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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 sub-gingival 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 (TRLs) 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.30 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	%	n	%	
Sex									
Male	30	25.9	19	20	6	8.7	55	19.6	0.018[1]
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[1]
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[2]
2 to 4 times BMMW	7	6.6	19	21.3	15	26.3	41	16.3	

4 to 10 times BMMW	4	3.8	3	3.4	4	7	11	4.4	
Total	106	100	89	100	57	100	252	100	
Smoking habit									
Never smoked	71	61.2	63	66.3	59	85.5	193	68.9	0.002[1]
Ex-smoker	45	38.8	32	33.7	10	14.5	87	31.1	
Total	116	100	95	100	69	100	280	100	
Schooling									
Illiterate	10	8.8	1	1.1	1	1.4	12	4.3	0.000[2]
Incomplete elementary	29	25.4	16	16.8	5	7.2	50	18	
Complete elementary	18	15.8	12	12.6	5	7.2	35	12.6	
Incomplete high school	9	7.9	6	6.3	8	11.6	23	8.3	
Complete high school	37	32.5	40	42.1	26	37.7	103	37.1	
Incomplete university	2	1.8	5	5.3	3	4.3	10	3.6	
Complete university	6	5.3	7	7.4	11	15.9	24	8.6	
Uncertain	2	1.8	7	7.4	10	14.5	19	6.8	
No response	1	0.9	1	1.1	0	0	2	0.7	
Total	114	100	95	100	69	100	278	100	

[1]Pearson chi-square test; [2]likelihood ratio test; *BMMW- Brazilian monthly minimum wage; statistically significant difference: p<0.05

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	

Healthy controls	69	1.9 ± 0.3	1.2	2.8	
Total	280	2.3 ± 0.6	1.2	5.2	
Clinical attachment loss					
Diabetes	116	3.9 ± 1.7	1.6	10.2	0.000
Periodontitis	95	3.5 ± 1.7	1.6	11.9	
Healthy controls	69	2.1 ± 0.4	1.3	3.8	
Total	280	3.3 ± 1.6	1.3	11.9	
Bleeding index (%)					
Diabetes	116	11.6 ± 14.3	0.0	100.0	0.000
Periodontitis	95 A	15.4 ± 14.0	0.0	50.0	
Healthy controls	69 B	5.1 ± 6.7	0.0	36.4	
Total	280	11.3 ± 13.3	0.0	100.0	
Plaque index (%)					
Diabetes	116 A	26.0 ± 25.4	0.0	100.0	0.003
Periodontitis	95 A	25.0 ± 22.9	0.0	100.0	
Healthy controls	69 B	16.4 ± 19.8	0.0	100.0	
Total	280	23.3 ± 23.5	0.0	100.0	

*BMMW- Brazilian monthly minimum wage; Nonparametric Kruskal-Wallis test; statistically significant difference: p<0.05.

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)**.

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/Alelles/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), 0.780	0.22 (0.02-1.16, 0.052
HWE	X2=3.544	X2=2.299	X2=2.070			

	p=0.060	p=0.129	p=0.150			

*Significant p-value.

HWE: Hardy-Weinberg Equilibrium; OR: Odds Ratio; CI: Confidence Interval

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[1]
	Mild				Moderate				Severe						
	Localized		Generalized		Localized		Generalized		Localized		Generalized				
	N	%	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	30	100	0.037**
Female	13	15.1	7	8.1	5	5.8	32	37.2	0	0	29	33.7	86	100	
Total	15	12.9	7	6	7	6	40	34.5	1	0.9	46	39.7	116	100	
Income															
> 2 times BMMW*	14	14.7	7	7.4	7	7.4	36	37.9	0	0	31	32.6	95	100	0.201
2 to 4 times BMMW	1	14.3	0	0	0	0	1	14.3	1	14.3	4	57.1	7	100	
4 to 10 times BMMW	0	0	0	0	0	0	1	25	0	0	3	75	4	100	
Total	15	14.2	7	6.6	7	6.6	38	35.8	1	0.9	38	35.8	106	100	
Smoking habit															
Never smoked	9	12.7	5	7	7	9.9	25	35.2	0	0	25	35.2	71	100	0.075
Ex-smoker	6	13.3	2	4.4	0	0	15	33.3	1	2.2	21	46.7	45	100	
Total	15	12.9	7	6	7	6	40	34.5	1	0.9	46	39.7	116	100	
Duration of diabetes															
≤ 5 years	6	14.3	1	2.4	3	7.1	13	31	1	2.4	18	42.9	42	100	0.608
5 to 10 years	2	7.4	3	11.1	1	3.7	13	48.1	0	0	8	29.6	27	100	
> 10 years	7	14.9	3	6.4	3	6.4	14	29.8	0	0	20	42.6	47	100	
Total	15	12.9	7	6	7	6	40	34.5	1	0.9	46	39.7	116	100	
Insulin use															
Yes	6	15.4	1	2.6	1	2.6	11	28.2	0	0	20	51.3	39	100	0.268
No	9	11.7	6	7.8	6	7.8	29	37.7	1	1.3	26	33.8	77	100	
Total	15	12.9	7	6	7	6	40	34.5	1	0.9	46	39.7	116	100	

[1]likelihood ratio test; *BMMW- Brazilian monthly minimum wage; **statistically significant difference: p<0.05

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			Total	p-value	CP			Total	p-Value
	AA	AG	GG			AA	AG	GG		
	N (%)	N (%)	N (%)	N (%)		N (%)	N (%)	N (%)		
Severity										
Mild	12 (17.9)	8 (17.4)	1 (50.0)	21 (18.3)	0.552	11 (19.3)	3 (10.3)	2 (22.2)	16 (16.8)	0.249
Moderate	29 (43.3)	17 (37.0)	1 (50.0)	47 (40.9)		15 (26.3)	13 (44.8)	1 (11.1)	29 (30.5)	
Severe	26 (38.8)	21 (45.7)	0 (0.0)	47 (40.9)		31 (54.4)	13 (44.8)	6 (66.7)	50 (52.6)	
Extent										
Localized	16 (23.9)	6 (13.0)	0 (0.0)	22 (19.1)	0.225	15 (26.3)	6 (20.7)	3 (33.3)	24 (25.3)	0.719
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)	
Classification										
Mild - Localized	9 (13.4)	5 (10.9)	0 (0.0)	14 (12.2)	0.507	9 (15.8)	1 (3.4)	2 (22.2)	12 (12.6)	0.181
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)	
Moderate - Localized	6 (9.0)	1 (2.2)	0 (0.0)	7 (6.1)		3 (5.3)	4 (13.8)	1 (11.1)	8 (8.4)	
Moderate - Generalized	23 (34.3)	16 (34.8)	1 (50.0)	40 (34.8)		11 (19.3)	9 (31.0)	0 (0.0)	20 (21.1)	
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)	
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)	
Total	67 (100.0)	46 (100.0)	2	115 (100.0)		57 (100.0)	29 (100.0)	9 (100.0)	95 (100.0)	
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	
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	

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

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 (-336) in the DC-SIGN gene in individuals with DM2 and CP, non-diabetic individuals with CP and healthy controls as well as the relationship with the susceptibility to CP were investigated in the present study. Individuals with the GG genotype were found to have less susceptibility to the concomitant development of DM2 and CP.

As a key molecule in the innate and adaptive immune response, DC-SIGN plays an important role in the recognition of a large number of pathogens of interest to public health, such as viruses [40,42,43] parasites, [44] bacteria [45] and fungi, [46] participating in the inflammatory response and activation of T cells, [47,48] which can be increased in individuals with CP [20]. Studies report that the key etiological agent of CP (the bacterium *Porphyromonas gingivalis*) infects myeloid and dermal dendritic cells through DC-SIGN [49-51] and that the glycoprotein Mfa1 of the pathogen can bond to the receptor, triggering immuno-stimulatory effects [50] and assisting in the systemic dissemination of the bacterium to atherosclerotic plaque, which implies cardiac risk [52].

In the present study, the GG genotype was associated with less susceptibility to the development of DM2+CP. 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

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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