-value <0.0.5.

The individual proportions of European, African, and Amerindian genetic ancestry were estimated using STRUCTURE software 2.3.3., assuming three parental populations (Europeans, Africans, and Amerindians) and using 200,000 runs for the burn-in period and 200,000 Markov chain Monte Carlo repetitions after burning (33, 34, 35, 36). An association test between CP and HC subjects following an adjustment for population stratification was performed using the STRAT software program with 10,000 simulations (33, 34, 35, 36).

4. RESULTS

The clinical and demographic variable parameters of the CP and HC samples are provided in Table 1. The CP and HC samples included in this study were mainly composed of females. The average age was 37.0.7 ± 2.1.7 years for the CP group and 24.2.8 ± 0.4.2 years for the HC group, and the difference between these groups was significant (p=0.0.3).

Regarding genomic ancestry, the ethnic composition of the CP group was 23% African, 38% European and 39% Amerindian, and the ethnic composition of the HC group was 18% African, 57% European and 25% Amerindian. These results revealed significant differences in African (p=0.0.04), European (p<0.0.01) and Amerindian (p<0.0.01) ancestry between the CP group and the HC group (Table 1). Figure 1 shows the individual estimates of the interethnic mixture between cases and controls.

Table 2 presents the allele and genotype frequencies for three IL-10 SNPs and the multiple logistic regression analyses after correction for age and ethnicity between the HC and CP groups. There were no significant differences between *IL-10*$^{*-1082}$*G>A* and *IL-10*$^{*-819}$*C>T*. The *IL-10*$^{*-592}$*C>A* SNP was significantly different for genotype *IL-10*$^{*-592}$*CA* between the HC and CP groups (*p*=0.0.1; OR=4.2.4), whereas the allele frequencies were corrected by structure populations. In addition, the differences were statistically significant ($p_{strat=}$7E^{-22}). However, this locus did not meet Hardy–Weinberg equilibrium criteria in the CP group and was not included in the analysis.

In the analysis of the three polymorphisms of the anti-inflammatory cytokine IL-10 (*IL-10*$^{*-1082}$*G>A, IL-10*$^{*-819}$*C>T* and *IL-10*$^{*-592}$*C>A*), seven haplotypes (ATA, ACT, ACC, GTC, GCA, GTA, and GCC) and the risk haplotype ATA were more frequent in the CP group (0.4.23) than in the HC group (0.1.31). The wild-type haplotype was more frequent in the HC group (0.3.26) than in the CP group (0.1.16). The haplotypes showed a block of LD (Figure 2; Table 3) in the CP patients and the HC group for the loci G-1082A/C-819T (D'=0.9.5, p<0.0.01) and C-819T/C-592A (D'=0.9.5, p<0.0.01).

Table 1. Clinical and demographic variables for chronic cases of periodontitis in comparison with controls

	Case N (%)	Control N (%)	p-value
Gender (M/F)[1]	20/35	58/92	0.871
Age[2]	37.07± 2.17	24.28±0.42	0.03
African ethnicity[3]	0.23	0.18	**0.003**
European ethnicity [3]	0.38	0.57	**<0.001**
Amerindian ethnicity [3]	0.39	0.25	**<0.001**

[1] Fisher´s exact test; [2] Student´s *t* test; [3] Mann – Whitney test

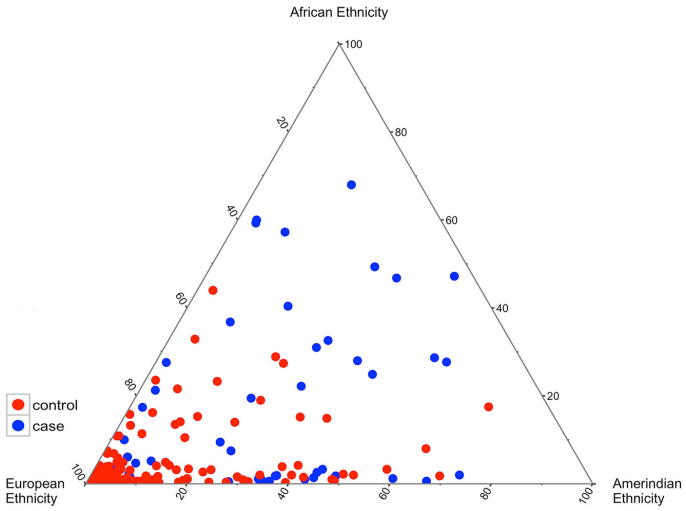

Figure 1. Representation of individual estimates of interethnic admixture. Each subject is represented by a colored dot, and their location on the graph corresponds to their mixing ratios.

This result suggests that the haplotypes may explain more of the association than the individual SNPs. Permutation analysis of the global differences between haplotype frequencies between the two groups (CP and HC) showed that a set of haplotypes was differentially distributed and indicates that these differences were not random (P_{global}=0.0.05 - Table 3).

Additionally, the multiple comparisons corrected test revealed that carriers of the *IL10*$^{*-1082}$*A*-containing haplotypes (ATA, ACT, and ACC) were associated with risk factors for CP (*p=0.0.46*, OR=3.4.59). This finding remained true after correction for population stratification (p_{strat}=2.4E^{-15}) (Table 3).

Table 4 summarizes the distribution of the genotypes and IL-10 production profiles with their respective frequencies. The distribution of the IL-10 production profiles with their respective frequencies is summarized in Table 5. In multiple logistic regression analyses, genotypes significantly associated with a low-production profile (*p*=0.0.4; OR=2.7.7) exhibited an increased risk of CP, whereas genotypes associated with a high-production profile (*p*=0.0.3; OR=0.2.8) were associated with a decreased risk of CP.

Figure 3 presents the OR (odds ratio) values obtained between the CP and HC groups in relation to the distinct level of ancestry composition. Figure 3a shows that the greater the European contribution is between groups, the lower is the risk of developing CP. These results are expected because this ethnic group is more frequent in the HC group (Table 1). Additionally, Figure 3b shows that the higher the Amerindian contribution is, the higher is the risk of developing CP. These results are expected because this ethnic group is more frequent in the CP group (Table 1). These analyses were performed for the two ethnic components with differences greater than 10% between the HC and CP groups.

5. DISCUSSION

The ancestry data revealed significantly different distributions of African ethnicity (23.7% *versus* 18.4%), European ethnicity (38% *versus* 58.2%) and Amerindian ethnicity (38.3% *versus* 23.4%) between the CP and HC groups. These results suggest a loss of contribution from European genes and an increased contribution from African ancestry genes in the case group compared with the control population

Table 2. Genotype distributions between cases and controls

Genotypes	Case N (%)	Control N (%)	p-value [a]	p_{strat} [b]	OR (IC95%) [a]
IL10 G-1082A 1082[a]					
GG	5 (9.1)	18 (12.0)			1
GA	14 (25.5)	61 (40.7)	0.85		1.10 (0.37 -3.22)
AA	36 (65.5)	71 (47.3)	0.37		0.58 (0.18- 1.88)
allele G	0.21	0.32			
allele A	0.79	0.67			
HWE	5.355	1.617			
IL10 C-819T					
CC	19 (34.5)	72 (48)			1
CT	25 (45.5)	59 (39.3)	0.72		0.84 (0.34 – 2.09)
TT	11 (20)	19 (12,7)	0.09		0.31 (0.08 - 1.21)
allele C	0.57	0.68			
allele T	0.43	0.32			
HWE	4.186	1.855			
IL10 C-592A					
CC	0 (0.0)	48 (32.0)			1
CA	42(76.4)	83 (55.3)	0.01	$7E^{-22}$	4.24 (1.33 – 13.52)
AA	13 (23.6)	19 (12.7)	0.14		0.37 (0.1 - 1.38)
allele C	0.38	0.60			
allele A	0.62	0.40			
HWE	$5.32E^{-8}$	$2.52E^{-31}$			

[a] Multiple logistic regression adjusted for independent variables age and ethnicity; [b] p-value adjusted by population structure using strat.

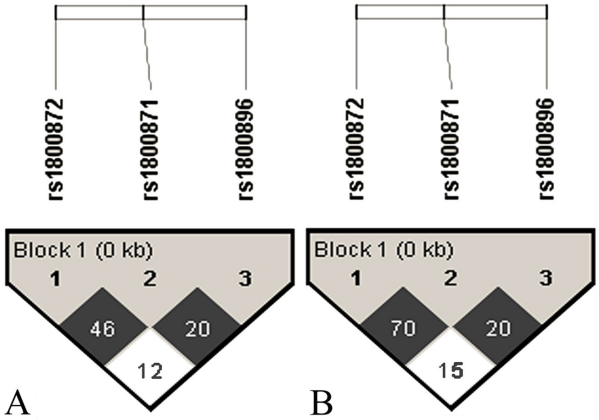

Figure 2. Pairwise linkage disequilibrium (LD) structure of the IL-10 gene SNPs. Panel (A) represents the LD of the IL-10 SNPs in the CP group. Panel (B) represents LD of the IL-10 SNPs in the HC group.

from northern Brazil (25). Furthermore, individuals with higher proportions of European ancestry had a decreased risk of developing the disease, whereas individuals with a higher contribution of Amerindian ancestral genes exhibited a disease susceptibility risk (Figure 2).

Due to the proportional differences in ancestry between the CP and HC groups (Table 1), haplotype association analyses corrected by population stratification were performed using STRAT software (Table 3). After population structure correction, the IL-10 haplotype that conferred a low production profile exhibited a significant risk for developing CP. Similarly, the IL-10 haplotypes that conferred a high production profile were associated with protection in the CP group. These results suggest that IL-10 haplotypes formed by the polymorphisms A-1082G, C-819T and C-592A are

Table 3. Analysis of haplotype by multiple logistic regression in cases versus controls

SNP			Frequency of haplotypes		$p_{global**}$	p_{strat} [b]	p-value [a]	OR (IC95%)
1082	819	592	Case % (N)*	Control % (N)*				
G	T	C	0.010 (1)	-	**0.005**			1
G	C	A	0.090 (13)	0.037 (7)				
G	T	A	-	0.192 (71)				
G	C	C	0.116 (10)	0.326 (89)				
A	T	A	0.423 (47)	0.131 (26)		**2.4E^{-15}**	**0.046**	3.459
A	C	T	0.103 (8)	0.045 (17)				(1.025-11.677)
A	C	C	0.258 (31)	0.269 (90)				

* Counts of haplotypes; ** p-value obtained by permutation test in software PHASE v2.1.1 for global differences between haplotypic frequencies; [a] Multiple logistic regression adjusted for independent variables age and ethnicity; [b] p-value adjusted by population structure using strat.

Table 4. Analysis of genotype profiles of the production of IL-10 in cases versus controls using multiple logistic regression

Genotypes	Case	Control
	Case% (N)*	Control% (N)*
Low production	36 (65.5)	36 (24.0)
ATA/ATA	11 (20)	7(4.7)
ATA/ACA	2 (3.6)	-
ATA/ACC	17 (30.9)	12 (8.0)
ACA/ACC	6 (10.9)	7 (4.7)
ACC/ACC	0 (0.0)	10 (6.7)
Average production	14 (25.5)	61 (40.7)
ATA/GCC	5 (9.1)	-
ATA/GTC	1	-
ACC/GCC	-	23 (15.3)
ACC/GCA	8	
ACA/GCC	-	10 (6.7)
GTA/ACC	-	28 (18.7)
High production	5 (9.1)	53 (35.3)
GCA/GCC	5 (9.1)	7 (4.7)
GTA/GTA	0 (0.0)	12 (8.0)
GTA/GCC	0 (0.0)	19 (12.7)
GCC/GCC	0 (0.0)	15 (10.0)

*Counts of haplotypes [a] Multiple logistic regression adjusted for independent variables age and ethnicity.

closely related and serve as risk and protection factors for CP in admixed populations.

Genetic polymorphisms may modify the biological pathways of periodontitis. The action of multiple genes can contribute to the relative risk of susceptibility and severity, but these effects may be confounded by the influence of population substructure (7, 15, 37). We analyzed the genetic contribution of Africans, Europeans and Amerindians of an admixed population using a panel of 48 AIMs that are capable of precisely distinguishing with low statistical error and cost (25). This control was important for analyses in our admixed population samples and for consolidating the susceptibility and severity of CP in relation to the SNPs investigated.

The three IL-10 SNPs (-592/-819 and -819/-1082) are in LD (Figure 1) and represent three major haplotypes (GCC, ACC and ATA) (15, 19). In our study, we identified seven haplotypes: ATA, ACT, ACC, GTA, GCA, GCC and GTC. Scarel-caminaga *et al.* 2004 (7) showed the ATA haplotype was predominant in individuals with CP, whereas the ACC haplotype was

Table 5. Analysis of haplotype of the IL-10 production profiles in case versus control

Genotypes	Case % (N)*	Control % (N)*	p [a]	OR (IC95%)
Average production	14 (25.5)	61 (40.7)		1
Low production	36 (65.5)	36 (24.0)	**0.05**	**2.77 (1 - 7.88)**
High production	5 (9.1)	53 (35.3)	**0.04**	**0.28 (0.09 – 0.90)**

*Counts of haplotypes ; [a] Multiple logistic regression adjusted for independent variables age and ethnicity; [b] p-value adjusted by population structure using strat

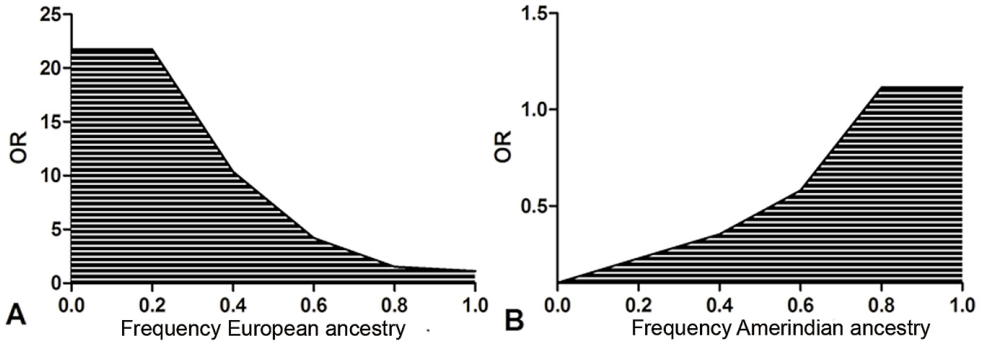

Figure 3. (A) The presence of events comparing 150 individuals in the HC group based on five categories of European genetic ancestry (> 20%, > 40%, > 60%, > 80%, > 100%). (B) The presence of events comparing 55 patients in the CP group based on five categories of Amerindian genetic ancestry (> 20%, > 40%, > 60%, > 80%, > 100%) that were statistically significant in the analysis (p <0.0.5). The Y and X axes represent the odds ratio (OR) and the percentage of categories of ancestry, respectively. In A, the OR decreases with increasing European ancestry. In B, the OR increases with a larger Amerindian contribution.

more common in the control group using a Brazilian population similar to our data. The authors also found non-significant, rare haplotypes: GTA, ATC, GTC and ACA. On the other hand, Cullinan *et al.* (38) investigated the association between IL-10 gene polymorphisms and periodontal disease progression in an Australian population. They reported that individuals having either the ATA/ACC or the ACC/ACC genotype experienced less periodontal disease progression after 5 years than did individuals with other genotypes. In European populations, the IL-10 haplotype ATA frequency (−1,082, −819, −592) was significantly different between vascular disease (VD) patients with CP and VD patients without CP, indicating protection against CP (39).

In the present study, we observed a predominance of the ATA haplotype in the CP group and the GCC haplotype in the HC group. This finding was statistically significant in a multiple logistic regression model adjusted for age and ethnicity (p=0.0.46). Furthermore, individuals with the risk haplotypes ATA, ACT and ACC exhibited a 3.5-fold increase in CP risk (p=2.4E^{-15}; OR=3.4.59) (Table 3).

The genotypes *IL-10*$^{*-1082}$*GG*, *IL-10*$^{*-1082}$*GA* and *IL-10*$^{*-1082}$*AA* correspond to high, intermediate and low producers of the cytokine IL-10, respectively. These genotypes are solely responsible for phenotype, independent of nucleotide variation at positions -819 and -592. Therefore**,** ATA/ATA, ACC/ATA, ATA/ACA, ACA/ACC and ACC/ACC were classified as low IL-10 production phenotypes; GCC/ACC, GCC/ATA, ATA/GTC, ACC/GCA, ACA/GCC and GTA/ACC were classified as intermediate IL-10 production phenotypes; and GCA/GCC, GTA/GTA, GTA/GCC and GCC/GCC were classified as high IL-10 production phenotypes (40). Low IL-10 production causes an excess of inflammatory cytokines in the periodontal lesions in the chronic form of this disease. This inflammation is a putative risk indicator of the progression of periodontal disease (38). However, the haplotype profiles for high IL-10 production conferred significant protection against CP; therefore, individuals who are high producers of IL10 are most likely protected against CP due to the anti-inflammatory cytokines that negatively regulate the immune response against periodontopathogenic bacteria (7).

We showed that the low IL-10 production phenotype (ATA/ATA, ACC/ATA, ATA/ACA, ACA/ACC and ACC/ACC genotypes) significantly increases the risk of developing CP (p=0.0.4; OR=2.7.7). In contrast, the high IL-10 phenotype (GCA/GCC, GTA/GTA, GTA/GCC and GCC/GCC genotypes) protects against CP (p=0.0.3; OR=0.2.8). This result supports previous studies that demonstrate that the high production of IL-10 promotes the anti-inflammatory immune response,

which is important for protection against the bacteria that cause periodontitis.

6. ACKNOWLEDGMENTS

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7. REFERENCES

1. D. F. Kinane, M. Peterson, P. G. Stathopoulou: Environmental and other modifying factors of the periodontal diseases. *Periodontology 2000* 40, 107–119 (2006) DOI: 10.1111/j.1600-0757.2005.00136.x

2. J. Meyle, I. Chapple: Molecular aspects of the pathogenesis of periodontitis. *Periodontology 2000 69,* 7–17 (2015) DOI: 10.1111/prd.12104

3. P. Peterson, O. Hiroshi: The global burden of periodontal disease: towards integration with chronic disease prevention and control. *Periodontology 2000* 60, 15–39 (2012) DOI: 10.1111/j.1600-0757.2011.00425.x

4. P. Dahiya, R. Kamal, R.Gupta, R. Bhardwaj, K Chaudhary, S Kaur: Reactive oxygen species in periodontitis. *J Indian Soc Periodonto*. 17(4), 411-416 (2013) DOI: 10.4103/0972-124X.118306

5. P. M. Preshaw: Host response modulation in periodontics. *Periodontoly 2000* 48, 92-110 (2008) DOI: 10.1111/j.1600-0757.2008.00252.x

6. V. Deo, M. L. Bhongade: Pathogenesis of peg riodontitis: role of cytokines in host response. *Dent Today* 29(9), 60-2, 64-6 (2010)

7. R. M. Scarel-Caminaga, P.C. Trevilatto, A.P. Souza, R. B. Brito, L. E. Camargo, S. R. Line: Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis. *J Clin Periodontol*.31, 443–448 (2004) DOI: 10.1111/j.1600-051X.2004.00500.x

8. W. T. Y. Loo, C. Fan, L. Bai, Y. Yue, Y. Dou, M. Wang, H. Liang, M. N. B. Cheung, L. W. C. Chow, J. Li, Y. Tian, L. Qing: Gene polymorphism and protein of human pro- and anti-inflammatory cytokines in Chinese healthy subjects and chronic periodontitis patients. *Journal of Translational Medicine* 10(Suppl 1), S8 (2008) DOI: 10.1186/1479-5876-10-S1-S8

9. P. R. Lowe, H. F. Galley, A. Abdel-Fattah, N. R Webster. Influence of interleukin-10 polymorphisms on interleukin-10 expression and survival in critically ill patients. *Crit. Care Med.* 31, 34–38 (2003) DOI: 10.1097/00003246-200301000-00005

10. A. Franke, T. Balschun, T. H. Karlsen, J. Sventoraityte, S. Nikolaus, G. Mayr, F. S. Domingues, M. Albrecht, M. Nothnagel, D. Ellinghaus, C. Sina, C. M. Onnie, R. K. Weersma, P.C. Stokkers, C. Wijmenga, M. Gazouli, D. Strachan, W. L. McArdle, S. Vermeire, P. Rutgeerts, P. Rosenstiel, M. Krawczak, M. H. Vatn, IBSEN study group, C. G. Mathew, S. Schreiber: Sequence variants in *IL10*, *ARPC2*, and multiple other loci contribute to ulcerative colitis susceptibility. *Nat. Genet.* 40, 1319–1323 (2008) DOI: 10.1038/ng.221

11. Q. Zhang, B. Chen, F. Yan, J. Guo, X. Zhu, S. Ma, W. Yang: Interleukin-10 inhibits bone resorption: a potential therapeutic strategy in periodontitis and other bone loss diseases. *BioMed research international.* 2014 (2014) DOI: 10.1155/2014/284836

12. H. Y. Chen, W. C. Chen, C. M Hsu, F. J. Tsai, C. H. Tsai: Tumor necrosis factor α, CYP 17, urokinase, and interleukin 10 gene polymorphisms in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis. *European Journal of Obstetrics Gynecology and Reproductive Biology* 122(1), 73–78 (2005) DOI: 10.1016/j.ejogrb.2005.02.003

13. H. Sasaki, Y. Okamatsu, T. Kawai, R. Kent, M. Taubman, P. Stashenko: The interleukin-10 knockout mouse is highly susceptible to Porphyromonas gingivalis-induced alveolar

bone loss. *Journal of Periodontal Research* 39(6), 432–441 (2004)
DOI: 10.1111/j.1600-0765.2004.00760.x

14. A. Stabholz, W. A. Soskolne, L. Shapira. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontology 2000* 53, 138-153 (2010)
DOI: 10.1111/j.1600-0757.2010.00340.x

15. P. Garcia, D. Alencar, P. Pinto, N. Santos, C. Salgado, V. A. Sortica, M. H. Hutz, Â. Ribeiro-dos-Santos, S. Santos: Haplotypes of the IL10 gene as potential protection factors in leprosy patients. *Clin Vaccine Immunol.* 20(10), 1599-603 (2013)
DOI: 10.1128/CVI.00334-13

16. H. Bai, D. Jing, A. Guo, S. Yin: Association between interleukin 10 gene polymorphisms and risk of type 2 diabetes mellitus in a Chinese population. *J Int Med Res.* 23,42(3), 702-710 (2014)

17. E. Crawley, R. Kay, J. Sillibourn, P. Patel, I. Hutchinson, P. Woo. Polymorphic haplotypes of the interleukin 10 5′ flanking region determine variable interleukin 10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis & Rheumatism.* 42(6), 1101-1108 (1999)
DOI: 10.1002/1529-0131(199906)42:6<1101::AID-ANR6>3.0.CO;2-Y

18. K. Koss, J. Satsangi, G. C. Fanning, K. I. Welsh, D. P. Jewell: Cytokine (TNFalpha, LTalpha and IL-10) polymorphisms in inflammatory bowel diseases and normal controls: differential effects on production and allele frequencies. *Genes and immunity.* 1(3), 185-190 (2000)
DOI: 10.1038/sj.gene.6363657

19. L. Zeng, W. Gu, K. Chen, D. Jiang, L. Zhang, D. Du, P. Hu, Q. Liu, S. Huang, J. Jiang: Clinical relevance of the interleukin 10 promoter polymorphisms in Chinese Han patients with major trauma: genetic association studies. *Crit. Care* 13, R188 (2009)
DOI: 10.1186/cc8182

20. A. J. P. Smith, S. E. Humphries. Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine Growth Factor Rev.* 20, 43–59 (2009)
DOI: 10.1016/j.cytogfr.2008.11.006

21. T. Eder, R. Mayer, U. Langsenlehner, W. Renner, P. Krippl, T. C. Wascher, K. Pummer, K. S. Kapp: Interleukin-10 (ATA) promoter haplotype and prostate cancer risk: a population-based study. *Eur. J. Cancer* 43, 472-475 (2007)
DOI: 10.1016/j.ejca.2006.11.003

22. T. Palha, L. Gusmão, E. Ribeiro-Rodrigues, J. F. Guerreiro, A. Ribeiro-Dos-Santos, S. Santos: Disclosing the genetic structure of Brazil through analysis of male lineages with highly discriminating haplotypes. *PLoS One* 7(7), e40007 (2012)
DOI: 10.1371/journal.pone.0040007

23. S. D. J. Pena, G. Di Pietro, M. Fuchshuber-Moraes: The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PloS One* 6(2), e17063 (2011)
DOI: 10.1371/journal.pone.0017063

24. H. Tang, T. Quertermous, B. Rodriguez, S. L. Kardia, X. Zhu, A. Brown, J. S. Pankow, M. A. Province, S. C. Hunt, E. Boerwinkle, N. J. Schork, N. J. Risch: Genetic structure, self-identified race/ethnicity, and confounding in case-control association studies. *Am J Hum Genetic.* 76(6), 268-275 (2005)
DOI: 10.1086/427888

25. N. P. C. Santos, E. M. Ribeiro-Rodrigues, A. K. C. Ribeiro-dos-Santos, R. Pereira, L. Gusmão, A. Amorim, J. F. Guerreiro, M. A. Zago, C. Matte, M. H. Hutz, S. E. Santos: Assessing Individual Interethnic Admixture and Population Substructure Using a 48–Insertion-Deletion (INSEL) Ancestry-Informative Marker (AIM) Panel. *Human Mutation* 31(2), 184-90 (2010)
DOI: 10.1002/humu.21159

26. J. Sambrook, E.F. Fritsch, T.Maniatis. Molecular cloning a laboratory manual. Eds: Cold Spring Harbor press, New York (1989)

27. M. Stephens, N. J. Smith, P. A. Donnelly: New statistical method for haplotype reconstruction from population data. *Am J Hum Genet.* 68, 978-89 (2001)
DOI: 10.1086/319501

28. M. Stephens, P. A. Donnelly: Comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet.* 73, 1162-9 (2003)
DOI: 10.1086/379378

29. M. Stephens, P. Scheet: Accounting for decay of linkage disequilibrium in haplotype inference and missing data imputation. *Am J Hum Genet.* 76, 449-62 (2005)
DOI: 10.1086/428594

30. S. B. Gabriel, S. F. Schaffner, H. Nguyen, J. M. Moore, J. Roy, B. Blumenstiel, J. Higgins, M. DeFelice, A. Lochner, M. Faggart, S. N. Liu-Cordero, C. Rotimi, A. Adeyemo, R. Cooper, R. Ward, E. S. Lander, M. J. Daly, D. Altshuler: The structure of haplotype blocks in the human genome. *Science* 296(5576), 2225–2229 (2002)
DOI: 10.1126/science.1069424

31. R. C. Lewontin: On measures of gametic disequilibrium. *Genetics* 120(3), 849-852 (1988)

32. D. J. Schaid, C. M. Rowland, D. E. Tines, R. M. Jacobson, G. A. Poland: Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet.* 70(2), 425–434 (2002)
DOI: 10.1086/338688

33. J. K. Pritchard, M. Stephens, P. Donnelly: Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959 (2000)

34. J. K. Pritchard, M. Stephens, N. A. Rosenberg, P. Donnelly: Association mapping in structured populations. *Am. J. Hum. Genet.* 67, 170–181 (2000)
DOI: 10.1086/302959

35. D. Falush, M. Stephens, J. K. Pritchard: Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164, 1567–1587 (2003)

36. D. Falush, M. Stephens, J. K. Pritchard: Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes* 7, 574-578 (2007)
DOI: 10.1111/j.1471-8286.2007.01758.x

37. K.F. Hu, K.C. Huang, Y. P. Ho, Y. C. Lin, K. Y. Ho, Y. M. Wu, Y. H. Yang, C. C. Tsai: Interleukin-10 (–592 C/A) and interleukin-12B (+16974 A/C) gene polymorphisms and the interleukin-10 ATA haplotype are associated with periodontitis in a Taiwanese population. *J Periodont Res.* 44, 378–385 (2009)
DOI: 10.1111/j.1600-0765.2008.01116.x

38. M. P. Cullinan, B. Westerman, S. M. Hamlet, J. E. Palmer, M. J. Faddy, G. J. Seymour, P. G. Middleton, J. J. Taylor. Progression of periodontal disease and interleukin-10 gene polymorphism. *J Periodontal Res.* 43, 328–33 (2008)
DOI: 10.1111/j.1600-0765.2007.01034.x

39. Z. Armingohar, J. J. Jørgensen, A. K. Kristoffersen, K. Schenck, K. Dembic: Polymorphisms in the interleukin-10 gene and chronic periodontitis in patients with atherosclerotic and aortic aneurysmal vascular diseases. *Journal of oral microbiology* 7 (2015)
DOI: 10.3402/jom.v7.26051

40. A. Plothow, R. Benvenutti, F. L. Contieri, M. G. Bicalho: Frequencies at three polymorphic sites of interleukin-10 gene promoter in Brazilian renal recipients. *Transplant Proc.* 35, 2908-2910 (2003)
DOI: 10.1016/j.transproceed.2003.10.013

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