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Analysis of *GLT6D1* and *CDKN2BAS* gene polymorphisms in Brazilian patients with advanced periodontitis

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Abstract: Gene polymorphisms can predispose to periodontal disease, as demonstrated by the well-documented association between aggressive periodontitis and single nucleotide polymorphisms (SNPs) such as rs153745 in the *GLT6D1* gene and rs3217992 in the *CDKN2BAS* gene. The purpose of this study was to evaluate the presence of these SNPs in Brazilian patients with advanced periodontitis (stages III/IV, Grade B/C) vs. healthy controls. A total of 100 patients with periodontitis (Group BC) were enrolled. Of these, 51 patients were classified as stage III and 49 patients were classified as stage IV, and 52 were Grade B (Group B) and 48 were Grade C (Group C). The control Group consisted of 61 healthy subjects. DNA samples extracted from buccal epithelial cells were used to genotype the SNPs rs1537415 (GLT6D1) and rs3217992 (CDKN2BAS) by real-time quantitative PCR. No significant differences in polymorphism frequency were found between the control Group and each of the patient groups (BC, B, or C), and Group B did not differ from Group C. In conclusion, the evaluated SNPs had no significant influence on the prevalence of periodontal disease in the sampled Brazilian population.

Keywords: Periodontitis; Polymorphism, Genetic; Polymorphism, Single Nucleotide.

Introduction

Periodontitis is an inflammatory condition caused by the presence of periodontopathogens,[1] and its phenotype is determined by both the individual genetic background and environmental factors.[2] The condition is currently classified into 4 stages (I–IV) according to disease severity and treatment complexity, and into 3 grades (A, B, C) based on progression rate and risk factors.[1]

Staging denotes the severity and extent of a patient's disease based on the measurable amount of periodontal loss resulting from periodontitis and assesses the specific factors that may be attributed to the complexity of case management. The severity increases from stage I to IV; stage III and IV represent the most severe forms of periodontitis, with interdental clinical attachment loss ≥ 5 mm and radiographic bone loss extending to the middle third of root and beyond. On the other hand, grading is related to the rate of periodontitis progression, responsiveness to standard

therapy, and potential impact on systemic health. Grade A patients exhibit a low rate of progression and no systemic risk factors, Grade B is associated with a moderate rate of progression, and Grade C is associated with rapid progression and systemic risk factors.[1]

Periodontal tissue destruction results from a cascade of biological events, such as the production of pro- and anti-inflammatory cytokines, growth factors, and enzymes,[3] which may be influenced by environmental (*e.g.*, smoking), systemic (e.g., diabetes), and genetic factors.[4] Human and animal studies have shown that genetic factors impairing immune and inflammatory responses can affect the occurrence of periodontitis.[4]

Genetic polymorphisms are normal minor variations in the nucleotide sequence occurring inside or outside genes throughout the genome.[4] The most common variations involve a single base in the DNA and are referred to as single nucleotide polymorphisms (SNP). SNPs may or may not have an effect on the expressed phenotype.[4,5] During inflammatory responses to exogenous stimuli, certain polymorphisms may increase or decrease the risk of developing a phenotype of a particular disease.[4] The genetic factors associated with periodontitis have not been completely identified, but some genes have been associated with aggressive forms of periodontitis in genome-wide association studies (a very efficient method of identifying genetic variants associated with a high risk of development of specific diseases) and candidate gene studies.[6-11]

An increasing body of evidence is pointing to an association, in certain populations, between periodontitis and polymorphisms in the *CDKN2BAS* (CDKN2B antisense RNA 1)[6-8] and *GLT6D1* (glycosyltransferase 6 domain-containing protein 1) genes.[10,11]

CDKN2BAS is a non-protein-coding RNA gene, formerly called ANRIL, located within the chromosomal region 9p21.3.[6,7] Noncoding RNAs such as *ANRIL* have been implicated in a variety of cellular pathways and stress responses and may influence gene transcription through mechanisms that may include RNA interference, gene silencing, chromatin remodeling, or DNA methylation.[12] Polymorphisms affect the *CDKN2BAS* gene expression, which is associated with genetic susceptibility to cardiovascular disease and other inflammatory pathologies, including several cancers, intracranial aneurysm, type 2 diabetes, periodontitis, Alzheimer's disease, and endometriosis.[6-9]

GLT6D1 is a protein-coding gene with a range of poorly mapped functions.[10,11] *GLT6D1* is located at chromosome 9q34.3 and encodes a protein belonging to a family of proteins that are characterized by a glycosyltransferase domain-1.[10] It is expressed in leukocytes, the gingiva, and testis, with a significantly stronger gene expression in gingival connective tissue than in the gingival epithelium.[10] Associations between some polymorphisms in this gene and periodontitis have been investigated.[10,11]

More research is needed to clarify the potential relationship between these SNPs and periodontal diseases, especially studies in Brazilian populations. Knowledge of variations in the distribution of genotypes among populations is important to our current understanding of the etiopathogenesis of periodontitis and may consequently contribute to the identification of individuals at risk and the establishment of an early diagnosis. In this study, we evaluated the correlation between advanced periodontitis (stages III and IV, grades B and C) and the presence of polymorphisms in the genes *GLT6D1* and *CDKN2BAS* in a sample of Brazilian patients and healthy controls.

Methodology

This was a cross-sectional study involving 100 patients with periodontitis and 61 healthy controls recruited at the Periodontology Clinic of the School of Pharmacy, Dentistry, and Nursing of the Federal University of Ceará (UFC), Fortaleza, Ceará, Brazil. The study protocol was previously approved by the institutional ethics committee (protocol #098/11), and all participants gave their informed written consent.

Study population

The recruited subjects were initially classified into chronic and aggressive periodontitis.[13] However, following the publication in 2018 of the new

periodontitis classification criteria,[1] the patients were reclassified into stages I, II , III, or IV and grades A, B, or C, based on clinical data (clinical attachment loss, probing depth, tooth mobility, furcation involvement, number of remaining teeth, and radiographic bone loss) and clinical patterns suggestive of periods of rapid progression and/or early onset of periodontitis and the correlation between plaque deposits and periodontal destruction. In the present study, all patients were classified as stage III or IV and Grade B or C. Therefore, the sample was divided into "Group B" (stage III or IV, Grade B), "Group C" (stage III or IV, Grade C), and "Group BC" (stage III or IV, Grade B or C).

The control Group consisted of healthy individuals with at least 24 teeth (except third molars), no sites with probing depth and clinical attachment loss ≥ 3 mm, and a gingival index[14] of <10%. The exclusion criteria were periodontal treatment within the previous 6 months, use of antibiotics within the previous 3 months, smoking, and systemic changes capable of interfering with periodontal health.

Periodontal clinical examination

All completely erupted permanent teeth (except third molars) were evaluated with a periodontal probe (PCP-UNC 15, Trinity, São Paulo, Brazil), and the following parameters were registered: plaque index (PI),[14] gingival index (GI),[14] probing depth (PD), clinical attachment loss (CAL), tooth mobility, and furcation involvement. PD and CAL were taken at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual). All clinical parameters were evaluated by a single examiner. The reproducibility of the PD and CAL measurements was assessed by calculating intraclass correlation coefficients (ICC). The agreement between repeated measurements was high (ICC > 0.80).

DNA sampling and analysis of genetic polymorphisms

DNA was extracted from buccal epithelial cell obtained with a 3% glucose mouth rinse for 2 min.[15] Two samples were collected from each patient. The samples were placed in falcon tubes and centrifuged at 3,000 g for 10 min. The supernatant was discarded, and 500 µL of extraction buffer (10Mm Tris, 0.5 M EDTA, 0.5% SDS) was added to the pellet containing cells. Then, 5 µL of proteinase K (20 mg/ml) was added to the pellet. This solution was mixed by vortex for 5 s at moderate speed and incubated at 55 °C overnight. After that, 500 µL of 7.5 M ammonium acetate (or 8M + 1mM EDTA) was added, vortexed for 5 s at high speed, and centrifuged at 14,000 g for 10 min.[16] The remaining supernatant was distributed equally (± 450 µL each) in microtubes and 540 µL isopropanol was added and centrifuged at 14,000 g for 5 min. The supernatant was removed and 1.000 µL 70% ethanol was added and then vortexed for 5 s at moderate speed and centrifuged at 14,000 g for 5 min. The supernatant was carefully removed and discarded with a micropipette, leaving the pellet of DNA. Following 1 h at room temperature, 50 µL of TE was added to the remaining pellet, and the DNA concentration was determined by spectrophotometry (Nanodrop 2000, Thermo Scientific, DE, USA), with purity estimated by the 260/280 nm OD ratio, followed by dilution to 10 ng/µL, and the solution was stored at -20°C until ready for real-time PCR .[16]

Genetic polymorphisms were analyzed by real-time quantitative PCR. The SNPs rs1537415 (GLT6D1) and rs3217992 (CDKN2BAS) were genotyped by predesigned TaqMan SNP assays (Applied Biosystems, Foster City, USA). DNA was amplified in a volume of 13 µL containing 1 µL genomic DNA (10 ng/µL), 6.25 µL Master Mix 2x (Applied Biosystems), 0.63 µL 20x TaqMan Assay Mix (Applied Biosystems), and 4.63 µL RNA/DNase-free water (Applied Biosystems). The amplifications were performed in a StepOne real-time PCR system (Applied Biosystems) using 96-well plates. The genotypes were distinguished based on their unique fluorescence. These analyses were performed at the Laboratory of Molecular Genetics of the School of Dentistry in Araraquara (UNESP-SP).

Statistical analysis

The normality of the data distribution was verified using the Shapiro-Wilk test. The groups were compared in terms of age using ANOVA and the Tukey post-hoc test. Clinical variables were evaluated using the *t*-test for independent samples

or the Mann-Whitney test. Pearson's chi-squared test was employed for categorical variables. The distribution of polymorphisms across the groups was tested according to the Hardy–Weinberg principle, whereas differences in allele and genotype frequency were analyzed using the chi-squared test. All analyses were performed using the software IBM SPSS Statistics v.25.0 (IBM Corp., Armonk, NY, EUA). The level of statistical significance was set at 5% ($p < 0.05$).

Results

Fifty-one patients were classified as periodontitis stage III and 49 patients were classified as periodontitis stage IV, totaling 100 periodontitis patients (Group BC), of whom 52 were Grade B and 48 were Grade C. DNA amplification for the analysis of the SNP rs3217992 failed in 3 patients from Group B and 3 patients from Group C, whereas DNA amplification for analysis of the SNP rs15374151 failed in 1 sample from each Group. The control Group included 61 healthy subjects, but 7 samples failed to amplify for analysis of the SNP rs3217992. From these patients, who presented at least 24 teeth, all periodontal clinical parameters were collected similarly to the other participants. However, these patients did not present sites with PD and CAL ≥ 3 mm, as well as plaque or gingival index > 10% (Table 1).

Table 1 shows the demographic and clinical data of the study participants. The mean age was similar in the control Group and Group C, but significantly lower in these two groups than in Group B. Moreover, Group C displayed higher PD and interproximal CAL values, and more bleeding on probing and teeth with CAL ≥ 5 mm than did Group B.

The allele and genotype frequencies of the *GLT6D1* and *CDKN2BAS* polymorphisms are shown in Table 2. There was a Hardy–Weinberg disequilibrium ($p < 0.05$) in the genotype frequency distribution of the polymorphism in the *CDKN2BAS* gene in Group C, as well in Group BC. The results of the

Table 1. Characteristics of the study population

Variable	Controls	Group B	Group C
	n = 61	n = 52	n = 48
Age (years; mean ± SD)	28.1 ± 10.0	43.4 ± 9.2*,**	27.5 ± 6.1
Male n (%)	18 (29.5)	22 (42.3)	16 (33.3)
Female n (%)	43 (70.5)	30 (57.7)	32 (66.7)
Number of teeth (mean ± SD)	§	22 ± 5.2	25.9 ± 1.9**
GI (% sites; mean ± SD)	< 10%	14.2 ± 9.5	12.0 ± 9.3
PI (% sites; mean ± SD)	< 10%	34.9 ± 15.4	33.3 ± 17.3
PD (mm; mean ± SD)	NE	2.8 ± 0.6	3.3 ± 0.6**
CAL (mm; mean ± SD)	NE	3.5 ± 1.0	3.7 ± 0.9
Interproximal CAL (mm; mean ± SD)	NE	3.8 ± 1.0	4.4 ± 1.2**
Proportion of teeth CAL ≥ 5 mm (mean %)	-	50.8	60.8**
Proportion of sites PD ≥ 5 mm (mean %)	-	15.4	25.4**
Proportion of sites PD ≥ 6mm (mean %)	-	7.4	12.5**
Proportion of sites PD ≥ 7 mm (mean %)	-	4.3	7.8**
Proportion of sites CAL ≥ 5 mm (mean %)	-	32.2	31.3
Proportion of sites CAL ≥ 6 mm (mean %)	-	14.4	19.6
Proportion of sites CAL ≥ 7 mm (mean %)	-	10.5	13.8
BoP (% sites; mean ± SD)	NE	31.2 ± 15.3	44.3 ± 16.3**

SD: standard deviation; NE: not evaluated; PD: probing depth; CAL: clinical attachment loss; BoP: bleeding on probing; PI: plaque index[14]; GI: gingival index[14], *$p < 0.05$ Group B vs. controls;,**$p < 0.05$ Group B vs. Group C; §at least 24.

Table 2. Frequency of alleles and genotypes of polymorphisms in the GLT6D1 and CDKN2BAS genes: comparison between patients and controls.

Variable	Controls	%	Group B	%	p-value	Group C	%	p-value	Group BC	%	p-value
GLT6D1 gene (rs1537415)	n = 61		n = 51			n = 47			n = 98		
Alleles											
C	46	37.7	34	33.3	0.5892	35	37.2	0.9435	69	35.2	0.7404
G	76	62.3	68	66.7		59	62.8		127	64.8	
Genotypes											
GG	22	36.1	21	41.2	0.7198	20	42.6	0.6265	41	41.8	0.8617
CG	32	52.4	26	51.0	0.8761	19	40.4	0.2467	45	45.9	0.4158
CC	7	11.5	4	7.8	0.7515	8	17.0	0.4179	12	12.2	0.6843
Hardy–Weinberg Equilibrium (p-value)	0.362		0.2936			0.4297			0.9487		
CDKN2BAS gene (rs3217992)	n = 54		n = 49			n = 45			n = 94		
Alleles											
C	64	59.3	58	59.2	0.9912	61	67.8	0.2761	119	63.3	0.6187
T	44	40.7	40	40.8		29	32.2		69	36.7	
Genotypes											
CC	17	31.5	14	28.6	0.8310	17	37.8	0.5313	31	33.0	0.9933
CT	30	55.5	30	61.2	0.6894	27	60.0	0.6875	57	60.6	0.7383
TT	7	13.0	5	10.2	0.7638	1	2.2	0.0682	6	6.4	0.3820
Hardy–Weinberg Equilibrium (p-value)	0.2685		0.0614			0.0122*			0.0031*		

*There was a Hardy–Weinberg disequilibrium ($p < 0.05$) in the genotype frequency distribution of the polymorphism in the CDKN2BAS gene in the Group C, as well in the Group BC.

comparison of the allele frequencies of groups B and C and the controls did not show a statistically significant difference (Table 2); the same occurred when comparing Group B with Group C (Table 3).

Discussion

This study was originally designed to compare the presence of the gene polymorphisms rs1537415 (GTL6D1) and rs3217992 (CDKN2BAS) in patients with chronic and aggressive periodontitis and healthy controls. However, with the introduction of new classification criteria in 2018, periodontitis is no longer classified into chronic and aggressive, but into stages and grades based on specific clinical parameters and factors that affect clinical management, prognosis and, possibly, oral and systemic health.[1,17]

All patients were therefore reclassified as stage III or IV periodontitis based on the findings of interproximal CAL ≥ 5 mm, PD ≥ 6 mm, tooth mobility ≥ 2, class II or III furcation involvement, the number of remaining teeth, and radiographic bone loss.[1,17]

Given the cross-sectional design of the study, no direct data on disease progression was collected. Thus, the grading of the patients (A, B, or C) was based on indirect evidence of progression. Smoking habits and systemic disease were exclusion criteria and were therefore not included in the analysis. Since none of our patients presented high plaque indices combined with low levels of destruction, the entire sample was graded B or C. Specifically, patients with substantial periodontal destruction combined with low plaque indices and clinical patterns suggestive of rapid progression and/or early onset of disease

Table 3. Frequency of alleles and genotypes of polymorphisms in the GLT6D1 and CDKN2BAS: genes comparison betwen Groups B and C.

Gene (SNP)	Group B	Group C	p-value
GLT6D1 (rs1537415) alleles			
C	34	35	0.6734
G	68	59	
GLT6D1 (rs1537415) genotypes			
GG	21	20	0.8902
CG	26	19	0.3174
CC	4	8	0.2216
Hardy–Weinberg *p*-value	0.2936	0.3543	
CDKN2BAS (rs3217992) alleles			
C	58	61	0.2847
T	40	29	
CDKN2BAS (rs3217992) genotypes			
CC	14	17	0.3851
CT	30	27	1.0000
TT	5	1	0.2061
Hardy–Weinberg *p*-value	0.0614	0.0122	

(such as incisive/molar pattern) were graded "C." When the level of destruction was compatible with the plaque indices (and in the absence of other evidence to support a grading of A or C), patients were graded "B."[1]

The patients were organized in groups according to Grade, not stage. While the stage depends on the severity and extent of the disease at the time of presentation, the Grade provides information on biological features of the disease, such as specific clinical patterns suggestive of periods of rapid progression and/or early onset of disease and the relationship between plaque index and periodontal destruction,[1,17] as previously required for the diagnosis of aggressive periodontitis,[13,18] which was the original focus of this study.

Patients with Grade C periodontitis (or with findings previously interpreted as aggressive periodontitis) appear to have a more destructive immunoinflammatory response to periodontal pathogens.[1,19,20] In fact, there is evidence suggesting that genetic polymorphisms in genes involved in immunoinflammatory reactions can modulate the occurrence of periodontitis.[2,4,21] The fact that, in our study, Group C showed a greater mean of bleeding on probing and PD, despite presenting a lower mean age, may be related to this.

The choice of the genes *GLT6D1* and *CDKN2BAS* for this investigation was based on the well-documented association between the polymorphisms of these genes and aggressive periodontitis in various populations.[6,9-11] For example, a *GLT6D1* polymorphism was strongly associated with aggressive periodontitis in a genome-wide association study involving 1758 German and Dutch patients.[10] The study suggested that a rare variant of the SNP rs1537415 was associated with aggressive periodontitis through the reduction of the binding affinity of GATA-3—an important factor in the development, activation, proliferation, and function of T cells.[10] Likewise, a candidate gene study revealed an association between the genetic variant of GLT6D1 and aggressive periodontitis in a Sudanese cohort of 132 patients with periodontitis and 136 healthy controls.[11] In contrast, a study enrolling 200 patients from Southeastern Brazil with aggressive periodontitis and 190 patients with chronic periodontitis found no statistically significant association between the SNP rs1537415 and periodontitis.[22] This finding is compatible with our results for patients from Northeastern Brazil, suggesting that the SNP rs1537415 may not be associated with periodontitis in at least two different Brazilian populations.

The choice of CDKN2BAS as a candidate gene in this study is justified by the confirmed association between the polymorphisms in this region and coronary artery disease.[23] Moreover, some studies have found associations between periodontal disease and cardiovascular disease.[24] These two complex conditions are characterized by changes in the immunoinflammatory response and share a range of environmental and genetic risk factors.[24] CDKN2BAS is a noncoding RNA gene whose actions are not yet fully understood,[25] but appears to be part of a regulatory network integrating glucose and fatty acid metabolism and immune responses (CAMTA1/VAMP3 locus, ADIPOR1, and C11ORF10).[25]

Working on German subjects, Schaefer et al.[6] were the first to correlate CDKN2BAS polymorphisms with both generalized and localized aggressive

periodontitis, a finding replicated for aggressive and chronic periodontitis in other European populations.[7] Three of the 51 CDKN2BAS SNPs observed in German and Dutch subjects (rs3217992, rs518394 and rs1360590) have been associated with periodontitis, especially the first of these.[7] Likewise, significant associations with the SNP rs1333048 have been reported for Caucasians in Germany and North Ireland,[8] and among Turks,[9] supporting the hypothesis of a causal link between CDKN2BAS polymorphisms and aggressive periodontitis in different populations. However, there are uncertainties about the variants and mechanisms involved. Interestingly, in our patients, the SNP rs3217992 was not significantly associated with stage III/IV, Grade B/C periodontitis, not even when Grade B and C were analyzed separately. This is in agreement with the results of another Brazilian study evaluating the possible connection between the SNP rs3217992 and aggressive periodontitis.[22] Thus, currently available evidence does not point to a significant influence of the SNP rs3217992 (CDKN2BAS) on the presence of advanced periodontal disease in subjects from Southeastern and Northeastern Brazil.

In contrast with the other discussed reports, in the present study, there were no statistically significant differences in the GLT6D1 and CDKN2BAS polymorphisms frequencies, and this result could be due to the fact that genotype and allele frequencies commonly vary among different ethnic populations, and a genetic risk factor for disease susceptibility in one population may not be a risk factor in another population.[11,22] Moreover, the non-significant association could have also been as a result of the sample size, which may be considered small for a genetic study. On the other hand, despite the relatively small number of evaluated samples, our sampling criteria were carefully designed to ensure a clinically homogeneous sample of patients with severe phenotypes and early onset disease, thereby increasing the statistical power of the study.[2]

In conclusion, in our sample of Brazilian subjects, the polymorphisms rs1537415 (GLT6D1) and rs3217992 (CDKN2BAS) were not significantly associated with stage III/IV and Grade B/C periodontitis when compared to healthy controls, and grades B and C did not differ significantly.

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol. 2018 Jun;45(20 Suppl 20):S162-70. https://doi.org/10.1111/jcpe.12946
2. Vaithilingam RD, Safii SH, Baharuddin NA, Ng CC, Cheong SC, Bartold PM, et al. Moving into a new era of periodontal genetic studies: relevance of large case-control samples using severe phenotypes for genome-wide association studies. J Periodontal Res. 2014 Dec;49(6):683-95. https://doi.org/10.1111/jre.12167
3. Michalowicz BS, Diehl SR, Gunsolley JC, Sparks BS, Brooks CN, Koertge TE, et al. Evidence of a substantial genetic basis for risk of adult periodontitis. J Periodontol. 2000 Nov;71(11):1699-707. https://doi.org/10.1902/jop.2000.71.11.1699
4. Kinane DF, Shiba H, Hart TC. The genetic basis of periodontitis. Periodontol 2000. 2005;39(1):91-117. https://doi.org/10.1111/j.1600-0757.2005.00118.x
5. Kinane DF, Hart TC. Genes and gene polymorphisms associated with periodontal disease. Crit Rev Oral Biol Med. 2003;14(6):430-49. https://doi.org/10.1177/154411130301400605
6. Schaefer AS, Richter GM, Groessner-Schreiber B, Noack B, Nothnagel M, El Mokhtari NE, et al. Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. PLoS Genet. 2009 Feb;5(2):e1000378. https://doi.org/10.1371/journal.pgen.1000378
7. Schaefer AS, Richter GM, Dommisch H, Reinartz M, Nothnagel M, Noack B, et al. CDKN2BAS is associated with periodontitis in different European populations and is activated by bacterial infection. J Med Genet. 2011 Jan;48(1):38-47. https://doi.org/10.1136/jmg.2010.078998
8. Ernst FD, Uhr K, Teumer A, Fanghänel J, Schulz S, Noack B, et al. Replication of the association of chromosomal region 9p21.3 with generalized aggressive periodontitis (gAgP) using an independent case-control cohort. BMC Med Genet. 2010 Aug;11(1):119. https://doi.org/10.1186/1471-2350-11-119

9. Schaefer AS, Bochenek G, Manke T, Nothnagel M, Graetz C, Thien A, et al. Validation of reported genetic risk factors for periodontitis in a large-scale replication study. J Clin Periodontol. 2013 Jun;40(6):563-72. https://doi.org/10.1111/jcpe.12092
10. Schaefer AS, Richter GM, Nothnagel M, Manke T, Dommisch H, Jacobs G, et al. A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. Hum Mol Genet. 2010 Feb;19(3):553-62. https://doi.org/10.1093/hmg/ddp508
11. Hashim NT, Linden GJ, Ibrahim ME, Gismalla BG, Lundy FT, Hughes FJ, et al. Replication of the association of GLT6D1 with aggressive periodontitis in a Sudanese population. J Clin Periodontol. 2015 Apr;42(4):319-24. https://doi.org/10.1111/jcpe.12375
12. Jarinova O, Stewart AF, Roberts R, Wells G, Lau P, Naing T, et al. Functional analysis of the chromosome 9p21.3 coronary artery disease risk locus. Arterioscler Thromb Vasc Biol. 2009 Oct;29(10):1671-7. https://doi.org/10.1161/ATVBAHA.109.189522
13. American Academy of Periodontology. Parameter on aggressive periodontitis. J Periodontol. 2000;71(5):867-9. https://doi.com/10.1902/jop.2000.71.5-S.867
14. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J. 1975 Dec;25(4):229-35.
15. Trevilatto PC, Line SR. Use of buccal epithelial cells for PCR amplification of large DNA fragments. J Forensic Odontostomatol. 2000 Jun;18(1):6-9.
16. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J. 2007;18(2):148-52. https://doi.org/10.1590/S0103-64402007000200012
17. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. J Clin Periodontol. 2018 Jun;45(20 Suppl 20):S149-61. https://doi.org/10.1111/jcpe.12945
18. Brito LF, Taboza ZA, Silveira VR, Furlaneto FA, Rosing CK, Rego RO. Aggressive periodontitis presents a higher degree of bilateral symmetry in comparison with chronic periodontitis. J Oral Sci. 2018;60(1):97-104. https://doi.org/10.2334/josnusd.16-0669
19. Nibali L. Aggressive periodontitis: microbes and host response, who to blame? Virulence. 2015;6(3):223-8. https://doi.org/10.4161/21505594.2014.986407
20. Nibali L, Donos N, Henderson B. Periodontal infectogenomics. J Med Microbiol. 2009 Oct;58(Pt 10):1269-74. https://doi.org/10.1099/jmm.0.012021-0
21. Silveira VR, Pigossi SC, Scarel-Caminaga RM, Cirelli JA, Rêgo R, Nogueira NA. Analysis of polymorphisms in Interleukin 10, NOS2A, and ESR2 genes in chronic and aggressive periodontitis. Braz Oral Res. 2016 Oct;30(1):e105. https://doi.org/10.1590/1807-3107BOR-2016.vol30.0105
22. Taiete T, Casati MZ, Stolf CS, Corrêa MG, Santamaria MP, Andere NM, et al. Validation of reported GLT6D1 (rs1537415), IL10 (rs6667202), and ANRIL (rs1333048) single nucleotide polymorphisms for aggressive periodontitis in a Brazilian population. J Periodontol. 2019 Jan;90(1):44-51. https://doi.org/10.1002/JPER.18-0071
23. Schunkert H, Götz A, Braund P, McGinnis R, Tregouet DA, Mangino M, et al.. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation. 2008 Apr;117(13):1675-84. https://doi.org/10.1161/CIRCULATIONAHA.107.730614
24. Blaizot A, Vergnes JN, Nuwwareh S, Amar J, Sixou M. Periodontal diseases and cardiovascular events: meta-analysis of observational studies. Int Dent J. 2009 Aug;59(4):197-209.
25. Bochenek G, Häsler R, El Mokhtari NE, König IR, Loos BG, Jepsen S, et al. The large non-coding RNA ANRIL, which is associated with atherosclerosis, periodontitis and several forms of cancer, regulates ADIPOR1, VAMP3 and C11ORF10. Hum Mol Genet. 2013 Nov;22(22):4516-27. https://doi.org/10.1093/hmg/ddt299