

## Influence of IL-1 gene polymorphism on the periodontal microbiota of HIV-infected Brazilian individuals

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**Abstract:** This study investigated the association of IL-1A (+4845) and IL-1B (+3954) gene polymorphism with the subgingival microbiota and periodontal *status* of HIV-infected Brazilian individuals on highly active antiretroviral therapy (HAART). One hundred and five subjects were included in the study, distributed into 2 HIV groups [29 chronic periodontitis (CP+) and 30 periodontally healthy (H+)] and 2 non-HIV groups (29 CP- and 17 H- patients). IL-1A and B were genotyped by PCR and restriction enzyme digestion. Thirty-three bacterial species were detected by checkerboard. Overall, we observed a prevalence of the allele 2 in the IL-1A and IL-1B polymorphism at 30.5% and 25.7%, respectively. Only 11.4% of all patients were composite genotype-positive, and 75% of those were HIV-infected. No significant associations between polymorphism of the IL-1 gene and periodontitis or HIV infection were observed. Likewise, no significant differences in the frequency and counts of any bacterial species were found between individuals with and without allele 2 (IL-1A or IL-1B). The data indicated that the IL-1 gene polymorphism is neither associated with periodontal destruction nor with high levels of subgingival species, including putative periodontal pathogens in HIV Brazilian individuals on HAART.

**Descriptors:** Periodontitis; HIV; Interleukin-1; Genetic polymorphism; Microbiology.

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## Introduction

Since the beginning of the AIDS epidemic, studies have reported a higher prevalence and severity of periodontitis in HIV patients.<sup>1,2</sup> However, the establishment of a highly active antiretroviral therapy (HAART) has resulted in a significant improvement in the periodontal *status* of HIV patients.<sup>3</sup> For instance, Gonçalves *et al.*<sup>3</sup> (2005) indicated that only a small proportion of immunodeficient HIV-infected subjects under HAART presented periodontitis. Thus, other risk factors may influence the susceptibility for chronic periodontitis associated with HIV infection.<sup>4,5</sup> Regarding genetic factors, the IL-1 gene cluster is strongly associated with many disorders involving chronic inflammatory diseases, cancer and autoimmunity.<sup>6</sup> This cluster is composed of genes coding for the IL-1 agonists (IL-1A and IL-1B), and the gene for IL-1 receptor antagonist, IL-1RN.<sup>7</sup> A specific genetic variation in these genes that results in a composite genotype comprising allele 2 of IL-1A plus allele 2 of IL-1B has been associated with an increased risk for severe periodontitis.<sup>8-12</sup> In addition, it has been demonstrated that polymorphism in the locus -889 and +3954 of IL-1A and IL-1B genes, respectively, could be a risk factor for chronic periodontitis in the Brazilian population.<sup>13,14</sup> Genotype positive individuals are more frequently colonized by high counts of periodontal pathogens, mainly in deep periodontal pockets.<sup>15</sup> However, the influence of this polymorphism on the subgingival microbiota of HIV-infected subjects remains unclear.<sup>4</sup> Therefore, the purpose of the current study was to determine the influence of IL-1 gene polymorphism on the subgingival microbiota of HIV-infected Brazilian individuals on HAART.

## Material and Methods

### Subject population

A convenience sample of 105 subjects was recruited from the Clementino Fraga Filho University Hospital and School of Dentistry, Federal University of Rio de Janeiro (UFRJ), Brazil. Information regarding demographic features, general health, HIV infection history, and laboratory data were obtained from the patients' medical records. Patients were over 21 years of age and presented at least 20 teeth.

They were distributed into 2 HIV groups [29 chronic periodontitis (CP+), and 30 periodontally healthy (H+) individuals] and 2 HIV-seronegative groups [29 chronic periodontitis (CP-), and 17 periodontally healthy (H-) subjects]. The chronic periodontitis patients presented at least 3 sites with pocket depth (PD)  $\geq$  5 mm and/or clinical attachment level (CAL)  $\geq$  4 mm, whereas the periodontally healthy individuals showed no sites with PD > 3 mm and/or CAL > 4 mm and less than 10% of sites or 18% of teeth with bleeding on probing (BOP). All HIV-infected patients were on HAART for at least 2 years. In addition, they were taking trimethoprim and sulfamethoxazole as prophylaxis for *Pneumocystis carinii* pneumonitis. The exclusion criteria included pregnancy, nursing, diabetes, auto-immune diseases, necrotizing periodontal diseases, a requirement for premedication with antibiotics for periodontal examination, and periodontal therapy within the last 6 months prior to the recruitment. The study protocol was approved by the Review Committee for Human Subjects, Clementino Fraga Filho University Hospital, UFRJ (CEP 166/03; CONEP 789/2004).

### Clinical measurements and therapeutic procedures

Clinical measurements [PD, CAL, BOP, plaque accumulation (PL)] were recorded at six sites per tooth in all teeth of all patients by two trained examiners, using a conventional manual periodontal probe (Hu-Friedy, Chicago, IL, USA).<sup>3</sup>

### Isolation of genomic DNA

Genomic DNA was isolated and purified from mouthwash samples of each subject as described by Laine *et al.*<sup>16</sup> (2000).

### IL-1 genotyping

For a subject to be positive for the composite genotype, he/she should carry at least one copy of allele 2 at the IL-1A (+4845) locus plus one allele 2 at the IL-1B (+3954) locus of the IL-1 gene cluster.<sup>8</sup> Genotyping was performed employing PCR and restriction fragment length product (RFLP) techniques, as previously described.<sup>15</sup>

## Microbiological assessment

During the clinical examination, individual subgingival biofilm samples were taken from the 6 deepest sites of each subject with chronic periodontitis, and 6 randomly selected sites of subjects with periodontal health, using sterile curettes. The presence and levels of 33 bacterial species (Figures 1 and 2), ordered according to the microbial complexes, were determined by the checkerboard DNA-DNA hybridization method.<sup>17</sup>

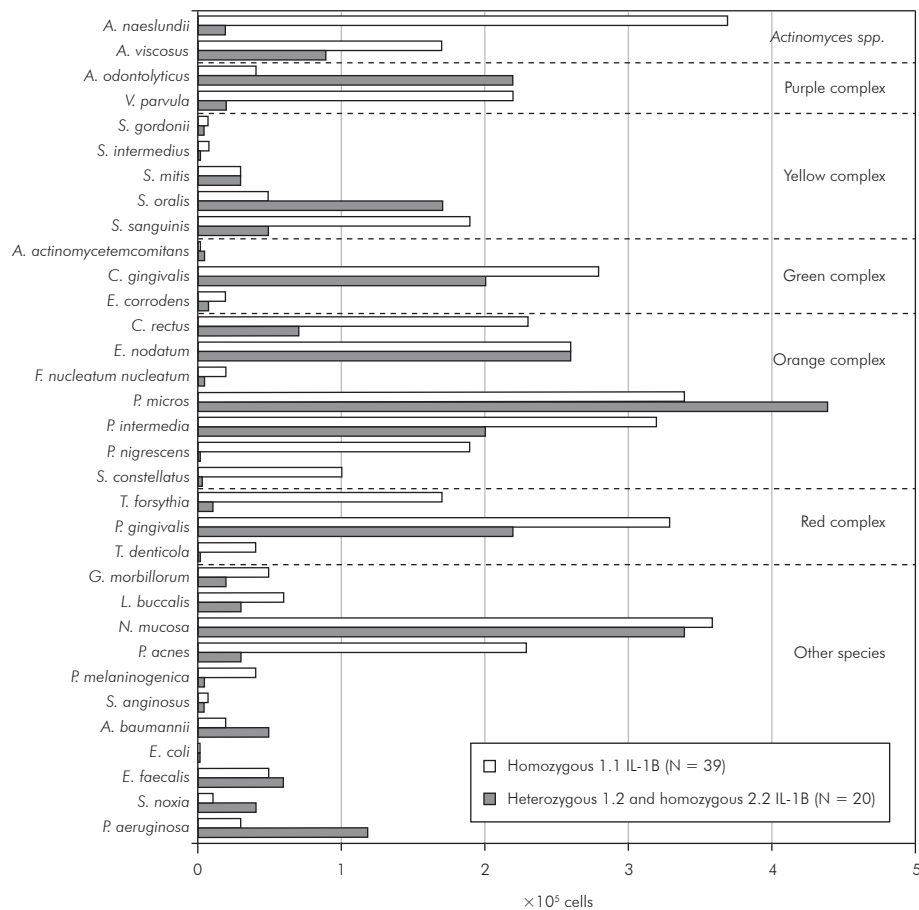
## Statistical analyses

Statistically significant differences were sought using the Kruskal-Wallis, Mann-Whitney and Chi-square tests. A logistic regression (Backward method) was performed to evaluate the possible influence of several co-variables. The statistical significance level established was 5% for all analyses.

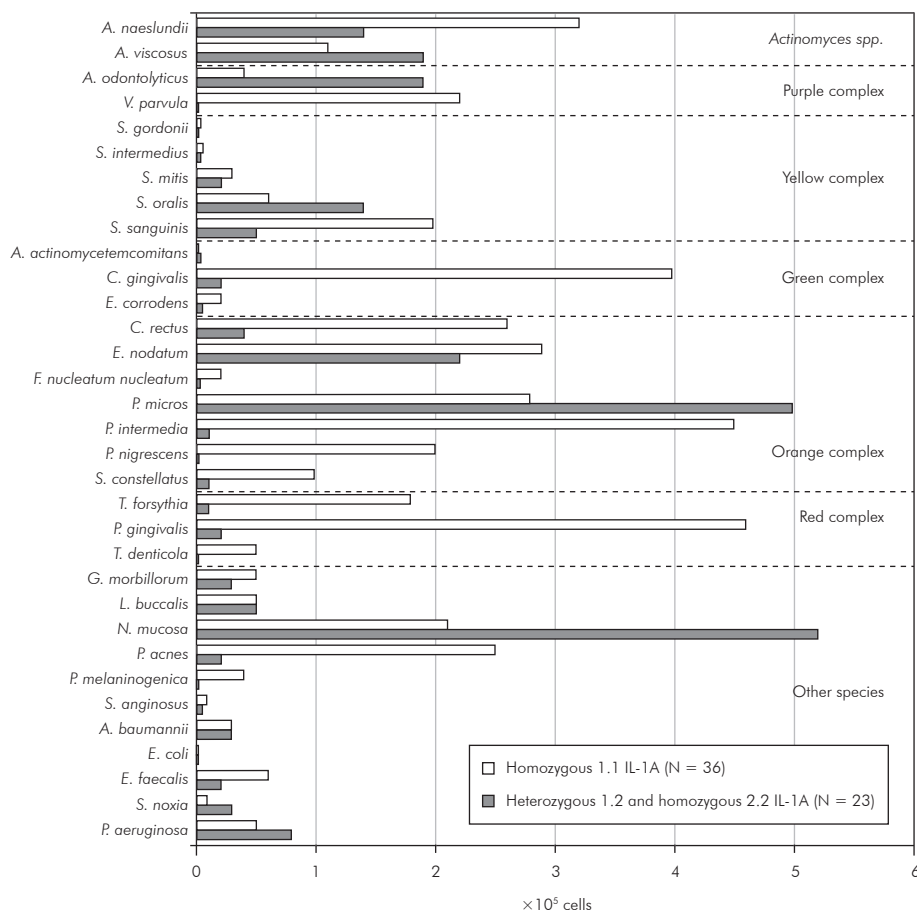
## Results

### Demographic, clinical and laboratorial features of the study population

Statistically significant differences among groups were observed for all periodontal parameters and age ( $p < 0.01$ ), but not for gender and tobacco use. When pairs of groups were compared, individuals with chronic periodontitis presented significantly more disease than periodontally healthy subjects, regardless of the HIV-infection status ( $p < 0.01$ ; Mann-Whitney test). Interestingly, CP- showed significantly more periodontal destruction than CP+ ( $p < 0.01$ ). In contrast, H+ subjects presented a significantly higher mean percentage of sites with BOP than H- subjects ( $p < 0.01$ ) (data not shown). The data of the logistic regression are presented in Table 1. Approximately 70% of the outcome chronic periodontitis was explained by this model. This model was explained by age ( $p = 0.015$ ) and BOP ( $p < 0.001$ ). In addition, HIV infection presented a



**Figure 1** - Mean counts ( $\times 10^5$  bacterial cells) of 33 bacterial species in individuals homozygous 1.1 and carriers of allele 2 (heterozygous 1.2 and homozygous 2.2) for IL-1B gene from a group of 59 HIV and non-HIV-infected patients. No significant differences were found for any bacterial species between groups ( $p > 0.05$ ; Mann-Whitney test, adjusted for multiple comparisons).



**Figure 2** - Mean counts ( $\times 10^5$  bacterial cells) of 33 bacterial species in individuals homozygous 1.1 and carriers of allele 2 (heterozygous 1.2 and homozygous 2.2) for IL-1A gene from a group of 59 HIV and non-HIV-infected patients. No significant differences were found for any bacterial species between groups ( $p > 0.05$ ; Mann-Whitney test, adjusted for multiple comparisons).

**Table 1** - Logistic regression model for outcome chronic periodontitis in 105 subjects (46 HIV-seronegative and 59 HIV-seropositive) of the population studied.

Co-variables	$\beta$	SE	OR	P-value	95% CI
$R^2 = 0.678$					
HIV-infection	-2.3	0.8	0.1	0.005	0.018 – 0.50
Age	0.11	0.04	1.1	0.015	1.02 – 1.21
Smoker	1.15	0.662	3.1	0.081	0.86 – 11.6
BOP	0.12	0.03	1.2	< 0.001	1.07 – 1.19

$\beta$  = Regression coefficient,  $P$  refers to statistical significance, SE = Standard Error, OR = Odds ratio, CI = Confidence Interval, and BOP = Bleeding on probing.

significant inverse association ( $\beta = -2.3$ ;  $p = 0.005$ ) with periodontitis.

### Genotyping data

Table 2 shows the distribution of the IL-1 alleles and genotypes in the study population according to periodontal status and HIV infection. For the IL-1B alleles, no significant differences in the frequency of homozygous and heterozygous genotypes, as well as in the carriage rate of allele 2, were found

among groups, although the homozygous genotype for the 1.1 composite tended to be more prevalent in this population (74%). Regarding the IL-1A gene, a significant over-representation of the allotype 2.2 (40.7%) was observed in HIV subjects, whereas the allotype 1.1 appeared significantly more often in HIV-seronegative individuals (82.6%), regardless of periodontal status ( $p < 0.01$ ; Chi-square test). When the frequency of the composite genotype comprising allele 2 of both IL-1A and IL-1B genes was deter-

**Table 2** - Distribution of IL-1 alleles and genotypes in HIV-seropositive and HIV-seronegative subjects with chronic periodontitis or periodontal health.

Genetic features	HIV- seronegative		HIV- seropositive	
	Periodontal health	Chronic Periodontitis	Periodontal health	Chronic Periodontitis
N	17	29	30	29
IL-1A + 4845 (%)*				
1.1	13 (76.5)	25 (86.2)	19 (63.3)	16 (55.2)
1.2	4 (23.5)	0	0	0
2.2	0	4 (13.8)	11 (36.7)	13 (44.8)
IL-1B + 3954 (%)				
1.1	14 (82.4)	21 (72.4)	24 (80)	19 (65.6)
1.2	2 (11.8)	8 (27.6)	3 (10)	5 (17.2)
2.2	1 (5.8)	0	3 (10)	5 (17.2)
IL-1A Allele 2 (%)	4 (23.5)	4 (13.8)	11 (36.7)	13 (44.8)
IL-1B Allele 2 (%)	3 (17.6)	8 (27.6)	6 (20)	10 (34.5)
Positive genotype (%)	2 (11.8)	1 (3.4)	4 (13)	5 (17.2)

\*Refers to statistically significant differences among groups ( $p < 0.01$ ; Chi-square test).

mined, only 12 (11.4%) of the 105 subjects evaluated were genotype-positive.

### Microbiological data

In order to evaluate the relationship between IL-1 polymorphisms and the composition of the subgingival microbiota, 59 patients presenting both genetic and microbiological data were included. Due to the small sample size, the mean percentage of colonized sites and the mean counts  $\times 10^5$  (levels) of each bacterial species between genotype-positive ( $N = 9$ ) and -negative ( $N = 50$ ) individuals were compared for the whole group, regardless of HIV infection or periodontal status. In addition, we examined the possible influence of the carriage rate of allele 2 for the IL-1A and IL1-B gene on the prevalence and levels of the bacterial species. Figures 1 and 2 present the mean levels of the bacterial species in subjects homozygous for allele 1 and allele 2 carrier individuals for the IL1-B and IL-1A gene, respectively. In general, genotype-positive subjects (data not shown) and individuals heterozygous (1.2) and homozygous (2.2) for IL1B (Figure 1) and IL-1A (Figure 2) tended to present lower mean levels of several species including some members of the orange complex and red complex than genotype-negative and homozygous (1.1) subjects. In contrast, other species, such

as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Selenomonas noxia* showed a trend to be detected in higher counts in genotype-positive and allele 2 carrier individuals.

### Discussion

Conflicting data on the association between genetic polymorphisms and HIV infection with an increased risk for severe periodontitis in distinct populations have been reported.<sup>3,8,12,18-22</sup> Moreover, the role of these possible risk factors on the subgingival microbiota of periodontitis patients remains unclear. Based on that, this study investigated the relationship between IL-1 genotypes, periodontal status and the subgingival microbiota of HIV-infected individuals under HAART. A low frequency of composite genotype was found in this study population (11.4%), contrasting with other reports that showed frequencies ranging between 26% and 48%.<sup>8-12,15</sup> Low frequencies for this composite genotype were also reported in Chinese (2.3%) and African-Americans (8-14%).<sup>18,19</sup> Seventy-five percent of the genotype-positive subjects were HIV-seropositive. However, no significant associations between composite genotype, HIV infection and periodontal clinical parameters were found in this study. Price *et al.*<sup>4</sup> (1999) reported that the periodontal parameters

of HIV patients carrying allele 2 of IL-1A and/or IL-1B were not significantly affected. In the present study, a strikingly high frequency of individuals homozygous (2.2) for the IL-1A gene was observed in the HIV group (40.7%), whereas the carriage rate of allele 2 for the IL-1B gene was 27.1%. Armitage *et al.*<sup>18</sup> (2000) reported the same frequency for the IL-1A allele 2, while a much lower frequency for allele 2 of the IL-1B gene (3.3%) was observed. Moreira *et al.*<sup>13,14</sup> (2005, 2007) showed a prevalence of 28% and 37.8% for the allele 2 of the IL-1B and IL-1A gene, respectively, in HIV-seronegative Brazilians with chronic periodontitis, while we observed similar results (31.3% and 29.3%, respectively) (data not shown). Other investigations reported frequencies over 40% for both allele 2 in HIV-infected<sup>23,24</sup> and non-HIV-infected individuals.<sup>8,9</sup> Despite the fact that the prevalence of HIV patients with periodontitis carrying alleles 2 was quite high, this group showed less periodontal destruction than non-HIV subjects with periodontitis. In addition, Price *et al.*<sup>24</sup> (2004) suggested that IL1 A genotyping may identify HIV-infected individuals at greatest risk of virological failure. Interestingly, H+ subjects presented a significantly higher mean percentage of sites with BOP than H- subjects. Lang *et al.*<sup>11</sup> (2000) showed a high prevalence of BOP in IL-1 genotype-positive patients, suggesting that some of these subjects may present a genetically determined hyper-inflammatory profile. Genetic polymorphisms also have been associated with high levels of members of the red and orange complexes.<sup>15</sup> The over-production of these cytokines might directly affect the growth and/or virulence of bacterial species,<sup>25</sup> or result in an increased inflammatory response at the periodontal site that, in turn, may favor more pathogenic bacteria.<sup>9,10,15</sup> However, in the present investigation, genotype-positive individuals showed a tendency to harbor lower mean counts of most species, including putative periodontal pathogens. Other authors have reported similar results.<sup>26</sup> Similar findings were observed for patients carrying allele 2 of the IL-1A or IL-1B gene. These conflicting data may be in part due to the fact that most of the individuals with a positive genotype and/or carrying an allele 2 for IL-1A and IL-1B were HIV- infected. These subjects

presented lower mean pocket depth and attachment level, and consequently lower levels of members of the orange and red complexes, than HIV-seronegative subjects. Furthermore, one could argue that the decreased counts of subgingival species in HIV individuals may result from the regular use of antimicrobials, such as trimethoprim and sulfamethoxazole.<sup>3</sup> HAART may also have an inhibitory effect on the pathogenic periodontal microbiota and *Candida* aspartic proteinases.<sup>27,28</sup> Finally, differences in ethnic background among populations may be responsible for different results. A model of logistic regression was carried out and it was observed that gender and IL-1 genotyping did not influence the prevalence of chronic periodontitis in the studied population. In addition, HIV infection presented a significant inverse association with the outcome chronic periodontitis. These findings confirm our results showing HIV-infected individuals with significantly less periodontal destruction than non-HIV patients with periodontitis, and lower subgingival levels of periodontal pathogens in subjects carrying allele 2 at IL-1A and IL-1B, which were more frequent in the HIV group. In this case, the HAART can have a protector effect among HIV-infected patients with periodontal disease.

## Conclusion

Polymorphisms in the IL-1 gene cluster did not associate with the presence of severe chronic periodontitis and periodontal pathogens in this HIV-infected Brazilian population under HAART. Since our investigation presented a limited number of study participants, these results should be interpreted with caution. Future longitudinal studies with larger subject populations may be needed to confirm the present findings.

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