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# Variants in interferon gamma inducible protein 16 (*IFI16*) and absent in melanoma 2 (*AIM2*) genes that modulate inflammatory response are associated with periodontitis

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ABSTRACT

*Objective:* Evaluate the association of genetic variants of the interferon gamma inducible protein 16 (*IFI16)* and absent in melanoma 2 (*AIM2)* genes with periodontitis.
*Methods:* The study involved 117 individuals with periodontitis and 389 without periodontitis, all Brazilians, miscegenated. Individuals with periodontitis presented at least 4 teeth with ≥ 1 site with probing depth ≥ 4 mm; clinical attachment level ≥ 3 mm on the same site and bleeding upon stimulus. Genotyping was performed using the Infinium Multi-Ethnic AMR/AFR-8 Bead Chip focused on Hispanic and African American populations with approximately 2 million markers of the human genome. Multivariate logistic regression was performed to identify associations in additive, dominant and recessive models adjusted for covariates age, obesity, mouth breathing, flossing, asthma, and ancestry.
*Results:* In *IFI16*, the rs75985579-A is positively associated with periodontitis in the additive (Odds Ratio adjusted (ORadjusted) 2.65, 95% confidence interval (CI):1.25–5.60, p value: 0.007) and dominant models (ORadjusted 2.56, 95%CI:1.13–5.81, p value: 0.017). In *AIM2*, the rs76457189-G, is associated negatively with periodontitis in two genetic models evaluated, additive (ORadjusted 0.21, 95%CI:0.05–0.94, p value: 0.022) and dominant (ORadjusted 0.21, 95%CI:0.05–0.94, p value: 0.022).
*Conclusions:* These results have shown that variants in the *IFI16* and *AIM2* genes are associated with periodontitis. Individuals with at least one A (adenine) allele of the rs75985579 (*IFI16*) are more than twice as likely to have periodontitis, while individuals with the G (guanine) allele of rs76457189 (*AIM2*) are less likely to be diagnosed with periodontitis, providing a negative association with periodontitis.

## 1. Introduction

Periodontitis is characterized by exaggerated inflammation of the connective tissues that support the tooth, resulting in loss of connective tissue, supporting alveolar bone, and eventually teeth. In clinically healthy patients, there is a symbiosis between microbiome and host (Suárez et al., 2020). In periodontitis, the intense inflammatory process is stimulated by a subgingival biofilm that is not periodically removed,

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favoring the development of keystone pathogens. Species such as *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia,* and *Fusobacterium nucleatum, among others,* influence the environment by releasing antigenic molecules, lipopolysaccharides, and virulence factors that stimulate a more intense and prolonged host response. In this context, there is increased development of pathobionts, establishing local dysbiosis (Hajishengallis, 2015; Meyle & Chapple, 2015; Zhang et al., 2020).

The proposal to establish only one factor responsible for dysbiosis, a key pathogen, has already been replaced by the polymicrobial concept, which involves a pathogenic microbial community, influenced by behavioral, environmental, and genetic risk factors (Avula & Chakravarthy, 2022; Deng et al., 2017; Nath & Raveendran, 2013). The pathogenesis of periodontitis may be related to different systemic diseases and various environmental factors, such as diet, smoking, stress, oral hygiene, among others; which, associated with genetic factors, are related to the development and severity of this disease at a certain point in life (Aljehani, 2014; Arboleda et al., 2019; Borojevic, 2012; Preshaw et al., 2012; Wadia, 2020; Wankhede et al., 2017). Therefore, genetics, lifestyle, and environmental factors jointly influence the inflammatory immune response that regulates the composition of the biofilm, creating a phenotype that hinders the success of preventive and treatment strategies (Meyle & Chapple, 2015).

In the inflammatory immune response, IFI16 (interferon gamma inducible protein 16) and AIM2 (absent in melanoma 2) proteins play a multifaceted role. They are members of the AIM2-like receptor family that act in innate immunity, are translated and co-localized in the cytosol, recognizing autologous bacterial, viral, or double-stranded deoxyribonucleic acid (dsDNA) fragments (Wang et al., 2018). Activated AIM2 is associated with the caspase recruitment domain and Caspase 1 proteins for the formation of inflammasome with expression of interleukins IL-1β, IL-18 and IL-33 and may cause pyroptosis (Vanhove et al., 2017). IFI16 has α and β isoforms with action wider than AIM2, strongly induced by interferon γ (IFN-γ) and to a lesser extent by interferon α. There is evidence that this protein is a transcription regulator for activating nuclear factor of kappa-light-chain-enhancer in B-cells (NF-κB) activity giving its pro-inflammatory characteristics (Choubey, 2012; Riva et al., 2020). IFI16 acts on the nucleus and cytosol and binds preferentially to double-stranded, as it also binds to single-stranded DNA structures and cruciform DNA (Choubey, 2012; Riva et al., 2020). Its role in the formation of cytosolic inflammasome occurs preferably with viral dsDNA, but not exclusively. It has been proven that the reduction of cytosolic IFI16 is related to the inhibition of interferon β production, therefore, it is an important DNA sensor that mediates the IFN type I response (Unterholzner et al., 2010).

IFI16 promotes the formation of the AIM2 inflammasome, competes with it for binding to the DNA fragment and heterodimerizes to it (Marchesan, 2020; Marchesan et al., 2020). These three actions modulate inflammation and are fundamental for the homeostasis of the tissue environment. A study demonstrated the role of IFI16 and AIM2 inflammasomes in periodontitis in a murine model, by blocking the formation of inflammasome, it reduced 50% of alveolar bone loss (Marchesan, 2020).

For more than 50 years, researchers have looked for answers to why some individuals are at higher risk of developing periodontitis than others (Gandhi & Kothiwale, 2012). Given the complexity of periodontitis, studies were extended beyond the well-established microbial cause (Gandhi & Kothiwale, 2012). Host genetic factors are often being cited as determinants for susceptibility to periodontitis. Genetic variants in associated genes, when present simultaneously, bring greater power over the result of worsening or providing protection against the disease (Lopes et al., 2020). The candidate genes *IFI16* and *AIM2* were selected as they are biologically associated genes due to the homology and co-expression of these proteins (Szklarczyk et al., 2019) and because there have been associations of genetic variants of these genes with increased clinical parameters of periodontitis and high levels of microorganisms in the subgingival plaque (Marchesan et al., 2017).

It is possible that there are increased risks in patients with inheritable elements of susceptibility and that diseases of different etiologies and complexities may be positively or negatively affected by high-risk or even common genetic variants (Gandhi & Kothiwale, 2012). This study investigates the association of genetic variants on the *IFI16* and *AIM2* genes with the presence of periodontitis.

## 2. Material and methods

### 2.1. Studied population

This is a cross-sectional study, nested in the Program for Control of Asthma in Bahia (ProAR) cohort in Salvador / Bahia – Brazil. This Program aims to investigate risk factors, endophenotypes, and biomarkers of severe asthma. Data for the study was collected from January 2013 to November 2014, in a convenience sample from 1179 unrelated individuals over eighteen years of age, genotyped. This study received National Research Ethics Commission (CONEP) approval with nº 15782, Presentation Certificate for Ethical Appreciation (CAAE) nº 25000.013834/2010–96 and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Written informed consent was obtained from each subject. All participants answered a structured questionnaire, through interviews, with information related to socioeconomic and demographic characteristics, lifestyle, and previous oral treatments (Soledade-Marques et al., 2018). Health history, such as hypertension, cardiovascular disease, kidney disease, and diagnosis of asthma were self-reported. Anthropometric measurements were taken, and the oral conditions of the participants were also verified by a trained periodontist. Anthropometric data were collected to evaluate obesity (WHO, 2000), including body weight, height, and the Body Mass Index (BMI) was calculated from the height and weight data (Gorman et al., 2012). BMI was categorized as $< 25$ kg/m$^2$ (without excess weight) and $\geq 25$ kg/m$^2$ (overweight/obese).

### 2.2. Definition of periodontitis

The oral examination was performed by a single trained periodontist (KSM) (Soledade-Marques et al., 2018). Intra-examiner reliability of recession and probing depth measurements was assessed using the Bland e Altman method (0.067 and 0.071, respectively) in 10% of the sample. The diagnosis of periodontitis occurred after checking at six sites of each tooth, excluding the third molars: recession measurement, probing depth, clinical attachment level, and bleeding on probing. Individuals with periodontitis presented at least 4 teeth with $\geq 1$ site with probing depth $\geq 4$ mm; clinical attachment level $\geq 3$ mm on the same site and bleeding upon stimulus (Gomes-Filho et al., 2018).

### 2.3. DNA extraction and genotyping

DNA extraction was performed from the peripheral blood samples according to the protocols of the QIAGEN kit (Gentra Puregene Blood Kit, Hilden, Germany). The samples were genotyped using the Multi-Ethnic Global Array, the Infinium Multi-Ethnic AMR/AFR-8 Bead Chip focused on Hispanic and African American populations with approximately 2 million markers of the human genome (www. ilumina.com). For this study, genetic information of variants in *IFI16* and *AIM2* genes was extracted between positions 158969766–159024491 and 159032274 – 159046556 (GRCh37), respectively from chromosome 1 (www.ncbi.nlm.nih.gov).

### 2.4. Data quality verification

In the quality control of participants, 528 individuals who were not evaluated for periodontitis were excluded. To avoid the occurrence of inflated data resulting in type I error, blood relatives ($n = 10$), duplicate

identification (n = 46) and data inconsistency (n = 41) which encompasses evaluation of anomalies, sex inconsistency, as well as individuals that did not present a minimum of 90% genotyping were also excluded. After checking these criteria, 506 adults made up the database (Fig. 1).

After extracting the information from the variants available in the database, the single nucleotide variants (SNV) that did not appear in at least 90% of the individuals, those that presented lower allele frequency (MAF) lower than 1% and markers with Hardy - Weinberg balance (HWE) less than 5% were excluded.

### 2.5. In silico analysis

*In silico* analyzes of bioinformatics for inference functional activity, molecular structure, and metabolic pathways of human proteins IFI16 and AIM2 were verified on the NextProt (Zahn-Zabal et al., 2020) (https://www.nextprot.org) and GeneCards (Stelzer et al., 2016) (https://www.genecards.org). The functions of the statistically significant SNV were verified in the NCBI (www.ncbi.nlm.nih.gov) and ENSEMBL database (Howe et al., 2021) (https://www.ensembl.org), as well as the putative regulatory potential in the RegulomeDB platform (Boyle et al., 2012) (https://regulomedb.org). The impact of variants and 1000 Genomes Phase 1 frequencies was analyzed on the platforms HaploReg version 4.1 (Ward & Kellis, 2012) (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php). Multi-tissue eQTL comparison was obtained from the GTEx portal (https://www.gtexportal.org/). Aggregation analysis based on the linkage disequilibrium was demonstrated with software Haploview® 4.2 (Barrett et al., 2005) (https://www.broadinstitute.org/haploview/haploview). The association of periodontitis with allele blocks was verified in the SNPSTATS platform (Solé et al., 2006) (https://snpstats.net). The results of this haplotype association and linkage disequilibrium were plotted in snp.plotter package (Luna & Nicodemus, 2007) in R version 4.0.3 (https://cran.microsoft.com/snapshot/2019–10–09/web/packages/snp.plotter/index.html).

### 2.6. Cytokine analysis

The serum concentration of Eotaxin-1 (Boström et al., 2015), tumor necrosis factor α (TNF-α), IFNγ, and the interleukins IL-1β, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A (Yucel-Lindberg & Båge, 2013), were measured in 296 samples using the HCYTOMAG-60 K assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Of the analyzed samples, 91 were from individuals with periodontitis and 205 from individuals without periodontitis. The minimum detectable concentration of each analyte was determined by the value of the detection limit of each analyte plus 2 times the standard deviation: Eotaxin-1 (6.8 pg/mL), TNF-α (1.1 pg/mL), IFN- γ (1.1 pg/mL), IL-10 (1.6 pg/mL), IL-12 (p70) (1.0 pg/mL), IL-13 (1.9 pg/mL), IL-17A (1.2 pg/mL), IL-1β (1.0 pg/mL), IL-5 (0.7 pg/mL), IL-6 (1.3 pg/mL), and IL-8 (0.7 pg/mL) (Fernandes et al., 2022).

### 2.7. Statistical analysis

The chi-square test was used to compare the groups with and without periodontitis in relation to independent variables. The best model for adjusting covariates was decided after binary logistic regression with the Hosmer Lemershow test performed in Statistical Package for the Social Sciences (SPSS) version 20.0 (Kremelberg, 2014). The results were compared with the Akaike information criterion stepwise algorithm (StepAIC) test in R version 4.0.3 (Z. Zhang, 2016). The appropriate model for adjustment in the association tests included the covariates age, obesity, mouth breathing, flossing, asthma, and ancestry (Daya et al., 2019) were added to the model in order to avoid the occurrence of type II error. The association tests between genetic variants in the *AIM2* and *IFI16* genes and the presence of periodontitis were performed in PLINK version 1.90 (Purcell et al., 2007) (https://zzz.bwh.harvard.edu/plink) by means of multivariate logistic regression in additive, dominant and recessive models, with 95% confidence interval (95% CI). The association measurement between variants and phenotype was expressed as odds ratio (OR) and 95% CI. The p-values and 10,000-fold permutational p-value were considered statistically significant when less than or equal to 0.05.

The gene-gene interaction was performed in SNPStats platform (Solé et al., 2006) (https://snpstats.net) through a logistic regression model between *IFI16* and *AIM2* with periodontitis, using the risk alleles of rs75985579-A and rs76457189-G, respectively, having as covariates age, obesity, mouth breathing habit, use of dental floss, presence of asthma and principal component of ancestry.

The influence of *IFI16* and *AIM2* genetic variants on cytokine production was verified. As a quality control, cytokines for which the detection limit was not reached in at least 90% of the individuals were excluded from the analysis, as well as the analyzes that presented in some group n < 5 were not performed. The normality of continuous variables was certified by the Kolmogorov-Smirnov test and due to the non-normal distribution, the non-parametric Mann Whitney test was applied. Analyzes were performed on SPSS version 20.0 (Kremelberg, 2014) and the graph in R version 4.0.3 (R: The R Project for Statistical Computing, n.d.).

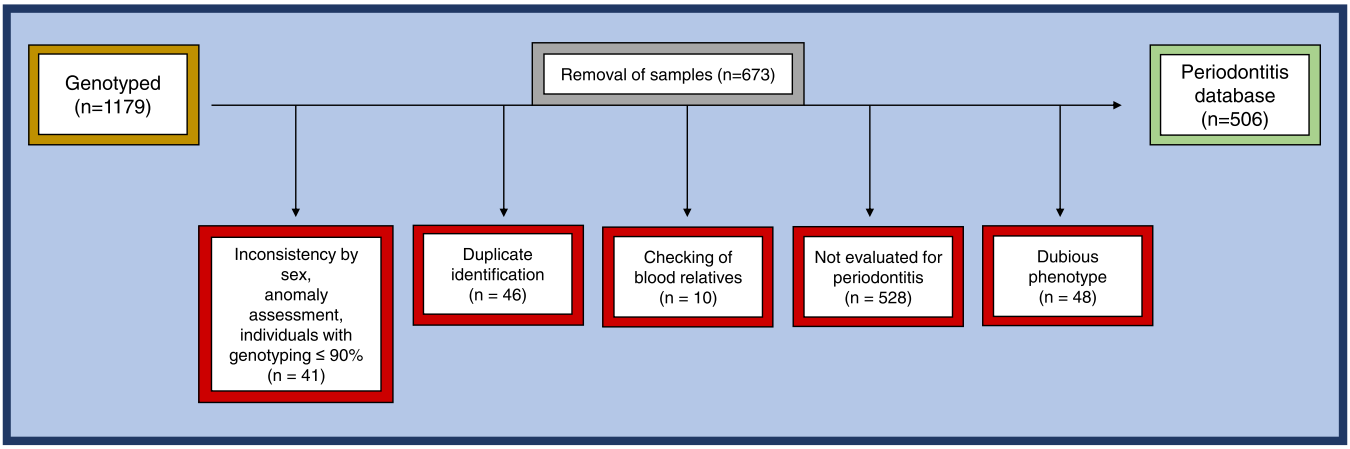


**Fig. 1.** Evaluation and verification of the quality of the sample to compose the periodontitis database. Of the 1179 participants, 673 were removed due to sex inconsistency, anomaly assessment, absence or low rate of genotyping, duplicate identity, blood relatives, not being analyzed for periodontitis, confirmation of phenotype. In this study, 506 adults made up the database.

## 3. Results

### 3.1. Studied population

The sample was of convenience, that is, all individuals from PROAR cohort who had data related to genotyping and who met the previously described quality criteria, totaling 506 individuals (85 males and 421 females), 117 with periodontitis and 389 without periodontitis were included in the sample of this study. Table 1 presents socioeconomic-demographic, related to lifestyle, general and oral health characteristics of the sample. As can be seen in this table, statistically significant differences between individuals with and without periodontitis were found in age, schooling level $\leq$ 4 years, overweight, obesity, diagnosis of asthma, hypertension, renal diseases, and cardiovascular diseases, visit to a dentist, daily mouthwash use, use of dental floss, mouth-breathing habit, and tooth loss ($p < 0.05$).

### 3.2. Description of the variants

From the total of 73 SNV in *IFI16* gene, thirty-four were excluded by the MAF and three by the HWE test. In *AIM2* gene from 37 variants, eighteen and two SNV were excluded, respectively, by the same criteria. None variant was excluded due to low genotyping of individual (mind > 0.1) or low genotyping of variant (geno > 0.1). After quality control, the study evaluated 36 variants on *IFI16* gene and 17 variants on *AIM2* gene (Supp. Table 1).

### 3.3. Associations between variants on IFI16 and AIM2 with periodontitis

Table 2 summarizes the statistically significant associations between variants on *IFI16* and *AIM2* with periodontitis. The other genetic variants analyzed were not associated with periodontitis (Supp. Table 1). In *IFI16*, the rs75985579-A was positively associated with periodontitis in the additive (ORadjusted 2.65, 95% CI 1.25–5.60, p value 0.007) and dominant (ORadjusted 2.56, 95%CI 1.13–5.81, p value 0.017) models. In *AIM2*, the rs76457189-G was associated with periodontitis in both, the additive and dominant models (ORadjusted 0.21, 95% CI 0.05–0.94, p value 0.022).

### 3.4. Functional impact of periodontitis-associated variants

The rs75985579-A and rs76457189-G are in introns. The rs75985579-A in *IFI16* had a frequency of 3% (MAF) in this population and its chromatin state is of strong transcription. In the 1000 Genomes Phase 1 Frequencies, the minor allele was frequent between 0% (Africans) to 9% (Europeans). The rs76457189-G in *AIM2* showed a frequency of about 2% (MAF) in this population. At 1000 Genomes Phase 1 Frequencies, it was found in between 2% and 4% of the reference populations. This variant has a significant regulatory potential with 2b score in the RegulomeDB (Table 3). This means that it can affect transcription factors binding, deoxyribonuclease Footprint, and deoxyribonuclease peak.

The regulatory characteristics of the analyzed variants and the tissues in which their possible regulatory effect have been indicated are presented in Table 4. Additionally, the table shows the presence of interferon regulatory factor (Irf) and Sodium/calcium exchanger, Nkx3, motif sequences that can change the accessibility of proteins to this region of DNA. Comparison of multi-tissue eQTL in whole blood of 670 showed a p-value of 2.0 × 10–6 for *IFI16* rsrs76457189. No significant eQTL was found for *AIM2* rs75985579 in tissue All eQTL Tissues.

**Table 1**
Distribution of socioeconomic demographic, related to lifestyle, general health, and oral health characteristics according to the presence of periodontitis (n = 506).

| Characteristics | Without periodontitis (n = 389) | | With periodontitis (n = 117) | | p-value* |
|---|---|---|---|---|---|
| | n | % | n | % | |
| > 39 years old | 253 | 65.0 | 88 | 75.2 | 0.040 |
| Women | 327 | 84.1 | 94 | 80.3 | 0.345 |
| Schooling level $\leq$ 4 years | 325 | 83.5 | 80 | 68.4 | 0.000 |
| Overweight[1] | 265 | 68.1 | 91 | 77.8 | 0.045 |
| Obesity[2] | 102 | 26.2 | 47 | 40.2 | 0.004 |
| Asthma | 219 | 56.3 | 95 | 81.2 | 0.000 |
| Hypertension | 99 | 25.4 | 43 | 36.8 | 0.017 |
| Renal diseases | 1 | 0.3 | 3 | 2.6 | 0.013 |
| Cardiovascular diseases | 10 | 2.6 | 8 | 6.8 | 0.029 |
| Never or $\geq$ 1 year/dentist | 204 | 52.4 | 76 | 65.0 | 0.017 |
| Daily mouthwash | 48 | 12.3 | 22 | 18.8 | 0.020 |
| Use of dental floss | 221 | 56.8 | 45 | 38.5 | 0.000 |
| Mouth-breathing habit | 228 | 58.6 | 91 | 77.8 | 0.000 |
| Lost tooth | 335 | 86.1 | 110 | 94.0 | 0.021 |

*Note:* *Pearson's chi-square test. [1]Dichotomized in BMI < and $\geq$ 25. [2]Dichotomized in BMI < and $\geq$ 30.

### 3.5. Haplotypes analysis

The degree of linkage disequilibrium in pairs was calculated for each pair of SNV. Considering determination coefficient $r^2 > 0.8$ as strong linkage disequilibrium (Santos Coelho et al., 2021), we did not find any pairs with SNV that were associated with periodontitis. However, association analyzes were performed in blocks of 2, 3 and 4 SNV always containing the SNV associated with periodontitis.

In blocks with 2 *IFI16* alleles the association with periodontitis was maintained in all 30 probabilities tested (Table 5). In blocks with 3 alleles, the global p-value was significant in 6 haplotype groups (Fig. 2A). Blocks with 4 alleles did not maintain at global p-values significance.

In the *AIM2* gene, the association with periodontitis was maintained only in 2 blocks of 2 SNV and 7 blocks of 3 SNV. Fig. 2B presents part of these results, at a small distance from the base pairs, with a linkage disequilibrium image below.

### 3.6. Gene-gene interaction

In the interaction between variants in the *IFI16* and *AIM2* genes, we found that the presence of 2 risk alleles increases by more than four times the chances of having periodontitis compared to individuals who have 1 or none of the risk alleles (ORadjusted = 4.61; 95%CI = 1.03 – 20.59; p-value = 0.017) (Table 6).

### 3.7. Influence of variants in AIM2 and IFI16 genes in the cytokine production

To analyze the association of genetic variants and cytokine production, a subsample with 296 patients who had data on cytokine levels were analyzed. Genotype individuals with at least one A allele showed higher eotaxin production than individuals carrying two G alleles ($median_{GG}$ = 40.13 pg/mL, Q1 = 33.76 pg/mL, Q3 = 46.52 pg/mL; $median_{GA + AA}$ = 67.94 pg/mL, Q1 = 49.61 pg/mL, Q3 = 96.03 pg/mL). There is a difference between the medians of the production values of the eotaxin-1 variable with the genotype variable in the dominant model of the rs75985579 polymorphism in *IFI16* gene. (Fig. 3). The cytokines TNF-α, IFNγ, IL-1β, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17A did not show significant results No association was found between the detection levels of the analyzed cytokines and the genotypes of the rs76457189 variant of the *AIM2* gene.

## 4. Discussion

This is the first study to evaluate the association of variants in the *IFI16* and *AIM2* genes with periodontitis in Brazilians, miscegenated, to the best of our knowledge. Among individuals of this sample, genetic

**Table 2**
Statistically significant associations between variants on *IFI16* and *AIM2* gene with the presence of periodontitis.

| Gene | SNV | Model | Geno | Without periodontitis (%) | With periodontitis (%) | OR adjusted | 95%CI | p perm |
|---|---|---|---|---|---|---|---|---|
| *IFI16* | rs75985579 | | GG | 372(95.63) | 105(89.74) | | | |
| | | ADD | AG | 17(4.37) | 10(8.55) | 2.65 | 1.25–5.60 | 0.007 |
| | | | AA | 0 | 2(1.71) | | | |
| | | DOM | GG | 372(95.63) | 105(89.74) | 2.56 | 1.13–5.81 | 0.017 |
| | | | AA+AG | 17(4.37) | 12(10.26) | | | |
| *AIM2* | rs76457189 | | AA | 363(93.56) | 115(98.29) | | | |
| | | ADD | GA | 25(6.44) | 2(1.71) | 0.21 | 0.05–0.94 | 0.022 |
| | | | GG | 0 | 0 | | | |
| | | DOM | AA | 363(93.56) | 115(98.29) | 0.21 | 0.05–0.94 | 0.022 |
| | | | GG+ GA | 25(6.44) | 2(1.71) | | | |

*Note:* 95%CI: confidence interval 95%. AA: adenine-adenine. ADD: aditive model. AG or GA: adenine-guanine. *AIM2*: absent in melanoma 2 gene. DOM: dominant model. Geno: genotype. GG: guanine-guanine. *IFI16*: interferon gamma inducible protein 16 gene. Model: logistic regression model. ORadjusted: odds ratio adjusted. p perm: permutational p-value in 10,000 times. SNV: single nucleotide variant.

**Table 3**
Functional analysis of *IFI16* and *AIM2* genes.

| Gene | SNV | MAF | A1 | A2 | Consequence | Regulome DB (score) | Chromatin state | HWE |
|---|---|---|---|---|---|---|---|---|
| *IFI16* | rs75985579 | 0.031 | A | G | intron | 7[a] (0.18412) | Strong transcription | 1 |
| *AIM2* | rs76457189 | 0.022 | G | A | intron | 2b[b] (0.7869) | Quiescent/low | 1 |

*Note:* [a]Prediction for SNV from RegulomeDB with score = 7: other and [b]2b: transcription factor binding + any motif + deoxyribonuclease footprint + deoxyribonuclease peak. A: adenine. A1: minor allele. A2: ancestral allele. *AIM2*: absent in melanoma 2 gene. G: guanine. HWE: Hardy Weinberg equilibrium. *IFI16*: interferon gamma inducible protein 16 gene. MAF: minor allele frequency. SNV: single nucleotide variant.

**Table 4**
In silico regulatory analysis of *IFI16* and *AIM2* genes.

| Gene | SNV | Regulatory Feature[a] | Protein bounds[b] | Motifs changed[c] | Epigenomics | | |
|---|---|---|---|---|---|---|---|
| | | | | | Osteoblast Primary Cells[d] | Adult Dermal Fibroblast Primary Cells[e] | Primary mononuclear cells from peripheral blood[f] |
| *IFI16* | rs75985579 | End of the flanking region | | Irf | H3K27ac_Enh | | H6K9ac_Pro |
| *AIM2* | rs76457189 | Regulatory region variant | p300 | Nkx3 | 2_TssAFlnk<br>2_PromU<br>H3K4me1_Enh<br>H3K4me3_Pro<br>H3K27ac_Enh | 7_Enh<br>14_EnhA2<br>H3K4me1_Enh<br>H3K4me3_Pro<br>H3K27ac_Enh | 15_EnhAF |

*Note:* a: source - https://www.ensembl.org. b to f: https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php. 2_PromU: promotor upstream TSS. 2_TssAFlnk: TSS active flanker. 7_Enh: enhancer. 14_EnhA2: active enhancer. 15_EnhAF: active enhancer flank. *AIM2*: absent in melanoma 2 gene. H3K4me1_Enh: metylation of lysine 4 on histone 3 with enhancer function. H3K4me3_Pro: metylation of lysine 4 on histone 3 with promoter function. H3K27ac_Enh: acetylation of lysine 27 on histone 3 with enhancer function. H6K9ac_Pro: acetylation of lysine 9 on histone 6 with promoter function. *IFI16*: interferon gamma inducible protein 16 gene. Irf: interferon regulatory factors. Nkx3: protein coding gene. p300:transcriptional coactivator. SNV: single nucleotide variant.

variants rs75985579 (allele A) in the *IFI16* gene presented almost three times greater chance of having the presence of periodontitis, in contrast, rs76457189 (allele G) in the *AIM2* gene reduced the chances of having the diagnosis of periodontitis by approximately 80%. In association studies, it has been shown that there is an unexpected correlation when alleles are inherited together, forming haplotypes (Barrett et al., 2005). Thus, we found, by gene-gene interaction evaluation, that if the polymorphic alleles of each gene are inherited together, the probability of the presence of periodontitis increases more than four times.

The *IFI16* and *AIM2* genes have already been studied and related to clinical and microbiological aspects that are used in the diagnosis, monitoring and research of periodontitis. The study selected haplotypes (rs6940 and rs1057028) in *IFI16* from a genome-wide association scan (GWAS) in 4910 European American individuals and associated them with an increase in the percentage of clinical parameters of periodontitis and levels of periodontal microorganisms (Marchesan et al., 2017). In the same study, the rs2814770 on *AIM2* presented a suggestive association with periodontitis by upregulation in the periodontium of patients (Marchesan et al., 2017). These 3 variants (rs6940, rs1057028, rs2814770) were present in our study, but without statistically significant association with periodontitis, probably due to difference in the genetic composition of the studied populations and the limited population size.

The *IFI16* and *AIM2* genes have been associated as candidate genes for periodontitis in 3 case-control studies (Li et al., 2020; Marchesan et al., 2017; Offenbacher et al., 2016). A genome-wide association study used principal component analysis to examine *loci* associated with periodontal microbial complexes associated with the *IFI16* gene compared to the Socransky microbial complex, as well as association *IFI16* and *AIM2* with six periodontal pathogens, both in the region of the top SNV rs1633266. Variants in these genes have been shown to lead to alterations in the processing of invasive intracellular oral pathogens (Offenbacher et al., 2016). *Porphyromonas gingivalis* and other oral pathogens activate inflammasomes formed with AIM2 and induce cleavage of pro IL-1β and pro IL-18 into its active forms IL-1β and IL-18, respectively (Park et al., 2014). Excess of IL-1β is associated with periodontal destruction through inflammatory cell death, pyroptosis, to reduce the replicative niche of intracellular pathogens (Park et al., 2014). The IL-18 is a proinflammatory cytokine that induces the expression of IFN-γ and TNF and impairs the expression of IL-10 (de

**Table 5**
Association measurements between *IFI16* haplotypes, formed by the minor allele (A) of rs75985579 and the ancestral alleles of the studied variants, with periodontitis.

| SNV | Ancestral allele | Freq | ORadjusted (95%CI) | p-value | Global p |
|---|---|---|---|---|---|
| rs4657616 | A | 0.031 | 2.62 (1.24–5.55) | 0.012 | 0.039 |
| rs856060 | A | 0.031 | 2.59 (1.22–5.47) | 0.013 | 0.027 |
| rs2276404 | A | 0.031 | 2.64 (1.24–5.64) | 0.012 | 0.04 |
| rs146748131 | A | 0.031 | 2.57 (1.22–5.43) | 0.014 | 0.014 |
| rs12756557 | G | 0.031 | 2.72 (1.29–5.77) | 0.009 | 0.026 |
| rs856064 | G | 0.031 | 2.66 (1.24–5.69) | 0.012 | 0.04 |
| rs142942227 | G | 0.031 | 2.67 (1.26–5.64) | 0.011 | 0.037 |
| rs866484 | G | 0.031 | 2.54 (1.19–5.43) | 0.017 | 0.034 |
| rs16841500 | G | 0.031 | 2.73 (1.29–5.79) | 0.0091 | 0.022 |
| rs12057410 | G | 0.031 | 2.68 (1.26–5.70) | 0.01 | 0.038 |
| rs140895207 | A | 0.031 | 2.69 (1.27–5.70) | 0.0099 | 0.025 |
| rs861318 | G | 0.031 | 2.63 (1.23–5.60) | 0.013 | 0.04 |
| rs1057027 | A | 0.031 | 2.67 (1.25–5.70) | 0.011 | 0.04 |
| rs1057028 | T | 0.031 | 2.66 (1.25–5.68) | 0.012 | 0.04 |
| rs74122227 | A | 0.03 | 2.82 (1.31–6.05) | 0.0083 | 0.023 |
| rs16841532 | A | 0.031 | 2.68 (1.26–5.71) | 0.011 | 0.039 |
| rs72709518 | A | 0.031 | 2.68 (1.27–5.67) | 0.01 | 0.034 |
| rs1633264 | G | 0.031 | 2.68 (1.27–5.67) | 0.01 | 0.031 |
| rs1772408 | G | 0.031 | 2.70 (1.26–5.77) | 0.011 | 0.038 |
| rs1633266 | A | 0.031 | 2.67 (1.25–5.70) | 0.011 | 0.04 |
| rs3754460 | A | 0.031 | 2.63 (1.24–5.58) | 0.012 | 0.04 |
| rs142854172 | T | 0.031 | 2.65 (1.25–5.61) | 0.011 | 0.04 |
| rs115506051 | A | 0.031 | 2.61 (1.24–5.53) | 0.012 | 0.034 |
| rs2793843 | G | 0.031 | 2.67 (1.26–5.65) | 0.011 | 0.035 |
| rs3018316 | A | 0.031 | 2.67 (1.26–5.67) | 0.011 | 0.039 |
| rs73023727 | A | 0.03 | 2.70 (1.27–5.77) | 0.01 | 0.03 |
| rs116744790 | A | 0.031 | 2.65 (1.25–5.60) | 0.011 | 0.04 |
| rs74122246 | A | 0.03 | 2.81 (1.31–6.05) | 0.0083 | 0.023 |
| rs59710606 | G | 0.031 | 2.63 (1.25–5.57) | 0.012 | 0.04 |
| rs6940 | A | 0.031 | 2.62 (1.23–5.54) | 0.012 | 0.037 |

*Note:* 95%CI: confidence interval 95%. A: adenine Freq: haplotype frequency. G: guanine. Global p: global test for interaction. ORadjusted: odds ratio adjusted for covariables. SNV: single nucleotide variant.

Andrea et al., 2020). IL-18 was essential for antimicrobial peptide production and epithelial proliferation in response to injury (Vanhove et al., 2017).

In periodontitis, there is an imbalance in the expression of pro-inflammatory and anti-inflammatory cytokines, chemokines, arachidonic acid metabolites and proteolytic enzymes in a cascade of events susceptible to complex modulatory effects (Wu et al., 2016; Yucel-Lindberg & Båge, 2013; W. Zhang et al., 2018). We checked eotaxin-1, TNF-α, IFNγ, and the interleukins IL-1β, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A that participate in this process. In the inflammatory process, cells of the Th1 profile are activated by IL-1α and IL-1β; secrete IL-2, IFN-γ that increase the synthesis of TNF-α by macrophages; and proliferate with IL-12 stimulation. The differentiation of Th2 profile cells involves multiple signals that culminate in the induction of the GATA3 transcription factor that promotes the transcription of IL-1, IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 generating a humoral response, mainly of an anti-inflammatory nature, inhibiting the proliferation and activity of IL-12 and TNF-α, blocking the activity of Th1 cells. Regulatory T cells arise in the presence of TGF-β and secrete IL-10 and more TGF-β. Also, there is increased expression of IL-17, which is a marker of increased presence of Treg and Th17 cells. Th17 cells, in addition to secreting family 17 interleukins, modulate the activities of the innate system through the regulation of macrophages and neutrophils, stimulate the production of TNF-α, IL-6, IL-1β and PGE2, inducing the differentiation and activation of osteoclasts and increased expression of RANK receptor ligand (RANKL) by osteoblasts (Hajishengallis, 2015; Meyle & Chapple, 2015; Yucel-Lindberg & Båge, 2013). We found a statistically significant difference between the median values for eotaxin-1 production and the presence of one or two A alleles of rs75985579 in the *IFI16* gene. Eotaxin-1 has chemoattractant action for immune cells through its C-C chemotherapy receptor motif, CCR3. One study detected the presence of eotaxin-1 in gingival fibroblasts stimulated with pro-inflammatory cytokines, however, the expression of this chemokine is proportional to the longer time and the greater intensity of the inflammatory stimulus in these cells (Boström et al., 2015), a fact that reinforces our finding.

The strength with which these genetic variants will impact the individual is related to the probability and intensity in the regulation of gene transcription, in the stimulation of innate immunity, as well as in the tissues in which they are frequently expressed (Choubey, 2012; Lloyd et al., 2018; S. Zhang et al., 2020). The intronic SNV analyzed in *IFI16* presents the chromatin state in strong transcription and is located at the end of the flanking region. The Irf motif, in *IFI16*, is an important site to modulate the inflammatory response. Irf transcription factors are a family of interferon regulatory factors. Irf3 and NF-kB are activated by IFI16 for the expression of interferon type I (IFN-β) (Marchesan et al., 2017; Unterholzner et al., 2010). In *in silico* platforms, there is no expression of these gene in gingival tissues, however, one study showed a greater expression of IFI16 inflammasomes in gingival tissues in mice (Marchesan, 2020). Much higher levels of inflammasome components were detected in the gingival tissues of patients with chronic periodontitis in a case-control study (Park et al., 2014). Although rs75985579 does not show linkage disequilibrium with SNV with more relevant functions, the formation of haplotypes between up to 4 variants in the *IFI16* gene in this population did not interfere in the association with the predisposition to the disease.

In the *AIM2* gene, the intron rs76457189-G is situated in a regulatory region and has a quiescent/low chromatin state that agrees with the high methylation of osteoblast primary cells and adult dermal fibroblast primary cells. Studies on the interaction of the *AIM2* gene with the structural motifs of Nkx3 may help to understand how genetic variants of *AIM2* respond in the presence of periodontitis. In vitro, there is a lower expression of Nkx3 in tissues with a high presence of pro-inflammatory cytokines such as TNF and IL-1β (Antao et al., 2021), while the AIM2 inflammasome induces the differentiation of pro-IL-1β into IL-1β. However, much remains to be understood about the relationship between this transcription factor and the *AIM2* gene, especially in periodontitis.

Understanding how the IFI16 and AIM2 proteins interact is fundamental for this interpretation of the results of this study. Previous studies have highlighted the role of inflammasomes in periodontitis, particularly the interaction between IFI16 and AIM2 proteins and inflammasomes (Marchesan, 2020; Marchesan et al., 2020; Wang et al., 2018). AIM2 has both inflammasome-dependent and inflammasome-independent actions (Vanhove et al., 2017). *AIM2* knockout mice had greater inflammation than wild-type mice. This may have occurred because the independent actions of the inflammasome converge with the dependent ones, preventing uncontrolled inflammation, maintaining homeostasis. In inflammasome-independent actions, it is suggested that AIM2 inhibits the phosphorylation of the serine/threonine kinase-AKT, when it is bound to DNA-dependent protein kinase (DNA-PK) (Vanhove et al., 2017). This restriction results in a cascade of reactions involving phosphoinositol 3 (PI3) kinase, mammalian target of rapamycin (mTOR) and other transcription factors that interrupt cell proliferation and survival (Vanhove et al., 2017). However, because it has a multifaceted role in the inflammatory process, in the absence of physical interaction with DNA-PK, the AIM2 protein can phosphorylate the AKT protein kinase (Vanhove et al., 2017). The inflammasome-dependent actions of AIM2 are modulated by IFI16 which inhibits the conversion of pro-caspase to caspase, interfering with the formation of pro-inflammatory cytokines (Marchesan et al., 2020; Wang et al., 2018; Yucel-Lindberg & Båge, 2013). Therefore, the presence of genetic variants in the *IFI16* and *AIM2* genes are significant for the imbalance of homeostasis in the periodontal region.

This study has an important construction of scientific knowledge, since the therapies used in the control of periodontitis are not successful in all the cases, and for these, individualized therapies, such as

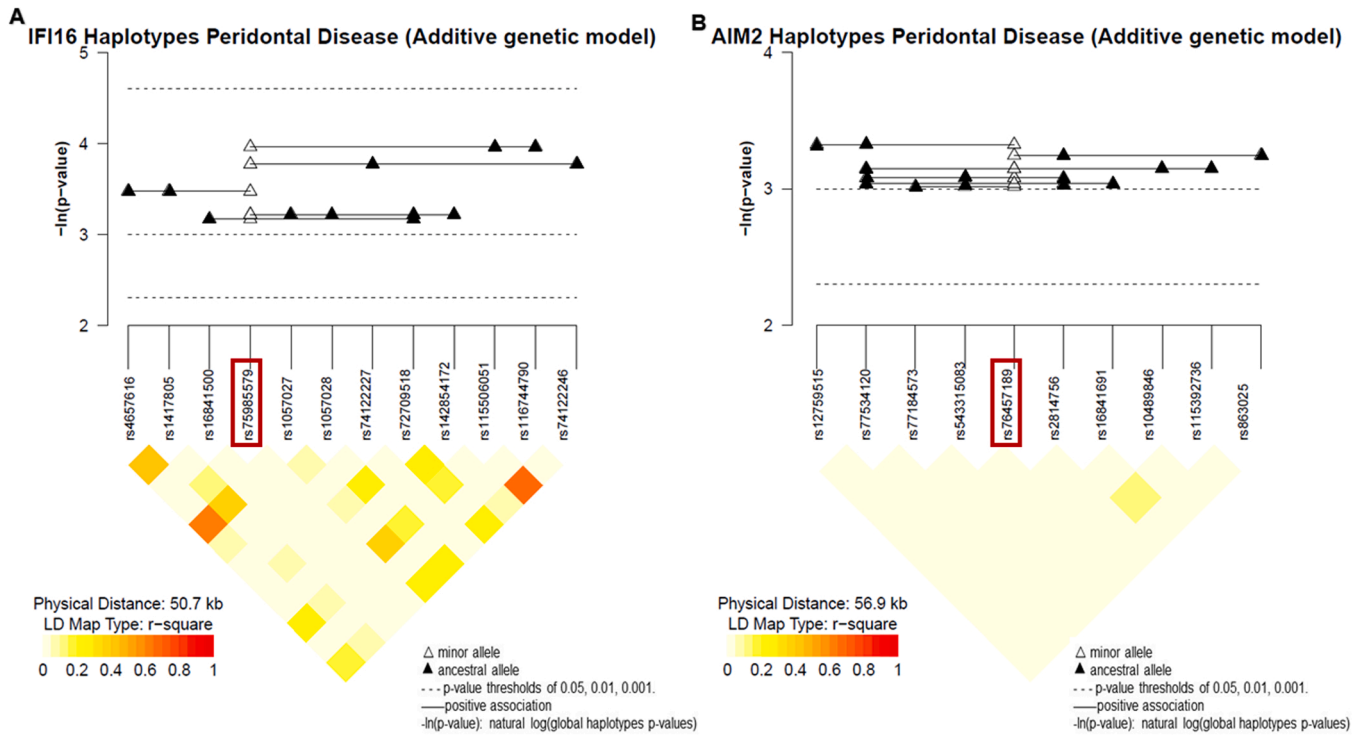


**Fig. 2. :** Association between blocks of 3 SNV in the *IFI16* (A) and *AIM2* (B) genes with periodontitis. The transparent triangles represent the minor allele of the variant associated with periodontitis, while the bold triangles are the ancestral alleles of the other variants present in the analyzed population. The line joining the triangles represents the presence of an association between each block and periodontitis with p values between 0.01 and 0.001. Image generated through the SNPStats.

**Table 6**
Association measurement between the concomitant presence of polymorphic alleles of variants rs75985579 and rsrs76457189 of genes IFI16 and AIM2, respectively, with the presence of periodontitis.

| Number of alleles | Risk alleles | Without periodontitis (%) | With periodontitis (%) | ORadjusted | IC95% | p value |
|---|---|---|---|---|---|---|
| ≤ 1 | G or A or none | 25(6.8) | 2(1.8) | | | |
| 2 | G and A | 343(93.2) | 108(98.2) | 4.61 | 1.03–20.59 | 0.017 |

*Note:* 95%CI: confidence interval 95%. A: adenine. G: guanine. ORadjusted: odds ratio adjusted.

therapeutic inhibition of the inflammasomes, might bring benefits greater than current conventional ones (Marchesan, 2020). However, some weaknesses were observed, such as the low frequency of the minor allele and a population with a restricted size that is reflected in the result borderline of the interaction analysis between the *AIM2* and *IFI16* genes that are so reported in the literature (Marchesan, 2020; Marchesan et al., 2017; Vanhove et al., 2017; Wang et al., 2018). However, we recognize that sample size may be the main factor responsible for the loss of statistical power.

## 5. Conclusion

The presence of the A allele of rs75985579 in the *IFI16* gene was associated with periodontitis and the eotaxin-1 production. In contrast, in the *AIM2* gene, the presence of the G allele of rs76457189 was associated with a lower chance of developing periodontitis. This variant is in a regulatory region and its chromatin state favors high methylation of osteoblasts and dermal fibroblasts. The joint inheritance of the two minor alleles increases the probability of having periodontitis by more than four times. This study will add to current knowledge of studies already carried out and inform future studies, that are needed to assess *IFI16* and *AIM2* as possible targets for new treatments of periodontitis.

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## CRediT authorship contribution statement

**Marcia Otto Barrientos** – (1) The conception and design of the study, analysis and interpretation of data, (2) drafting the article, (3) final approval of the version to be submitted. **Álvaro A. Cruz** – (1) The conception and design of the study, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Helena M.P. Teixeira** - (1) Analysis and interpretation of data, (2) drafting the article, (3) final approval of the version to be submitted. **Hátilla dos Santos Silva** - (1) Analysis and interpretation of data, (2) drafting the article, (3) final approval of the version to be submitted. **Isaac Suzart Gomes-Filho** – (1) The conception and design of the study,

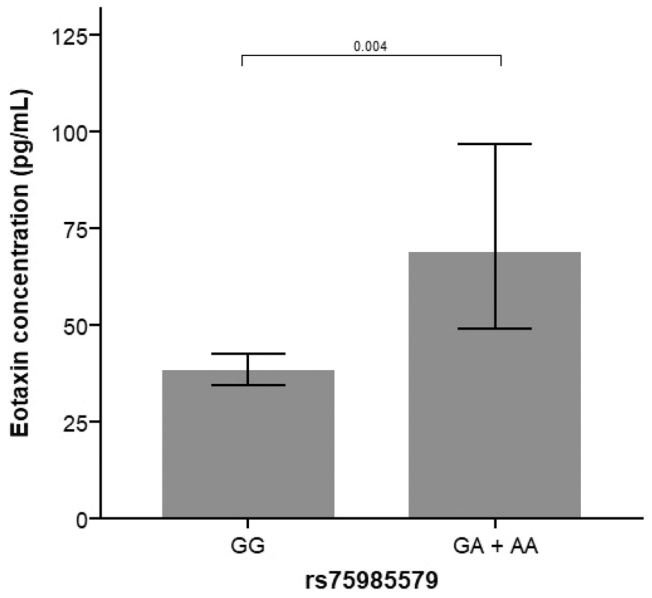


**Fig. 3. :** Influence of rs75985579 of the *IFI16* gene in eotaxin-1 production. Subsample of 296 individuals. Individuals carrying one or two A alleles of the rs75985579 variant have higher concentrations of eotaxin than individuals carrying two G alleles of the same variant. P value equal to 0.004 Mann Whitney test. Boxplot data refer to median and interquartile range.

(2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Soraya Castro Trindade** – (1) The conception and design of the study, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Kaliane Rocha Soledade**- (1) Acquisition of data, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Jamille Souza Fernandes** – (1) Acquisition of data, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Cinthia Vila Nova Santana** (1) Acquisition of data, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Gabriela Pimentel Pinheiro** – (1) Acquisition of data, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Adelmir Souza-Machado** - (1) The conception and design of the study, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Ryan dos Santos Costa** – (1) The conception and design of the study, analysis and interpretation of data, (2) drafting the article, revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Camila A. Figueiredo** – (1) The conception and design of the study, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. **Tatiane Teixeira M. Carletto Oliveira** – (1) The conception and design of the study, analysis and interpretation of data, (2) drafting the article, revising it critically for important intellectual content, (3) final approval of the version to be submitted.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2023.105640.

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