Single Nucleotide Polymorphism (Rs4804803) in the DC-SIGN Promoter Region Cd209, and Implications Regarding the Susceptibility to Chronic Periodontitis in Individuals with Type 2 Diabetes Mellitus

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Abstract

Chronic periodontitis (CP) is a disease caused by an impaired immune response to oral bacteria and is often found in individuals with type 2 diabetes mellitus (DM2). Dendritic cells are involved in CP and genetic polymorphisms in the DC-SIGN receptor may modulate susceptibility to the disease. The aim of the study was to investigate the distribution of a single nucleotide polymorphism in the DC-SIGN in individuals with DM2 and CP, non-DM2 individuals with CP and healthy controls and its association with CP in a sample of population. 280 individuals (116 with DM2+CP, 95 with CP and 69 healthy controls) were genotyped using real-time PCR with allele-specific probes. Significant differences (p<0.05) were found among the groups with regard to socio-epidemiological variables, as well as clinical-epidemiological variables. With regard to allelic and genotypic distribution, the GG genotype was significantly more frequent among the healthy individuals compared to those with DM2+CP, suggesting less susceptibility to DM2+CP (p=0.030). The AG genotype was also associated with a lower bleeding index compared to the AA genotype in healthy individuals (p=0.016). This is the first record of an association between a variant in DC-SIGN and susceptibility to DM2 and CP.

Keywords: Type 2 diabetes mellitus; Chronic periodontitis; Single nucleotide polymorphism; DC-SIGN

Introduction

Periodontitis is a multifactor, infectious, inflammatory disease caused by an impaired immune response to oral bacteria [1,2] which in turn, stimulates a local inflammatory reaction and immune activation [3] causing damage to connective and bone tissue [4]. This disease can present in two forms: aggressive and chronic [5].

Chronic periodontitis (CP) is characterized by a long period of exposure to periodontal pathogens [6] resulting in the buildup of dental biofilm, with slow, progressive damage to the dental support structures [7]. Numerous factors have been implicated in the risk of the development of this condition, such as tobacco smoking, diabetes mellitus, stress and medications [8,9].

The susceptibility to the development of periodontitis is threefold greater in the diabetic population compared to the non-diabetic population [10] especially when glycemic control is poor [11]. CP is considered the sixth most common complication of type 2 diabetes mellitus (DM2) [12]. Moreover, there is a bidirectional relationship between the two conditions, with one affecting the control of the other [13,14]. Periodontal tissues are the most affected oral tissues in DM2 [15] as the state of hyperglycemia can directly alter the subgingival microbial flora, impairing cell function, altering the
metabolism of collagen [16] and promoting vascular changes [17]. Although bacterial infection in CP does not differ between diabetic and non-diabetic individuals, a differentiated immune response is found in diabetic individuals, in whom the development of antibodies against periodontal pathogens may be impaired [18].

The immune pathogenesis of CP has been associated with the negative regulation of toll-like receptors (TLRs) and populations of effector T cells [19], which are also associated with the mechanism of action of dendritic cells. Organized lymphoid aggregates containing immune conjugates of dermal dendritic cells, CD4+ T lymphocytes and B cells can be found in the oral mucosa of infected individuals [20]. Interestingly, an intense infiltrate of dendritic cells expressing the dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) receptor is found in the lamina propria, along with evidence that dendritic cells in lesions seems to move toward capillaries. These facts suggest that the specific microbiota in the oral mucosa may target dendritic cells in the lamina propria, guiding the responses of effector T cells [21,22].

The type C lectin DC-SIGN, which is coded by the CD209 gene in chromosome 19 (19p13.2-3) [23], is a pattern-recognition and adhesion molecule expressed in dendritic cells and some types of macrophages that is involved in the endocytosis of microbial antigens in peripheral tissues (through bonding to ICAM-2 in endothelial cells) and the mediation of the immune response (through bonding to ICAM-3+ T cells in lymph nodes) [22,24]. Studies report an increase in DC-SIGN receptors in dendritic cells in the oral mucosa of individuals with CP [25,26] as well as their key involvement in the induction of the immune response against numerous pathogens through the modulation of immune activation induced by TLRs [27].

Besides the factors cited above, susceptibility to CP may also be associated with genetic variability, suggesting an important role of the host genome in the modulation of the susceptibility to the disease [28,29]. Thus, the aim of the present study was to investigate the distribution of the single nucleotide polymorphism (SNP) rsrs4804803 A>G (-336) in the DC-SIGN gene among individuals with DM2 and CP, non-diabetic individuals with CP and healthy controls and its association with susceptibility to CP in a population in the state of Pernambuco, Brazil.

Methods

Study design and target population

A case-control study arm was developed, composed of a clinical arm conducted at the Endocrinology Clinic of Agamenon Magalhães Hospital and the clinic of the Postgraduate Program in Dentistry of Universidade Federal de Pernambuco (state of Pernambuco, Brazil) and a laboratorial arm conducted at the Molecular Biology Laboratory of the Postgraduate Program in Dentistry and the Molecular Biology Sector of the Keizo Asami Immunopathology Laboratory of Universidade Federal de Pernambuco.

The study population comprised 116 individuals with a diagnosis of type 2 diabetes melitus and chronic periodontitis (DM2+CP), 95 non-diabetic individuals diagnosed with chronic periodontitis (CP) (case groups) and 69 individuals without either condition (control group) recruited from the Endocrinology Clinic of the hospital and the clinic of the Postgraduate Program in Dentistry of the university between November 2015 and November 2016. All individuals were from the state of Pernambuco and were included based on the following eligibility criteria:

Inclusion criteria: For all groups, the individuals needed to be at least 35 years of age and have at least eight natural teeth (excluding those indicated for extraction). Individuals in the DM2+CP group needed to have DM2 as well as a clinical diagnosis of CP. 30 Individuals in the CP group needed to have a clinical diagnosis of CP. Individuals with neither of these two conditions were included in the group of healthy controls.

Exclusion criteria: Individuals having taken antibiotics in the previous six months, those who made chronic use of anti-inflammatory agents, those with conditions that compromised systemic immunity, pregnant or lactating women, individuals having been submitted to periodontal treatment in the previous six months, smokers and individuals wearing an orthodontic appliance were excluded from the study.

Clinical aspects

CP was characterized by the presence of inflammation (bleeding on probing), an increase in probing depth and clinical attachment loss, following the recommendations of the American Association of Periodontology [30]. The diagnosis was based on different clinical and radiographic findings, which were used to classify severity (mild, moderate and severe) and extent (localized or generalized).

A periogram was created for each individual, with data on visible plaque, probing depth, bleeding on probing, clinical attachment loss, mobility and furcation involvement. Six sites were probed for each tooth: mesio-vestibular, medio-vestibular, disto-vestibular, mesio-lingual, medio-lingual and disto-lingual. The examination was performed under artificial light using an odontoscope and University of North Carolina millimeter probe (Trinity®). The examiners wore individual protective equipment. Three examiners and assistants who had undergone training and calibration exercises (Kappa agreement coefficients 0.80) performed the clinical examinations and recorded the individual findings [31].

After the clinical examination, saliva was collected in sterile Falcon tubes (15 mL). For such, the individual was instructed to spit for a period of three minutes. The material collected was stored at -20°C for subsequent isolation of the genetic material.

Isolation of genetic material, choice of polymorphism and genotyping

DNA was extracted from saliva samples using commercial genomic DNA purification kits (Wizard® Promega), following
The manufacturer’s protocol for blood samples. The material was quantified using NanoDrop (Thermo Fischer®) and kept was at -20°C until analysis.

The choice of the polymorphism was based on the impact of the variant on gene expression [32] in previous associations with other infectious, inflammatory diseases and on a minimum allele frequency of 0.1 in reference populations (Utah [USA] residents with Northern and Western European ancestry and Yoruba in Ibadan, Nigeria) deposited in the 1000 Genomes databank [33]. For the present study, the SNP rs4804803 A>G located in the promoter region (position -336) of the CD209 or DC-SIGN gene was chosen.

Genotyping was performed using real-time polymerase chain reaction (PCR) analyses with allele-specific probes (TaqMan®) in an ABI 7500 thermal cycler (Applied Biosystems®).

Ethical considerations
All procedures employed in the present study received approval from the human research ethics committees of the Center for Health Sciences of Universidade Federal de Pernambuco (certificate number: 1310208) and Agamenon Magalhães Hospital (certificate number: 1368830).

Statistical analyses
Allele and genotype frequencies were calculated using direct counts. Adherence of the genotype distribution to Hardy-Weinberg equilibrium in each group was determined using the chi-square test. Fisher’s exact test was used to test possible associations using contingency tables (2 × 2) in R program [34]. For all analyses, 95% confidence intervals (CI) were calculated and a p-value <0.05 was considered indicative of statistical significance. The likelihood ratio test for independence was used to determine associations with genotype when it was not possible to use Pearson’s chi-square test (IBM SPSS Statistics 20.0 trial version, IBM, Armonk, NY, USA).

Results
Two hundred eighty individuals participated in the present study: 116 (41.5%) in the DM2+CP group (mean: age 58.2 ± 9.7 years; range: 20 to 80 years), 95 (33.9%) in the CP group (mean age: 51.1 ± 9.6 years; range: 35 to 76) and 69 (24.6%) in the group of health controls (mean age: 49.6 ± 10.7 years; range: 35 to 77). The largest portions of the groups were female (74.1%, 80% and 91.3%, respectively), married (64.7%, 48.4% and 44.9%, respectively), had a household income up to two times the Brazilian monthly minimum wage (89.6%, 75.3% and 66.7%, respectively), were non-smokers (61.2%, 66.3% and 85.5%, respectively) and had a complete high school education (32.5%, 42.1% and 37.7%, respectively). Significant differences among the groups were found for all of these variables (p<0.05) (Table 1). Significant differences among groups were also found for the clinical variables (number of teeth, probing depth, clinical attachment loss, bleeding index and plaque index) (Table 2).

Table 1 Socio-epidemiological profile of individuals involved in study (categorical variables).

| Categorical variables | Individuals | Total | p-value |
|-----------------------|-------------|-------|---------|
|                       | DM2+CP | CP  | Healthy |       |
|                       | n     | %   | n       | %     | n     | %   |       |
| Sex                   |        |     |         |       |       |     |       |
| Male                  | 30    | 25.9| 19      | 20    | 6     | 8.7 | 55    | 19.6 | 0.018<sup>1</sup> |
| Female                | 86    | 74.1| 76      | 80    | 63    | 91.3| 225   | 80.4 |       |
| Total                 | 116   | 100 | 95      | 100   | 69    | 100 | 280   | 100  |       |
| Marital status        |        |     |         |       |       |     |       |
| Married               | 75    | 64.7| 46      | 48.4  | 31    | 44.9| 152   | 54.3 | 0.017<sup>1</sup> |
| Single                | 23    | 19.8| 31      | 32.6  | 19    | 27.5| 73    | 26.1 |       |
| Divorced              | 7     | 6   | 8       | 8.4   | 14    | 20.3| 29    | 10.4 |       |
| Widowed               | 10    | 8.6 | 10      | 10.5  | 4     | 5.8 | 24    | 8.6  |       |
| No response           | 1     | 0.9 | 0       | 0     | 1     | 1.4 | 2     | 0.7  |       |
| Total                 | 116   | 100 | 95      | 100   | 69    | 100 | 280   | 100  |       |
| Income                |        |     |         |       |       |     |       |
| < 2 times BMMW*       | 95    | 89.6| 67      | 75.3  | 38    | 66.7| 200   | 79.4 | 0.003<sup>2</sup> |
| 2 to 4 times BMMW     | 7     | 6.6 | 19      | 21.3  | 15    | 26.3| 41    | 16.3 |       |
### Table 2: Clinical-epidemiological profile of individuals studied (quantitative variables).

| Quantitative variables | N    | Mean ± SD | Minimum | Maximum | p-value |
|------------------------|------|-----------|---------|---------|---------|
| **Age (years)**        |      |           |         |         |         |
| Diabetes               | 116  | 58.2 ± 9.7| 35.0    | 80.0    | 0.000   |
| Periodontitis          | 95   | 53.0 ± 9.6| 35.0    | 76.0    |         |
| Healthy controls       | 69   | 49.6 ± 10.7| 35.0   | 77.0    |         |
| Total                  | 280  | 54.3 ± 10.5| 35.0   | 80.0    |         |
| **Income (x BMMW*)**   |      |           |         |         |         |
| Diabetes               | 106  | 1.6 ± 1.2 | 0.0     | 10.0    | 0.004   |
| Periodontitis          | 89   | 1.6 ± 1.3 | 0.0     | 7.0     |         |
| Healthy controls       | 57   | 2.2 ± 1.5 | 1.0     | 7.0     |         |
| Total                  | 252  | 1.7 ± 1.3 | 0.0     | 10.0    |         |
| **Number of teeth**    |      |           |         |         |         |
| Diabetes               | 116  | 15.8 ± 5.6| 8.0     | 28.0    | 0.000   |
| Periodontitis          | 95   | 17.7 ± 5.6| 8.0     | 29.0    |         |
| Healthy controls       | 69   | 20.2 ± 5.8| 8.0     | 28.0    |         |
| Total                  | 280  | 17.5 ± 5.9| 8.0     | 29.0    |         |
| **Probing depth**      |      |           |         |         |         |
| Diabetes               | 116  | 2.4 ± 0.7 | 1.3     | 5.2     | 0.000   |
| Periodontitis          | 95   | 2.3 ± 0.6 | 1.3     | 4.4     |         |
Table 3 Allele and genotype distribution of single nucleotide polymorphism (SNP) in DC-SIGN gene (rs4804803) among individuals with type 2 diabetes mellitus and chronic periodontitis (DM2+CP), non-diabetic individuals with chronic periodontitis (CP) and healthy individuals in a population from the state of Pernambuco, Brazil.

| SNPs/Aleles/Genotypes | Individuals | Fisher’s exact test OR (95% CI), p-value |
|------------------------|-------------|----------------------------------------|
|                        | DM2+CP n=115 | CP n=95 | Healthy n=69 | DM2+CP vs. Healthy | CP vs. Healthy | DM2+CP vs. CP |
| rs4804803 (-336) A/G    |             |         |             |                   |               |              |
| A                      | 180 (78.3)   | 145 (76.3) | 102 (73.9)  | Reference         | Reference      | Reference     |
| G                      | 50 (21.7)    | 45 (23.7)   | 36 (26.1)   | 0.79 (0.47-1.33), 0.374 | 0.88 (0.51-1.51), 0.697 | 0.89 (0.55-1.45), 0.641 |
| AA                     | 67 (58.3)    | 58 (61.1)   | 40 (58.0)   | Reference         | Reference      | Reference     |
| AG                     | 46 (40.0)    | 29 (30.5)   | 22 (31.9)   | 1.25 (0.63-2.51), 0.521 | 0.91 (0.43-1.92), 0.861 | 1.37 (0.74-2.57), 0.306 |
| GG                     | 2 (1.7)      | 8 (8.4)     | 7 (10.1)    | 0.17 (0.02-0.97), 0.030 | 0.79 (0.23-2.78), 0.785 | 0.22 (0.02-1.16), 0.052 |
| HWE                    | X2=3.544     | X2=2.299    | X2=2.070    |                   |               |              |

Significant differences were found with regard to the allele and genotype distribution of the SNP rs4804803A/G (-336). The GG genotype was significantly more frequent in the healthy individuals (10.1%) than those with DM2+CP (1.7%) (OR=0.17; 95% CI: 0.02 to 0.97; p=0.030) and was therefore considered a protection factor (Table 3). Moreover, genotype distribution did not deviate from Hardy-Weinberg equilibrium.

Regarding the classification of periodontitis in the DM2+CP group, an association was only found for sex, as a greater number of individuals with severe, generalized periodontitis were male (56.7%). No statistically significant associations were found with regard to the other variables (income, smoking habit and duration of diabetes) (Table 4).

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Table 4 Classification of chronic periodontitis according to sex, smoking habit, income and duration of diabetes among individuals with type 2 diabetes mellitus and chronic periodontitis in a population from the state of Pernambuco, Brazil.

| Variables                  | Classification of Chronic Periodontitis | Total     | p-value<sup>1</sup> |
|----------------------------|----------------------------------------|-----------|----------------------|
|                            |                                        |           |                      |
|                            | **Mild**                               | **Moderate** | **Severe**      |
|                            | **Localized**                          | **Generalized** | **Localized** | **Generalized** | **Localized** | **Generalized** |
|                            | N  | %   | N  | %   | N  | %   | N  | %   | N  | %   | N  | %   |
| Sex                        |    |     |    |     |    |     |    |     |    |     |    |     |
| Male                       | 2  | 6.7 | 0  | 0   | 2  | 6.7 | 8  | 26.7| 1  | 3.3 | 17 | 56.7|
| Female                     | 13 | 15.1| 7  | 8.1 | 5  | 5.8 | 32 | 37.2| 0  | 0   | 29 | 33.7|
| Total                      | 15 | 12.9| 7  | 6   | 7  | 6   | 40 | 34.5| 1  | 0.9 | 46 | 39.7|
| Income                     |    |     |    |     |    |     |    |     |    |     |    |     |
| > 2 times BMMW<sup>*</sup> | 14 | 14.7| 7  | 7   | 7  | 7   | 36 | 37.9| 0  | 0   | 31 | 32.6|
| 2 to 4 times BMMW          | 1  | 14.3| 0  | 0   | 0  | 0   | 1  | 14.3| 1  | 14.3| 4  | 57.1|
| 4 to 10 times BMMW         | 0  | 0   | 0  | 0   | 0  | 0   | 1  | 25  | 0  | 0   | 3  | 75  |
| Total                      | 15 | 14.2| 7  | 6.6| 7   | 6.6| 38 | 35.8| 1  | 0.9 | 38 | 35.8|
| Smoking habit              |    |     |    |     |    |     |    |     |    |     |    |     |
| Never smoked               | 9  | 12.7| 5  | 7   | 7  | 9.9 | 25 | 35.2| 0  | 0   | 25 | 35.2|
| Ex-smoker                  | 6  | 13.3| 2  | 4.4 | 0  | 0   | 15 | 33.3| 1  | 2.2 | 21 | 46.7|
| Total                      | 15 | 12.9| 7  | 6   | 7  | 6   | 40 | 34.5| 1  | 0.9 | 46 | 39.7|
| Duration of diabetes       |    |     |    |     |    |     |    |     |    |     |    |     |
| ≤ 5 years                  | 6  | 14.3| 1  | 2.4 | 3  | 7.1 | 13 | 31  | 1  | 2.4 | 18 | 42.9|
| 5 to 10 years              | 2  | 7.4 | 3  | 11.1| 1  | 3.7 | 13 | 48.1| 0  | 0   | 8  | 29.6|
| > 10 years                 | 7  | 14.9| 3  | 6.4 | 3  | 6.4 | 14 | 29.8| 0  | 0   | 20 | 42.6|
| Total                      | 15 | 12.9| 7  | 6   | 7  | 6   | 40 | 34.5| 1  | 0.9 | 46 | 39.7|
| Insulin use                |    |     |    |     |    |     |    |     |    |     |    |     |
| Yes                        | 6  | 15.4| 1  | 2.6 | 1  | 2.6 | 11 | 28.2| 0  | 0   | 20 | 51.3|
| No                         | 9  | 11.7| 6  | 7.8 | 6  | 7.8 | 29 | 37.7| 1  | 1.3 | 26 | 33.8|
| Total                      | 15 | 12.9| 7  | 6   | 7  | 6   | 40 | 34.5| 1  | 0.9 | 46 | 39.7|

<sup>1</sup><sup>**</sup>p=0.060<sup>1</sup> p=0.129<sup>1</sup> p=0.150

<sup>*</sup>Significant p-value.
HWE: Hardy-Weinberg Equilibrium; OR: Odds Ratio; CI: Confidence Interval

No significant associations were found between the genotype distribution of SNP rs4804803 and the severity, extent or classification of periodontitis in either the DM2+CP group or CP group (Table 5). However, the bleeding index was...
associated with genotype distribution in the group of health controls, as individuals with the AG genotype had a lower mean percentage of bleeding (2.49 ± 3.79) compared to those with the AA genotype (6.74 ± 7.81); this difference was statistically significant (p=0.016) (Table 6).

Table 5 Genotype distribution of variant rs4804803 of DC-SIGN gene according to severity, extent and classification of periodontitis among individuals with type 2 diabetes mellitus and chronic periodontitis (DM2+CP) and non-diabetic individuals with chronic periodontitis (CP) in a population from the state of Pernambuco, Brazil.

| Periodontitis | DM2+CP | CP | Healthy controls |
|---------------|--------|----|------------------|
|               | AA     | AG | GG  | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| Severity      |        |    |     | Total |       |       |       |       |       |       |       |       |       |
| Mild          | 12 (17.9) | 8 (17.4) | 1 (50.0) | 21 (38.3) | 11 (19.3) | 3 (10.3) | 2 (22.2) | 16 (16.8) | 0.552 |
| Moderate      | 29 (43.3) | 17 (37.0) | 1 (50.0) | 47 (74.9) | 15 (26.3) | 13 (44.8) | 1 (11.1) | 29 (30.5) | 0.249 |
| Severe        | 26 (38.8) | 21 (45.7) | 0 (0.0) | 47 (74.9) | 31 (54.4) | 13 (44.8) | 6 (66.7) | 50 (52.6) | 0.719 |
| Extent        |        |    |     | Total |       |       |       |       |       |       |       |       |       |
| Localized     | 16 (23.9) | 6 (13.0) | 0 (0.0) | 22 (19.1) | 15 (26.3) | 6 (20.7) | 3 (33.3) | 24 (25.3) | 0.225 |
| Generalized   | 51 (76.1) | 40 (87.0) | 2 (100.0) | 93 (80.9) | 42 (73.7) | 23 (79.3) | 6 (66.7) | 71 (74.7) | 0.719 |
| Classification|        |    |     | Total |       |       |       |       |       |       |       |       |       |
| Mild - Localized | 9 (13.4) | 5 (10.9) | 0 (0.0) | 14 (22.2) | 9 (15.8) | 1 (3.4) | 2 (22.2) | 12 (22.6) | 1.000 |
| Mild - Generalized | 3 (4.5) | 3 (6.5) | 1 (50.0) | 7 (6.1) | 3 (5.3) | 2 (6.9) | 0 (0.0) | 5 (5.3) | 0.526 |
| Moderate - Localized | 6 (9.0) | 1 (2.2) | 0 (0.0) | 7 (8.1) | 3 (5.3) | 4 (13.8) | 1 (3.4) | 8 (4.8) | 0.181 |
| Moderate - Generalized | 23 (34.3) | 16 (34.8) | 1 (50.0) | 40 (49.1) | 11 (19.3) | 9 (31.0) | 0 (0.0) | 20 (21.1) | 0.056 |
| Severe - Localized | 1 (1.5) | 0 (0.0) | 0 (0.0) | 1 (0.9) | 4 (7.0) | 1 (3.4) | 0 (0.0) | 5 (5.3) | 1.000 |
| Severe - Generalized | 25 (37.3) | 21 (45.7) | 0 (0.0) | 46 (40.0) | 27 (47.4) | 12 (41.4) | 6 (66.7) | 45 (47.4) | 1.000 |
| Total          | 67 (100.0) | 46 (100.0) | 2 (100) | 115 (100.0) | 57 (100.0) | 29 (100.0) | 9 (100.0) | 95 (100.0) | 1.000 |

Statistical significance: p<0.05

Table 6 Mean probing depth, clinical attachment level, bleeding index and plaque index according to genotype distribution of SNP rs4804803 of DC-SIGN gene among individuals with type 2 diabetes mellitus and chronic periodontitis (DM2+CP), non-diabetic individuals with chronic periodontitis (CP) and healthy individuals in a population from the state of Pernambuco, Brazil.

| Genotypes | DM2+CP | CP | Healthy controls |
|-----------|--------|----|------------------|
|           | N      | Mean ± SD | p-value | N      | Mean ± SD | p-value | N      | Mean ± SD | p-value |
| Probing depth |        |        |        |        |        |        |        |        |        |        |
| AA        | 67     | 2.37 ± 0.56 | ref. | 57     | 2.32 ± 0.61 | ref. | 40     | 1.87 ± 0.31 | ref. |
| AG        | 46     | 2.58 ± 0.77 | 0.285 | 29     | 2.29 ± 0.53 | 0.684 | 22     | 1.88 ± 0.30 | 0.389 |
| GG        | 2      | 2.10 ± 0.71 | 0.567 | 9      | 2.22 ± 0.43 | 0.801 | 7      | 1.77 ± 0.37 | 0.455 |
| Total     | 115    | 2.45 ± 0.66 | 95     | 2.30 ± 0.57 | 69     | 1.86 ± 0.31 | 69     | 2.14 ± 0.44 | 0.719 |
| Clinical attachment loss |        |        |        |        |        |        |        |        |        |
| AA        | 67     | 3.90 ± 1.82 | ref. | 57     | 3.57 ± 1.79 | ref. | 40     | 2.10 ± 0.42 | ref. |
| AG        | 46     | 3.97 ± 1.46 | 0.385 | 29     | 3.28 ± 1.14 | 0.468 | 22     | 2.10 ± 0.40 | 0.924 |
| GG        | 2      | 2.89 ± 0.35 | 0.431 | 9      | 3.97 ± 2.54 | 0.808 | 7      | 2.45 ± 0.62 | 0.056 |
| Total     | 115    | 3.91 ± 1.67 | 95     | 3.52 ± 1.70 | 69     | 2.14 ± 0.44 | 69     | 2.14 ± 0.44 | 1.000 |
**Discussion**

CP is a destructive form of periodontal disease that is frequently found in individuals with DM2. It is initiated and maintained by an impaired immune reaction to oral bacteria that culminates in damage to connective and bone tissue [1,11,13]. The inflamed gingival tissue is characterized by a large quantity of cellular sub-populations, [35] such as dendritic cells, which are involved in periodontal disease [26,36,37] as well as the capture and presentation of antigens [38]. Through the DC-SIGN receptor, dendritic cells may be targeted by oral pathogens, which modulate the cellular immune response [21,22]. Polymorphisms in DC-SIGN regulatory regions are related to a change in levels of gene expression [39-41] and, consequently, in the susceptibility to different diseases, such as CP and DM. Thus, the distribution of the SNP rs4804803 A>G located in the promoter region of DC-SIGN (position -336) affects the bonding to the transcription factor Sp1 and in vivo studies have related the presence of the A allele to an increased expression of the receptor [40,53].

Studying individuals with DM2 and healthy controls in a population from northeastern Brazil for SNPs rs735239 and rs4804803 of DC-SIGN, da Silva et al. [41] found greater susceptibility to the development of DM1 related to the G allele and the GG and AG genotypes of SNP rs735239 (-871) as well as G-G allelic (rs735239-rs4804803) and AA-GG genotypic (rs735239-rs4804803) combinations, which is in partial agreement with the present results involving individuals with DM2 and CP. One may hypothesize that individuals with the GG genotype have low DC-SIGN expression [40,53] and consequently, a smaller number of receptors to interact with Mfal1 from *P. gingivalis*, thereby modulating susceptibility through an anti-inflammatory immune response [47]. Indeed, the immune-modulatory effect of DC-SIGN is associated with the type of bond [41].

An association was also found between the classification of periodontitis and sex, as a greater proportion of males with DM2 and CP was diagnosed with severe generalized periodontitis. This finding allows one to infer that, in the population studied, women demonstrated greater care with regard to oral health. Studies involving Chinese [54] and German [55] populations report similar results.

**Conclusion**

In the population analyzed, individuals with DM2 and the GG genotype (rs4804803) of DC-SIGN demonstrated less susceptibility to the development of CP. Despite the limitations of the study (lack of expression assays, number of variants studied and small sample size), this is the first record of an association between a variant in DC-SIGN and susceptibility to the development of CP among individuals with DM2. Studies should be conducted addressing other variants with a larger

| Bleeding index (%) | AA  | 67 | 11.69 ± 12.34 | ref. | 57 | 16.09 ± 14.16 | ref. | 40 | 6.74 ± 7.81 | ref. |
|-------------------|-----|----|----------------|------|----|----------------|------|----|----------------|------|
|                   | AG  | 46 | 11.70 ± 17.20 | 0.573| 29 | 14.03 ± 13.14 | 0.528| 22 | 2.49 ± 3.79 | 0.016*|
|                   | GG  | 2  | 11.57 ± 11.93 | 0.871| 9  | 15.50 ± 17.36 | 0.556| 7  | 3.89 ± 4.67 | 0.375|
| Total             |     | 115| 11.69 ± 14.36 |      | 95 | 15.41 ± 14.05 |      | 69 | 5.09 ± 6.73 |      |

| Plaque index (%)  | AA  | 67 | 25.89 ± 23.87 | ref. | 57 | 25.32 ± 24.43 | ref. | 40 | 17.39 ± 19.58 | ref. |
|-------------------|-----|----|----------------|------|----|----------------|------|----|----------------|------|
|                   | AG  | 46 | 26.13 ± 26.90 | 0.582| 29 | 24.10 ± 21.64 | 0.989| 22 | 16.50 ± 22.39 | 0.61 |
|                   | GG  | 2  | 40.00 ± 56.57 | 0.943| 9  | 25.86 ± 18.28 | 0.449| 7  | 10.02 ± 12.18 | 0.293|
| Total             |     | 115| 26.23 ± 25.44 |      | 95 | 25.00 ± 22.89 |      | 69 | 16.36 ± 19.81 |      |

*Statistical significance: p<0.05, p-value comparing each genotype with reference value in groups
number of individuals and in other populations to enable a better understanding of the role of this receptor in chronic periodontitis.

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