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MARCIA OTTO BARRIENTOS

ESTUDO DE ASSOCIAÇÃO GENÉTICA PARA PERIODONTITE EM INDIVÍDUOS DE SALVADOR/BA

TESE DE DOUTORADO

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MARCIA OTTO BARRIENTOS

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Tese apresentada ao Programa de Pós-graduação em Imunologia, da Universidade Federal da Bahia como requisito parcial para obtenção do título de Doutora em Imunologia.

Orientador: Prof. Dr. Ryan dos Santos Costa

Coorientadora: Profa. Dra. Tatiane de Oliveira Teixeira Muniz Carletto

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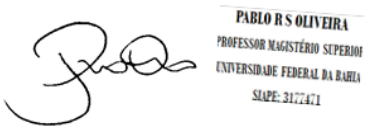

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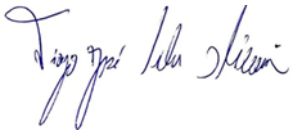

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Dedico

Ao meu companheiro e amor de toda vida, Juan e as preciosidades que carregam meu DNA mitocondrial, Aileen Marián e Janine.

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Ao Autor e mantenedor da Vida que colocou em mim sonhos, planos e que satisfaz os desejos do meu coração, antes mesmo de que eu os conheça.

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Aos pesquisadores que construíram o alicerce desta pesquisa e aos que já

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Mais que epígrafe...

Eu não tive a genialidade para criar este texto, mas ao lê-lo no dia 22/10/2018, quando ainda estava preparando o projeto de pesquisa para a admissão neste programa de doutorado, o salvei com o título: Prólogo mais interessante que já li. Ele me representa.

"E se, ao invés de ter dito, Deus tivesse escrito ...
[0]No princípio o tudo era nada e o nada era tudo o que existia. Então, Deus escreveu:

$$V = H_0.r$$

... e de um único ponto do nada, surgiu o novo tudo, com suas dimensões espaciais e temporais; e Ele viu que era bom.
[1]O novo tudo ainda era muito parecido com o nada do antigo tudo. Então, Deus escreveu:

$$\vec{\nabla}\cdot\vec{E}=\frac{\rho}{\varepsilon_0} \qquad \vec{\nabla}\cdot\vec{B}=0$$

$$\vec{\nabla}\times\vec{E}=-\frac{\partial\vec{B}}{\partial t} \qquad \vec{\nabla}\times\vec{B}=\mu_0\vec{J}+\varepsilon_0\mu_0\frac{\partial\vec{E}}{\partial t}$$

... e fez-se a luz. E Ele viu que isso também era bom: primeiro dia.
[2]A luz era maravilhosa, mas não havia o que ser iluminado. Então, Deus escreveu:

$$\hat{\mathrm{H}}\psi(x)=\mathrm{E}\psi(x)$$

$$F=\frac{mMG}{d^2}$$

... e se formaram as poeiras cósmicas que se uniram gerando corpos celestes, inclusive um denominado por Ele de "Terra"; e ele viu que isso era bom: Segundo dia.
[3]Mas àquele novo corpo não caberia uma eternidade.... Então Deus escreveu:

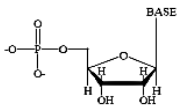
$$H_2 + O_2 \rightarrow H_2O$$

... e fez-se a água. Deus escreveu ainda:

... e aos aglomerados destes, chamou de continentes; e ele viu que isso era bom: Terceiro dia.
[4]Mas as Suas criações ainda caminhavam isoladas e não se encontravam em um ponto comum. Então Deus escreveu:

$$4\ {}^{1}H + e^{-} \rightarrow {}^{4}He + 2\upsilon + 6\gamma$$

... e fizeram-se as estrelas, iluminando e aquecendo seus arredores por todo o Universo, inclusive o corpo chamado Terra. Ele viu que isso era bom: Quarto dia.
[5]Vendo que tudo ainda era tão triste, Deus escreveu:



... e fez-se a vida e toda a sua diversidade. Deus viu que isso era bom: Quinto dia.
[6]Deus sentia que sua obra ainda estava incompleta. Então, Deus moldou uma criação especial e, ao invés de escrever ou simplesmente dizer, Ele deu Seu coração e amor a estes seres, que foram diferenciados entre homens e mulheres. Ele viu tudo quanto fizera e era muito bom. Sexto dia.
[7]Ao sétimo dia, após completar a obra, descansou, abençoou, santificou e se esqueceu (ou não...) de contar ao ser humano o que ele era e como funcionava o novo tudo...

... e fez-se a CIÊNCIA.

Para ler mais:

[0]A lei de Hubble descreve os movimentos galácticos e é a base da Teoria da Expansão do Universo. Vide: Hawking, S.: *"O Universo numa Casca de Noz",* 2ª ed.; Mandarim, São Paulo, 2002, pp. 76-77.

[1]As equações de Maxwell são extensões das leis de Gauss, da indução de Faraday e de Ampère e descrevem o comportamento da luz, bem como sua interação com a matéria. Vide: Halliday, D.; Resnick, R.; Walter, J. *"Fundamentos de Física – v.3:Eletromagnetismo"*, 4ª ed.; LTC Editora, Rio de Janeiro, 1996, pp. 309-316.

[2]A equação de Schrödinger descreve as evoluções temporais de sistemas físicos definidos pela Mecânica Quântica, que caracterizam a matéria. Vide: McQuarie, D.A.; Simon, J.D. *"Physical Chemistry: A molecular approach"* University Science Books, Sausalito, EUA, 1997, pp. 73-75. A Lei da Gravitação Universal de Newton define a força de atração entre dois corpos devido às suas massas. Vide: Halliday, D.; Resnick, R.; Walter, J. *"Fundamentos de Física – v.1:Mecânica",* 4ª ed.; LTC Editora, Rio de Janeiro, 1996, pp. 86-87.

[3]A água foi primeiramente formada pela reação dos gases hidrogênio e oxigênio, iniciada por raios elétricos. Vide: Atkins, P.; Jones, L. *"Princípios de Química: Questionando a Vida Moderna e o Meio Ambiente"*, 3ª ed.; Bookman Editora, São Paulo, 2006. E mais: Teixeira, W.; de Toledo, C.M.; Fairchild, T.R.; Taioli, F. (organizadores); *"Decifrando a Terra"*, Oficina de Textos, São Paulo, 2000. A crosta terrestre é formada principalmente pelos elementos Si e O que formam, dentre outras, as estruturas dos tectossilicatos. Vide: Schirver, D.F.; Atkins, P.W. *"Química Inorgânica",* 3ª ed., Bookman Editora, São Paulo, 2003, pp. 395-400. E mais: Teixeira, W.; de Toledo, C.M.; Fairchild, T.R.; Taioli, F. (organizadores); *"Decifrando a Terra"*, Oficina de Textos, São Paulo, 2000.

[4]A energia das estrelas provém de 4 átomos de hidrogênio formando um átomo de hélio e partículas subatômicas. Vide: Halliday, D.; Resnick, R.; Walter, J. *"Fundamentos de Física – v.4: Ótica e física moderna",* 4ª ed.; LTC Editora, Rio de Janeiro, 1996, pp. 285-289.

[5]Os ribonucleotídeos se unem formando os ácidos ribonucléicos (RNA), um dos componentes essenciais à autoreplicagem de moléculas, propriedade na qual se baseia a vida. Vide: Voet, D.; Voet, J.G.; Pratt, C.W. *"Fundamentos de Bioquímica"*, Artmed editora, São Paulo, 2002, p. 43 – 50.

[6]Inúmeras formas do amor de Deus são narradas na Bíblia Sagrada. Vide: Peterson, E.H.; *"A Mensagem: Bíblia em Linguagem Contemporânea"*, Editora Vida, São Paulo, 2011.

[7]O sétimo dia Ele nos deixou como incentivo ao conhecimento e à busca de Seus maiores mistérios. Vide: Timm, A.R. *"O Sábado na Bíblia*", Casa Publicadora Brasileira, Tatuí, 2010."

RESUMO

INTRODUÇÃO: A periodontite é um processo inflamatório consequente da disbiose do biofilme bacteriano estimulado por fatores modificáveis, relacionados ao estilo de vida, ou não modificáveis, nos quais variantes genéticas se enquadram. OBJETIVO: Realizar estudo de associação genética para identificar variantes que estejam associadas com a periodontite em uma população de Salvador. METODOLOGIA: Estudo transversal (n=506) desenvolvido em participantes do Programa para Controle de Asma na Bahia, classificados com presença (n=117) ou ausência (n=389) de periodontite, de acordo com critérios de Gomes Filho et al.(2007). Genotipagem foi realizada usando Illumina Multi-Ethnic Global Array (MEGA, Illumina), que inclui mais de 1,5 milhões de variantes. Foram realizados estudos para o melhor modelo de ajuste das análises, dos indicadores de risco, de associação positiva ou negativa de genes candidatos e estudo de ampla varredura genômica para associação com a periodontite. RESULTADOS: O melhor modelo de ajuste para regressão logística inclui as variáveis idade, escolaridade, obesidade, respiração pela boca, uso do fio dental e asma. Obesidade, o não uso de fio dental e asma são indicadores de risco para desenvolvimento da periodontite. Alelo A no rs75985579 do gene *IFI16* está associado positivamente e alelo G no rs76457189 do gene *AIM2* está associado negativamente com periodontite. A interação entre essas variantes apresentou que a presença dos 2 alelos de risco aumenta em mais de quatro vezes as chances de ter periodontite em comparação com indivíduos que possuem 1 ou nenhum dos alelos de risco (ORajustado = 4,61; IC 95% = 1,03 - 20,59; valor p = 0,017). No estudo de ampla varredura genômica, há associação estatisticamente significativa entre rs10496038-T do gene *RTN4*, rs58327429-C e rs67797971-A do gene *LINC02505* e associação sugestiva de 130 variantes com a presença de periodontite em uma população de Salvador. No cromossomo 2, um haplótipo de dois loci (rs10496038-rs74410951) pertencente aos genes *RTN4* e *MTIF2*, respectivamente, tem sido associado a periodontite. No cromossomo 3, dois haplótipos de dois loci (rs74635888-rs11706761, rs114884128-rs73162961) e um haplótipo de cinco loci (rs710479-rs710480-rs850306-rs115314220-rs9990329) foram associados à periodontite. No cromossomo 5, indivíduos que herdam conjuntamente os alelos G e C do haplótipo de dois loci (rs57620661-rs73054303) do gene *CTNND2* aumentam em 2,64 vezes as chances de desenvolver periodontite. Na interação entre genes, herdabilidade de 5 alelos de risco ou mais de variantes que apresentaram alto desequilíbrio de ligação está positivamente associada a cinco vezes mais para as chances de um indivíduo desenvolver periodontite em algum momento da vida. Estes resultados são o alicerce para muitos outros estudos de confirmação de associação em outras populações, bem como de alvos terapêuticos para o tratamento e controle da periodontite.

Palavras-chave: GWAS. Genes candidatos. Periodontite. Imunogenética. Polimorfismo de nucleotídeo único.

ABSTRACT

INTRODUCTION: Periodontitis is an inflammatory process resulting from bacterial biofilm dysbiosis stimulated by modifiable, lifestyle-related or non-modifiable factors, which include genetic variants. OBJECTIVE: To carry out a genetic association study to identify variants associated with periodontitis in a population of Salvador. METHODOLOGY: Cross-sectional study (n = 506) developed in participants of the Asthma Control Program in Bahia, who were classified as having the presence (n = 117) or absence (n = 389) of periodontitis, according to criteria by Gomes Filho et al. (2007). Genotyping was performed using Illumina Multi-Ethnic Global Array (MEGA, Illumina), which includes more than 1.5 million variants. The studies were performed for the best fit model of the analyses, risk indicators, positive or negative association of candidate genes and a large genomic scanning study for association with periodontitis. RESULTS: The best-fit model for logistic regression included the variables age, education, obesity, mouth breathing, flossing and asthma. Obesity, no flossing and asthma are risk indicators for the development of periodontitis. The A allele at rs75985579 of the *IFI16* gene is positively associated and the G allele at rs76457189 of the *AIM2* gene is negatively associated with periodontitis. The interaction between these variants presented that the presence of the 2 risk alleles increases the chances of having periodontitis by more than four times 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). In the genomic wide association study, there is a statistically significant association between rs10496038-T in *RTN4* gene, rs58327429-C and rs67797971-A in *LINC02505* gene and suggestive of 130 variants with the presence of periodontitis in a population of Salvador. On chromosome 2, a two-locus haplotype (rs10496038-rs74410951) belonging to the *RTN4* and *MTIF2* genes, respectively, has been associated with periodontitis. On chromosome 3, two two-loci haplotypes (rs74635888-rs11706761, rs114884128-rs73162961) and one five-loci haplotype (rs710479-rs710480-rs850306-rs115314220-rs9990329) have been associated with periodontitis. On chromosome 5, individuals who jointly inherit the G and C alleles of the haplotype of two loci (rs57620661-rs73054303) of the *CTNND2* gene increase by 2.64 times the chances of developing periodontitis. In the interaction between genes, the heritability of 5 risk alleles or more of variants that showed high linkage disequilibrium is positively associated with more than five times the chances of an individual developing periodontitis at some point in life. These results are the basis for many other studies to confirm the association in other populations, futhermore, therapeutic targets for the treatment and control of periodontitis.

Keywords: GWAS. Candidate Gene. Periodontitis. Immunogenetics. Polymorphism, Single Nucleotide.

LISTA DE ILUSTRAÇÕES

REFERENCIAL TEÓRICO

Figura 1 Complexo de Socransky acrescentado de outros microrganismos do microbioma oral que vivem em simbiose no ambiente supra e subgengival mas que em condições adversas interagem desequilibradamente provocando a destruição dos tecidos periodontais. 31

Figura 2 Panorama dos genes candidatos para periodontite estudados em população brasileira publicados até out/2022. 38

CHAPTER 2

Figure 1 Evaluation and verification of the quality of the sample to compose the periodontitis database. 78

Figure 2 Association between blocks of 3 SNV in the *IFI16* (A) and *AIM2* (B) genes with periodontitis. 79

Figure 3 Influence of rs75985579 of the *IFI16* gene in eotaxin-1 production (n = 296). 80

CHAPTER 3

Figure 1 Manhattan plot of GWAS results in a population of Salvador/Brazil. 99

Figure 2 QQ-plot for the analysis of periodontitis using 506 non-parents. 100

Figure 3 Top SNV on chromosome 2 (rs10496038) associated to periodontitis. 111

Figure 4 Top SNV on chromosome 4 (rs58327429 and rs67797971) associated to periodontitis. 112

Figure 7 Interaction network of significant and suggestive association proteins in GWAS for periodontitis. 113

Figure 8 Genetic variants associated with periodontitis, located on chromosome 3 that show linkage disequilibrium (n = 506). 114

Figure 9 Association of rs10496038 of the *RTN4* gene in eotaxin-1 production and association of rs58327429 of the *LINC02505* gene in IL-12 production. 116

LISTA DE TABELAS

CHAPTER 1

Table 1	Characteristics of the study population (n = 506) in absolute and relative frequencies.	53
Table 2	Verification of collinearity statistics between independent variables.	55
Table 3	Coding of Categorical Variable Parameters (n = 506).	55
Table 4	Variables not in the equation.	56
Table 5	Verification of the best fit model between independent covariates.	56
Table 6	Analysis results for comparison (n = 506).	56

CHAPTER 2

Table 1	Distribution of socioeconomic demographic, related to lifestyle, general health, and oral health characteristics according to the presence of periodontitis (n=506).	80
Table 2	Statistically significant associations between variants on *IFI16* and *AIM2* gene with the presence of periodontitis.	81
Table 3	Functional analysis of *IFI16* and *AIM2* genes.	81
Table 4	*In silico* regulatory analysis of *IFI16* and *AIM2* genes.	81
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.	82
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.	83
Supp. Table 1	Variants expressed in *IFI16* and *AIM2* genes after quality control.	83

CHAPTER 3

Table 1	Socioeconomic and demographic characteristics of the study group for periodontitis (n = 506).	93
Table 2	Characteristics related to the general health conditions of the studied group for periodontitis (n = 506).	93
Table 3	Characteristics related to oral health status and lifestyle habits of patients studied for periodontitis (n = 506).	95
Table 4	Periodontitis GWAS by logistic regression adjust for age, obesity, breathe through the mouth, flossing, asthma, and genetic ancestry (CRCh37) (n = 506).	98
Table 5	Functional annotations of SNV tops with association with periodontitis.	104
Table 6	Analysis of genetic pathways of genes suggestively associated with periodontitis using the VEGAS2 platform. The p values are stratified by the populations studied.	113
Table 7	Haplotypic association between chromosome 2 variants with the presence of periodontitis (n = 506).	115
Supp. 1	Haplotypic association between chromosome 3 variants with the presence of periodontitis (n = 506).	123
Supp. 2	Haplotypic association between chromosome 5 variants with the presence of periodontitis (n = 506).	124
Supp. 3	Interaction between a risk allele of each of the 7 genes that form the haplotypes associated with periodontitis (n = 506).	124

LISTA DE ABREVIATURAS E SIGLAS

95% CI	Intervalo de confiança de 95%
A	Adenina
A1	Alelo alternativo/menor alelo
A2	Alelo de referência/selvagem
AC083864.3	Gene baseado em clones
ADAMTS9-AS2	Iniciação da tradução eucariótica (ADAM) metalopeptidase com trombospondina tipo 1 motif 9 RNA anti-sentido 2
ADRL	Proteína receptora de adiponectina
AIM2	Ausente no melanoma 2
AIRE	Regulador autoimmune
AKT	Serina/treonina quinase
ALR	Receptor tipo ausente no melanoma 2 (AIM2)
AMR/AFR-8	Populações hispânicas e afro-americanas
AP	Proteína ativadora
APC	Célula apresentadora de antígeno
ARIC	Risco de aterosclerose nas comunidades
ARID3A	Domínio de interação rico em adenine e timina (AT-Rich) em 3A
ASAP	Repetição de anquirina e domínio homólogo a pleckstrina (PH)
ASB	Repetição de anquirina e caixa supressora da sinalização de citocinas
ASC	Speck-Like associada à apoptose contendo caspase de ativação e domínios de recrutamento ou “Apoptosis-associated Speck-like Containing A CARD”
ASCL	Família “Achaete-scute” com “basic Helix-Loop-Helix”, fator de transcrição
ATF	Ativando o fator de transcrição
ATL	Atlastina GTPase
BACH	Domínio “bric-a-brac, tramtrack, amplo complexo” (BTB) e “cap 'n' collar” (CNC) homólogo

BARX	Barh-Like homeobox
BATF	Fator básico de transcrição do zipper de leucina ativando o fator de transcrição
BAX	BCL2 associado X, regulador de apoptose
BCL	Regulador de apoptose
BDP	B duplo prime, subunidade do RNA polymerase III fator de iniciação de transcrição IIIB
BHLHE	Membro básico da família helix-loop-hélice
BLD	Sangue
BMI	Índice de massa corporal
BMP	Fator de diferenciação do crescimento
BRN	Cérebro
BRST	Células primárias mioepiteliais da mama
BTBD	Domínio contendo “bric-a-brac, tramtrack, amplo complexo” (BTB)
C	Citosina
CAAE	Certificado de Apresentação para Apreciação Ética
CAAPA	Consórcio sobre asma entre populações de ascendência africana nas Américas
CACD	Distrofia coroidal areolar central
CART	Transcrição regulamentada por cocaína e anfetamina
CASC	Suscetibilidade ao câncer
CASP	Caspase
CCDC85A	Domínio de bobina enrolada contendo 85A
CCR	C-C motivo receptor de quimioterapia
CDC5	Ciclo de divisão celular 5
CDP	Peptídeo natriurético
CDX	Homeobox tipo caudal
CEBPA e B	Proteína alfa e beta de ligação ao intensificador CCAAT
CEP	Proteína centrossomal
CFOS	Fos proto-oncogene, subunidade do fator de transcrição proteína ativadora 1
ChIP-Seq	Imunoprecipitação da cromatina seguida de

	sequenciamento
CHR	Cromossomo
circRNAs	Rnas circulares
CIZ	Proteína de dedo de zinco
CJUN	Jun proto-oncogene, subunidade do fator de transcrição proteína ativadora 1
CMYC	Mielocitomatose proto-oncogene, fator de transcrição básico helix-loop-helix (BHLH)
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
COHRA1	Centro de pesquisas bucal em Appalachia
CONEP	Comissão Nacional de Ética em Pesquisa
CTCF	CCCTC-fator de ligação
CTNND	Catenina (proteína associada à caderina) delta
CXCR	C-X-C motivo receptor de quimioterapia
DBX	Desenvolvimento de homeobox cerebral
DEC	Deletado no câncer de esôfago
DEFA1A3	Defensina alfa 1 e alfa 3
df	Graus de liberdade
DMRT	Duplosex e mab-3 fator de transcrição relacionado
DNA	Ácido desoxirribonucleico
DNA-PK	DNA dependente de proteina kinase
DNAse	Desoxiribonuclease
DRDR	Registro odontológico e repositório de DNA
dsDNA	DNA dupla fita
DTHD	Domínio de morte contendo
DUXL	Dupla proteína homeobox
DYRK	Fosforilação de tyrosina de dupla especificidade regulada quinase
E2A	Regulador chave dos rearranjos do gene imunoglobulina κ
E2F	Fator de transcrição E2F
EBF	Fator de transcrição EBF
EFCAB4B	Liberação de cálcio regulador de canal ativado 2A

EGR	Resposta de crescimento inicial
ELF	E74 como fator de transcrição ETS
Enh	“Enhancer” ou potencializador
EnhA	Potencializador ativo
EnhAc	Possível potenciador h3k27ac primário
EnhAF	Flanco do potencializador ativo
EPIGEN	Epidemiologia Genômica de Doenças Complexas em Coortes Brasileiras de Base Populacional
ERALPHA	Receptor de estrogênio alfa
ERBB4	Receptor tirosina quinase
ESC	Célula-tronco embrionária
ESDR	Célula-tronco mesenquimal derivada de H1
ESR	Receptor de estrogênio
ETS	Reguladores de transcrição dependentes de sinal
EWSR1	Proteína de ligação da região 1 do ponto de interrupção do sarcoma de Ewing
Exp(B)	Coeficiente de regressão logística
FAPESB	Fundação de Amparo à Pesquisa do Estado da Bahia
FAT	Tecido adiposo
FEV1	Volume expiratório forçado em um segundo
FMN	Formina
FOSL	FOS ligante, subunidade do fator de transcrição proteína ativadora 1
FOX	Forkhead Box. Família de proteínas onde letras e números depois do prefixo Fox, caracteriza sua função específica ou tecido de expressão. Ex: Foxa - Forkhead Box a
FOXP	Caixa de forquilha P
Freq	Frequência
FRMD6-AS2	Domínio FERM contendo 6 RNA antisense 2
FXR	Homólogo autossômico de ribonucleoproteína mensageiro X frágil
G	Guanina
GATA	Fator de transcrição Eritróide ou proteína de ligação GATA

GC	Gene candidato
GCNF	Subfamília de Receptores Nucleares 6 Grupo A Membro 1
GENO	Genótipo
GI	Gastrointestinal
GLI	Dedo de zinco da família de oncogenes associados ao glioma
Global p	Teste global para interação
GLT6D1	Domínio glicosiltransferase 6 contendo 1
Gm397	Membro da família Zscan4 de proteínas de dedo de zinco contendo domínio SCAN.
GR	glicina-arginina motivo ou “motif glycine-arginine”
GTEX	Expressão genótipo-tecido
GWAS	Estudos de ampla varredura genômica
GYG	Glicogenina
H0	Hipótese nula
H1	Hipótese alternativa
H3K27ac Enh	Acetilação da lisina 27 na histona 3 com função enhancer
H3K4me1 Enh	Metilação da lisina 4 na histona 3 com função enhancer
H3K4me3 Pro	Trimetilação da lisina 4 na histona 3 com função promoter
H3K9ac Pro	Acetilação da lisina 9 na histona 3 com função promoter
Hand	Expressões derivadas do coração e da crista neural
HBP	Proteína de Ligação à heparina
HCHS/SOL	Estudo da Saúde Comunitária Hispânica
HDAC	Histona deacetilase
HEN	Intensificador semelhante à visão anormal letal embrionário
HEY	“Hairy” (peludo) e potenciador de família relacionada com divisão, membro da família hélice-alça-hélice básica. Fator de transcrição com motivo YRPW
HIV	Vírus da imunodeficiência humana
HLTF	Fator de transcrição tipo helicase
HMG-IY	Proteína do grupo de alta mobilidade
HNF	Fator hepatócito nuclear
Homez	Codificação homeobox e leucina zipper

HOX	Homeobox
HRT	Ventrículo esquerdo
HSF	Fatores de choque térmico
HWE	Equilíbrio de Hardy-Heinberg
IFI16	Proteína indutível por interferon gama 16
IFN-α/β/γ	Interferon alfa, beta e gama
IK	Fatores de transcrição de dedo de zinco restritos a linfoides que são considerados reguladores mestres da diferenciação de linfócitos (IKAROS)
IL	Interleucina
IMUNOBIO	Laboratório de Imunofarmacologia e Biologia Molecular
IPSC	Células-tronco pluripotentes induzidas
IRF	Fator regulador do interferon
ISL2	Homeobox da proteína de ligação do potenciador do gene da insulina com a proteína muscular de transporte nucleocitoplasmático 2
JUND	Proto-oncogene jund, subunidade de fator de transcrição proteína ativadora 1
KLF7	Proteína nuclear Krüppel-like fator 7
LBP	Proteína de ligação de lipopolissacarídeo
LD	Desequilíbrio de ligação
LHX	Proteína muscular de transporte nucleocitoplasmático (LIM) homeobox
LINC	RNA de codificação não proteico longo intergênico – ncRNA
LIV	Fígado
Lmo2-complex	Complexo do domínio proteína muscular de transporte nucleocitoplasmático (LIM) “only” 2
lncRNAs	RNAs não codificantes longos
LNG	Linha celular de fibroblastos pulmonares fetais
LOC	RNA intergênico longo não codificante
LPS	Lipopolissacarídeo
LRP1B	Proteína relacionada ao receptor lipoproteína de baixa

	densidade 1B
LUN	Proteína rica em arginina/serina top1, liga ligase de ubiquitina E3
LYRM4	Leucine/tyrosine/arginine motif proteins
MAC	Contagem dos menores alelos
Maf	Homólogo do oncogene fibrossarcoma musculoaponeurótico
MAF	Frequência do menor alelo
MASP1	Serina protease 1 associada à lectina de ligação à manose
MAX	Fator X associado ao MYC
MDS2	Síndrome mielodisplásica 2 associada à translocação
MEF2	Fator de aprimoramento do miócito
mind	Comando utilizado no software Plink para excluir indivíduos com mais de 10% de genótipos ausentes
miRNAs	MicroRNAs
MMP	Metaloproteinase
MODEL	Modelo de regressão logística
MRG	Relacionado ao inibidor de crescimento derivado da mama
MTIF2	Fator de iniciação translacional mitocondrial 2
MTND1P5	NADH codificado mitocondrialmente: Ubiquinona Oxidorredutase Subunidade Central 1 Pseudogene 5
mTOR	Alvo mamífero da rapamicina
MUS	Músculo esquelético
MXI	Interagente do fator X associado a mielocitomatose (MYC), proteína de dimerização
MYC	Mielocitomatose pro-oncogene, fator de transcrição basic helix-loop-helix (bHLH)
MYF	Fator Miogênico
MYR	Fator regulatório de mielina
MZF	Dedo de zinco mieloide
NANOG	Fator de transcrição em células-tronco embrionárias
NCR2	Receptor de Disparo de Citotoxicidade Natural 2
NCX	Trocador de sódio/cálcio

NELL1	Fator de crescimento epidérmico neural-like 1
NF-E2	Fator Nuclear, Eritróide 2
NF-κB	Fator Nuclear Kappa B
ng / µL	Nanograma por microlitro
NK	Assassino natural ou “natural killer”
NKX	Homeobox classe “natural killer”
Nogo-A	Também conhecido como RTN4 (Reticulon-4)
NOS	Óxido nítrico sintase
NPY	Neuropeptídeo Y
Nr2f2	Subfamília de receptores nucleares 2 grupo F membro 2
NRF	Fator respiratório nuclear
NRSF	Fator silenciador restritivo neural
OBOX	Homeobox específico para oócitos
OPG	Osteoproteferina
OR	Razão de chances ou “Odds ratio”
OR3A2	Receptor olfativo família 3 subfamília A membro 2
OR3A4P	Receptores olfativos 3 subfamília A membro 4 pseudogene
OSF2	Periostina
OVRY	Ovário
p perm	Valor de p permutacionado 10.000 vezes
p300	Coativador de transcrição
PANC	Ilhotas pancreáticas ou ilhotas de Langerhans
PAX	Caixa emparelhada
PBX	Fator de transcrição de leucemia de células pré-B
PGE2	Prostaglandina E2
PHACTR1	Regulador de Fosfatase e Actina 1
PI3	Fosfoinositol 3
PLAG1	Gene de adenoma pleomórfico 1 dedo de zinco
PLCNT	Placenta
POU	Um domínio de ligação de DNA bipartido, consistindo em duas regiões altamente conservadas, amarradas por um ligante variável
PPAR	Receptor Ativado por Proliferador de Peroxissoma

PRDM1	Proteína 1 de dedo de zinco de domínio relacionado à patogênese ou proteína 1 de maturação induzida por linfócitos B
PRISMA	Itens de relatório preferidos para revisões sistemáticas e meta-análises
ProAR	Programa de controle de asma da Bahia
PromU	Promotor upstream tss
PTF1-beta	Fator de Transcrição 1a Associado ao Pâncreas
PTPRD	Receptor de Proteína Tirosina Fosfatase Tipo D
PU.1	Fator de transcrição hematopoiética de ação em células linfóides e mielóides
PYCARD	Domínio pirina e domínios de ativação e recrutamento de caspases
QQ-plot	Gráfico quantil-quantil
R^2	Coeficiente de determinação
RAD21	Componente complexo de coesina RAD21. Proteína envolvida no reparo de quebra de fita dupla de DNA
RANK	Receptor ativador do fator nuclear kappa-B também conhecido como membro da superfamília do receptor do fator de necrose tumoral 11
RANKL	Ligante do receptor ativador do fator nuclear kappa-B, também conhecido como membro da superfamília do ligante do fator de necrose tumoral 11
RNA	Ácido ribonucleico
RNASET2	Ribonuclease T2
RORγt	Receptor gama nuclear órfão relacionado ao receptor de ácido retinoico
RP11	RNAs longos não codificantes associados a superpotenciadores (RP = retinite pigmentosa)
RP1-90J4.1	Retinite pigmentosa 1, RNA não proteico intergênico longo
RPAP3	Proteína 3 associada à RNA polimerase II
RTN4	Reticulon-4
RXR::LXR	Receptor X retinóide dimerizado com o receptor X hepático

RXRA	Receptor alfa do retinoide X
SAV1	Família salvador domínio WW contendo proteína 1
SEPT7	GTPase citoesquelética formadora de filamentos homóloga ao ciclo de divisão celular 10
SETDB1	Domínio SET bifurcado histona lisina metiltransferase 1
SF1	fator de emenda 1 ou “ splicing factor 1”
SHIP	Estudo de saúde na Pomerânia
SHIP-Trend	Coorte independente conduzida entre 2008 e 2012 dentro do estudo de saúde da Pomerânia
SHISA9	Membro da família shisa 9
Sig.	Valor de p
SIGLEC5	Ácido siálico ligando Ig como lectina 5
Sin3Ak-20	Anticorpo policlonal, correpressor interagindo com HDAC1, N-corR, SMRT e MeCP2.
SIX5	Distrofia Miotônica homeobox 5
SKIN	Tecido cutâneo
SLC	Superfamília de transportadores de soluto
SMAD3	Membro da família mães contra decapentaplegia, drosofila, homologia de 3 ou “ mothers against decapentaplegic, drosophila, homolog of 3”
SMC3	Manutenção estrutural dos cromossomos 3
SNTG1	Sintrofina gama 1
SNV	Variante de nucleotídeo único
SOX	Fator de transcrição da caixa região determinante do sexo Y
SP	São Paulo
SP1	Fator de transcrição SV40 promotor-1
SPDEF	Domínio apontou S-adenosil metionina contendo fator de transcrição ETS
SPLN	Baço
SPSS	Pacote Estatístico para as Ciências Sociais
SREBP	Proteína de ligação ao elemento regulador de esterol
SRF	Fator de resposta ao soro

STAT	Transdutor de sinal e ativador de transcrição
StepAIC	Algoritmo stepwise de critério de informação Akaike
STRING	Análise de enriquecimento funcional das redes de interação proteína-proteína
STRM	Células cultivadas de condrócitos derivadas de células-tronco mesenquimais
T	Timina
TAL	Fator de transcrição do leucemia linfocítica aguda de células T 1
TATA	Sequência de DNA não codificante formado pelas bases nitrogenadas Timina e Adenina repetidas
TBC1D22A	Tre-2, Bub2 e Cdc16 1 Membro da família de domínio 22A
TCF	Fator de Transcrição Específico de Células T
TCI/CCI IL-4	Haplótipo formado pelos polimorfismos -590(T/C), +33(T/C) e número variável de repetição em tandem(I/I) no gene da interleucina-4
TEF	Fator de alongamento de tradução ou fator de elongação
TGF-β	Fator de transformação do crescimento beta
TGIF	Fator induzido pelo fator de transformação do crescimento-β homeobox 1
Th	Linfócitos T “helper” auxiliares
THAP	Domínio de proteínas associadas a tanatos contendo proteína associada à apoptose
THYM	Timo
TIMP	Inibidor de metaloproteinases
TLR	Receptor igual a pedágio ou “Toll like receptor”
TNF-α	Fator de necrose tumoral alfa
TR4	Subfamília de receptores nucleares 2 grupo C membro 2
Treg	Linfócito T regulador
TSNAX-DISC1	Fator X associado à translina e interrompido na esquizofrenia 1
TssAFlnk	Flanqueadora ativa TSS
TxEnh5	Transcrito 5' preferencial e potencializador

USF	Fator de transcrição a montante
UTAT39	RNA de codificação não proteico longo intergênico 1541
VEGAS2	Evolução viral de sequências de atuação genética 2
VIF	Fator de inflação de variação
WWOX	Domínio WW contendo oxidorredutase
X^2	Qui quadrado
YY	Fator de transcrição yin-yang
ZBTB	Dedo de zinco e domínio contendo “bric-a-brac, tramtrack, amplo complexo”
ZEB	Homeobox de encadernação de dedo de zinco
ZEC	Proteína dedo de zinco 628
ZFC	Proteína dedo de zinco
ZFX	Proteína de dedo de zinco ligada ao X
ZIC	Membro da família das proteínas dedo de zinco
ZNF	Proteína dedo de zinco

SUMÁRIO

1.	**INTRODUÇÃO GERAL**	28
2	**REVISÃO DE LITERATURA**	30
2.1	PATOGÊNESE E EPIDEMIOLOGIA DA PERIODONTITE	30
2.2	A IMUNOLOGIA DA PERIODONTITE	34
2.3	ESTUDOS DE GENES CANDIDATOS	36
2.4	ESTUDOS DE AMPLA ASSOCIAÇÃO GENÔMICA PARA PERIODONTITE	40
3.	**OBJETIVOS**	42
3.1	OBJETIVO PRINCIPAL	42
3.2	OBJETIVOS SECUNDÁRIOS	42
	CHAPTER 1 - MANUSCRIPT 1: A STATISTICAL MODEL TO EVALUATE FACTORS ASSOCIATED WITH PERIODONTITIS.	43
	ABSTRACT	43
	KEYWORDS	44
1	INTRODUCTION	44
2	METHOD	44
2.1	**Study Population**	45
2.2	**Dependent variable: Periodontitis**	45
	Independent variables	45
2.3	**Statistical Analysis**	45
3	RESULTS	46
4	DISCUSSION	49
	ACKNOWLEDGEMENT	51
	REFERENCES	51
	ANNEXES	51
	CHAPTER 2 - MANUSCRIPT 2: GENETIC VARIANTS IN *IFI16* AND *AIM2* GENE MODULATE PERIODONTAL INFLAMMATORY RESPONSE.	57

	ABSTRACT	57
	KEYWORDS	58
1	INTRODUCTION	58
2	MATERIAL AND METHODS	60
2.1	**Studied Population**	60
2.2	**Definition of Periodontitis**	61
2.3	**DNA extraction and Genotyping**	61
2.4	**Data Quality Verification**	62
2.5	**Cytokine Analysis**	62
2.6	***In Silico* Analysis**	63
2.7	**Statistical Analysis**	63
3	RESULTS	64
3.1	**Studied Population**	65
3.2	**Description of the variants**	65
3.3	**Associations between variants on IFI16 and AIM2 with periodontitis**	65
3.4	**Functional impact of periodontitis-associated variants**	66
3.5	**Analysis of Haplotypes**	66
3.6	**Gene-Gene Interaction**	67
3.7	**Influence of variants in AIM2 and IFI16 genes in the cytokine production**	67
4	DISCUSSION	68
5.	CONCLUSION	71
	ACKNOWLEDGEMENTS AND FUNDINGS	72
	REFERENCES	72
	Supplemental files	73
	CHAPTER 3 – MANUSCRIPT 3: GWAS IDENTIFIES GENES ASSOCIATED WITH PERIODONTITIS IN A POPULATION FROM SALVADOR/BA/BRAZIL	86
	ABSTRACT	86
	KEYWORDS	87
1	INTRODUCTION	87

2	METODOLOGY	88
2.1	**Participants and Studies**	88
2.2	**Phenotype definition**	89
2.3	**Genotyping and quality control**	89
2.4	**Imputation Strategy**	90
2.5	**GWAS**	90
2.6	**Funtional annotation**	91
2.7	**Funtional analysis**	91
2.8	**Visualization enrichment**	92
2.9	**Analysis of signaling pathways**	92
2.10	**Analyzes on the influence on the expression of cytokines**	93
3	RESULTS	93
3.1	**Participants**	93
3.2	**GWAS results**	97
3.3	**Functional annotations and analysis**	99
3.4	**Visualization enrichment**	111
3.5	**Analysis of signaling pathways**	112
3.6	**Analyzes on the influence on the expression of cytokines**	116
4	DISCUSSION	116
	REFERENCES	121
	SUPPLEMENTS	123
	CONCLUSÃO GERAL	125
	REFERÊNCIAS DA INTRODUÇÃO	125

1 INTRODUÇÃO GERAL

A periodontite é consequência de um processo inflamatório iniciado no tecido gengival que, se não tratada, resulta na inflamação nos tecidos mais profundos, alterando a homeostase óssea causando perda de dentes(1). Expõe aos indivíduos em verdadeira desvantagem biológica, funcional e inclusive, psicossocial(2). A etiologia, com o olhar molecular, traz como principal agente, a resposta imunológica do hospedeiro em interações complexas com o biofilme bacteriano 788em disbiose(3). No entanto, a multifatorialidade é a característica tônica que a classifica como integrante do quadro de doenças complexas(1,3).

A complexidade da periodontite envolve fatores relacionados ao comportamento do paciente que podem ser modificáveis com ajuste no estilo de vida e cuidados pessoais. Saúde sistêmica do paciente, hábitos de vida e fatores ambientais podem ou não ter influência epigenética. Ademais, disbiose dos periodontopatógenos, características anatômicas do local e motivos genéticos somam à resposta inflamatória imune do hospedeiro complementando o grau complexidade da periodontite(3,4).

Na periodontite, o intenso processo inflamatório é estimulado por um biofilme não removido periodicamente, favorecendo o desenvolvimento de colônias bacterianas. Espécies como, *Fusobacterium nucleatum,* influenciam o ambiente ao liberar moléculas antigênicas, lipopolissacarídeos, fatores de virulência que estimulam uma resposta mais intensa e prolongada do hospedeiro. Neste contexto, ocorre o desenvolvimento aumentado de patógenos, tendo como patógenos chave ou de maior virulência o *Porphyromonas gingivalis*, *Treponema denticola* e *Tannerella forsythia* instaurando o desequilíbrio na microflora oral, ou disbiose local(3,5,6).

A resposta imunológica na periodontite não se limita ao periodonto, devido a disseminação sistêmica de bacterias transitórias e mediadores pró-inflamatórios seus efeitos deletérios podem acometer órgãos, condições sistêmicas ou exacerbar outras doenças crônicas, sobretudo as de envolvimento inflamatório. Asma e atopia(7–11), infarto agudo do miocárdio(12), câncer de cabeça e pescoço(13), síndrome do ovário policístico(14)stress(15), mal de Parkinson(16), obesidade(17–19), diabetes(20,21), dentre outras como demência, reações adversas na gravidez,

doença renal crônica, artrite reumatóide e síndrome metabólica(22) apresentaram associação com periodontite.

A associação significativa ou sugestiva de genes com a periodontite foi crescente na últimas décadas através de estudos de ampla varredura genômica (GWAS) e de genes candidatos (GC)(23–27). O primeiro GWAS para periodontite registrado associou significativamente o alelo G da variante rs1537415 do gene *GLT6D1* com periodontite agressiva sem o ajuste de covariáveis e associação sugestiva após o ajuste para tabagismo, diabetes e gênero(28). Desde então, há diversidade nos resultados de cada GWAS com poucas validações entre grupos de diferentes ancestralidades(24).

Um grande número de publicações associam fatores genéticos à predisposição e gravidade da periodontite(29–34). Nestas, se inclui a recente descoberta da associação da periodontite à expressão gênica espécie-específica da microbiota oral(35). Por outro lado, buscam-se confirmações para a herdabilidade da periodontite. Uma revisão sistemática seguiu o protocolo PRISMA e registrou o estudo focado na herdabilidade da periodontite entre os anos 1969 – 2018. Selecionaram 23 estudos envolvendo gêmeos, famílias e GWAS que forneceram dados de herdabilidade. Subdividiram em grupos para metanálises pois havia muita variação entre os mais de 50.000 participantes. Ficou registrado que parte significativa da variação fenotípica da periodontite na população se deve à genética e que a herdabilidade tende a ser maior em características graves de início precoce e indivíduos mais jovens. Não foram encontradas concordâncias em todos os aspectos, fato justificado por ser um estudo em populações heterogêneas, abrangendo um longo período e sem sistematização quanto a classificação da periodontite(36).

Recentes revisões têm ocorrido para a padronização e atualização da classificação da periodontite(37–39). O objetivo é uma classificação que considere a avaliação de características relacionadas à periodontite, como: estágios de gravidade, complexidade, extensão, distribuição e características biológicas da doença, onde a evidência ou risco para progressão rápida, resposta ao tratamento e impactos sistêmicos sejam considerados. Nesse sentido, quando submetidos à um exame periodontal, o indivíduo tem avaliado o índice de recessão e hiperplasia gengival, profundidade de sondagem de sulco/bolsa, nível de inserção clínica, índice de sangramento à sondagem. A perda de suporte do osso alveolar, principal

característica da periodontite, pode ser avaliada radiograficamente(39).

Diante da complexidade da patologia, consequências crônicas e graves que esta acomete, evidências sugestivas de herdabilidade, falta de marcadores para diagnóstico, diversidade de resultados entre estudos e poucas confirmações interraciais é que este estudo se propôs a realizar uma GWAS em uma população de Salvador, Bahia/Brasil. Para tal, foi realizado um estudo para definir o melhor modelo de ajustes das análises (capítulo 1), bem como a verificação de associação de genes candidatos com o desfecho (capítulo 2) e a apresentação de como a GWAS foi realizada e seus resultados, estão no capítulo 3.

2 REVISÃO DA LITERATURA

2.1 PATOGÊNESE E EPIDEMIOLOGIA DA PERIODONTITE

A periodontite é caracterizada por inflamação exagerada dos tecidos conectivos que sustentam o dente, resultando em perda de tecido conjuntivo, do osso alveolar de suporte, e, eventualmente, de dentes. No paciente clinicamente saudável há uma simbiose entre microbioma e hospedeiro(40). Os microrganismos que colonizam o microbioma são divididos em grupos que se relacionam. O complexo de Socransky é uma organização e classificação, em 5 grandes complexos, da comunidade bacteriana em placas subgengivais (41). Na figura 1, o primeiro complexo é o vermelho da extrema direita, as bactérias que o compõe são as principais responsáveis pelo desenvolvimento de biofilmes (41,42). O segundo e o terceiro possuem um grupo central de bactérias fortemente relacionadas a subespécies destas mesmas bactérias. O quarto complexo inclui a *Actinobacillus actinomycetemcomitans* sorotipo A (em verde) e o quinto complexo está em preto. Demais bactérias, actinomicetos e que aparecem fora das formas geométricas, possuem pouca ou nenhuma relação com os outros 5 grandes complexos(41).

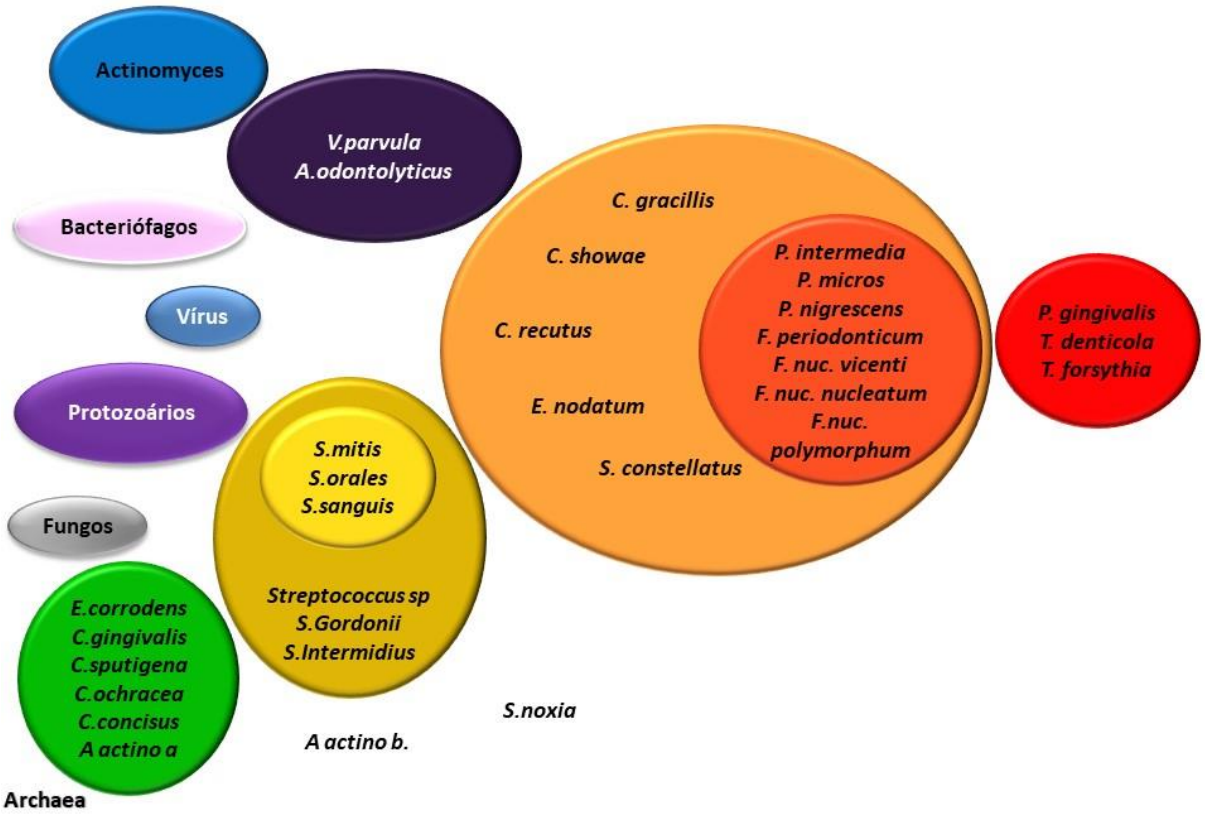


Figura 1: Complexo de Socransky acrescentado de outros microrganismos do microbioma oral que vivem em simbiose no ambiente supra e subgengival mas que em condições adversas interagem desequilibradamente provocando a destruição dos tecidos periodontais. Cores mais quentes representam microrganismos de maior importância na instalação da periodontite.

Sem reduzir a importância das comunidades bacterianas, pelo sequenciamento ribossômico 16S confirma-se no metatranscrito periodontal a presença de vírus, incluindo bacteriófagos, archaea, protozoários e fungos participando desta simbiose microbiótica que ocorre entre os próprios microrganismos e destes com o hospedeiro (40,43). No microbioma observam-se microrganismos permantes e transitórios; patogênicos e não patogênicos, todos modulam a resposta imune. Dentre os microrganismos não patogênicos, há os autobiontes que são os principais mantenedores da homeostase e em adição à estes, há os patobiontes, que são microrganismos regularmente em simbiose que apresentam patogenicidade transitória(40,43,44).

A proposta de estabelecer apenas um fator responsável pela disbiose,

patógeno chave, já está substituída pelo conceito polimicrobiano que envolve uma comunidade microbiana patogênica, influenciada por fatores de risco comportamentais, ambientais e genéticos(42,44,45). No entanto, a literatura apresenta as bactérias periodontopatogênicas do complexo vermelho de Socransky como as mais virulentas, sobretudo *P. gingivalis*, pois mesmo em baixo número podem influenciar a virulência de toda a comunidade. A *P. Gingivalis* apresenta uma comunicação diferenciada com outros microrganismos, com alta sinergia, que favorece a transição para a patogenicidade. Outras espécies bacterianas podem ser iguais ou até mais ativas no processo que conduz à doença no periodonto e devem ser investigadas(42). A virulência se torna um fator importante sobretudo quando o hospedeiro está susceptível(40).

Se for permitido o acúmulo de biofilme sem sua regular remoção, resultará na mudança das típicas comunidades de microrganismos, processo denominado disbiose da microbiota oral. Esta disbiose será conduzida por bactérias patogênicas que irão alterar o biofilme levando a uma resolução alterada da inflamação local(40). No estágio inicial, os fatores de risco exercem pouca ou moderada influência e não há evidência de efeitos epigenéticos; porém, dependendo desses fatores de risco, alguns pacientes progridem para a periodontite. Neste caso, há uma inflamação crônica destrutiva com dano tecidual, procedente da disbiose estabelecida e das respostas imunes inflamatórias exageradas, em que a resposta do hospedeiro à infecção, é um fator determinante da extensão e gravidade da doença(3,46).

A periodontite se desenvolve lentamente, ao longo de anos, ou apresenta desenvolvimento rápido; ambas podem levar à perda dos elementos dentários comprometidos. Pacientes diabéticos insulinodependentes, imunodeprimidos, com síndrome de Down ou com outras patologias sistêmicas estão mais susceptíveis ao desenvolvimento da periodontite(37,39). A literatura cita relações observadas entre a periodontite com outras doenças distais. Esta relação é justificada, em parte, pela translocação microbiana oral a outros sistemas, que mesmo não sendo a causa direta, são responsáveis por alterações fisiológicas que podem acontecer com a disbiose deste novo sítio(40,42).

Atualmente, é preocupante o comprometimento de indivíduos sistemicamente sadios e até mesmo jovens na evolução da periodontite, sendo uma grande causa das perdas de dentes do adulto(47). A terapia mecânica e a antibioticoterapia sistêmica são tratamentos bem estabelecidos para a periodontite, porém, uma

parcela de pacientes com periodontite não responde a estes tratamentos(48–50). A falta de resposta aos tratamentos convencionais pode ser atribuída ao fato de que 80% do risco do dano tecidual na periodontite depende da resposta imune inflamatória do hospedeiro. Esta resposta varia de acordo com aspectos biológicos e psicossociais nas diferentes fases da vida, interagindo com predisposição genética(3).

Na periodontite há a presença de fatores de risco modificáveis e não modificáveis, dentre estes estão comportamentais, ambientais, genéticos, bem como há evidências de fatores de risco epigenéticos. A patogênese da periodontite pode estar relacionada a distintas doenças sistêmicas e diversos fatores ambientais como dieta, tabagismo, estresse, higiene bucal dentre outros; que associados a fatores genéticos estão relacionados ao desenvolvimento e severidade desta doença em um determinado momento da vida(15,32,51,52). Portanto, genética, estilo de vida e fatores ambientais influenciam conjuntamente na resposta imune inflamatória que regula a composição do biofilme, criando um fenótipo que dificulta o sucesso de estratégias preventivas e de tratamento(3).

A periodontite enquadra-se dentre as doenças complexas e multifatoriais por poder apresentar uma progressão rápida ou lenta, possuir múltiplos agentes etiológicos, relacionar-se a outras doenças sistêmicas e ser influenciada por genes e suas variantes(30,53–55). Uma das hipóteses propostas sobre as bases genéticas de doenças complexas diz que, há variantes genéticas comuns em todas as populações mas que, individualmente a susceptibilidade genética às doenças complexas tem grande influência(56). Estas variantes de nucleotídeo único (SNV) ocorrem quando há a substituição de uma base nucleotídica por outra(57,58). Na literatura há SNV relacionadas tanto à proteção da doença quanto a predisposição e severidade da periodontite (34,59,60). Embora seja improvável que todas as variantes de um genoma estejam envolvidos com a susceptibilidade à periodontite, variantes alélicas em múltiplos genes podem ser considerados determinantes genéticos a susceptibilidade diferencial à periodontite(61).

Observa-se que há diferenças na periodontite entre as populações, envolvendo etnias. Estudos mostram que, por exemplo, a periodontite agressiva é mais prevalente na África e em populações de ascendência Africana e é menos prevalente em caucasianos na Europa e América do Norte(62). Este risco diferencial para a periodontite parece estar relacionado com elementos hereditários de

susceptibilidade e variantes genéticas.(62) Entretanto, reconhece-se que há poucos estudos epidemiológicos para periodontite em populações africanas e asiáticas(63). No Brasil, que apresenta uma população miscigenada, dados epidemiológicos do Ministério da Saúde apontam que os problemas periodontais aumentam de acordo com a idade(64). A presença de cálculo dental e sangramento é mais frequente entre os adolescentes e as formas mais graves da periodontite acomete os adultos de 35 a 44 anos. Nas regiões brasileiras os dados foram heterogêneos. As regiões Norte e Nordeste apresentaram as piores condições periodontais em todas as idades e grupos etários(64).

Considerando outros fatores demográficos, entre sexos, a maioria dos estudos não apresentam diferencial de prevalência(63). A susceptibilidade aumenta com a idade, porém tem sido o principal motivo de perda de elemento dentário em adultos jovens(65). Parece que há concenso que a maior dificuldade em estudos epidemiológicos para a periodontite está na carência de consistência metodológica(2,55,63,65–67).

2.2 A IMUNOLOGIA DA PERIODONTITE

O processo inflamatório se inicia como uma resposta à presença agressiva de moléculas como lipopolissacarídeos (LPS), peptideoglicanos, proteases e toxinas produzidas pelas bactérias patogênicas presentes no biofilme ou que são subprodutos de sua degradação. A primeira resposta celular, como primeira barreira, ocorre a chegada dos neutrófios no fluido crevicular no sulco subgengival. Havendo a infiltração bacteriana no tecido, outras células imunes circulantes, como mastócitos, monócitos/macrófagos também invadem o tecido liberando enzimas lisossomais (3,5). Células *natural killer* (NK) e células dendríticas, mediadores da imunidade inata, induzem a ativação de linfócitos T em resposta aos agonistas tipo *tool like receptors* (TLR) através da produção de IFN-α/β/γ e a resposta humoral ocorre através da invasão dos linfócitos B e T(40). Há produção de mediadores inflamatórios, incluindo citocinas, quimiocinas, metabólitos do ácido araquidônico e enzimas proteolíticas que coletivamente contribuem para a destruição dos tecidos, incluindo a ativação de vias degradativas para a reabsorção óssea(68).

Em homeostase, os neutrófilos conseguem conter o desafio microbiano ao gerarem fibras extracelulares, formando armadilhas que imobilizam, desarmam e matam os patógenos. Porém, em quantidades excessivas, os neutrófilos deixam de ser defensores e passam a ser perpetradores, participando da destruição dos tecidos periodontais devido ao excesso de substâncias inflamatórias e enzimas degradadoras de tecidos(5). Dentre estas enzimas degradatórias, encontram-se as metaloproteinases de matriz (MMPs) que ao participarem da homeostasia local atuam na migração celular, ativam de células imunes, atuam na cicatrização de tecidos dentre outras funções(5,40).

Uma pequena quantidade de MMPs são produzidas por microrganismos, o principal destaque está em que os microrganismos ativam células imunes circulantes a produzirem mais MMPs. Em quantidades exacerbadas, as MMPs conduzem o rompimento da matriz extracelular e a destruição de células hígidas do hospedeiro(40). As extremidades de moléculas fragmentadas de colágeno, proteoglicanos, glicosaminoglicanos e glicoproteínas funcionam como epítopos de autoantígenos que são apresentadas por células apresentadoras de antígeno (APC) gerando resposta de células T. Ao ocorrer a interação dos autoantígenos com receptores de células T, estes passam a ser denominados de autoanticorpos e são mais comuns em estágios avançados de periodontite não controlada(3,4,40).

Uma interação complexa entre vários tipos de células, mediadores inflamatórios proteicos e receptores geram um sistema em cascata. LPS, autoantígenos, padrões moleculares associados a patógenos estimulam os mastócitos a liberar aminas vasoativas e fator de necrose tumoral alfa (TNF-α) pré formado(68). Estes farão um *feedback* positivo em células do tecido gengival que liberarão mediadores inflamatórios como interleucinas (IL1β, IL6 e IL8), prostaglandinas (PGE2) e MMPs. Em resposta à presença destes mediadores, mais linfócitos e macrófagos são recrutados para o tecido(5). Com a invasão de macrófagos e linfócitos, APCs ativam células Th0, que se diferenciam em Th1, Th2, Th17, Treg. A quantidade e o tipo de antígenos, bem como os mediadores inflamatórios, conduzirão qual a subpopulação e quantidade de linfócitos T helper que será formada(3,5,68).

Células de perfil Th1 são ativadas por IL1α e IL1 β; secretam IL2, IFN-γ que aumentam a síntese de TNF-α pelos macrófagos; e se proliferam com o estímulo de IL12. A diferenciação de células de perfil Th2 envolve múltiplos sinais que culminam

na indução do fator de transcrição GATA3 que promove a transcrição de IL1, IL4, IL5, IL6, IL9, IL10 e IL13 gerando um resposta humoral sobretudo de caráter anti-inflamatório com inibição da proliferação e atividade da IL12 e TNF-α, bloqueando a atividade das células Th1(68). Além disto, células Th2 estão envolvidas com as respostas humorais de imunoglobulinas. As células T regulatórias surgem na presença de TGF-β e secretam IL10 e mais TGF-β, bem como aumentam a expressão de FOXP3. Na periodontite, ocorrem um aumento na expressão de FOXP3 e de IL17 que são marcadores do aumento da presença de células Treg e Th17 nestas condições do tecido periodontal(68). Células Th17 diferenciam-se a partir de estímulos da IL23 e secretam IL17A, IL17B, IL17C, IL17D, IL17E ou IL25, IL17F e expressam RORγt. Ficou evidente que mesmo sendo secretada pelo sistema adaptativo, as interleucinas da família 17, modulam as atividades do sistema inato através da regulação de macrófagos e neutrófilos, estimulam a produção de TNF-α, IL6, IL1β e PGE2 induzindo a diferenciação e ativação de osteoclastos e aumentando a expressão do ligante do receptor RANK (RANKL) por osteoblastos(3,5,68).

Neste mecanismo, o RANKL produzido pelos osteoblastos se ligará ao receptor RANK nos osteoclastos, resultando na diferenciação (osteoclastogênese) e ativação dos osteoclastos, conduzindo à reabsorção óssea característica da periodontite. A osteoprotegerina (OPG) é a proteína reguladora do RANKL, que impede a ligação RANKL-RANK nos osteoclastos. No mecanismo da periodontite, há a estimulação da produção do RANKL e a inibição da OPG(68). Esta resposta imunológica resulta na degradação dos tecidos ósseos periodontais por estimulação do mecanismo de remodelação esquelética que envolve o receptor RANK, seu ligante RANKL e a OPG mas que foi originada pelos múltiplos fatores já descritos(68).

2.3 ESTUDOS DE GENES CANDIDATOS

Estudos de genes candidatos utilizam SNV de genes cuja função se relaciona com o fenótipo, verificando se há uma frequência estatisticamente significativa maior nos casos comparados aos controles(61). A bioinformática prevê

que certas variantes de nucleotídeo único, especialmente as codificantes não-sinônimas em exons, possam gerar algum efeito no gene afetado, quer seja alterar a função protéica a até mesmo imprimir um efeito deletério(6). Outra forma de estudar estes efeitos é através de blocos de haplótipos, onde se reduz a quantidade de testes e consequentemente, associações espúrias(69). Estudos de haplótipos e genes candidatos individuais não devem ser apresentados como único fator genético determinante para o desfecho e sim, apenas como parte do risco genético pois vários genes atuam em conjunto e o resultado da associação destas ações podem contribuir para a predisposição ou a proteção da doença. O efeito de um haplótipo sobre o desfecho pode ter uma resposta divergente da resposta de cada SNV que o compõe(61).

A frequência da variante genética pode variar consideravelmente entre diferentes coortes. Até hoje no Brasil, a grande maioria dos estudos publicados são de associação de genes candidatos com a periodontite, também foram encontrados ensaios de expressão gênica e validações de GWAS na população brasileira(24,70). Em uma das validações, foram verificados 20 SNV em uma coorte do Rio de Janeiro (n = 359) e outra de Porto Alegre (n = 1460). A variante rs1477403 localizada em 16q22.3 foi a única que apresentou associação na coorte de Porto Alegre (p = 0,05)(70). Três variantes em 21q22.11 apresentaram tendência de associação (p < 0,05) com periodontite crônica na coorte do Rio de Janeiro(70). A validação de GWAS realizada em Araraquara/SP foi realizada com 714 pacientes maiores de 30 anos. Sete SNV foram verificados e os SNV nos genes *NPY, IL37* e *NCR2* apresentaram associação para suscetibilidade à periodontite moderada ou grave; enquanto o marcador no gene *TLR9* foi associado com menor chance de desenvolver periodontite grave(24).

Nos últimos 20 anos, foram publicados mais de 20 estudos gene candidato para periodontite, realizados na população brasileira. Pode-se observar na figura 2 que, como um quebra cabeça sendo montado, as análises até o presente momento ainda não concluem vias genéticas verdadeiramente associadas à periodontite e a quantidade de genes que não apresentaram associação com o desfecho proposto (cinza claro) está praticamente a mesma dos genes que obtiveram alguma associação (vermelhos e verdes).

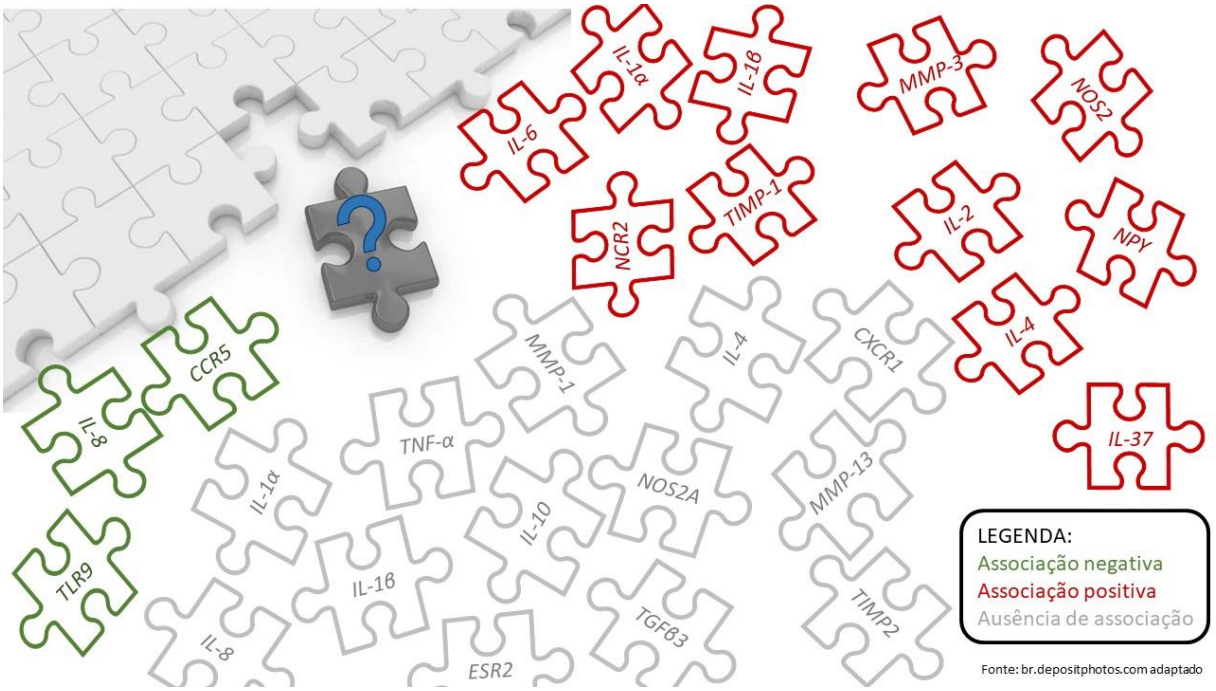


Figura 2: Panorama dos genes candidatos para periodontite estudados em população brasileira publicados até out/2022. As cores apresentam o resultado da associação da periodontite com o menor alelo da variante em estudo.

Os genes das interleucinas foram os mais estudados na população brasileira. Um estudo realizado com 163 brasileiros caucasianos encontrou que a presença do alelo T da variante *IL1α* (-889) aumenta as chances de desenvolvimento de periodontite crônica, tanto em fumantes quanto em não fumantes(71). Na mesma época, porém em uma população menor composta por portadores de HIV, não foi observada associação entre a variante *IL1α* (-889) e periodontite agressiva em pacientes brasileiros(72). Aproximadamente 2 anos após, uma população de indivíduos infectados por HIV não apresentaram associação entre destruição periodontal e variantes em *IL1α* ou *IL1β*(73). Para o gene *IL1β*, em 129 indivíduos de Minas Gerais se verificou que a presença do alelo T no *locus* +3954 pode ser um fator de risco para periodontite crônica(74). Este mesmo *locus* foi verificado em outro estudo consecutivo com o dobro da população anteriormente estudada, sendo constatado que, periodontopatógenos do complexo vermelho de *Socransky*, individualmente e aditivamente, modulam os níveis de IL1β nos tecidos doentes de pacientes com periodontite crônica, não fumantes(75). A presença do alelo polimórfico em rs1143634 de *IL1β* apresentou-se como protetor para o desenvolvimento de periodontite e diabetes melittus 2, sobretudo em mulheres(20).

O gene *IL2*, no *locus* -330 (T>G) no modelo dominante, foi associado à gravidade da doença periodontal(76). Este estudo foi incorporado a uma metanálise com mais 4 outros estudos internacionais desta variante, onde não foram encontradas associações com periodontite crônica(77). Três estudos pequenos (n = 39, 62 e 12) e um de maior significância amostral (n = 894) foram realizados para variantes e haplótipos do gene *IL4.* No primeiro, a terapia periodontal foi igualmente eficaz, independente da carga genética da *IL4* do indivíduo. O haplótipo TCI/CCI *IL4*, foi associado ao aumento dos níveis de bactérias periodontopatogênicas, mas esse *background* genético não influenciou a resposta ao tratamento periodontal não cirúrgico(78). No segundo, haplótipos *IL4* de suscetibilidade à periodontite crônica estão associados aos níveis de proteína IL4, mas não com os desfechos clínicos da terapia periodontal(78). No terceiro, as variantes genéticas em *IL4* associadas à positiva ou negativamente com a periodontite crônica estão diretamente associadas à influência da resposta das células imunes aos periodontopatógenos(79). Em estudo mais recente e significativo, a susceptibilidade para desenvolver periodontite e *diabetes mellitus* foi verificada na variante rs224320, sendo encontrada que a presença do alelo T aumenta em 80% as chances de desenvolvimento destas patologias(20)

Variantes no gene *IL6* foram verificadas em 3 estudos. Estudo em caucasianos brasileiros concluiu que o alelo C da variante em *IL6* (-174) está associada a susceptibilidade para periodontite crônica(80). Outro estudo confirmou que o a presença do mesmo alelo do mesmo SNV *IL6* (-174) está associado a gravidade da periodontite em indivíduos brasileiros(81). Em estudo mais amplo (n = 894), se observou que homens com o genótipo CC do rs1800795 em *IL6* são menos propensos a desenvolver periodontite e diabetes mellitus tipo 2(20).

Um haplótipo em *IL8*, formado por 3 variantes foi associado à susceptibilidade à periodontite crônica, embora não tenha sido encontrada nenhuma associação individual(82). Em estudo subsequente, este haplótipo não foi associado aos níveis de citocinas IL8 nem com parâmetros clínicos periodontais. No entanto, na periodontite crônica, o haplótipo em *IL8* e concentração de IL8 mostraram associação positiva com os níveis de volume de fluido crevivular gengival nos pacientes estudados(83). Na sequência da confirmação deste haplótipo, não foram encontradas diferenças significativas entre pacientes com ou sem o haplótipo *IL8* ATC/TTC para o resultado da terapia periodontal não cirúrgica e níveis de IL8(84).

Para o receptor da IL8, CXCR1, a variante rs2234671 não está associada à susceptibidade à periodontite crônica na população estudada(85).

Outras associações genéticas que foram estudadas na população brasileira tendo como desfecho a periodontite em suas diferentes classificações estão: *IL10*(27), *TNF-α*(26,72), metaloproteinases *MMP-1*(86) e *MMP-3*(86,87), *TIMP1*(87), *NOS2*(88) e *NOS2A*(27), *ESR2*(27) e *CCR5Δ32*(89). Recomenda-se que SNV identificadas através de uma abordagem essencialmente estatística sejam verificados biologicamente *in vivo*(6).

2.4 ESTUDOS DE AMPLA ASSOCIAÇÃO GENÔMICA PARA PERIODONTITE

A associação dos genes predisponentes a doenças complexas pode ser verificada através dos estudos de ampla associação genômica (GWAS)(90). O GWAS verifica se o alelo de uma variante genética é encontrado com frequência significativamente diferente entre indivíduos com periodontite versus indivíduos saudáveis(91). O GWAS amplia a visão para até milhões de marcadores em uma pequena amostra de DNA, identifica as variantes no DNA associadas a uma doença, no entanto, considera-se que estas variantes identificadas marcam uma região do genoma que pode influenciar para a predisposição ou para a proteção da doença estudada. Os dados devem ser devidamente tratados para evitar correlações falso positivas pois, sozinhos, não podem especificar quais genes são causais(62,92).

Estudos de GWAS se esforçam para reduzir a estratificação populacional, pois está dentre os fatores que causam diferentes achados genéticos dos GWAS para um mesmo desfecho. O primeiro GWAS de periodontite publicada foi estudada em uma população alemã e replicada em um painel de holandeses(28). Com população similar, o GWAS que identificou variantes em *SIGLEC5* e *DEFA1A3* foi realizada em amostra caso-controle mesclada entre alemães e holandeses(93). Outro GWAS foi realizado em indivíduos da Pomerânia Ocidental, alemães, oriundos de dois estudos transversais independentes (SHIP and SHIP Trend)(94). Mais recentemente, um GWAS estudou uma população isolada de italianos(95) e outro uma população espanhola(96).

Nas publicações de estudos americanos encontramos cinco GWAS com

desfecho associado a periodontite, inflamação periodontal e inflamação gengival grave, realizados na coorte longitudinal de risco de aterosclerose nas comunidades (ARIC), em população autorreferida branca de americanos europeus, sendo um com replicação insatisfatória em uma população afro-americana(60,97–100). Em outros GWAS com população americana observa-se que um recrutou do Registro Odontológico e Repositório de DNA (DRDR) da Faculdade de Medicina Dentária da Universidade de Pittsburgh(70) e o outro recrutamento ocorreu no Centro de Pesquisa em Saúde Bucal em Appalachia (COHRA1), que recrutou famílias do oeste da Pensilvânia e norte da Virgínia Ocidental, com exclusão de 110 hispanos caucasianos(101). Posteriormente, foi publicada um GWAS com população exclusivamente participante do Estudo da Saúde Comunitária Hispânica (HCHS/SOL)(102).

Por serem a vanguarda das ciências médicas, os GWAS foram maiormente realizados em europeus ou americanos mas não exclusivamente. Estudo caso controle, realizado entre 2760 japoneses com diagnóstico positivo para periodontite e 15158 japoneses saudáveis para esta patologia, não identificou associação genômica significativa para a periodontite, apenas 2 *loci* com associação sugestiva(103). Uma revisão sobre estudos de associação genética de susceptibilidade à periodontite agressiva em afro-americanos e outras populações de ascendência africana publicado em 2018, apresenta estudos com forte associação de genes candidatos em periodontite agressiva(62).

Este trabalho possui o potencial de proporcionar novas descobertas dentre os estudos de periodontite. Na população brasileira, a cidade de Salvador foi apontada como a população mais miscigenada que outras cidades do sudeste e sul do Brasil(104). O estudo referência EPIGEN Brazil Iniciative descreve que, em média, indivíduos desta população apresentam 50,8% de ancestralidade africana e 42,9% de ancestralidade europeia(104). O presente estudo se justifica por evidências de herdabilidade, ausência de marcadores de diagnóstico, dos distintos resultados de GWAS na literatura e escassas confirmações, sobretudo em populações latino-americanas miscigenadas. A literatura científica carece de um estudo de GWAS para periodontite nesta população.

3 OBJETIVOS

3.1 OBJETIVO PRINCIPAL

Investigar a associação entre variantes genéticas e periodontite em uma população de Salvador - Bahia, Brasil.

3.2 OBJETIVOS SECUNDÁRIOS

3.2.1 Estabelecer o grupo adequado de covariáveis para o ajuste das análises de associação genética verificando indicadores de risco para o desenvolvimento da periodontite.

3.2.2 Avaliar a associação de variantes genéticas relacionadas à periodontite, em um estudo de ampla varredura genômica (GWAS);

3.2.2 Realizar estudo de associação de genes candidatos envolvidos na patogênese da periodontite em comparação com controles saudáveis;

3.2.3 Realizar estudo *in silico* do impacto funcional das variantes associadas com periodontite.

CHAPTER 1 - MANUSCRIPT 1:

A STATISTICAL MODEL TO EVALUATE FACTORS ASSOCIATED WITH PERIODONTITIS

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ABSTRACT

The objective of this study was to search for factors associated with periodontitis through an adequate logistic model. A cross-sectional study (n=506) observed that the independent variables older age, sex, lower education, obesity, being a mouth breather, not using dental floss daily, being a smoker or ex-smoker, and the presence of asthma; are indicators of risk of periodontitis. Associations were measured by the Chi-square test and binary logistic regression, adjusted. The suitability of the model was tested by the Hosmer-Lemeshow test. Comparison of results was performed by StepAIC analysis. Older age, lower education level, obesity, mouth breathing, no use of dental floss and presence of asthma make up the most appropriate model ($p<0.001$). In the population studied, a composite of variables, including older age, lower education, obesity, mouth breathing, flossing and asthma were the most fit to adjust for the logistic regression analysis. In addition, obesity, not using dental floss and the presence of asthma are associated with the development of periodontitis.

KEYWORDS**:** Periodontitis. Obesity. Asthma. Logistic Models.

INTRODUCTION

The inflammatory process of periodontal tissues has been dimensionally studied, both for its local and systemic consequences and for its multifactorial origins. Dysbiosis of dental biofilm microenvironments stimulates an innate host response that, in a cascade effect, results in activation of the adaptive response with stimulation of osteoclasia[1,2]. The loss of dental elements associated with a variety of systemic conditions is recognized as a public health problem worldwide[3–5].

Periodontal disease reduces the quality-of-life score, it is more prevalent in the adult population, which brings ingrained and irregular habits of oral hygiene[6]. Multiple factors participate in the proximal/distal paradigm in the involvement with the disease[6]. Obesity[7,8], smoking habit[9], stress[10], age[11,12], sex[13,14], diabetes[15], asthma[15,16], even social class and race/ethnicity[17,18] was studied as predisposing factors. The fact is, that frontiers have expanded in the observation that genetic factors are associated, as a tie, with other factors, providing a predictive addition to the outcome[17,19,20].

Over the past decade, significant studies have explored the genetic bases for the susceptibility and severity of periodontal disease[17,19,21–23]. However, there are difficulties in estimating probabilities in complex diseases such as periodontal disease. Each predisposing factor can have multiple genetic determinants and the adjustment of analyzes using all predisposing factors, without criteria, can generate falsely robust associations [24,25].

Having an adequate logistic model for the adjustment of genetic analyzes in periodontal disease will help us to obtain greater precision of the genetic effect on the outcome[25]. Therefore, this study aims to find factors associated with the development of periodontal disease in a population of Salvador, Bahia, Brazil, through an adequate logistic model.

METHODS

Study Population

A cross-sectional study in patients verified for periodontitis, performed through the Programa de controle da asma na Bahia (ProAR) in Salvador / Bahia – Brazil. The database consisted of 506 adult individuals of both genders and with the requirement of them having at least 4 teeth in their mouths. Related individuals were excluded from the study. Ethical approval was obtained by the National Research Ethics Committee of Brazil (CONEP), according to the Declaration of Helsinki, with the Certificate of Presentation for Ethical Assessment (CAAE) number 5000.013834/2010-96. Information on personal characteristics, dental and health history was provided. Written informed consent was obtained from everyone.

Dependent variable: Periodontitis

The patients were diagnosed with periodontitis according to the criteria by Gomes-Filho et al[26,27] which consists of simultaneously presenting at least four teeth with one or more sites with probing depth ≥4 mm, clinical attachment loss ≥3 mm and bleeding on probing at the same site. A single examiner calibrated in 10% of the sample performed the analysis[27,28].

Independent variables

The population was verified in 37 characteristics divided into social, self-reported comorbidities, dental history, health habits (Table 1).

Statistical Analysis

Using the SPSS® v.20.0.0 software, the associations of the binary independent variables were verified using the chi-square test; non-binary variables were tested using Fisher's exact test. The presence of outliers was verified through frequency analysis, and the existence of multicollinearity between the variables through the variance inflation factor (VIF) and tolerance. For binary logistic regression, the stepwise–backward method was adopted to avoid type II error, using the likelihood ratio statistics for testing the global null hypothesis and the Akaike information criterion to determine the genetic model of inheritance. The Hosmer-Lemeshow test verified the model's adequacy. To compare the results, the StepAIC analysis was performed using the R® v.4.0.3 software for the same variables. The results with the values of $p < 0.05$ were considered as significant.

RESULTS

The Table 1 summarizes characteristics of the study population. The population was predominantly composed of women (83.20%) over 39 years old (67.39%), overweight (70.36%), with less than or equal to four years of schooling (80.04%), self-reported brown or black (83.20%). The ratio between groups with (n = 117) and without (n = 389) periodontitis was approximately 1:3, respectively.. The presence of asthmatics was significant in the sample (62.05%) because the study was carried out among ProAR patients, their companions and people who seek this referral center. In the dental history it was observed that most (55.34%) had been without dental care for over a year or had never even been to the dentist. Most of them were mouth breathers (63.04%), had malocclusion (71.54%) and had lost at least one permanent tooth (87.94%). 57.9% of this population declared the habit of brushing their teeth at least 3 times a day and 68.38% reported that they change their toothbrush at most every 4 months.

Quantitative association was evaluated between periodontal disease and the following independent dichotomous variables that make up the characteristics of the participants and their medical history: Age of participants, education level,

overweight, obesity, hypertension, kidney disease, heart disease and asthma. These characteristics and the cutoff follow the study carried out by Soledade-Marques et al.[27]. As for oral history and health habits, it was observed that the frequency of visits to the dentist, loss of permanent teeth, presence of gingivitis, mouth breathing and flossing at least once a day are also associated to periodontitis.

Frequency of physical activity, flossing and mouthwash, as well as alcohol use and smoking-related habits, reasons for tooth loss and time of tooth loss were classified as non-binary nominal and ordinal variables. In the Fisher exact test, they were significantly associated with periodontal disease, but they were not appropriate for logistic regression.

In the data quality control, the presence of outliers in the model was verified and the variables kidney disease presence and gingivitis were also excluded because their frequencies in the case or control groups compromise the reliability of the results. In the logistic regression, the multicollinearity between the variables is tested to avoid an overfit, checking the tolerance and the VIF.. The condition for the variables not to have high correlation is to have tolerance >0.1 and VIF values <10. A second multicollinearity test was performed, excluding the variables overweight, hypertension, presence of heart disease and time since the last dental appointment (Table 2); model that was used to continue the analyses. The sex variable remained in the analyzes as it participated as covariates in a sequence of studies[17,19,20,22,23].

To confirm the presence of multicollinearity in the independent variable obesity, overweight and hypertension, tolerance and VIF were tested. The values were identical in these variables, but the p value was less than 0.05 for the obesity and greater than 0.05 for the other 2 variables. We understand that if the variables flossing and time of last visit to the dentist are used together, her will over-adjust the analysis, as both represent the care that the patient has with his oral health. The SPSS INDICATOR coding standard was used with the choice of the last category as a base. Table 3 shows the codes of categorical variables for interpreting the results of the logistic regression.

Binary logistic regression was performed using the Hosmer & Lemeshow goodness test to verify whether the following variables - age, sex, schooling, obesity, mouth breathing, flossing, asthma, current or previous smoking habit are risk indicators of development of periodontal disease. As the logistic regression was exploratory, that is, to find a model to adjust the data, the stepwise - backward

method was adopted to avoid type II errors.

A prediction was made with an accuracy of 100% for cases without periodontitis and 0% for the presence of periodontitis. In this condition, if in each case the answer was NO, 76.9% of the answers would be correct.

In the equation variables, the Wald test was equal to 129,827. Exp value (B) equal to 0.301 with significance less than 0.001 with 1 degree of freedom. Evaluate the model as adequate. For Variables Not in the Equation (Table 4), the Overall Statistics in the last row represent the chi-square statistic. A p-value < 0.001 means that adding one or more of these variables to the model will significantly affect its predictive power, that is, it is necessary to proceed to find the ideal model[29].

Model 3 containing the variables age, schooling, obesity, mouth breathing, flossing and presence of asthma was significant [$X^2_{(1)}$ = 52.208; p < 0.001 (Block and Model) with 6 degrees of freedom; R^2 Nagelkerke = 0.148; Hosmer-Lemeshow test p = 0.982 with 7 degrees of freedom] with a prediction of 77.1% of correct answers (Table 5).

The variables obesity, flossing and presence of asthma were significant risk indicators of periodontitis with the following results. Absence of obesity is among the protective factors to prevent the development of periodontitis (OR = 0.595; 95% CI = 0.376 - 0.941; p-value = 0.027), the same occurs with the absence of asthma (OR = 0.388; 95% CI = 0.224 - 0.673; p-value = 0.001). Absence of daily flossing habit was a significant risk indicator of predisposition to periodontitis (OR = 1.956; 95% CI = 1.243 – 3.077; p-value = 0.004) (Table 6). The variables age, schooling and breathe through the mouth were not significant in the prediction.

StepAIC analysis[30] was performed to verify whether the following variables - age, sex, schooling, overweigth, obesity, hypertension, time to visit the dentist, mouth breathing, flossing, presence of asthma and smoke - were the best covariate model for the dependent variable, presence of periodontitis. The model containing the variables age, obesity, breathe through the mouth, flossing and asthma was the smallest model for this proposal.

The variables obesity, flossing and asthma were significant associated factors of periodontal disease with the following results: The presence of obesity is positively associated with the development of periodontal disease (OR = 1.756; IC 95% = 1.115 – 2.767; p-value = 0.015), the same is true for the presence of asthma (OR = 2.719; IC 95% = 1.578 – 4.686; p-value < 0.001). Regular flossing was a significant indicator

of protection for the development of periodontitis (OR = 0.477; IC95% = 0.306–0.741; p-value = 0.001) (Table 6). The variables age, mouth breather was not significant in the prediction.

DISCUSSION

The adequate logistic model for the analyzes of a population studied with periodontal disease in Salvador, Bahia/Brazil contains the variables age, schooling, obesity, breathe through the mouth, flossing and presence of asthma. Authors support the use of these variables in other compositions, depending on the population studied[11,17,19,20,25]. Of these variables, three were significantly positively or negatively associated with the presence of periodontitis. It has been statistically shown that being obese or asthmatic and don't floss are factors associated with periodontitis.

A pattern consistent with the association between obesity and periodontitis pointed to insulin resistance derived from a chronic inflammatory state and oxidative stress as the probable reasons for this association[7,8]. The descriptive data of this study showed the same proportion of obese and hypertensive individuals in the case group and a difference <1% in the control group, indicating the possibility of overfitting due to overlapping data. Hypertension and obesity are chronic diseases, and obesity is a risk factor for hypertension, but there is no evidence to the contrary[31].

Soledade-Marques et al. observed an association between severe asthma and periodontitis, with the frequency of periodontitis being higher in individuals in the case group compared to controls, as well as in participants with periodontitis outcome with a 3 times greater risk of developing severe asthma[27]. The same research team presented results that propose that dysbiosis in the subgingival biofilm, demonstrated by *Prevotella intermedia* levels, is associated with severe asthma[28]. Additionally, an association between asthma and chronic periodontitis suggests that further periodontal deterioration in the asthma group may occur due to increases in IgE in the gingival tissue.

A diseased periodontium has a microbial profile with an unbalanced proportion

of colonizing species. The absence of plaque removal generates responses modulated by dysbiotic bacteria[2]. Studies presented a positive association between adequate and regular use of dental floss to remove newly formed dental plaque and the reduction of periodontal disease [32–35]. Unlike other variables, in our study, the association between daily flossing and periodontal disease occurred even with similar proportions of daily flossing and non-flossing, with results of 52.6% for yes and 47.4% for not using it.

Covariates need to be associated with the outcome without high intercorrelations between these independent variables to avoid overfitting. In verifying the existence of multicollinearity between variables, a VIF equal to or greater than 5 and a tolerance of 0.2 or less are sufficient to explain the presence[36]. All independent variables fit these parameters, however, due to similarity and cause/effect, the results of the second multicollinearity test were used, in which the results are close to 1, suggesting a positive decision to reduce these variables. The stepwise - backward method to avoid type II error, not rejecting a hypothesis if it is false, was used because the forward method is more likely to exclude indicators involved in suppressor effects, where one indicator has a significant effect only when the other the variable is kept constant, with greater chances of type 2 error[36].

The Wald Test is equivalent to the T Test, in that any indicator in the model that has a statistically significant Wald value (above the default removal criterion of 0.1) will be removed. However, this statistic can sometimes be unreliable, and the probability value is preferable. Exp(B) is the value of OR. If the model is not adequate, the value of B will be close to zero and the value of $p > 0.05$, in this case, the model was adequate. The Nagelkerke R^2 overestimates in samples with a large group for cases[24], which did not occur in this sample, as we have 3:1, control:case.

The StepAIC in R analysis aimed to simplify the model without compromising performance and was compared with binary logistic regression performed in SPSS with the Hosmer-Lemeshow test, in the Backward model with the same covariates used in the logistic regression. The AIC value (Akaike information) is useful to select the best balance of the genetic model to be used, avoiding proxy variables. When performing the association analysis, the maximum probability estimate must be chosen for a model to serve as an adjustment, which is the one with the lowest AIC[30].

Some factors overlap but are not identical; the choice of confounders is theoretical based on the outcome, study design, and population characteristics. In

many cases, the decision to include or exclude is based on physiological, social, group-specific, or causal reasons[25]. However, it is necessary to consider that the individual's susceptibility, as well as populations aspects, strongly influence the severity and presence of periodontitis[3,5] . Thus, due to the gap in the literature[25], this study presented a practical and accurate statistical model for the selecting the covariates or confirming those theoretically selected, as well as for verifying risk indicators for the development of diseases. The advantage of having a model is the safety, speed, and reduction of bias in the analysis. The limitation of this study is that the data used were high frequency extracted from a database with a asthmatics and, consequently, impair the extrapolation of the results to other populations.

CONCLUSIONS

This study indicates the variables age, schooling, obesity, breathing through the mouth, not flossing and asthma are the best choices for adjusting the logistic regression analysis and that obesity, not flossing and the presence of asthma are risk factors for the development of periodontal disease. It is suggested that the model be validated to populations of different sizes and with different independent variables.

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Table 1: Characteristics of the study population (n = 506) in absolute and relative frequencies.

Characteristics	**Without**	**With**	**p-value***

	periodontitis (%) n = 389	periodontitis (%) n = 117	
> 39 years	253 (65.0)	88 (75.2)	0.04
Women	327 (84.1)	94 (80.3)	0.345
Schooling ≤ 4 years	325 (83.5)	80 (68.4)	<0.001
Black or mixed self-reported race	328 (84.3)	93 (79.5)	0.220
Self-reported comorbidities			
Overweight (BMI ≥ 25)**	265 (68.1)	91 (77.8)	0.045
Obesity (BMI ≥ 30)**	102 (26.2)	47 (40.2)	0.004
Hypertension	99 (25.5)	43 (36.8)	0.017
Diabetes	18 (4.7)	9 (7.7)	0.196
Osteoporosis	13 (3.3)	5 (4.3)	0.633
Rhinitis	124 (31.9)	33 (28.2)	0.452
Presence of sexually transmitted diseases	5 (1.3)	0 (-)	0.218
Anemia presence	5 (1.3)	1 (0.9)	0.706
Kidney disease presence	1 (0.3)	3 (2.6)	0.013
Presence of cardiopathies	10 (2.6)	8 (6.8)	0.029
Hypercholesterolemia	43 (11.1)	15 (12.80)	0.599
Positive diagnosis of neoplasias	5 (1.3)	0 (-)	0.218
Liver disease	1 (0.3)	1 (0.9)	0.366
Presence of gastric diseases	39 (10.0)	14 (12.0)	0.548
Presence of other illnesses	54 (13.9)	16 (13.7)	0.955
Asthma	219 (56.3)	95 (81.2)	<0.001
Dental history			
Mouth breather	228 (58.6)	91 (77.8)	<0.001
Visit the dentist regularly***	202 (51.9)	50 (42.7)	0.081
Visit dentist ≥ 1 year or never	204 (52.4)	76 (65.0)	0.017
Oral hygiene orientation	310 (79.7)	91 (77.8)	0.654
Gengivitis	14 (3.6)	0 (-)	0.037
Lost permanent tooth	335 (86.1)	110 (94.0)	0.021
Presence of malocclusion	270 (69.4)	92 (78.6)	0.053
Presence of a soft tissue lesion	26 (6.7)	14 (12.0)	0.063
Any surgery	189 (48.6)	54 (46.2)	0.700
Health habits			
Be a smoker or have already smoked	129 (33.2)	47 (40.2)	0.163
Alcoholic beverages	135 (34.7)	47 (40.2)	0.280
Practice physical activity	129 (33.2)	43 (36.8)	0.472
Brush the teeth	387 (99.5)	116 (99.1)	0.674
Brush your teeth ≥ 3 times a day	229 (58.9)	64 (54.7)	0.423

Flossing	221 (56.8)	45 (38.5)	0.000
Uses mouthwash	148 (38.1)	52 (44.4)	0.215
Brush change in less than 4 months	269 (69.2)	77 (65.8)	0.496

*Pearson chi-square. **Measured comorbidities. ***One or more times a year.

Table 2: Verification of collinearity statistics between independent variables.

Variables	**Tolerance (1)**	**VIF (1)**	**Tolerance (2)**	**VIF (2)**
Age	0.865	1.155	0.975	1.026
Sex	0.944	1.059	0.957	1.045
Schooling	0.874	1.144	0.890	1.123
Obesity	0.789	1.267	0.953	1.049
Overweight	0.782	1.279	-	-
Mouth breather	0.820	1.219	0.832	1.202
Hypertension	0.802	1.247	-	-
Flossing	0.906	1.104	0.932	1.073
Visit dentist ≥ 1 year or never	0.926	1.080	-	-
Asthma	0.811	1.233	0.814	1.229
Be a smoker or have already smoked	0.968	1.033	0.970	1.031

VIF: Variance inflation factor

Table 3: Coding of Categorical Variable Parameters (n = 506).

Categorical variables		**Frequency**	**Parameter coding**
Do you smoke or have you already smoked?	No	330	1
	Yes	176	0
Sex	Female	421	1
	Male	85	0
Educational level in full years of study	More than 4 years	101	1
	Less than or equal to 4 years	405	0
Obesity	No	357	1
	Yes	149	0
Breathe through the mouth	No	187	1
	Yes	319	0
Presence of asthma	No	192	1
	Yes	314	0
Floss at least once a day	No	240	1
	Yes	266	0
Age in years	18 to 39	165	1
	Greater than 39	341	0

Table 4 – Variables not in the equation.

Step 0 - Variables	Score	df	Sig.
Age	4.238	1	0.040
Sex	0.890	1	0.345
Schooling	12.959	1	0.000
Obesity	8.425	1	0.004
Breathe through the mouth	14.181	1	0.000
Flossing	12.148	1	0.000
Presence of asthma	23.681	1	0.000
Smoke	1.948	1	0.163
Overall Statistics	50.533	8	0.000

df: degrees of freedom; sig.: p value.

Table 5: Verification of the best fit model between independent covariates.

Step	Hosmer-Lemeshow test			Model Summary			Porcentage correct (%)
	Chi-square	df	Sig.	-2 Log likelihood	Cox&Snell R Square	Nagelkerke R Square	
1	8.075	8	0.426	493.611	0.101	0.152	77.5
2	3.619	8	0.890	494.217	0.099	0.151	77.5
3	1.498	7	0.982	495.033	0.098	0.148	77.1
4	2.230	8	0.973	496.918	0.095	0.143	77.1
5	4.926	7	0.669	499.524	0.090	0.136	76.9

df: degrees of freedom; sig.: p value.

Table 6: Analysis results for comparison (n = 506).

Variables		Hosmer-Lemeshow test			StepAIC		
		OR	95%CI	p-value	OR	95%CI	p-value
Obesity	No	0.595	0.376 - 0.941	0.027			
	Yes				1.756	1.115 – 2.767	0.015
Asthma	No	0.388	0.224 - 0.673	0.001			
	Yes				2.719	1.578 – 4.686	<0.001
Flossing	No	1.956	1.243 – 3.077	0.004			
	Yes				0.477	0.306 – 0.741	0.001

95%CI: confidence interval 95%. OR: odds ratio.

CHAPTER 2 - MANUSCRIPT 2

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.

Keywords: Genetic variants. *AIM2*. *IFI16*. Gene. Periodontitis. Inflammasome.

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, 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; S. 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

1,179 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) n° 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 (World Health Organization, 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 < 25kg/m² (without excess weight) and ≥25kg/m² (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 ≥ 1 site with probing depth ≥ 4 mm; clinical attachment level ≥ 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 (Figure 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-60K 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.).

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 his 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 ≤ 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% - 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 x 10-6 for *IFI16* rsrs76457189. No significant eQTL was found for *AIM2* rs75985579 in tissue All eQTL Tissues.

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 (Figure 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. Figure 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. (Figure 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 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 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 *IFI-16* 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 *IFI-16* 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 IL1β (Antao et al., 2021), while the AIM2 inflammasome induces the differentiation of pro-IL1β into IL1β. However, much remains to be understood about the relationship between this transcription factor and the *AIM2* gene, especially in periodontitis.

Understanding how the IFI-16 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 IFI-16 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 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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Figure 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.

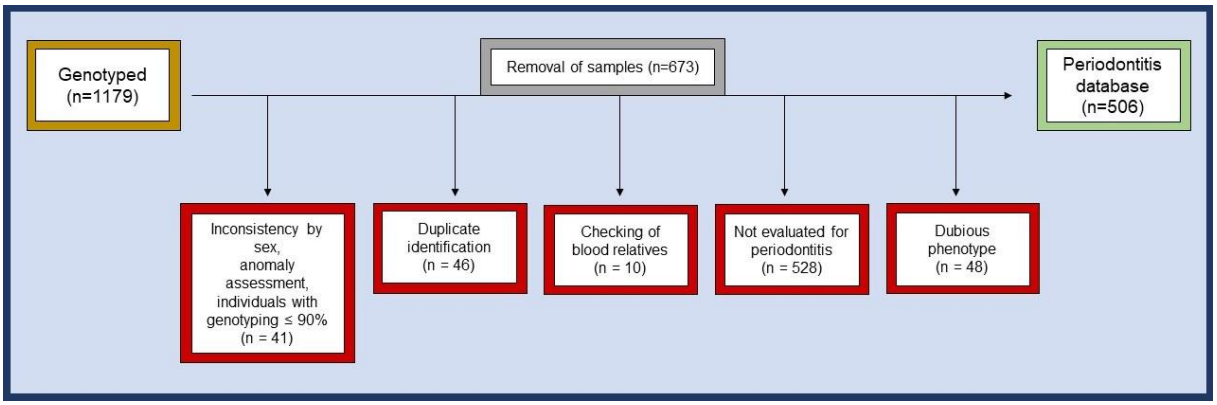

Figure 2:

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.

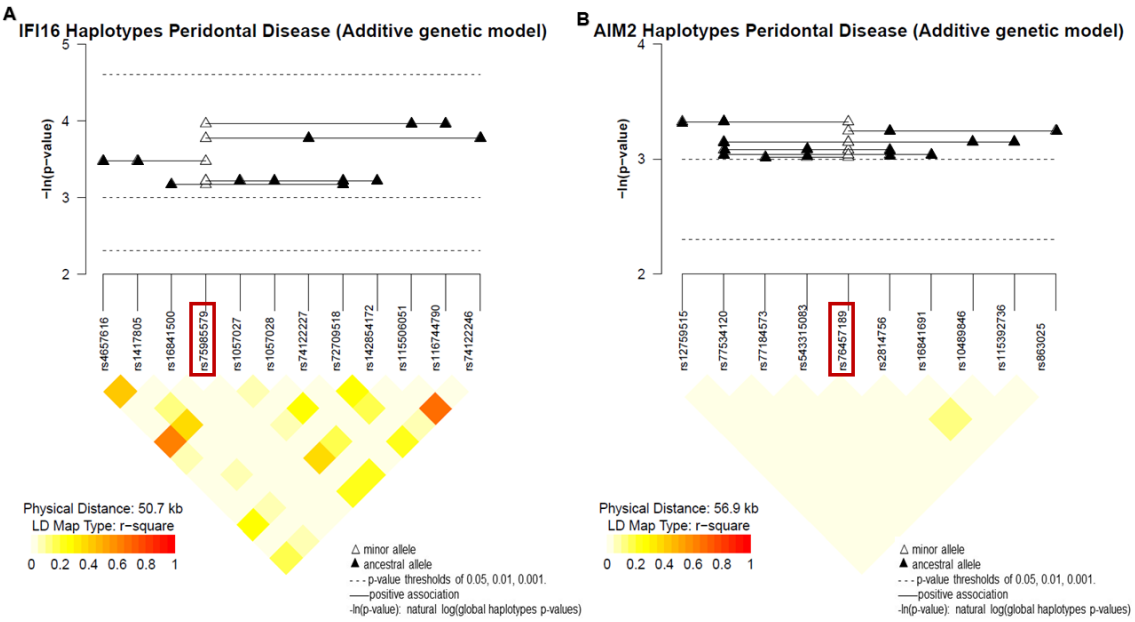

Figure 3:

Association 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.

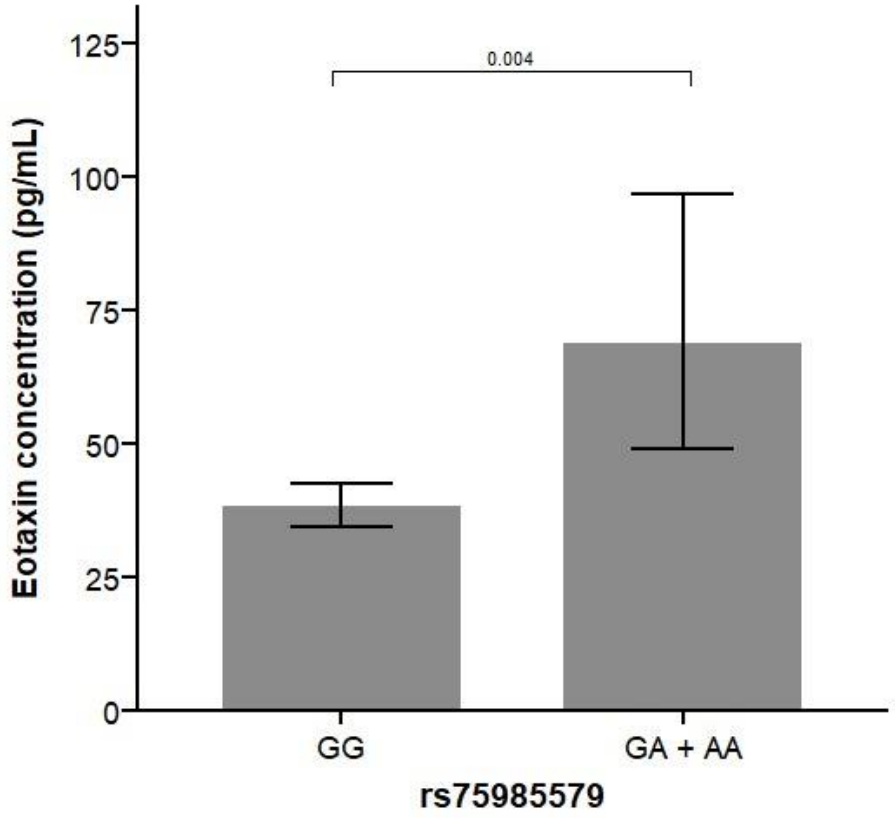

Table 1:

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 ≤ 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 ≥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 ≥ 25. [2]Dichotomized in BMI < and ≥ 30.

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	ADD	GG	372(95.63)	105(89.74)	2.65	1.25-5.60	0.007
			AG	17(4.37)	10(8.55)			
			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	ADD	AA	363(93.56)	115(98.29)	0.21	0.05-0.94	0.022
			GA	25(6.44)	2(1.71)			
			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 2_PromU H3K4me1_Enh H3K4me3_Pro H3K27ac_Enh	7_Enh 14_EnhA2 H3K4me1_Enh H3K4me3_Pro 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.

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. SNV: single nucleotide variant.

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.

Supplemental files

Supp. Table 1:

Variants expressed in *IFI16* and *AIM2* genes after quality control.

SNV	Position	A1	A2	MAF	GENO	HWE	Functional Consequense
				*IFI16**			
rs4657616	chr1:158971086	A	G	0.2263	30/169/307	0.2477	intron
rs4657618	chr1:158972490	A	G	0.4200	99/227/180	0.1244	intron
rs856060	chr1:158978137	A	G	0.0337	1/32/472	1	intron
rs9887904	chr1:158978596	C	A	0.1354	14/109/383	0.7603	intron
rs1417805	chr1:158979458	A	C	0.2609	34/196/276	0.8263	intron
rs2276404	chr1:158979950	A	G	0.2026	17/171/318	0.1457	5 Prime UTR Variant
rs146748131	chr1:158980213	A	T	0.01587	0/16/490	1	intron
rs12756557	chr1:158980534	G	A	0.1542	11/134/361	0.4541	intron
rs35904745	chr1:158981969	C	A	0.04947	1/48/457	1	intron
rs856064	chr1:158983285	A	G	0.4704	128/220/158	0.06532	intron
rs142942227	chr1:158984054	G	A	0.01587	0/16/490	1	intron
rs866484	chr1:158986477	G	C	0.4287	101/231/173	0.7648	missense - S (Ser) > T (Thr)
rs16841500	chr1:158986561	G	A	0.0287	0/29/477	1	intron
rs12057410	chr1:158988813	G	A	0.06223	2/59/445	0.5914	intron
rs75985579	chr1:158988992	G	A	0.0306	2/27/477	1	intron
rs140895207	chr1:158989844	A	G	0.0138	0/14/492	1	intron
rs148678235	chr1:158993690	G	A	0.0138	0/14/492	1	intron
rs861318	chr1:159002222	G	A	0.4081	87/239/180	0.294	intron
rs1057027	chr1:159002377	A	C	0.4059	85/240/180	0.2309	missense - R (Arg) > S (Ser)
rs1057028	chr1:159002389	T	A	0.4071	86/240/180	0.2309	missense - Y (Tyr) > N (Asn)
rs74122227	chr1:159002410	A	G	0.0158	1/14/491	1	missense - T (Thr) > A (Ala)
rs16841532	chr1:159003520	A	G	0.1502	11/130/365	0.6064	intron
rs72709518	chr1:159004900	A	G	0.0217	0/22/484	1	intron
rs1633264	chr1:159005585	G	A	0.0148	0/15/491	1	intron
rs1772408	chr1:159005649	G	A	0.3745	77/225/204	1	intron
rs1633266	chr1:159005977	A	G	0.3508	68/219/219	0.759	intron
rs3754460	chr1:159006846	A	G	0.1966	22/155/329	0.8307	intron
rs142854172	chr1:159014172	T	A	0.0208	0/21/485	1	intron
rs115506051	chr1:159016433	A	C	0.0178	0/18/488	1	intron
rs2793843	chr1:159016491	G	A	0.0484	3/43/460	0.1279	intron
rs3018316	chr1:159017958	A	C	0.2881	48/195/262	0.3916	intron
rs73023727	chr1:159020844	A	T	0.0188	0/19/487	1	intron
rs116744790	chr1:159021132	A	C	0.0148	0/15/491	1	intron
rs74122246	chr1:159021815	A	C	0.0158	1/14/490	1	missense - V (Val) > G (Gly)
rs59710606	chr1:159022779	G	A	0.0168	0/17/489	1	intron
rs6940	chr1:159024668	A	T	0.2085	19/173/314	0.3013	missense - T (Thr) > S (Ser)
				AIM2**			
rs74689714	chr1:159032484	A	C	0.0288	0/31/475	1	stop-lost - stop,GLU
rs12139815	chr1:159042416	C	A	0.0485	1/48/457	0.4948	intron
rs2276405	chr1:159043196	G	A	0.0201	0/13/493	1	missense - GLU,stop,GLN
rs12759515	chr1:159044590	A	C	0.0119	0/9/497	1	intron
rs77534120	chr1:159045762	A	G	0.0178	0/15/491	1	intron
rs77184573	chr1:159051634	A	G	0.0411	1/45/460	0.5914	intron
rs543315083	chr1:159057129	T	A	0.0301	0/31/475	1	intron
rs76457189	chr1:159061359	A	G	0.0215	0/27/478	1	intron
rs2814756	chr1:159074447	A	G	0.2651	46/167/293	0.2138	intron
rs12047287	chr1:159077121	A	G	0.2136	27/177/301	1	intron
rs16841691	chr1:159080243	G	A	0.1124	8/114/384	0.743	intron
rs10489846	chr1:159086711	A	G	0.1627	9/125/372	0.1327	intron

rs12130875	chr1:159093732	C	A	0.0978	3/94/409	0.6986	intron
rs191580157	chr1:159093992	T	A	0.0151	0/17/488	1	intron
rs115392736	chr1:159094844	C	A	0.0156	0/16/490	1	intron
rs2814779	chr1:159098713	G	A	0.2626	39/200/267	0.8533	intron
rs863025	chr1:159101523	G	A	0.0795	2/75/429	1	intron

Note: *IFI16 also known as PYHIN2; IFNGIP1; SNV: Single Nucleotide Variant; A1: ancestral allele; A2: minor allele; MAF: minor allele frequency; GENO: A2A2/A1A2/A1A1; HWE: Hardy–Weinberg equilibrium; ** Also known as PYHIN4.

CHAPTER 3 - MANUSCRIPT 3

GWAS IDENTIFIES GENES ASSOCIATED WITH PERIODONTITIS IN MIXED-RACE LATIN AMERICANS

Manuscript to be submitted to Journal Dental Research

ABSTRACT

Introduction – Periodontitis has multiple causal components that act through the interaction between environmental, epigenetic and, genetic factors. The disease varies from remissive to active state, without obvious or logical explanation. Genetic variants can determine a susceptibility to the development of the disease and therefore need to be better characterized. **Objective** – Identify genetic regions or pathways associated with periodontitis through a Genome-Wide Association Studies in a population of Salvador, Bahia, Brazil. **Methods** –This study included 389 controls and 117 cases with periodontitis from The Program for asthma control and allergic rhinitis in Bahia (ProAR). The samples were genotyped using the Illumina Infinium kit Multi-Ethnic AMR/AFR-8, imputed by CAAPA (Consortium on Asthma among African-ancestry Populations in the Americas) panel, Michigan Imputation Server (https://imputationserver.sph.umich.edu). We examined genome-wide associations ($p<5x10^{-8}$) and followed the most promising ones in pathway verification and marker identification. Subsequently, we performed annotations and functional analysis, as well as road analysis on *in silico* platforms. Linkage disequilibrium, haplotype analysis with outcome and gene-gene interaction. **Results** – We developed a GWAS in this population (λ = 1,015) and found a positive association between periodontitis and the presence of the T allele of rs10496038 in the *RTN4* (Reticulon 4) gene on chromosome 2 (ORadjusted = 33.27, CI95% = 10.29 – 107.63, p value = 4.88×10^{-9}) and the C allele of rs58327429 (ORadjusted = 4.13, CI95% = 2.51 – 6.81), p value = 2.36×10^{-8}) and the A allele of rs67797971 (ORadjusted = 4.07, CI95% = 2.46 – 6.72, p value = 4.35×10^{-8}) on the *LINC02505* (long intergenic non-protein coding RNA 2505 – ncRNA) gene on chromosome 4. High linkage disequilibrium were found in all chromosomes present in the result of this study. Of these variants, 5 haplotypic

blocks associated with periodontitis were located. The presence of 5 or more alternative alleles increases the chances of developing periodontitis by more than 5 times. **Conclusion -** This study is a pioneer in a Brazilian population and presented a list of genes associated and suggestively associated with periodontitis. Three significant genomic associations and another 130 suggestive associations ($10^{-6} > p > 5.10^{-8}$) were presented. The significant genome-wide success of the *RTN4* gene and the associations between variants establish it as a putative genetic risk factor for periodontitis. The results of this GWAS may favor studies of therapeutic targets. Further investigation of the *loci* presented here and the influence of these genes on periodontitis is recommended.

KEYWORD: GWAS. Polymorphism. Gene. Periodontitis.

1 INTRODUCTION

Periodontitis has multiple causal components common to chronic inflammatory diseases(1). Lifestyle, environmental factors, systemic conditions, dysbiosis, diet, aging are capable of epigenetically altering the individual(2). An individual's heredity is perhaps the most complex of factors, where genetic variants of protection or risk are presented as an accounting balance (3,4). These causal components do not contribute proportionately among individuals. The immune response to the chronicity of the disease varies between individuals, ranging from remissive to active state without obvious or logical explanation(1).

The first genetic risk site for periodontitis with Genome-wide statistical significance was published in 2009 in a German population studied between 2003-2008 and validated in a Dutch population(5). In this study, the G allele of the intronic variant rs1537415, located in the glycosyltransferase gene - *GLT6D1* - was significantly associated with aggressive periodontitis(5). Subsequent studies have shown variants significantly associated with periodontitis in several genes, such as *SIGLEC5* (sialic acid-binding immunoglobulin-like lectin 5)*, DEFA1A3* (defensin alpha 1 and alpha 3)(6), *EFCAB4B* **(**EF-hand calcium binding domain 4B**)**(7), one *locus* in the 1q42.2 region *TSNAX-DISC1* (translin associated factor X - disrupted in

schizophrenia 1)(8), *MTND1P5* (mitochondrially encoded NADH: ubiquinone oxidoreductase core subunit 1 pseudogene) on chromosome 8 and the LOC107984137 (long non-coding intergenic RNA) in chromosome 16, downstream of the *SHISA9* (shisa family member 9) gene(9).

Genome-wide association studies (GWAS) for periodontitis were initially developed and validated mainly in European populations(5,10,11). The first study in populations of different ancestry was a study to validate a GWAS performed in a European population, finding no statistical significance(12). GWAS performed at the University of Pittsburg resulted in 20 variant results suggestive of association. These variants were validated in 2 Brazilian groups: one from Rio de Janeiro and the other from Porto Alegre(13). The rs1477403 variant located at 16q22.3 was validated with suggestive significance in the Porto Alegre study. Three variants in 21q22.11 tended to be associated ($p < 0.05$) with chronic periodontitis in the Rio de Janeiro cohort(13). Another validation in a Brazilian population of GWAS was performed with 714 patients older than 30 years. Seven single nucleotide variants (SNV) were verified and the SNV in the *NPY* (Neuropeptide Y), *IL37* (interleukin 37) and *NCR2* (natural cytotoxicity triggering receptor 2) genes were associated with susceptibility to moderate or severe periodontitis, while the *TLR9* (toll like receptor 9) marker was associated with a lower chance of developing severe periodontitis(14).

This is the first GWAS performed for periodontitis in a Brazilian population, miscegenated and a strong African heritage. Therefore, the objective of this study was to identify variants, regions and genetic pathways associated with periodontitis through genome-wide association studies in a population from Salvador, Bahia, Brazil.

2 METODOLOGY

2.1 Participants and Studies

The Programa de Controle da Asma na Bahia (ProAR) in Salvador / Bahia - Brazil is a cohort existing since 2010 that, examined transversally more than 800

patients for the presence of periodontitis and completed the questionnaire by entering personal information and self-reports about their general and oral health status. After inclusion and exclusion criteria, genotyping and genetic quality control, 506 patients make up the periodontitis database. At the time, study participants were between 18 and 81 years old. The median is 45.5, respectively, for the original data, with a standard deviation of 12.77. Each participant expressed free and informed consent. This project received National Research Ethics Council - CONEP - approval with number 15782 - CAAE: 25000.013834/2010-96, in accordance with the Declaration of Helsinki.

2.2 Phenotype definition

Cases were defined as individuals with at least four teeth containing a site with a probing depth greater than or equal to 4 mm, clinical attachment level greater than or equal to 3 mm, and bleeding on probing at the same site.

Controls were people with at least 4 teeth in the mouth, analyzed for periodontitis, who had healthy periodontium or who had insufficient clinical criteria for inclusion in the case group. Patients were evaluated by a single trained periodontist and inter- and intra-examiner validity with kappa index (± 1 mm difference) in 10% of the sample. All teeth were evaluated at 6 sites, except for the third molars(15).

2.3 Genotyping and quality control

DNA was extracted from blood samples according to the Gentra Puregene® Blood Kit (Quiagen, Germany) protocol, stored at -30 °C at a concentration of 5ng / µL. Genotyping was performed using the Illumina Multi-Ethnic Global Array (MEGA, Illumina) (www. ilumina.com) which includes over 1.5 million variants.

In the quality control, blood relatives, inconsistency by sex, evaluation of anomalies, identification of duplicates, confirmation of the phenotype were verified. Individuals with genotyping less than or equal to 10%, absent variants in 10% or more of the subjects, alleles with a frequency less than 1% and variants that showed

Hardy-Weinberg equilibrium with p value <0.05 in controls were excluded from the analysis(16).

2.4 Imputation Strategy

For the GWAS an imputation was performed through the CAAPA (Consortium on Asthma among African-ancestry Populations in the Americas) panel, Michigan Imputation Server (https://imputationserver.sph.umich.edu). Previously, non-biallelic variants were excluded, with frequency of the minor allele less than $1x10^{-3}$, or or variants with deviation from Hardy-Weinberg equilibrium with p value < 0.05, individuals with a genotyping rate less than 98% and variants that were not present in the minimum in 98% of individuals. In addition, there was a quality control of the Michigan Imputation Server with the exclusion of incompatible alleles, invalid alleles (indels) and duplicated variants. In imputation quality control, 16,915 variants removed due to Hardy-Weinberg exact test, 5,156,655 variants removed due to minor allele threshold. Approximately 15 million genotyped and imputed SNV passed quality control filters and were included in the GWAS.

2.5 GWAS

The association statistic for each variant of the 22 autosomal chromosomes was calculated assuming an additive genetic model, adjusting for the dichotomized covariates: age, obesity, mouth breathing habit, flossing at least once a day, presence of asthma and the variable continuous principal component of ancestry 1. The adjustment covariates for the analyzes were decided after binary logistic regression with the Hosmer Lemershow test performed in SPSS (Statistical Package for the Social Sciences) version 20.0(17).

The GWAS was performed on the R platform version 3.6.3 (R Core Team, 2020), packages GWASTools, SeqVarTools, and GENESIS. Statistical significance was evaluated considering the Bonferroni criterion $\alpha = 0.05$ and the number of

genotyped variants verified in the GWAS (~15000000), statistical significance will be found in a p value lower than 3.33 x 10^{-9}. Due to the studied population being too small for a GWAS, we decided not to impose as much statistical rigor and applied a multi-test correction assuming 1 million independent tests, considering as a genomic significance p value $<5 \times 10^{-8}$. The p-value $<10^{-5}$ was defined to be the threshold of suggestive association.

Post-analysis procedures included generating Manhattan plots and quantile-quantile (Q-Q) plots with the genomic inflation factor (lambda) and detailed annotation of the context and gene functions.

2.6 Funtional Annotation

We used online information from the platform of the National Center for Biotechnology Information (NCBI-SNP) (https://www.ncbi.nlm.nih.gov), Ensembl(18) and Haploreg v 4.1(19) to locate the rsID and the gene where the variant is located or closest to it. The SeattleSeqAnnotation platform (https://snp.gs.washington.edu/SeattleSeqAnnotation137/) made it possible to convert all locations into rsID in a practical way. In the analysis of the regulatory potential of the variants, the variant biotype and the consequence on these same platforms were verified. The classification of regulatory potential was performed using the RegulomeDB platform(20).

2.7 Funtional Analysis

Enhancer histone tags, altered motifs, protein limits. Primary Osteoblast Cells, Primary Adult Dermal Fibroblast Cells, Primary peripheral blood mononuclear cells were selected to present the regulatory states of chromatin DNAse and histone ChIP-Seq (Roadmap Epigenomics Consortium, 2015), as they are cells somehow related to periodontal tissue. All this information was extracted from the Haploreg v4.1 platform(19), as it was about analyzing the impact of non-coding variants.

2.8 Visualization enrichment

LocusZoom v0.12 was used to visualize genomic regions of interest. The regions around the 3 significantly associated variants were visualized in Zoomplot(21). Highlighted is the top SNV and periodontitis-associated variants in this GWAS, in colors representative of the top SNV binding disequilibrium.

We also present the location of these variants and their genes in the chromosome images (https://www.ncbi.nlm.nih.gov/variation/view/). The violin-plot image shows the distribution of alleles of these variants in the whole blood of a reference population in GTEX Portal (https://www.gtexportal.org).

2.9 Analysis of signaling pathways

The network of interactions between the proteins of the genes resulting from this GWAS were verified in STRING® v 11.5(22), followed by the analysis of pathways in the VEGAS2 platform(23).

We verified the number of SNV within each chromosome that showed a suggestive or true association with periodontitis and the characteristics of local linkage disequilibrium (LD). The LD between the markers was estimated by the square of the correlation between two *loci* (r^2) using the Haploview 4.2® software(24) considering high correlation values greater than 0.8.

We also examined haplotype formation and its association with outcome, prioritizing SNV that showed high linkage disequilibrium. Haplotype frequencies and their association with periodontitis outcome were verified by means of logistic regression, adjusted for covariates age, obesity, mouth breathing, flossing, asthma, and main component of ancestry, using the SNPSTATS platform(25).

The gene-gene interaction was performed using a risk allele of each gene, from the variants that formed haplotypes with periodontitis. After data preparation, the SNPSTATS platform(25) was used for logistic regression. Analyzes were adjusted for

covariates age, obesity, mouth breathing, flossing, asthma, and main component of ancestry.

2.10 Analyzes on the influence on the expression of cytokines

We verified whether the presence of minor alleles in SNV associated with statistical significance with periodontitis influences the concentration of cytokines related to the inflammatory state. A subsample of this study population (n = 296) was analyzed using the HCYTOMAG-60K assay kit (Bio-Rad Laboratories, Hercules, CA, USA) to verify the serum concentration of Eotaxin-1, IFNγ (interferon γ), the following interleukins IL1β, IL5, IL6, IL8, IL10, IL12, IL13, IL17A and TNF-α (tumor necrosis factor α). The minimum detectable concentration of each analyte was determined by the detection limit value of each analyte plus 2 times the standard deviation: Eotaxin-1 (6.8 pg/ml), IFN-γ (1.1 pg/ml), IL 10 (1.6 pg/ml), IL12 (p70) (1.0 pg/ml), IL13 (1.9 pg/ml), IL17A (1.2 pg/ml), IL 1β (1.0 pg/ml), IL5 (0.7 pg/ml), IL6 (1.3 pg/ml), IL8 (0.7 pg/ml) and TNF-α (1.1 pg/ml). As quality control, analyzes in which the presence of genotypes was less than 5 in any of the groups, as well as cytokines whose results showed less than 90% of individuals above the detection limit, were excluded. After verifying normality using the Shapiro-Wilk test, the Mann Whitney hypothesis test was applied.

3 RESULTS

3.1 Participants

The database consisted of 389 controls and 117 cases studied for periodontitis. Black women (83.2%), over 40 years old[15] (67.4%), overweight (70.4%), with low education (80.0%), living in Salvador / Bahia / Brazil (97.0%) were the majority in this study. Asthma is the only comorbidity significantly present

(62.1%). With no soft tissue injuries (92.1%), no gingivitis (97.2%), loss of permanent teeth (87.9%), malocclusion (71.5%), and a significant habit of mouth breathing (63%), most of these participants have already received guidance on oral hygiene(79.2%). The descriptions of the participants, case and control, are presented in tables with socioeconomic and demographic characteristics (Table 1), general health conditions (Table 2), self-reported situations of oral health conditions and lifestyle habits (Table 3).

Table 1 – Socioeconomic and demographic characteristics of the study group for periodontitis (n = 506).

Socioeconomic and demographic characteristics	Control (%) n = 389	Case (%) n = 117	All (%) n = 506	p value*
Age (in years)				
18 - 39	136 (35.0)	29 (24.8)	165 (32.6)	0.04
> 39	253 (65.0)	88 (75.2)	341 (67.4)	
Sex				
Male	62 (15.9)	23 (19.7)	85 (16.8)	0.345
Female	327 (84.1)	94 (80.3)	421(83.2)	
Self-reported race/ethnicity				
Caucasian	61 (15.7)	24 (20.5)	85 (16.8)	0.220
Black or brown	328 (84.3)	93 (79.5)	421 (83.2)	
Level of education in years of study				
≤ 4	325 (83.5)	80 (68.4)	405 (80.0)	<0.001
> 4	64 (16.5)	37 (31.6)	101 (20.0)	
Household density (number of people)				
≤ 3	217 (55.8)	72 (61.5)	289 (57.1)	0.270
>3	172 (44.2)	45 (38.5)	217 (42.9)	
Marital status				
With partner	196 (50.4)	52 (44.4)	248 (49.0)	0.260
No partner	193 (49.6)	65 (55.6)	258 (51.0)	
City of residence				
Salvador/Bahia	375 (96.4)	116 (99.1)	491 (97.0)	0.125
Bahia interior	14 (3.6)	1 (0.9)	15 (3.0)	
Family income (in minimum wages)				
≤ 1	87 (22.4)	41 (35.0)	128 (25.3)	0.06
> 1	302 (77.6)	76 (65)	378 (74.7)	
Habitation				
Personal	316 (81.2)	92 (78.6)	408 (80.6)	0.532

Not own	73 (18.8)	25 (21.4)	98 (19.4)

*Chi-square test (control-case) significance level ≤ 0.05

Table 2: Characteristics related to the general health conditions of the studied group for periodontitis (n = 506).

Characteristics of health conditions	Control (%) n = 389	Case (%) n = 117	All (%) n = 506	p value*
Overweight (BMI)				
< 25	124 (31.9)	26 (22.2)	150 (29.6)	0.045
≥ 25	265 (68.1)	91 (77.8)	356 (70.4)	
Obesity (BMI)				
< 30	287 (73.8)	70 (59.8)	357 (70.6)	0.004
≥ 30	102 (26.2)	47 (40.2)	149 (29.4)	
Hypertension				
Presence	99 (25.4)	43 (36.8)	142 (28.1)	0.017
Absence	290 (74.6)	74 (63.2)	364 (71.9)	
Diabetes				
Presence	18 (4.7)	9 (7.7)	27 (5.3)	0.196
Absence	371 (95.4)	108 (92.3)	479 (94.7)	
Osteoporosis				
Presence	13 (3.3)	5 (4.3)	18 (3.6)	0.633
Absence	376 (96.7)	112 (95.7)	488 (96.4)	
Rhinitis				
Presence	124 (31.9)	33 (28.2)	157 (31.0)	0.452
Absence	265 (68.1)	84 (71.8)	349 (69.0)	
Presence of sexually transmitted diseases				
Presence	5 (1.3)	0 (-)	5 (1.0)	0.218
Absence	384 (98.7)	117 (100.0)	501 (99.0)	
Anemia presence				
Presence	5 (1.3)	1 (0.9)	6 (1.2)	0.706
Absence	384 (98.7)	116 (99.1)	500 (98.8)	
Kidney disease presence				
Presence	1 (0.3)	3 (2.6)	4 (0.8)	0.013
Absence	388 (99.7)	114 (97.4)	502 (99.2)	
Presence of cardiopathies				
Presence	10 (2.6)	8 (6.8)	18 (3.6)	0.029
Absence	379 (97.4)	109 (93.2)	488 (96.4)	
Hypercholesterolemia				
Presence	43 (11.1)	15 (12.80)	58 (11.5)	0.599

Absence	346 (88.9)	102 (87.2)	448 (88.5)	
Positive diagnosis of neoplasias				
Presence	5 (1.3)	0 (-)	5 (1.0)	0.218
Absence	384 (98.7)	117 (100.0)	501 (99.0)	
Liver disease				
Presence	1 (0.3)	1 (0.9)	2 (0.4)	0.366
Absence	388 (99.7)	116 (99.1)	504 (99.6)	
Presence of gastric diseases				
Presence	39 (10.0)	14 (12.0)	53 (10.5)	0.548
Absence	350 (90.0)	103 (88.0)	453 (89.5)	
Presence of other health alterations				
Presence	54 (13.9)	16 (13.7)	70 (13.8)	0.955
Absence	335 (86.1)	101 (86.3)	436 (86.2)	
Asthma				
Presence	219 (56.3)	95 (81.2)	314 (62.1)	0.000
Absence	170 (43.7)	22 (18.8)	192 (37.9)	

*Chi-square test (control-case) significance level ≤ 0.05. BMI: Body mass index.

Table 3: Characteristics related to oral health status and lifestyle habits of patients studied for periodontitis (n = 506).

Lifestyle habits and oral health status	Control (%) n = 389	Case (%) n = 117	All (%) n = 506	p value*
Oral health status				
Mouth-breathing habit				
Yes	228 (58.6)	91 (77.8)	319 (63.0)	<0.001
No	161 (41.4)	26 (22.2)	187 (37.0)	
Visit the dentist periodically				
Yes	202 (51.9)	50 (42.7)	252 (49.8)	0.081
No	187 (48.1)	67 (57.3)	254 (50.2)	
Time of last visit to the dentist				
Never or ≥ 1 year	204 (52.4)	76 (65.0)	280 (55.3)	0.017
≤ 1 year	185 (47.6)	41 (35.0)	226 (44.7)	
Oral hygiene guidance				
Already received guidance	310 (79.7)	91 (77.8)	401 (79.2)	0.654
Never received guidance	79 9 (20.3)	26 (22.2)	105 (20.8)	
Presence gingivitis				
Yes	14 (3.6)	0 (-)	14 (2.8)	0.037
No	375 (96.4)	117 (100.0)	492 (97.2)	
Lost tooth				

Yes	335 (86.1)	110 (94.0)	445 (87.9)	0.021
No	54 (13.9)	7 (6.0)	61 (12.1)	
Presence of malocclusion				
Yes	270 (69.4)	92 (78.6)	362 (71.5)	0.053
No	119 (30.6)	25 (21.4)	144 (28.5)	
Presence of a soft tissue lesion				
Yes	26 (6.7)	14 (12.0)	40 (7.9)	0.063
No	363 (93.3)	103 (88.0)	466 (92.1)	
Any surgery				
Yes	189 (48.6)	54 (46.2)	243 (48.1)	0.700
No	200 (51.4)	62 (53.4)	262 (51.9)	
Lifestyle habits	Control (%) n = 389	Case (%) n = 117	All (%) n = 506	p value*
Be a smoker or have already smoked				
Yes	129 (33.2)	47 (40.2)	176 (34.8)	0.163
No	260 (66.8)	70 (59.8)	330 (65.2)	
Use of alcoholic beverages				
Yes	135 (34.7)	47 (40.2)	182 (36.0)	0.280
No	254 (65.3)	70 (59.8)	324 (54.0)	
Perform physical activity				
Yes	129 (33.2)	43 (36.8)	172 (34.0)	0.472
No	260 (66.8)	74 (63.2)	334 (66.0)	
Brush the teeth				
Yes	387 (99.5)	116 (99.1)	503 (99.4)	0.674
No	2 (0.5)	1 (0.9)	3 (0.6)	
Brush your teeth ≥ 3 times a day				
Yes	229 (58.9)	64 (54.7)	293 (57.9)	0.423
No	160 (41.1)	53 (45.3)	213 (42.1)	
Use of dental floss				
Yes	221 (56.8)	45 (38.5)	266 (52.6)	<0.001
Do not use	168 (43.2)	72 (61.5)	240 (47.4)	
Uses mouthwash daily or occasionally				
Yes	148 (38.0)	52 (44.4)	200 (39.5)	0.215
Do not use	241 (62.0)	65 (55.6)	306 (60.5)	
Brush change				
< 4 months	269 (69.2)	77 (65.8)	346 (68.4)	0.496
> 4 months	120 (30.8)	40 (34.2)	160 (31.6)	

*Chi-square test (control-case) significance level ≤ 0.05

3.2 GWAS results

Genome-wide association analysis identified three SNV that reached the genome-wide study significance (p value < 5 × 10-8), an intronic *locus* in chromosome 2, gene *RTN4* (reticulon 4, rs10496038, ORadjusted (CI95%) = 33.27(10.29-107.63); p value = $4.88x10^{-9}$) and two intronic variants on chromosome 4, gene *LINC02505* (long intergenic non-protein coding RNA 2505 – ncRNA, rs58327429, ORadjusted (CI95%) = 4.13(2.51-6.81); p value = $2.36x10^{-8}$ and rs67797971, ORadjusted (CI95%) = 4.07(2.46-6.72); p value = $4.35x10^{-8}$). Figure 1A presented the Manhattan plot of the results (p-value < 5 × 10^{-8}) where SNV significantly associated are observed above the red line and between the red and blue lines, the suggestively associated SNV. The genotyped and imputed SNV genomic inflation factor was λ = 1,015 and the QQ-plot (Figure 2) suggested that there is a low possibility of false positive associations resulting from population stratification.

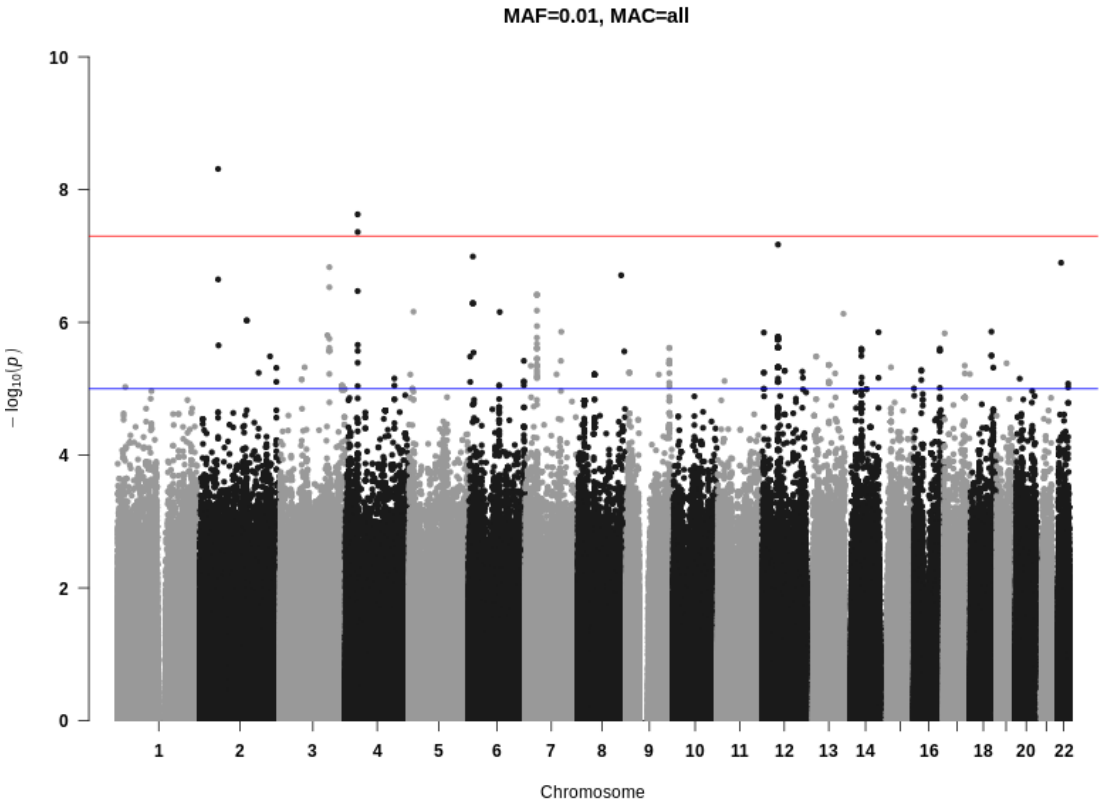

Figure 1. Manhattan plot of GWAS results in a population of Salvador/Brazil. Each

point represents a single nucleotide variant, with the x-axis showing the positions of the SNV on the chromosomes and the y-axis showing the measures of association (p value $-log_{10}(p)$).

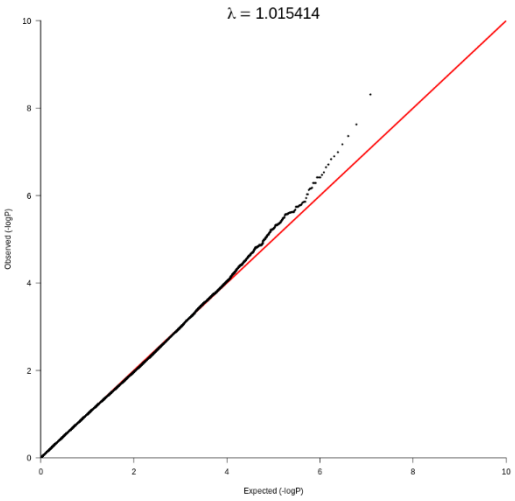

Figure 2. QQ-plot for the analysis of periodontitis using 506 non-parents. The analysis was adjusted for age, obesity, breathe through the mouth, flossing, asthma, and principal component of ancestry 1.

3.3 Functional annotations and analysis

The results and detailed information for the 3 significant SNV and the 130 *loci* in different genes with suggestive association, in the chromosome position, rsID, in the alternative and reference alleles, frequency of the smallest allele in the studied population, size of the influential association and the presence of the alternative allele with the outcome and the gene in which the variant is inserted or close are presented in Table 4.

Table 4. Periodontitis GWAS by logistic regression adjust for age, obesity, breathe through the mouth, flossing, asthma, and genetic ancestry (CRCh37) (n = 506).

Rank	Chr	Position (CRCh37)	rsID	A1/A2	MAF	ORadjusted (CI95%)	p-value	Nearest gene
1	2	55340963	rs10496038	T/C	0.016	33.27(10.29-107.63)	4.88E-09	*RTN4*
2	4	36514331	rs58327429	C/G	0.138	4.13(2.51-6.81)	2.36E-08	*LINC02505*
3	4	36512627	rs67797971	A/T	0.135	4.07(2.46-6.72)	4.35E-08	*LINC02505*
4	12	47939760	rs61918129	A/G	0.046	8.02(3.77-17.08)	6.74E-08	*RPAP3*
5	6	13161638	rs11963505	A/G	0.094	4.36(2.54-7.51)	1.02E-07	*PHACTR1*
6	8	131493952	rs113301484	T/C	0.016	41.95(10.27-171.36)	1.95E-07	*ASAP1*
7	2	55464700	rs74410951	G/T	0.018	18.40(6.11-55.42)	2.25E-07	*MTIF2*
8	4	36488422	rs12499402	G/A	0.138	3.59(2.20-5.86)	3.38E-07	*RP11-722M1.1*
9	6	13162296	rs78286825	T/C	0.076	4.42(2.48-7.90)	5.16E-07	*PHACTR1*
10	6	13161454	rs11968523	T/C	0.075	4.42(2.48-7.90)	5.16E-07	*PHACTR1*
11	6	13160167	rs111855278	A/G	0.075	4.42(2.48-7.90)	5.16E-07	*PHACTR1*
12	6	94272747	rs75379370	G/A	0.045	8.59(3.67-20.08)	6.98E-07	*LOC105377899*
13	2	141921867	rs2017825	C/T	0.038	9.03(3.75-21.76)	9.36E-07	*LRP1B*
14	2	141902393	rs75518785	A/G	0.038	9.03(3.75-21.76)	9.36E-07	*LRP1B*
15	7	36040923	rs74780979	A/C	0.039	9.83(3.92-24.69)	1.14E-06	*LOC105375233*
16	17	3214502	rs8076130	A/G	0.119	0.30(0.18-0.49)	1.47E-06	*OR3A2/ OR3A4P*
17	3	142944742	rs9850239	C/T	0.603	2.18(1.58-2.99)	1.57E-06	*SLC9A9*
18	12	47907832	rs61918078	A/G	0.040	7.44(3.27-16.92)	1.66E-06	*RP1-90J4.1*
19	12	47907883	rs73107470	G/A	0.040	7.44(3.27-16.92)	1.66E-06	*RP1-90J4.1*
20	12	47911622	rs75580793	T/C	0.040	7.44(3.27-16.92)	1.66E-06	*RP1-90J4.1*
21	12	47936650	rs61918094	C/T	0.040	7.38(3.25-16.76)	1.81E-06	*RPAP3*
22	12	47937748	rs61918095	T/G	0.040	7.38(3.25-16.76)	1.81E-06	*RPAP3*
23	12	47938538	rs73109412	A/G	0.040	7.38(3.25-16.76)	1.81E-06	*RPAP3*
24	12	47938011	rs61918096	T/G	0.039	7.38(3.25-16.76)	1.81E-06	*RPAP3*
25	12	47939616	rs61918128	A/C	0.040	7.38(3.25-16.76)	1.81E-06	*RPAP3*
26	4	36523572	rs11096812	T/A	0.104	3.94(2.23-6.94)	2.18E-06	*LINC02505*
27	2	56592089	rs73940694	T/C	0.042	6.57(3.01-14.34)	2.22E-06	*CCDC85A*

28	12	47921701	rs73107481	A/C	0.037	7.62(3.28-17.71)	2.39E-06	*RP1-90J4.1*
29	12	47922342	rs61918087	T/G	0.037	7.62(3.28-17.71)	2.39E-06	*RP1-90J4.1*
30	12	47922433	rs61918088	C/T	0.037	7.62(3.28-17.71)	2.39E-06	*RP1-90J4.1*
31	12	47923396	rs61918089	C/G	0.037	7.62(3.28-17.71)	2.39E-06	*RP1-90J4.1*
32	12	47925030	rs61918091	T/C	0.037	7.62(3.28-17.71)	2.39E-06	*RP1-90J4.1*
33	12	47925037	rs61918092	T/C	0.037	7.62(3.28-17.71)	2.39E-06	*RP1-90J4.1*
34	16	78658232	rs2738572	C/A	0.775	2.54(1.72-3.75)	2.49E-06	*WWOX*
35	14	51097975	rs73162990	T/C	0.020	12.89(4.45-37.38)	2.52E-06	*GYG1*
36	14	51098133	rs75303184	G/C	0.020	12.89(4.45-37.38)	2.52E-06	*ATL1*
37	14	51142708	rs7147836	G/C	0.031	9.28(3.66-23.52)	2.63E-06	*SAV1*
38	14	51104784	rs114863142	C/T	0.031	9.28(3.66-23.52)	2.63E-06	*SAV1*
39	14	51108191	rs28648926	A/G	0.031	9.28(3.66-23.52)	2.63E-06	*SAV1*
40	16	78601832	rs113856814	C/G	0.024	17.47(5.29-57.65)	2.67E-06	*WWOX*
41	16	78601665	rs111396690	G/T	0.024	17.47(5.29-57.65)	2.67E-06	*WWOX*
42	4	36511308	rs68114741	G/A	0.104	3.88(2.20-6.83)	2.69E-06	*LINC02505*
43	3	148690698	rs73162961	T/C	0.022	10.72(3.98-28.85)	2.69E-06	*RP11-680B3.2*
44	3	148679226	rs76715910	A/G	0.022	10.72(3.98-28.85)	2.69E-06	*RP11-680B3.2*
45	3	148677420	rs73162950	C/T	0.022	10.72(3.98-28.85)	2.69E-06	*GYG1*
46	6	15066636	rs3846946	G/T	0.275	0.43(0.30-0.61)	2.86E-06	*RP11-146I2.1*
47	14	51129087	rs28441486	A/G	0.079	3.96(2.22-7.07)	3.20E-06	*SAV1*
48	2	212289451	rs55901919	T/C	0.342	2.18(1.57-3.02)	3.25E-06	*ERBB4*
49	6	5063445	rs4298384	G/A	0.111	3.34(2.01-5.54)	3.28E-06	*LYRM4*
50	7	36040759	rs10265584	G/A	0.140	2.87(1.84-4.49)	3.54E-06	*LOC105375233*
51	7	36039189	rs56358543	T/A	0.140	2.87(1.84-4.49)	3.54E-06	*LOC105375233*
52	7	36037435	rs10242783	A/G	0.140	2.87(1.84-4.49)	3.54E-06	*SEPT7*
53	9	130877692	rs76420223	A/G	0.065	4.45(2.36-8.38)	3.77E-06	*SLC25A25-AS1*
54	6	167334847	rs9356543	T/G	0.521	2.13(1.54-2.93)	3.79E-06	*RNASET2*
55	4	36486453	rs186052079	A/T	0.158	2.89(1.84-4.55)	4.05E-06	*RP11-722M1.1*

56	13	68085861	rs1937520	A/T	0.604	0.46(0.33-0.64)	4.38E-06	*LINC00364*
57	13	68087632	rs1937517	G/T	0.604	0.46(0.33-0.64)	4.38E-06	*LINC00364*
58	13	68088772	rs9541138	C/A	0.603	0.46(0.33-0.64)	4.38E-06	*LINC00364*
59	13	68089412	rs1937516	A/G	0.607	0.46(0.33-0.64)	4.38E-06	*LINC00364*
60	17	63916387	rs6504375	C/G	0.055	4.79(2.45-9.34)	4.49E-06	*CEP112*
61	7	17512723	rs11980915	T/C	0.039	5.80(2.74-12.30)	4.51E-06	*LINC02889*
62	7	36027315	rs114578046	C/G	0.040	8.64(3.44-21.71)	4.54E-06	*LOC107986734*
63	7	36030630	rs76567141	A/G	0.040	8.64(3.44-21.71)	4.54E-06	*LOC107986734*
64	7	36026893	rs116233946	G/C	0.040	8.64(3.44-21.71)	4.54E-06	*LOC107986734*
65	15	33231544	rs1470677	T/C	0.135	6.99(3.04-16.07)	4.75E-06	*FMN1*
66	12	47915959	rs117014522	T/C	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
67	12	47918277	rs117606601	A/G	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
68	12	47918364	rs61918083	T/C	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
69	12	47918748	rs118036531	G/T	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
70	12	47918982	rs117835442	T/C	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
71	12	47919187	rs142480306	T/C	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
72	12	47919900	rs55700347	C/T	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
73	12	47919985	rs55701123	A/G	0.038	6.99(3.04-16.07)	4.76E-06	*RPAP3*
74	18	68975376	rs1026182	G/A	0.347	2.18(1.56-3.04)	4.80E-06	*UTAT39*
75	16	22733035	rs11648331	T/C	0.482	2.07(1.51-2.83)	5.23E-06	*MYR548D2*
76	12	68178287	rs555316142	A/G	0.013	23.31(6.00-90.49)	5.37E-06	LINC02421
77	12	68176183	rs183005059	T/C	0.013	23.31(6.00-90.49)	5.37E-06	*DYRK2*
78	16	22730983	rs12920706	T/C	0.482	2.07(1.51-2.83)	5.38E-06	*MYR548D2*
79	12	121515627	rs73214199	G/A	0.045	5.83(2.73-12.48)	5.52E-06	*LOC105378258*
80	9	9891195	rs28719869	C/T	0.031	8.64(3.40-21.94)	5.72E-06	*PTPRD*
81	9	9890548	rs10124543	C/G	0.031	8.64(3.40-21.94)	5.72E-06	*PTPRD*
82	9	9889163	rs112648615	G/C	0.032	8.64(3.40-21.94)	5.72E-06	*PTPRD*
83	9	130850879	rs6478815	A/G	0.878	3.13(1.91-5.12)	5.81E-06	*SLC25A25*

84	17	63921354	rs78044190	T/C	0.065	4.37(2.31-8.27)	5.89E-06	*CEP112*
85	3	148662114	rs114884128	T/C	0.021	10.51(3.80-29.11)	5.99E-06	*LOC107986045*
86	7	95161660	rs80300265	G/A	0.088	3.50(2.03-6.01)	6.07E-06	*ASB4*
87	8	50803806	rs13271238	G/A	0.087	3.43(2.01-5.86)	6.09E-06	*SNTG1*
88	8	50840042	rs66661683	C/T	0.090	3.43(2.01-5.86)	6.09E-06	*SNTG1*
89	8	50828356	rs68134005	A/G	0.090	3.43(2.01-5.86)	6.09E-06	*SNTG1*
90	8	50809667	rs72638364	A/G	0.089	3.43(2.01-5.86)	6.09E-06	*SNTG1*
91	8	50815776	rs17676467	A/C	0.091	3.43(2.01-5.86)	6.09E-06	*SNTG1*
92	8	50815672	rs12549517	A/G	0.091	3.43(2.01-5.86)	6.09E-06	*SNTG1*
93	5	4632253	rs190885	A/G	0.051	5.44(2.61-11.32)	6.10E-06	*LINC02114*
94	7	36025786	rs10277193	A/G	0.130	2.99(1.86-4.80)	6.13E-06	*LOC107986734*
95	7	36029834	rs11971834	C/T	0.150	2.78(1.78-4.34)	6.66E-06	*LOC107986734*
96	7	36035167	rs73087848	C/T	0.150	2.78(1.78-4.34)	6.66E-06	*AC083864.3*
97	14	52052021	rs6572778	G/A	0.915	3.58(2.06-6.25)	6.77E-06	*FRMD6-AS2*
98	7	36033091	rs10254864	G/A	0.137	2.87(1.81-4.55)	6.92E-06	*LOC107986734*
99	4	147385142	rs116137057	A/T	0.018	25.05(6.15-102.09)	7.00E-06	*SLC10A7*
100	12	47939856	rs61918130	A/G	0.038	6.71(2.93-15.40)	7.01E-06	*RPAP3*
101	20	11869478	rs117876230	T/C	0.017	21.87(5.69-84.00)	7.05E-06	*BTBD3*
102	3	64978671	rs74635888	T/G	0.013	27.12(6.41-114.65)	7.25E-06	*ADAMTS9-AS2*
103	3	65015822	rs11706761	G/T	0.013	27.12(6.41-114.65)	7.25E-06	*ADAMTS9-AS2*
104	6	167333290	rs2876012	T/C	0.516	2.07(1.50-2.85)	7.79E-06	*RNASET2*
105	6	167333602	rs4710142	T/C	0.516	2.07(1.50-2.85)	7.79E-06	*RNASET2*
106	13	68083114	rs2875530	G/T	0.604	0.47(0.34-0.66)	7.81E-06	*LINC00364*
107	13	68083418	rs2186134	C/T	0.604	0.47(0.34-0.66)	7.81E-06	*LINC00364*
108	12	47932980	rs61918093	A/G	0.039	6.61(2.89-15.12)	7.85E-06	*RPAP3*
109	6	5061580	rs9405253	A/G	0.115	3.17(1.91-5.26)	7.90E-06	*LYRM4* *LYRM4-AS1*
110	12	47899160	rs73107458	A/T	0.049	5.15(2.51-10.58)	7.90E-06	*RPAP3*
111	12	47902338	rs61918072	A/G	0.049	5.15(2.51-10.58)	7.90E-06	*RPAP3*

112	13	68096701	rs1937499	C/T	0.599	0.47(0.34-0.66)	8.16E-06	*LINC00364*
113	13	68093783	rs1937505	A/G	0.599	0.47(0.34-0.66)	8.16E-06	*LINC00364*
114	13	68091301	rs1937514	G/A	0.599	0.47(0.34-0.66)	8.16E-06	*LINC00364*
115	13	68091338	rs1937513	T/C	0.599	0.47(0.34-0.66)	8.16E-06	*LINC00364*
116	14	51064998	rs28489683	A/G	0.034	6.29(2.80-14.13)	8.24E-06	*ATL1*
117	22	47175815	rs17762038	A/G	0.063	3.75(2.10-6.71)	8.40E-06	*TBC1D22A*
118	6	167334382	rs10806874	A/G	0.519	2.06(1.50-2.84)	8.86E-06	*RNASET2*
119	6	92587093	rs6929276	G/T	0.488	2.03(1.48-2.77)	8.95E-06	*CASC6*
120	6	92589873	rs9359960	T/A	0.488	2.03(1.48-2.77)	8.95E-06	*CASC6*
121	4	147525100	rs77376908	C/G	0.016	23.66(5.85-95.59)	8.98E-06	*POU4F2*
122	3	187030480	rs115314220	C/T	0.896	3.44(1.99-5.94)	9.04E-06	*MASP1*
123	3	187030140	rs850306	C/G	0.896	3.44(1.99-5.94)	9.04E-06	*MASP1*
124	3	187027156	rs710479	C/T	0.896	3.44(1.99-5.94)	9.04E-06	*MASP1*
125	3	187027301	rs710480	G/A	0.896	3.44(1.99-5.94)	9.04E-06	*MASP1*
126	3	187035234	rs9990329	C/T	0.896	3.44(1.99-5.94)	9.04E-06	*MASP1*
127	4	36490832	rs66827186	C/T	0.161	2.75(1.76-4.30)	9.11E-06	*DTHD1*
128	9	130933819	rs11999486	T/A	0.038	5.66(2.63-12.16)	9.17E-06	*CIZ1*
129	1	23955450	rs9424389	A/G	0.219	2.42(1.64-3.57)	9.39E-06	*MDS2*
130	22	47181753	rs60370054	G/C	0.065	3.71(2.08-6.64)	9.50E-06	*TBC1D22A*
131	16	78656579	rs61408223	C/T	0.744	2.29(1.59-3.30)	9.70E-06	*WWOX*
132	5	10912320	rs73054303	C/T	0.026	9.36(3.47-25.23)	9.96E-06	*CTNND2*
133	5	10910387	rs57620661	G/C	0.026	9.36(3.47-25.23)	9.96E-06	*CTNND2*

A1: Alternative allele; A2: Reference allele; A: Adenine; AC083864.3: Homo sapiens chromosome 7 clone RP11-196O2, complete sequence; ADAMTS9-AS2: A desintegrina e metaloproteinase (ADAM) metallopeptidase with thrombospondin type 1 motif 9 antisense RNA 2; ASAP1: ADP-ribosylation factor (ARF) GTPase-activating protein with SH3 domain, ankyrin repeat and PH domain 1; ASB4: Ankyrin repeat and couple suppressor of cytokine signalling box containing 4; ATL1: Atlastin GTPase 1; BTBD3: "bric-a-brac, tramtrack, larage complex" (BTB) domain containing 3; CASC6: Cancer Susceptibility 6; CCDC85A: Coiled-Coil domain containing 85A; CEP112: Centrossomal protein 112; C: cytosine; Chr: Chromosome; CI95%: 95% confidence interval; CIZ1: Cyclin Dependent Kinase Inhibitor 1A Interacting Zinc Finger Protein 1; CTNND2: Catenin delta 2; DTHD1: Death Domain Containing 1; DYRK2: Dual Specificity Tyrosine Phosphorylation Regulated Kinase 2; ERBB4: Erythroblastic leukemia viral oncogene homolog 2 receptor tyrosine kinase 4; FMN1: Formin1; FRMD6-AS2: FERM (F for 4.1 protein, E for ezrin, R for radixin and M for moesin) domain containing 6 antisense RNA 2; G: guanine; GYG1: Glicogenin 1; LINC02114, LINC02421, LINC02505, LINC02889, LINC00364: Long Intergenic Non-Protein Coding RNA; LOC105377899, LOC105375233, LOC105378258, LOC107986045, LOC107986734: Long non-coding intergenic RNA; LRP1B: Low density lipoprotein receptor related protein 1beta; LYRM4: Leucine/tyrosine/arginine (LYR) motif containing 4; LYRM4-AS1: Leucine/tyrosine/arginine (LYR) motif containing 4 antisense RNA 1; MAF: minor allele frequency (alternative); MASP1: Mannose-binding lectin-associate serine protease 1; MDS2: Myelodysplastic Syndrome 2 Translocation Associated; MTIF2: Mitochondrial Translational Initiation Factor 2; ORadjusted: Odds ratio adjusted for age, obesity, breathe through the mouth, flossing, asthma, and genetic ancestry; OR3A2: Olfactory Receptor Family 3 Subfamily A Member 2; OR3A4: Olfactory Receptor Family 3 Subfamily A Member 4 Pseudogene; PHACTR1: Phosphatase And Actin Regulator 1; POU4F2: POU Class 4 Homeobox 2; PTPRD: Protein tyrosine phosphatase receptor type D; RNASET2: Ribonuclease T2; RP11-680B3.2, RP11-722M1.1, RP11-146I2.1: Retinitis pigmentosa 11, long non-coding RNAs associated with superenhancers; RP1-90J4.1: Retinitis pigmentosa 1, long intergenic non-protein

RNA; RPAP3: RNA polymerase II associated protein 3; RTN4: Reticulon-4; SAV1: Salvador family WW domain containing protein 1; SEPT7: Septin-7; SLC10A7: Solute Carrier Family 10 Member 7; SLC9A9: Solute Carrier Family 9 Member A9; SLC25A25: Solute Carrier Family 25 Member 25; SLC25A25-AS1: SLC25A25 Antisense RNA 1; SNTG1: Syntrophin Gamma 1; T: thymine; TBC1D22A: Tre-2, Bub2, and Cdc16 1 Domain Family Member 22A; UTAT39: Long intergenic non-protein coding RNA 1541; WWOX: WW domain containing oxidoreductase.

The main localization of genetic variants is intronic (n = 60). Table 5 presents the possible functional role of the identified SNV, of which we highlight that the Regulome DB score is composed of a score between 1 and 7, with the lower the score, the greater the probability that this variant is a regulatory variant. Tissues where transcription of target genes by DNA enhancer sequences, protein binding annotations, regulatory motifs has already been observed and we separated 3 tissues where conditions of methylation, acetylation or epigenetic alterations were observed and these tissues, which somehow, could cause changes in the periodontium.

Table 5: Functional annotations of SNV tops with association with periodontitis.

Rank	Chr	rsID	Nearest gene	Biotype	Consequense	Regulome DB	Enhancer histone marks	Motifs changed	Osteoblast Primary Cells	Adult Dermal Fibroblast Primary Cells	Primary mononuclear cells from peripheral blood	Proteins bounds
1	2	10496038	*RTN4*	Protein coding, Processed transcript	Intron Upstream transcript gene	2b	-	E2A, Mxi1, TAL1	H3K4me1 Enh H3K27ac Enh	H3K4me1 Enh	-	
2	4	58327429	*LINC02505*	lincRNA, processed pseudogene	Intron non coding transcript downstream gene	5		Arid3a, Dbx1, Fox, Foxf1, Foxi1, Foxj1, Foxl1, Foxp1, Ncx, Pou2f2				
3	4	67797971	*LINC02505*	lincRNA, processed pseudogene	Intron	7						
4	12	61918129	*RPAP3*		Intergenic	5	FAT, BLD	Pbx1				
5	6	11963505	*PHACTR1*	Protein coding, processed transcript	Intron non coding transcript	4		Maf				
6	8	113301484	*ASAP1*		Intergenic	5	ESDR, LNG	Znf143, p300				
7	2	74410951	*MTIF2*	Protein coding	Intron downstream gene	3a	SKIN	Cdc5, Irf	H3K27ac Enh			
8	4	12499402	*RP11-722M1.1*		Intergenic	3a		FXR, GR, Nrf1				ERALPHA A
9	6	78286825	*PHACTR1*	protein_coding, processed_transcript	Intron downstream transcript	7						
10	6	11968523	*PHACTR1*	protein_coding, processed_transcript	Intron downstream transcript	4		DMRT1, DMRT7, PAX6				
11	6	111855278	*PHACTR1*	protein_coding	Intron downstream transcript	5	BLD	Foxm1, GATA				
12	6	75379370	*LOC105377899*	open_chromatin_region	Intron	5	ESRD, GI, LNG	CCNT2, GATA				
13	2	2017825	*LRP1B*	protein_coding	Intron Upstream transcript	7		Dbx1, HNF4, Hoxa5, Hoxd8, Ncx				
14	2	75518785	*LRP1B*	protein_coding	Intron Upstream transcript	5		Cart1				

15	7	74780979	*LOC105375233*		Upstream transcript	5		EBF			
16	17	8076130	*OR3A2/ OR3A4P*		Intron Non coding transcript Upstream transcript	5		Mtf1			
17	3	9850239	*SLC9A9*		Intergenic	5		GATA, PLZF			
18	12	61918078	*RP1-90J4.1*		Intergenic	4		AP-2, Roaz			
19	12	73107470	*RP1-90J4.1*		Intergenic	3a		Mef2			
20	12	75580793	*RP1-90J4.1*		Intergenic	5		DMRT2, Evi-1, RXRA, p300			
21	12	61918094	*RPAP3*		Intergenic	5					
22	12	61918095	*RPAP3*		Intergenic	5	BRN, SPLN				
23	12	73109412	*RPAP3*		Intergenic	5		Evi-1, Rad21			
24	12	61918096	*RPAP3*		Intergenic	5	BLD, BRN, SPLN	HNF4, RXRA			
25	12	61918128	*RPAP3*		Intergenic	5	FAT, BLD	NRSF			
26	4	11096812	*LINC02505*	lincRNA	Intron	7		CEBPA, CEBPB, Foxj2, Isl2, Nkx6-1, p300			
27	2	73940694	*CCDC85A*	protein_coding	Intron	6		GR			
28	12	73107481	*RP1-90J4.1*	open_chromatin_region	Regulatory region	5	PLCNT	CEBPA, STAT, p300	19 DNAse	19 DNAse	
29	12	61918087	*RP1-90J4.1*		Intergenic	5		Cart1, E2F, Foxa, HMG-IY, NF-kappaB, Nanog, Pou2f2, Pou5f1, Sox, TATA, Tef, YY1			
30	12	61918088	*RP1-90J4.1*		Intergenic	4					
31	12	61918089	*RP1-90J4.1*		Intergenic	6		Fox, Foxa, Foxd3, Foxf1, Foxi1, Foxj1, Foxj2, Foxp1, Foxq1, Sox, Zfp105			
32	12	61918091	*RP1-90J4.1*		Intergenic	3a	BLD	AP-1, Barx1, GATA, HNF4, Hoxa5, Hoxb3, Hoxb6, Irf, Pou6f1, RXR::LXR, RXRA, TR4			
33	12	61918092	*RP1-90J4.1*		Intergenic	3a	BLD	AIRE-2, HNF4, Irf, Pou3f2, RXR::LXR, RXRA, SIX5, TCF4, TR4			
34	16	2738572	*WWOX*	Protein coding Nonsense mediated decay	Intron Downstream transcript	4	LNG, GI	HNF1		H3K4me1 Enh	
35	14	73162990	*GYG1*	Protein coding Retained intron Processed transcript	Intron	5	12 tissues	Ik-1	7 Enh, 14 EnhA2, H3K4me1 Enh, H3K4me3 Pro, H3K27ac Enh	7 Enh, 14 EnhA2, H3K4me1 Enh, H3K27ac Enh	H3K4me1 Enh
36	14	75303184	*ATL1*	Protein coding Nonsense mediated decay	Intron	4	LNG, BRN	Pax-4, Pou2f2			
37	14	7147836	*SAV1*		Intergenic	4	11 tissues		2 TssAFlnk, 2 PromU, H3K4me1 Enh, H3K4me3 Pro, H3K27ac Enh	7 Enh, 13 EnhA1, H3K4me1 Enh, H3K4me3 Pro, H3K27ac Enh	
38	14	114863142	*SAV1*	Protein coding	Intron	5	6 tissues	CTCF, Nanog, Obox3			
39	14	28648926	*SAV1*	Protein coding	Intron	5	BLD	Irf, PRDM1, STAT			
40	16	113856814	*WWOX*	Protein coding Nonsense mediated decay	Intron Downstream transcript	4	BRN	NRSF, Sin3Ak-20			
41	16	111396690	*WWOX*	Protein coding Nonsense mediated decay	Intron Downstream transcript	4	BLD	HEN1, Rad21			

42	4	68114741	*LINC02505*	lincRNA	Intron	6		Hltf, SF1				
43	3	73162961	*RP11-680B3.2*		Intergenic	5	LGN					
44	3	76715910	*RP11-680B3.2*	antisense	Upstream gene	5	MUS					
45	3	73162950	*GYG1*	antisense	Intron, non coding transcript	4	OVRY	AP-2, PLAG1				
46	6	3846946	*RP11-146I2.1*	lincRNA	Intron, non coding transcript	4	15 tissues	Foxd3, HMG-IY, Irf	7 Enh, 13 EnhA1, H3K4me1 Enh, H3K4me3 Pro, H3K27ac Enh	13 EnhA1, H3K4me1 Enh	18 EnhAc, H3K27ac Enh	
47	14	28441486	*SAV1*	Protein coding Retained intron	Intron Downstream gene	5						
48	2	55901919	*ERBB4*	Protein coding	Intron	5		Maf, Pou2f2				ERALPHA-A
49	6	4298384	*LYRM4*		Intron Downstream transcript	5	IPSC, LIV	Irf, PU.1, Pax-5, RAD21, STAT				
50	7	10265584	*LOC105375233*		Upstream transcript	5		Klf7, Znf143				
51	7	56358543	*LOC105375233*		Upstream transcript	5		DMRT2, Foxj1, HMG-IY, Maf				
52	7	10242783	*SEPT7*		Intergenic	6		Dbx1, Ncx, Nkx6-1, Pou3f1, Sox, TATA				
53	9	76420223	*SLC25A25-AS1*	Antisense Promoter	Intron	2b	6 tissues	E2A, EWSR1-FLI1, Foxo, Myf, PPAR, RXRA, STAT, Zfp281		18 EnhAc		USF1, ATF3, BHLHE40, FOSL2, FOXA1, FOXA2, HDAC2, HNF4A, HNF4G, JUND, P300, RXRA, SP1, TCF12, USF2, TCF4
54	6	9356543	*RNASET2*	protein_coding	Intergenic	6	ESDR, BLD, BRN	AP-1, Pou2f2, Pou5f1				
55	4	186052079	*RP11-722M1.1*		Intergenic	6		Cdx, Foxd3, Foxj1, Foxp1, Foxq1, Hltf, TATA, TEF				
56	13	1937520	*LINC00364*		Intergenic	7		Hbp1				
57	13	1937517	*LINC00364*		Intergenic	6		AP-1, CDP, CEBPB, CEBPD, Foxa, HNF1, OTX, Pou1f1, p300				
58	13	9541138	*LINC00364*		Intergenic	7	ESC, IPSC	AP-1, Zfp691				
59	13	1937516	*LINC00364*	Enhancer	Regulatory region	4	ESC, IPSC	GR				
60	17	6504375	*CEP112*	Protein coding Processed transcript Promoter flanking region	Intron Downstream transcript Upstream transcript	5	ESDR	Egr-1, Rad21				
61	7	11980915	*LINC02889*	lincRNA	Intron	4	BLD	Znf143				BATF, PU1
62	7	114578046	*LOC107986734*	enhancer	Intron	5	8 tissues	CTCF, RXRA, Sin3Ak-20		7 Enh, H3K4me1 Enh		
63	7	76567141	*LOC107986734*		Intron	6		CEBPA, CEBPB, GCNF, Nanog, Sox				
64	7	116233946	*LOC107986734*	enhancer	Intron	4	10 tissues			7 Enh, H3K4me1 Enh		
65	15	1470677	*FMN1*	Protein coding	Intron Downstream transcript	5		Foxp3, Hoxa10, Sox	H3K4me1 Enh, H3K27ac1 Enh			
66	12	117014522	*RPAP3*		Intergenic			Foxj2, GATA				
67	12	117606601	*RPAP3*		Intergenic	7						

68	12	61918083	*RPAP3*		Intergenic	6		BATF. Pou2f2				
69	12	118036531	*RPAP3*		Intergenic	6		AP-2, BCL, CEBPB, NF-kappaB				
70	12	117835442	*RPAP3*		Intergenic	-		Irf, Mrg				
71	12	142480306	*RPAP3*		Intergenic	5		GR, Hsf				
72	12	55700347	*RPAP3*		Intergenic	5		MZF1::1-4, Mrg, NF-kappaB, Tgif1				
73	12	55701123	*RPAP3*		Intergenic	5		Ascl2, CTCF, DEC, E2A, Ets, Lmo2-complex, Maf, Nkx2, Rad21, SMC3, SP1, TCF11::MafG, TCF12, UF1H3BETA, ZEB1, Zfp281				
74	18	1026182	*UTAT39*		Intergenic	5		BHLHE40, DEC, Egr-1, Myc, NF-E2, PTF1-beta, Sin3Ak-20				
75	16	11648331	*MYR548D2*	enhancer	Regulatory region, intergenic	3a	4 tissues	AP-4, Ascl2, E2A, HEN1, LBP-1, Myf, Pou2f2, Rad21, Sin3Ak-20, Smad3		DNAse		P300, RAD21
76	12	555316142	LINC02421		Intergenic	6						
77	12	183005059	*DYRK2*		Intergenic	7		NRSF, RXRA, Sin3Ak-20				
78	16	12920706	*MYR548D2*		Intergenic	4						
79	12	73214199	*LOC105378258*		Intron	5	BLD	CTCF, Hand1, LBP-1				
80	9	28719869	*PTPRD*	protein_coding	Intron Upstream transcript	7		GR, Myf, Irf, PU.1, SP1, UF1H3BETA				
81	9	10124543	*PTPRD*	protein_coding	Intron Upstream transcript	7		Duxl, SP1				
82	9	112648615	*PTPRD*	protein_coding	Intron Upstream transcript	4		P300				
83	9	6478815	*SLC25A25*	protein_coding	Intron Upstream gene	2b	11 tissues	DMRT2, Foxj1, Foxk1, Pou1f1	H3K27ac Enh	7 Enh, H3K4me1 Enh, H3K27acEnh	10 TxEnh5	
84	17	78044190	*CEP112*	Protein coding, Processed transcript	Intron, Downstream transcript Upstream transcript	7	STRM, SKIN	CTCF	18 Enh, H3K4me1 Enh, H3K27ac Enh	7 Enh, H3K4me1 Enh, H3K27ac Enh		
85	3	114884128	*LOC107986045*	antisense	Intron	6	LNG	Arid5a, Foxa				
86	7	80300265	*ASB4*	Protein coding	Intron Downstream gene	6	ADRL	HDAC2				
87	8	13271238	*SNTG1*		Intergenic	7		AP-2, ERalpha-a				
88	8	66661683	*SNTG1*	Protein coding Processed transcript	Intron, Upstream transcript	6		Arid5a, Foxa, Lhx3, Mef2, Pou2f2, Pou3f2				
89	8	68134005	*SNTG1*	Protein coding Processed transcript	Intron Upstream transcript	5	ESDR, PANC	Foxa				
90	8	72638364	*SNTG1*		Intergenic	7	LIV, GI	Pou2f2, Spdef				
91	8	17676467	*SNTG1*	lincRNA	Downstream gene	5		Pax-8, Pbx-1				
92	8	12549517	*SNTG1*	lincRNA	Downstream gene	7		Ika2, PMDN1				
93	5	190885	*LINC02114*	lincRNA	Intron, non coding transcript	5	ESDR	AP-1, EWSR1-FLI1,SREBP				
94	7	10277193	*LOC107986734*		Intron	5		AP-4, E2A, SRF, TAL1, YY1				

95	7	11971834	*LOC107986734*		intron	5		E2A, ELF1, Ets, HEN1, Maf, Mxi1, Myc, Myf, NRSF, Pax-4, Pou2f2, Sin3Ak-20, TAL1				
96	7	73087848	*AC083864.3*		Intergenic	4	5 tissues	Sin3Ak-20				
97	14	6572778	*FRMD6 FRMD6-AS2*	Protein coding Processed transcript Enhancer	Intron Upstream transcript Regulatory region	3a	ESC, IPSC	Gm397, Nanog, Pou1f1, Pou2f2, Pou5f1				
98	7	10254864	*LOC107986734*	enhancer	Regulatory region Upstream transcript	3a		ATF4, HEY1, TATA		H3K4me1 Enh		
99	4	116137057	*SLC10A7*	Protein coding Processed transcript	Intron, Downstream transcript Upstream transcript	5	HRT	Hoxa4, Mef-2, Pax-4, Zfp105				
100	12	61918130	*RPAP3*		Intergenic	5	FAT, BLD					
101	20	117876230	*BTBD3*	Protein coding Nonsense mediated decay	Upstream gene	5						
102	3	74635888	*ADAMTS9-AS2*	Antisense Promoter flanking region	Intron Regulatory region	4	13 tissues	Homez				
103	3	11706761	*ADAMTS9-AS2*		Intergenic	5		Myc, Myf, SP1, STAT, TATA, ZNF263				
104	6	2876012	*RNASET2*	Protein coding	Intron	7		CEBPA, Cdx, Zec, Zfp105				
105	6	4710142	*RNASET2*	Protein coding	Intron	5		GR				
106	13	2875530	*LINC00364*		Intergenic	5	ESC, BRN	Cdx2, HDAC2, Hoxc10, Hoxd10, Irf, p300				
107	13	2186134	*LINC00364*		Intergenic	5	ESC, BRN	Ascl2, E2A, HEN1, Lmo2-complex, Myf, NRSF				
108	12	61918093	*RPAP3*		Intergenic	5						
109	6	9405253	*LYRM4 LYRM4-AS1*	Processed pseudogene Promoter flanking region	Intron Downstream gene regulatory region	4	FAT, LIV, GI			DNAse		CEBPB, USF2, BHLHE40, FOXA1, FOXA2, HDAC2, HNF4A, HNF4G, P300, SP1, USF1, CMYC, MAX
110	12	73107458	*RPAP3*	Promoter flanking region	Regulatory region TF binding site	2b	10 tissues	GR, Ik-3, Nkx2	7 Enh, 13 EnhA1, H3K4me1 Enh, H3K27ac Enh	7 Enh, 15 EnhAF, H3K4me1 Enh, H3K27ac Enh		CFOS, CJUN
111	12	61918072	*RPAP3*		Intergenic	6	BRST	Egr-1, Ets, GATA, GR, Irf, NF-1, Nrf-2, PU.1, RXRA		H3K4me1 Enh, H3K27ac Enh		
112	13	1937499	*LINC00364*		Intergenic	5		Foxq1, HNF1, Hltf, Hoxc1, Hoxc9, Maf, Pou6f1				
113	13	1937505	*LINC00364*	Promoter flanking region	Regulatory region	4	5 tissues					
114	13	1937514	*LINC00364*		Intergenic	4	ADRL	Myc, Nkx2, SETDB1				EBF1
115	13	1937513	*LINC00364*		Intergenic	3a	ADRL	CACD, Irf, Klf4, Klf7, MZF1::1-4, SP1, TATA				
116	14	28489683	*ATL1*	Protein coding	Intron	6		Foxj1, Obox6, Pou2f2, Sox				
117	22	17762038	*TBC1D22A*	Protein coding Nonsense mediated decay Processed transcript	Intron	5	BLD, GI	Myc, Osf2, RXRA			H3K4me1 Enh, H3K9ac Pro	
118	6	10806874	*RNASET2; RP11-514O12.4*	Protein coding	Intron	6		Cdc5, Mef2, STAT, Sox				
119	6	6929276	*CASC6*		Intergenic	7						
120	6	9359960	*CASC6*		Intergenic	6		Pou2f2, SETDB1				

121	4	77376908	*POU4F2*		Intergenic	5	ESC	CIZ, Foxp1, NF-kappaB		
122	3	115314220	*MASP1*		Intergenic					H3K9ac Pro
123	3	850306	*MASP1*		Intergenic	4		MAZ, STAT		H3K9ac Pro
124	3	710479	*MASP1*		Intergenic	7		E2F, ELF-1, Maf, NF-E2		
125	3	710480	*MASP1*		Intergenic	3a		GLI, Rad21, Zfx, Zic, Znf143		
126	3	9990329	*MASP1*		Intergenic	5		BDP1, Hoxb13, Hoxd10, Maf, RAD21, STAT, p300		
127	4	66827186	*DTHD1*		Intergenic	5	ESC, SKIN	LUN-1		
128	9	11999486	*CIZ1*	Protein coding Processed transcript Retained intron	Intron	7	ESDR	GR, RXRA		
129	1	9424389	*MDS2*	Protein coding Processed transcript Promoter flanking region	Intron Regulatory region	2b	BLD, THYM	NRSF, SETDB1, Zfx		15 EnhAF, H3K9ac Pro
130	22	60370054	*TBC1D22A*	Protein coding Nonsense mediated decay Processed transcript	Intron	5	STRM	CTCF, Nanog, Nr2f2, Zbtb3		
131	16	61408223	*WWOX*	Protein coding Nonsense mediated decay	Intron Downstream transcript	5		THAP1		
132	5	73054303	*CTNND2*		Intergenic	6	BRN	Dbx1, Hoxd10, TATA		
133	5	57620661	*CTNND2*		Intergenic	5		Bach1, Pax-2		

10TxEnh5: Preferred and potentiating 5' transcript; 13EnhA1, 14EnhA2: Active enhancer; 15EnhAF: Active booster flank; 7Enh, 18Enh: Enhancer; 18EnhAc: Possible primary h3k27ac enhancer; 19DNAse: Desoxiribonuclease; 2 PromU: Promotor upstream tss; 2 TssAFlnk: TSS active flanker; 2b: Transcription factor binding + any motif + DNase Footprint + DNase peak; 3a: Transcription factor binding + any motif + DNase peak; 4: Transcription factor binding + DNase peak; 5: Transcription factor binding or DNase peak; 6: Motif hit; 7: Other; AC083864.3: Homo sapiens chromosome 7 clone RP11-196O2, complete sequence; ADAMTS9-AS2: A desintegrina and metaloproteinase (ADAM) metallopeptidase with thrombospondin type 1 motif 9 antisense RNA 2; ADRL: adiponectin receptor protein; AIRE-2: Autoimmune regulator 2; AP-1, AP-2, AP-4: Activator protein; Arid3a: AT-Rich interaction domain 3A; Arid5a: AT-Rich interaction domain 5A; ASAP1: ArfGAP With SH3 Domain, Ankyrin Repeat And PH Domain 1; ASB4: Ankyrin repeat and SOCS box containing 4; Ascl2: Achaete-Scute family BHLH transcription factor 2; ATF3: Activating transcription factor 3; ATF4: Activating transcription factor 4; ATL1: Atlastin GTPase 1; Bach1: BTB domain and CNC homolog 1; Barx1: BARX homeobox 1; BATF: Basic leucine zipper ATF-like transcription factor; BCL: B-Cell CLL/Lymphoma 2; BDP1: B Double Prime 1, subunit of RNA Polymerase III transcription initiation factor IIIB; BHLHE40: Basic helix-loop-helix family member E40; BLD: Blood; BRN: Brain; BRST: Primary myoepithelial cells of the breast; BTBD3: “Bric-a-brac, tramtrack, larage complex” (BTB) domain containing 3; CACD: Central areolar choroidal dystrophy: Cart1: Cartilage paired-class homeoprotein 1; CASC6: Cancer susceptibility 6; CCDC85A: Coiled-coil domain containing 85ª; CEBPB: CCAAT enhancer binding protein beta; CCNT2: Cyclin T2; CDC5: Cell division cycle 5; CDP: Phosphatidate cytidylyltransferase; Cdx, Cdx2: Caudal type homeobox 2; CEBPA e CENPAB: CCAAT enhancer binding protein alpha and beta; CEP112: Centrossomal protein 112; CFOS: Fos proto-oncogene, AP-1 transcription factor subunit; Chr: Chromossome; CIZ: Cas-interacting zinc finger protein; CIZ1: Cyclin Dependent Kinase Inhibitor 1A Interacting Zinc Finger Protein 1; CJUN: Jun Proto-Oncogene, AP-1 Transcription Factor Subunit; CMYC: Myelocytomatosis proto-oncogene, helix-loop-helix basic transcription factor (BHLH); CTCF: CCCTC-Binding Factor; CTNND2: Catenin delta 2; Dbx1: Developing Brain Homeobox 2; DEC: Deleted in esophageal cancer; DMRT1, DMRT2, DMRT7: Doublesex and mab-3 related transcription factor 2; Dnase: Dnase; DTHD1: Death domain containing 1; Duxl: Double homeobox B-like 1; DYRK2: Dual specificity tyrosine phosphorylation regulated kinase 2; E2A: Immunoglobulin enhancer-binding factor E12/E47; E2F: E2F transcription factor 1; EBF1: EBF, Early B cell factor 1; Egr-1: Early growth response 1; ELF1: E74 like ETS transcription factor 1; ERALPHA A: Estrogen nuclear receptor alpha; ERBB4: Erythroblastic leukemia viral oncogene homolog 2 receptor tyrosine kinase 4; ESC: Embryonic stem cell; ESDR: H1-derived mesenchymal stem cell; Ets: ETS proto-oncogene 2, transcription factor; Evi-1: Ecotropic virus integration site 1 protein homolog; EWSR1-FLI1: Ewing sarcoma breakpoint region 1-Fli-1 proto-oncogene, ETS: Transcription factor; FAT: Fat tissue; FMN1: Formin1; Fosl1, FOSL2: FOS like 2, AP-1 transcription factor subunit; Fox, Foxa, FOXA1, FOXA2, Foxf1, Foxi1, Foxj1, Foxk1, Foxm1, Foxo, Foxp1, Foxd3, Foxj2, Foxp3, Foxq1: Forkhead Box; FRMD6-AS2: FERM (F for 4.1 protein, E for ezrin, R for radixin and M for moesin) domain containing 6 antisense RNA 2; FXR: Farnesoid X receptor; GATA: GATA binding protein 1; GCNF: Germ cell nuclear factor; GI:Gastrointestinal; GLI: Zinc finger family of oncogenes associated with glioma; Gm397: Member of the Zscan4 family of SCAN domain-containing zinc finger proteins; GR: Motif glycine-arginine; GYG1: Glicogenin 1; H3K27ac Enh: Acetylation of lysine 27 to histone 3 with enhancer function; H3K27ac1 Enh: Acetylation 1 of lysine 27 to histone 3 with enhancer function; H3K4me1 Enh: Methylation of lysine 4 to histone 3 with enhancer function; H3K4me3 Pro: Trimethylation of lysine 4 to histone 3 with promoter function; H3K9ac Pro: Acetylation of lysine 9 to histone 3 with promoter function; Hand1: Heart and neural crest derivatives expressed 1; Hbp1: HMG-box transcription factor 1; HDAC2: Histone deacetylase 2; HEN1: Hua enhancer 1 homolog 1; HEY1: Hes related family BHLH transcription factor with YRPW motif 1; Hltf: Helicase like transcription factor; HMG-IY: High mobility group AT-hook 1; HNF1: Hepatic nuclear factor 1; HNF4: Hepatic nuclear factor 4; HNF4G: Hepatic nuclear factor 4G; Homez: Homeobox and leucine zipper encoding; Hoxd10, Hoxb13, Hoxa4, Hoxa5, Hoxd8, Hoxa10, Hoxc10, Hoxb3, Hoxb6: Homeobox; HRT: Left ventricle of the heart; Hsf: Heat shock transcription factor; Ik-1, Ik-3: IKAROS family zinc finger 1; IPSC: Induced pluripotent stem cells; Irf: Interferon regulatory factor; Isl2: Homeobox of insulin gene enhancer binding protein with muscle nucleocytoplasmic transport protein 2; JUND: JunD proto-oncogene, AP-1 transcription factor subunit; Klf4: Kruppel like factor 4; Klf7: Kruppel like factor 7; LBP-1: Lipopolysaccharide binding protein 1; Lhx3: Homeobox 3 nucleocytoplasmic transport protein (LIM) muscle; LINC02114: Long intergenic non-protein coding RNA 2114; LINC00364: Long intergenic non-protein coding RNA 0364; LIV: Liver; Lmo2-complex: Nucleocytoplasmic transport protein (LIM) domain complex “only” 2; LNG: Fetal lung fibroblast cell line; LOC107984137: RNA intergênico longo não codificante; LRP1B: Low density lipoprotein receptor related protein 1beta; LUN-1: Top1 arginine/serine rich protein, binds E3 ubiquitin ligase; LYRM4: Leucine/tyrosine/arginine (LYR) motif containing 4; MAC: Leucine/tyrosine/arginine (LYR) motif containing 4 antisense RNA 1; Maf: Homologous to the musculoaponeurotic fibrosarcoma oncogene; MASP1: Mannose-binding lectin-associate serine protease 1; MAX: X Factor associated with MYC; MDS2: Myelodysplastic syndrome 2 translocation associated: Mef2: Myocyte enhancement factor; Mrg: Mortality factor 4-like; Mtf1: Metal regulatory transcription factor 1; MTIF2: Mitochondrial translational initiation factor 2; MUS: Muscle tissue; Mxi1: Myxoma dynamin like GTPase 1; Myc: Myelocytomatosis viral oncogene homolog; Myf: Myogenic factor; MZF1::1-4: Myeloid zinc finger 1 dimerized with 1-4; Nanog: Transcription factor in embryonic stem cells; Ncx: Na(+)/Ca(2+) exchange protein; NF-E2: Nuclear factor, erythroid 2; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; Nkx2: Natural killer 2; Nkx6-1: Natural killer 6 transcription factor related, locus 1; Nr2f2: Nuclear receptor subfamily 2 group F member 2; Nrf1, Nrf2: Nuclear respiratory factor 1 and factor2; NRSF: Neural-restrictive silencer factor; Obox3, Obox6: Oocyte specific homeobox; OR3A2: Olfactory receptor family 3 subfamily A member 2; OR3A4: Olfactory receptor family 3 subfamily A member 4 pseudogene; Osf2: Osteoblast-specific transcription factor 2; Otx: Orthodenticle homeobox; OVRY: Ovary; p300: Transcription coactivator; PANC: Pancreas; Pax-2, Pax-4, Pax-5, PAX6, Pax8: Paired box; Pbx2: Pre-B-cell leukemia transcription factor 2; Pbx1:Pre-B-cell leukemia transcription factor 1; PHACTR1: Phosphatase and actin regulator 1; PLAG1: Pleiomorphic adenoma gene 1; PLCNT: Placenta; PLZF: Promyelocytic leukaemia zinc finger; PMDN1: Midasin AAA ATPase 1 protein; Pou1f1: POU domains, class 1, transcription factor 1; Pou2f2: POU domains, class 2, transcription factor 2; Pou2f3: POU domain, class 2, transcription factor 3; Pou3f1: POU domains, class 3, transcription factor 1; Pou3f2: POU domains, class 3, transcription factor 2; POU4F2: POU domains, class 4, transcription factor2; Pou5f1: POU domains, class 5, transcription factor 1; Pou6f1: POU domains, class 6, transcription factor 1; PPAR: Peroxisome proliferator activated receptor; PRDM1: Positive regulatory domain I-binding factor1; PTF1-beta: Transcription factor 1a associated with the pancreas; PTPRD: Protein tyrosine phosphatase receptor type D; PU.1: Hematopoietic transcription factor of action on lymphoid and myeloid cells; RAD21: RAD21 cohesin complex component. Protein involved in DNA double-strand break repair; RNASET2: Ribonuclease T2; RP11-680B3.2, RP11-722M1.1, RP11-146I2.1: Retinitis pigmentosa 11, long non-coding RNAs associated with superenhancers; RP1-90J4.1: Retinitis pigmentosa 1, long

intergenic non-protein RNA; RPAP3: RNA polymerase II associated protein 3; RTN4: Reticulon-4; RXR::LXR: Retinoid X receptor dimerized with hepatic X receptor; RXRA: Retinoid X receptor alpha; SAV1: Salvador family WW domain containing protein 1; SEPT7: Septin-7; SETDB1: SET domain bifurcated histone lysine methyltransferase 1; SF1: Splicing factor 1; Sin3Ak-20: Polyclonal antibody, corepressor interacting with HDAC1, N-corR, SMRT and MeCP2; Six5: Dystrophia myotonica-associated homeodomain protein 5; SKIN: Skin tissue; Smad3: Mothers against decapentaplegic homolog 3; SLC10A7: Solute Carrier Family 10 Member 7; SLC9A9: Solute Carrier Family 9 Member A9; SLC25A25: Solute Carrier Family 25 Member 25; SLC25A25-AS1: SLC25A25 Antisense RNA 1; SMC3: Structural maintenance of chromosomes 3; SNTG1: Syntrophin gamma 1; Sox: Transcription factor from the sex-determining region Y box; SP1: Specificity protein 1; Spdef: S-adenosyl metionin pointed domain containing ETS transcription factor; SPLN: spleen; SREBP: Sterol regulatory element-binding protein; SRF: Serum response factor; STAT: Signal transducer and activator of transcription; STRM: Cultured chondrocyte cells derived from mesenchymal stem cells; TAL1: T-Cell acute lymphocytic leukemia 1; TATA: Non-coding DNA sequence formed by the repeated nitrogenous bases Thymine and Adenine; TBC1D22A: Tre-2, Bub2, and Cdc16 1 Domain Family Member 22A; TCF11:::MafG: Transcription factor 11 dimerized with V-Maf avian musculoaponeurotic fibrosarcoma oncogene homolog G; TCF12: Transcription factor 12; TCF4: Transcription factor 4; Tef: Thyrotroph embryonic factor; Tgif1: Transforming growth factor-beta-induced factor homeobox 1; THAP1: Domain of tannate-associated proteins containing apoptosis-associated protein 1; THYM: Thymus; TR4: Testicular receptor 4; UF1H3BETA: Transcription factor-binding sites, motif; USF1: Upstream transcription factor 1; USF2: Upstream transcription factor 2, C-Fos interacting; UTAT39: Long intergenic non-protein coding RNA 1541; WWOX: WW domain containing oxidoreductase; YY1: Yin-yang transcription factor; Zbtb3: Zinc finger and “bric-a-brac, tramtrack, large complex” domain-containing protein 3; ZEB1: Zinc finger E-box binding homeobox 1; Zec: Zinc finger expressed In embryonal cells and certain adult organs; Zfp105, Zfp281, Zfp691: Zinc finger protein; Zfx: Zinc finger protein X-linked; Zic: Zinc finger of the cerebellum; Znf143, ZNF263: Zinc finger protein.

3.4 Visualization enrichment

Regional association (LocusZoom) graph of *RTN4* on chromosome 2 (Figure 3) *locus*, which presented an association sign (rs10496038) and from *LINC02505* on chromosome 4 which showed two signs of association with periodontitis (rs58327429 and rs67797971) (Figure 4). In both figures, part A is a region closer to the significantly associated variant on the chromosome and part B has a wider region. The different colors representing the SNV are plotted to reflect the linkage disequilibrium (r^2) around the associated SNV.

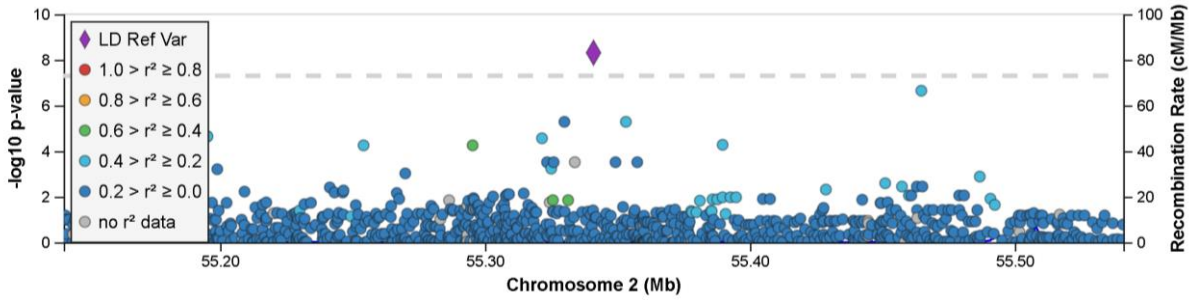

Figure 3. Top SNV on chromosome 2 (rs10496038) associated to periodontitis. The LocusZoom plot (https://my.locuszoom.org) shows the negative log P binding values and upper SNV binding disequilibrium patterns. The dotted line delimits the genomic significance (p value <5 × 10−8). Linkage disequilibrium, represented by colors in the legend, occurs between the top SNV and the variants in this population.

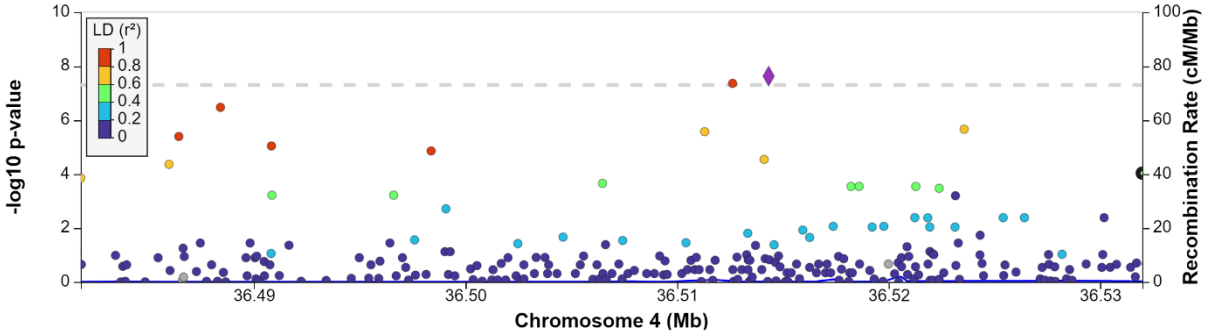

Figure 4. Top SNV on chromosome 4 (rs58327429 and rs67797971) associated to periodontitis. The LocusZoom plot (https://my.locuszoom.org) shows the negative log P binding values and upper SNV binding disequilibrium patterns. The dotted line delimits the genomic significance (p value <5 × 10−8). Linkage disequilibrium, represented by colors in the legend, occurs between the top SNV rs58327429 and the variants in this population.

3.5 Analysis of signaling pathways

The *RTN4* gene participates in the family of reticulons that are basic regulatory factors in the immune system and the central nervous system and is directly involved in neuroinflammation and neurodegeneration processes(26). Figure 6 presents a network of some protein interactions, whose genes were associated with periodontitis. Note that the RTN4 protein interacts with ATL1 (atlastin GTPase 1) with RNA coexpression score 0.08. Protein-protein interaction was obtained by co-immunoprecipitation assay with medium-grade detection confidence and these proteins are mentioned together in PubMed publications. The STRING Platform assigns this interaction a combined score of 0.802 out of a maximum of 1.0. Following the analysis for Table 6, it is observed that the *ATL1* gene follows two pathways. In one, it interacts with the *WWOX* (WW domain containing oxidoreductase) gene and this with the *TBC1D22A* (tre-2, bub2, and cdc16 1 domain family member 22A) gene in sequence with the *PHACTR1* (phosphatase and actin regulator 1) and *SAV1* (salvador family WW domain containing protein 1) genes. In the other, it interacts with the *LRP1B* (low density lipoprotein receptor related protein 1beta) gene and this one with the *PTPRD* (protein tyrosine phosphatase receptor

type D) gene.

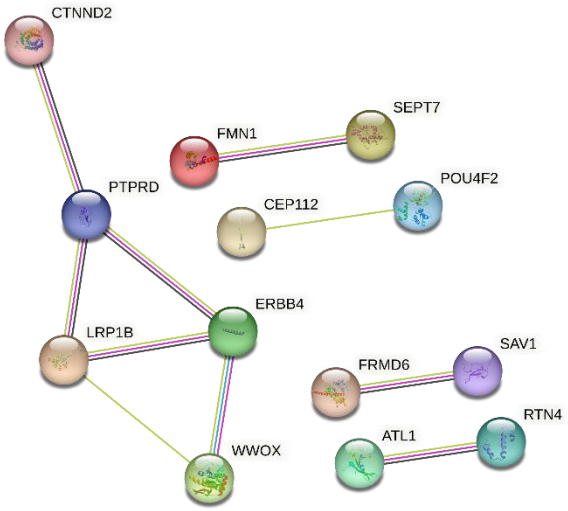

Figure 5: Interaction network of significant and suggestive association proteins in GWAS for periodontitis.

Table 6: Analysis of genetic pathways of genes suggestively associated with periodontitis using the VEGAS2 platform. The p values are stratified by the populations studied.

Gene	Pathway	European Pathwayp	Africaner Pathwayp	American Pathwayp
PHACTR1_SNTG1	Actin binding	1,54E+04	2,27E+04	3,34E+04
PHACTR1_SNTG1	Cytoskeletal protein binding	1,54E+04	2,27E+04	3,34E+04
PHACTR1_TBC1D22A	Enzyme regulator activity	4,74E+04	4,39E+04	6,12E+04
WWOX_TBC1D22A	Regulation of signal transduction	4,74E+04		6,12E+04
WWOX_TBC1D22A	Protein dimerization activity	4,74E+04		6,12E+04
LRP1B_PTPRD	Panther molecular function other receptor		1,54E+04	2,27E+04
SAV1_TBC1D22A	Identical protein binding			1,19E+05
LRP1B_ATL1	Membrane fraction			4,06E+03
LRP1B_ATL1	Insoluble fraction			4,06E+03
ATL1_WWOX	Golgi apparatus			4,06E+03
ATL1_WWOX	Cell death			4,06E+03
ATL1_WWOX	Death			4,06E+03

ATL1: Atlastin GTPase 1; LRP1B: Low density lipoprotein receptor related protein 1beta; PHACTR1: Phosphatase and actin regulator 1; PTPRD: Protein tyrosine phosphatase receptor type D; SAV1: Salvador family WW domain containing protein 1; SNTG1: Syntrophin Gamma 1; TBC1D22A: Tre-2, bub2, and cdc16 1 domain family member 22A; WWOX: WW domain containing oxidoreductase.

When verifying, focally, linkage disequilibrium (LD) between the chromosome 2 variants that participate in the results of this study, we observed that there is high

LD (r^2 = 77) between rs10496038 (*RTN4* gene) and rs74410951 (Mitochondrial Translational Initiation Factor 2*, MTIF2* gene). These variants are 123kb apart and some platforms present the rs74410951 variant belonging to the *RTN4* gene. We then checked for haplotype formation associated with periodontitis and found, despite the low haplotypic frequency, a significant positive association of this haplotype with the outcome (Supplement 1).

Two haplotypes from two loci (rs74635888-rs11706761, rs114884128-rs73162961) and one haplotype from five loci (rs710479-rs710480-rs850306-rs115314220-rs9990329) have been associated with periodontitis (Supplement 2). All these haplotypes are located on chromosome 3. In our sample rs74635888 and rs11706761 (r^2 = 1), and rs114884128 and rs73162961 (r^2 = 0.96) are in LD (Figure 7). The third and largest block of 8kb, in perfect LD (r^2 = 1) occurs between variants rs710479, rs710480, rs850306, rs115314220 and rs9990329 (Block1, Figure 7).

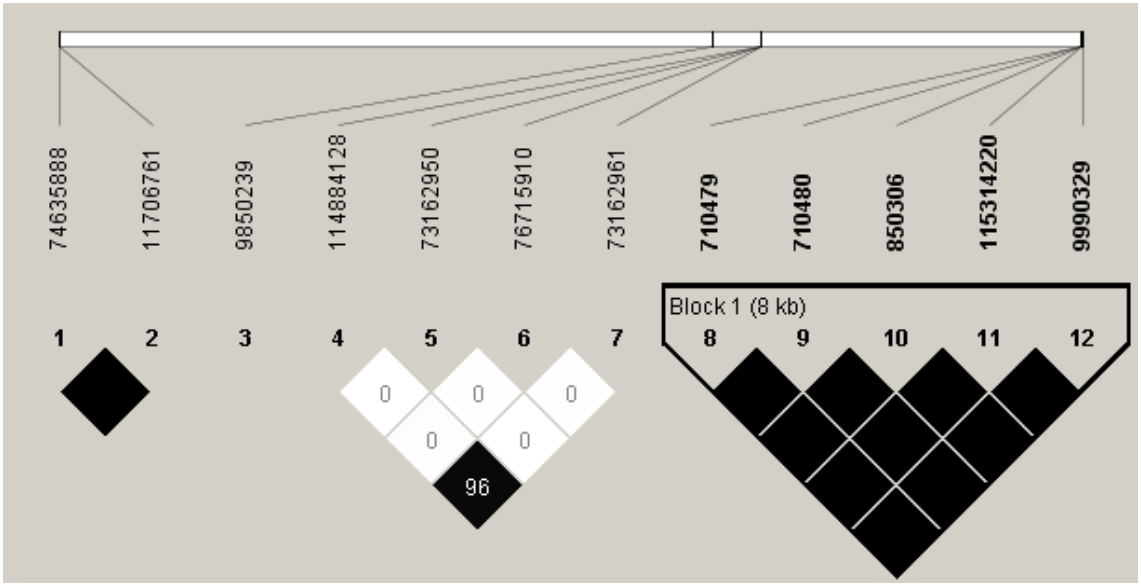

Figure 6: Genetic variants associated with periodontitis, located on chromosome 3 that show linkage disequilibrium. (n = 506)

On chromosome 4 there was the formation of a block with 6 SNV in high linkage disequilibrium ($r^2 \geq 60$). In this block are the variants rs67797971 and rs58327429 of the *LINC02505* gene associated with periodontitis in this study. The possibility of total or partial interactions of the haplotypes with the outcome were

verified and were not statistically significant.

There is a binary haplotype with perfect LD ($r^2 = 1$) on chromosome 5. It is formed by the rs57620661 and rs73054303 variants of the *CTNND2* (catenin delta 2) gene. Individuals who jointly inherit the G and C alleles of the respective SNV, increase the chances of developing periodontitis by 2.64 times (Supplement 3).

High and perfect LD haplotypes were found on chromosomes 6 to 9, 12 to 14, 16, 17 and 22, however, there were no haplotypes associated with periodontitis, among those with LD with $r^2>0.60$.

The results of the genes that formed the periodontitis-associated haplotypes were questioned about the joint heritability of the risk alleles. Through gene-gene interaction, does heritability positively or negatively influence the emergence of the outcome?

Seven alleles were considered, one of each of the following genes: *RTN4* and *MTIF2* of chromosome2; *ADAMTS9-AS2* (a desintegrina e metalloproteinase – ADAM - metallopeptidase with thrombospondin type 1 motif 9 antisense RNA 2), *LOC107986045*, *RP11-680B3.2* (Retinitis pigmentosa 11, long non-coding RNAs associated with superenhancers), *MASP1* on chromosome 3 and *CTNND2* on chromosome 5. Does having inherited 5 or more risk alleles together from these genes attribute a positive or negative association with periodontitis? Table 7 sets out the answers to this question.

Table 7: Interaction between a risk allele of each of the 7 genes that form the haplotypes associated with periodontitis (n = 506).

Number of alleles	Without periodontitis(%)	With periodontitis(%)	ORadjusted**	IC95%†	p value
≤4	359(92.3)	88(75.9)			
≥5	30(7.7)	28(24.1)	5.47	2.91 -10.27	<0.0001

ORadjusted: odds ratio adjusted for age, obesity, breathe through the mouth, flossing, asthma, and genetic ancestry. (95%CI): confidence interval 95%.

The heritability of 5 risk alleles or more, in the criteria already exposed, is positively associated in dimensions above five times of chance for the development of periodontitis at some point in the individual's life.

3.6 Analyzes on the influence on the expression of cytokines

When verifying the influence of the presence of the minor alleles in SNV associated with periodontitis, it was found that there is a difference between the medians of the eotaxin-1 concentration values with the rs10496038 variant, genotype in the dominant model, in the *RTN4* gene (p = 0.025) and there is a difference between the medians of the IL12 concentration values with the rs58327429 variant, genotype in the dominant model, in the *LINC02505* gene (p = 0.047).

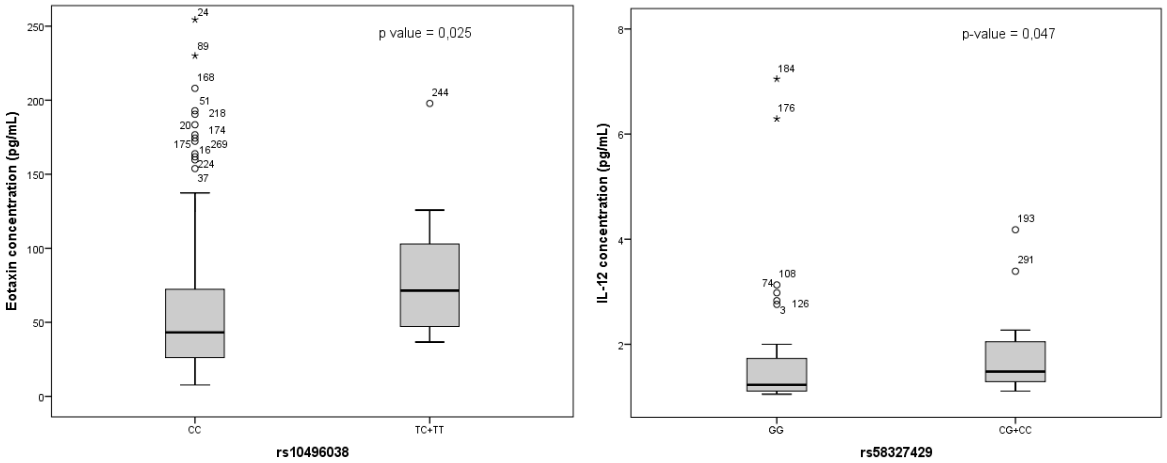

Figure 7: Association of rs10496038 of the *RTN4* gene in eotaxin-1 production and association of rs58327429 of the *LINC02505* gene in IL-12 production. Subsample of 296 individuals, 91 with periodontitis and 205 without periodontitis. Individuals carrying one or two T alleles of the rs10496038 variant of the *RTN4* gene have higher eotaxin concentrations than individuals carrying two C alleles of the same variant (p value = 0.025). Individuals carrying one or two C alleles of the rs58327429 variant of the *LINC02505* gene have higher concentrations of IL-12 than individuals carrying two G alleles of the same variant (p value = 0.047). Mann Whitney test. Boxplot data refer to the median and interquartile range.

This is the first GWAS for periodontitis performed in a highly mixed and mostly self-declared Afro-descendant Brazilian population. We found in this population a statistically significant association between PD and the presence of the T allele of the intronic variant rs10496038 in the *RTN4* gene (Reticulon 4) on chromosome 2 and the C allele of rs58327429 and the A allele of rs67797971 of the intronic variants in the *LINC02505* gene (long intergenic non-protein coding RNA 2505 - ncRNA) on chromosome 4. Other variants (n = 130) close to or inserted into another ~90 genes on 18 different chromosomes showed a suggestive association with periodontitis. Of these associations, only 12 were negative in terms of outcome and almost all are in the *LINC00364* gene (long intergenic non-protein coding RNA 364 - ncRNA).

The Reticulon 4 protein is responsible for preserving the structure of the endoplasmic reticulum tubules, these tubules establish contact with the mitochondria, as they surround them. A change in the balance between the tubules and laminae of the endoplasmic reticulum could alter this contact, which could affect the structure of mitochondria and even their function(27). The RTN4 protein does not seem to have a direct action on mitochondria, but it regulates the recruitment of BCL2 associated X (BAX) to the endoplasmic reticulum and mitochondrial membranes(27). The translocation of BAX to mitochondria activates caspases initiating cellular apoptotic pathways(28). RTN4 proteins, despite differentially regulating the ultrastructure of the endoplasmic reticulum, demonstrated surprisingly similar effects in the regulation of mitochondrial morphology(27). Functional analyzes presented that the rs10496038 variant in the *RTN4* gene has a high regulatory potential with methylation and acetylation action in osteoblasts and primary fibroblasts. The presence of genetic variants in the *RTN4* gene can influence the expression of the protein, which can alter its metabolic and cellular role.

The association between the presence of the *RTN4* gene allele and the presence of periodontitis presented high OR and 95% CI, which can be explained by its low frequency in the population. However, at the frontier of PD immunogenetics are studies involving miRNAs, circRNAs, exosomes and exogenous proteins released by cells from periodontal tissue(29). circ-Rtn4 derivated Exosomes (circ-reticulon 4) inhibited cytotoxicity and apoptosis in mouse osteoblastic cells(28). This

fact has not yet been proven in human periodontal cells, but there are studies associating exosomes derived from periodontal cells and circRNAs related to both homeostasis and regeneration, as well as tissue loss during periodontitis(29). Another study associated RTN4 protein (also known as Nogo-A) to osteoclastogenesis, in the formation phase of bone resorption cells, suggesting that it already regulates the expression of Nogo-A at the level of transcription(30). Observing these findings, we observed that *RTN4* can contribute both to homeostasis and regeneration, as well as to tissue loss due to cellular apoptosis during periodontitis. In the present study, the presence of the T allele in the rs10496038 variant of the *RTN4* gene was positively associated with the presence of periodontitis.

The results of the association of the *RTN4* gene with periodontitis are supported by the metabolic pathways discussed and presented in the results of this study. The interaction of the RTN4 protein with the ATL1 protein, also associated with periodontitis in this study, suggests that genetic variants in one or both genes may alter their functions(22). The *ATL1* gene, in two GWAS studies, is associated with severe chronic periodontitis in adult men(12) and together with *SAV1* gene presented association with probing depth in young adults(31). The sequence of interactions between *ATL1* - *WWOX* - *TBC1D22A* - *PHATR1* - *SAV1* genes in one pathway and *ATL1* - *LRP1B* - *PTPRD* in another pathway are also strong indications that polymorphisms in the *RTN4* gene or in any other sequential gene can influence regulatory activities, enzymes, signal transduction regulation, protein dimerization, actin binding to cytoskeletal proteins, among identical proteins that are crucial functions for the maintenance of cellular homeostasis, in addition, that genetic variants can even interfere positively or negatively in cell death(23).

Another evidence of the true association of the T allele of rs10496038 in the *RTN4* gene with periodontitis is the positive association of the haplotype rs10496038-T – rs74410951-G, *RTN4* and *MTIF2* respectively, with the presence of periodontitis. As well as the high association with the outcome, with the presence of 5 or more polymorphic alleles through gene-gene interaction. The influence of the *RTN4* gene on eotaxin expression points to the immunological role mentioned in the literature(26). We understand that a GWAS, by itself, is not sufficient to determine a genetic marker for the outcome, however, it points to regions of important associations that need to be studied in more details(32).

Variants significantly associated with periodontitis in the *LINC02505* gene showed high LD but, as haplotypes, no statistically significant association with periodontitis. However, the rs10496038 variant in the dominant model had an influence on IL12 concentration in a fraction of this same population. IL12 is identified as a cytokine involved in the immunoregulatory mechanisms of periodontitis with a strong pro-inflammatory role(33–35). The rs58327429 and rs67797971 of the *LINC02505* gene variants together were presented in open GWAS data from different GWAS with statistical significance in association with results on the order of < 10-5. Suicide attempt, smoking, predicted percentage of forced expiratory volume in 1 second (FEV1), left leg impedance are among the studies that the rs58327429 and rs67797971 variants in the *LINC02505* gene presented in the literature(36). Other variants such as rs61918094 from the *RPAP3* gene, rs73107481 and rs61918087 from the *RP1-90J4.1* gene appear in the same study in association with the presence of bacteria of the genus Alloprevotella and the third of these variants also appears in another study associated with bacteria of the genus Prevotella(36), whose species range from rare to totally common in the oral cavity(37,38).

Among the positive associations of haplotypes with periodontitis in this study, there are genetic variants in the *MASP1* and *CTNND2* genes. Downregulation of the *MASP1* gene, by DNA methylation in conditions of *diabetes mellitus*, suggests the inhibition of the classical and lectin pathways of the complement cascade causes dysbiosis in the oral microbiota favoring periodontitis(39). *CTNND2* gene is shown to participate in the maintenance of homeostasis and adhesion in periodontal ligament cells, conditions that, if altered, can also favor periodontitis(40,41).

Genes of this GWAS with suggestive association have already been reported in other studies with association with periodontitis or some related outcome. The Erb-b2 receptor tyrosine kinase 4 (ErbB4) has been shown to have a suggestive influence on periodontitis(42), in this study it was demonstrated that the protein is an important point of interaction between other proteins, whose genes are included in this GWAS. One study analyzed patients with abnormal Golgi glycosylation, finding SLC10A7 (Solute Carrier Family 10 Member 7) as a new genetic factor for bone mineralization and transport of glycoproteins to the extracellular matrix(43). Another study extracted and tested non-coding long ribonucleic acids (lncRNAs) from periodontal ligament stem cells, in the result, ADAMTS9-AS2 showed the ability to interact and modulate extracellular matrix activity during periodontal tissue

remodeling(44). In the present study, an ADAMTS9-AS2 haplotype was positively associated with the presence of periodontal disease. A recent study suggested the combined use of Neural Epidermal Growth Factor-Like 1 (NELL1) and Bone Morphogenetic Protein 9 (BMP9) proteins with the potential to promote alveolar bone regeneration in periodontitis(45). Likewise, studies can be carried out for specific populations, such as ours, developing therapeutic targets.

The small sample size for a GWAS did not fully limit the statistical power to find associations of statistical significance. However, to avoid understanding that the results of this study were false positives, we performed analyzes of LD, association of haplotypes with outcome, and gene-gene interaction. In all these analyzes we obtained responses with statistical association. In fact, some suggestive associations were intergenic, imputed and their genes are not yet relevant to oral health or periodontitis and this can be explained by the power of the study directly influenced by the sample size.

Many of the variants of this study appear in the literature pointing to other systemic, autoimmune, or central nervous system related diseases. Most SNV are rare variants, and these can potentially have the largest and most significant effects on periodontitis. More studies with large samples, preferably with the same miscegenated ancestry, are needed to determine the effect of these rare variants on periodontitis. In this study, many SNV had high odds ratios and a broad CI of 95% (3 times the OR). These large odds ratios and confidence intervals may be in part due to the small sample size. However, these variants may, in fact, make a major contribution to susceptibility to or protection against to periodontitis. This study is a pioneer in a Brazilian population and presented a list of genes associated and suggestively associated with periodontitis. Three significant genomic associations between periodontitis and the presence of the T allele of intronic rs10496038 in the *RTN4* gene (Reticulon 4) of chromosome 2 and the C allele of rs58327429 and the A allele of intronic rs67797971 in the *LINC02505* gene (non-protein coding long intergenic 2505 - ncRNA) from chromosome 4 and another 130 suggestive associations ($p < 10\text{-}6 > 5.10\text{-}8$) were presented. The significant genome-wide success of the *RTN4* gene and the associations between variants establish it as a putative genetic risk factor for periodontitis. The results of this GWAS may favor studies of therapeutic targets. Further investigations of the *loci* presented here and the influence of these genes on periodontitis are recommended.

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SUPPLEMENTS

Supplement 1: Haplotypic association between chromosome 2 variants with the

presence of periodontitis (n = 506).

Chr	Genes	Haplotype combination	Aleles	Chromosomal position	Frequence haplotypes	ORadjusted (95%CI)	p-value	Global haplotype association p-value
2	*RTN4-MTIF2*	rs10496038-rs74410951	T-G	55340963-55464700	0.0178	17.99 (5.54-58.48)	<0.0001	<0.0001

Chr: chromossome. ORadjusted: odds ratio adjusted for age, obesity, breathe through the mouth, flossing, asthma, and genetic ancestry. 95%CI: confidence interval 95%. RTN4: reticulon 4. MTIF2: translation initiation factor IF-2.

Supplement 2: Haplotypic association between chromosome 3 variants with the presence of periodontitis (n = 506).

Chr	Gene	Haplotype combination	Aleles	Chromosomal position	Frequence haplotype	ORadjusted (95%CI)	p-value	Global haplotype association p-value
3	*ADAMTS9-AS2*	rs74635888-rs11706761	A-T	64978671-65015822	0.0257	6.74 (2.70-16.80)	<0.0001	<0.0001
3	*LOC107986045-RP11-680B3.2*	rs114884128-rs73162961	C-T	148662114-148690698	0.0247	6.48 (2.57-16.37)	<0.0001	<0.0001
3	*MASP1*	rs710479-rs710480-rs850306-rs115314220-rs9990329	T-A-G-T-T	187027156-187027301-187030140-187030480-187035234	0.0949	2.91 (1.78-4.76)	<0.0001	<0.0001

Chr: chromossome. ORadjusted: odds ratio adjusted for age, obesity, breathe through the mouth, flossing, asthma, and genetic ancestry. 95%CI: confidence interval 95%. *ADAMTS9-AS2:* A desintegrina e metaloproteinase (ADAM) metallopeptidase with thrombospondin type 1 motif 9 antisense RNA 2. *LOC107986045:* Long non-coding intergenic RNA. *RP11-680B3.2:* Retinitis pigmentosa 11, long non-coding RNAs associated with superenhancers. *MASP1:* Mannose-binding lectin-associate serine protease 1.

Supplement 3: Haplotypic association between chromosome 5 variants with the presence of periodontitis (n = 506).

Chr	Gene	Haplotype combination	Aleles	Chromosomal position	Frequence haplotype	ORadjusted (95%CI)	p-value	Global haplotype association p-value
5	*CTNND2*	rs57620661-rs73054303	G-C	10910387-10912320	0.0267	2.64 (1.11-6.29)	0.029	0.034

Chr: chromossome. ORadjusted: odds ratio adjusted for age, obesity, breathe through the mouth, flossing, asthma, and genetic ancestry. 95%CI: confidence interval 95%. *CTNND2:* Catenin delta 2.

CONCLUSÃO GERAL

Esta tese apresentou o primeiro estudo de ampla varredura genômica em uma população brasileira, altamente miscigenada, maiormente autorreferida afro descente. Através de um GWAS, foi demonstrado que associação estatisticamente significativa entre os genes *RTN4* e *LINC02505* e a presença de periodontite em uma população de Salvador, Bahia/Brasil. Verificou-se o melhor modelo de ajustes das análises, encontrando que as variáveis idade, escolaridade, obesidade, hábito de respirar pela boca, uso de fio dental ao menos uma vez ao dia e presença de asma foi o melhor modelo de ajuste para as análises de regressão logística. A obesidade, o não uso de fio dental e a presença de asma são indicadores de risco para o desenvolvimento da periodontite. Também foi detectado que a presença do alelo A da variante rs75985579 do gene *IFI16* está associada positivamente com a presença de periodontite. No gene *AIM2*, a presença do alelo G da variante rs76457189 está associada negativamente com a presença de periodontite. Estes resultados são o alicerce para muitos outros estudos de confirmação de associação em outras populações e de alvos terapêuticos para o tratamento e controle da periodontite.

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