

Periodontitis association with IL-8 gene polymorphisms

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IL-8 and its polymorphisms are involved in multiple acute and chronic inflammatory processes including pathological changes to surrounding structures of the teeth called periodontal diseases or periodontitis. The aim of this manuscript was to systematically review studies from 2006 to 2021 on IL-8 polymorphisms and their association with periodontitis. Literature analysis was done following the PRISMA protocol guidance using articles not older than 15 years (2006-2021). The search was carried out using PubMed (MEDLINE), ScienceDirect and Wiley Online Library databases. For the focus question, the PICO (population (P), intervention (I), control (C), and outcome (O)) study design protocol was used, and the following question was formulated: are IL-8 gene polymorphisms associated with periodontitis? A total of 2422 articles were found at the beginning of the search. After applying the inclusion and exclusion criteria, screening, and full-text article exclusion with reasons, 31 studies were included in the analysis. In conclusion, IL-8 and its gene polymorphisms are associated with an increased risk of periodontal diseases.

Key words: periodontitis, IL-8, gene polymorphisms, serum level, literature review, PRISMA protocol guidance

Received: July 27, 2021; Revised: September 23, 2021; Accepted: November 16, 2021; Available online: December 13, 2021
<https://doi.org/10.5507/bp.2021.066>

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INTRODUCTION

Periodontitis is a chronic, multifactorial inflammatory disease of the periodontium, affecting up to 47% of adults over the age of 30 in the United States^{1,2}. In Europe, this disease is found in 5-20% of middle-aged adults, and this number increases up to 40% for older individuals³. What begins at first as a loss of connective tissue and gingiva, during the course of this disease leads to periodontal ligament loss, resorption of alveolar bone, and the exposure of tooth roots to bacteria⁴. This inflammation can ultimately lead to the tooth loss in the affected region⁵. Tooth loss leads to impaired oral health-related quality of life, and the region from which the teeth are lost can have an impact on the severity of the impairment⁶.

Back in the 1999, periodontitis was classified into 4 different forms: necrotizing, chronic, aggressive, and a manifestation of a systemic disease, but nowadays, a new 2018 classification has been adopted and approved⁷. Periodontitis is classified into three main forms: periodontitis, necrotizing periodontitis and periodontitis as a direct manifestation, each having a localised (<30% of teeth affected) or generalised (>30% of teeth affected) extent⁷. The primary cause of periodontitis are bacteria, which accumulate and colonise mouth tissues. However, multiple factors influence the aetiology of this disease: unhealthy lifestyle habits (such as smoking, drinking), systemic illnesses, calculus accumulation around teeth and restorations, bad oral hygiene and genetic factors⁸.

Various studies have been conducted where the gene polymorphisms were analysed, and some of them have been proven to have an association with periodontitis⁹⁻¹¹.

Today at least 65 genes are associated with this mouth disease⁷. Since periodontitis is an infectious disease, interleukin mediates inflammatory and immune responses, and this is one of the main reasons why polymorphisms of this gene may have an impact on the periodontitis pathogenesis¹². A commonly investigated interleukin-8 gene polymorphism is highly associated with periodontitis susceptibility¹³. Its mechanism is explained by the role of IL-8 in regulating inflammatory reaction to the infection caused by bacteria, as the increased expression of proinflammatory cytokines is highly dependent on the presence of bacterial metabolic products¹⁴. Various studies analyse different polymorphisms and their association with periodontitis, but mixed results regarding the disease dependency on IL-8 polymorphisms are being appraised¹².

The aim of this work was to carry out a systematic literature review with no limit to study design of all studies from 2006 to 2021, regarding IL-8 gene polymorphisms and their association with various types of periodontitis.

MATERIALS AND METHODS

The aim of the literature review

The aim of this review was to analyse studies from 2006 to 2021 on IL-8 polymorphisms association with periodontitis. The review was done according to the systematic review statement (PRISMA protocol) (ref.¹⁵).

Table 1. Gene polymorphisms associated with risk of periodontitis.

Gene	Name	Variant/DNA Marker	Ref.
IL-8	Interleukin-8	rs4073, A2767T, T11722T2, rs2234671, rs2230054, rs1126579, rs2227306, rs2227307, rs2227532, and T-738A	12
BAT1 intron	Spliceosome RNA helicase	rs11796, rs3130059	16
BAT5' untranslated region		rs2239527	16
NFKBIL1 intron	NFKB Inhibitor Like 1	rs2071591	16
LTA intron	lymphotoxin-a concentration	rs909253, rs1041981, rs2844482	16
LTA promoter	lymphotoxin-a concentration	rs2857708, rs2009658	16
IL-8 (CXCL8)	Interleukin-8	251(T>A) rs4073, 396(T>G) rs2227307, 781(C>T) rs2227306	17
CXCR2	C-X-C Motif Chemokine Receptor 2	+1208C/T, rs1126579	18
IL-8	Interleukin-8	-251A/T, rs4073	18
IL-8	Interleukin-8	-845 T/C, +781 C/T	19
IL-8	Interleukin-8	C1633T, rs1126580	
IL-8	Interleukin-8	A2767T, T(1)1722T(2), C781T, A251T, G396T, C1633T	20
IL-8	Interleukin-8	A(-251)/T(+396)/T(+781), T(-251)/G(+396)/C(+781)	21
IL-8	Interleukin-8	-251A/T, -845T/C	22
IL-8	Interleukin-8	-251A/T	23
CXCR2	C-X-C Motif Chemokine Receptor 2	+785C/T (rs2230054), +1208T/C (rs1126579), +1440A/G (rs1126580)	24
IL-8	Interleukin-8	-251	25
MMP-1	Matrix Metalloproteinase-1	-1067	25
MMP-9	Matrix Metalloproteinase-9	-1562	25
MMP-3	Matrix Metalloproteinase-3	-1171	25
IL-8	Interleukin-8	rs2227307 (+396), rs2227306 (+781), rs4073 (-251).	26
IL-8	Interleukin-8	-353T>A	27
IL-8	Interleukin-8	rs4073	28
IL-18	Interleukin-18	-607A, -137G	29
IL-8	Interleukin-8	rs4073, rs2227307, rs2227306	30
IL-8	Interleukin-8	rs4073	31
IL-8	Interleukin-8	ATC/TTC haplotype	32
IL-8 (CXCL8)	Interleukin-8	-251 T allele, -251 A allele	33
FPR1	Formyl peptide receptor 1	c576 T>C>G	35
CXCR2	C-X-C Motif Chemokine Receptor 2	+785(C/T), +1208(T/C), +1440(G/A)	36
IL-8	Interleukin-8	ATC/TTC, AGT/TTC	37
CXCR1	C-X-C Motif Chemokine Receptor 1	rs2234671	39
IL-8	Interleukin-8	ATC/TTC, AGT/TTC	40
IL-8	Interleukin-8	-845(T/C), -738(T/A)	41
IL-8	Interleukin-8	-251A/T	43
IL-8	Interleukin-8	ATC/TTC, ATT/TTC, rs4073	44
IL-8	Interleukin-8	rs4073	45

Table 2. Results and their synthesis.

Study	Year	Population/Problem/ Sample size	Intervention	Comparison	Outcome	Ref.
Liukkonen et al.	2018	Parogene study participants: n=455.	Saliva biomarkers level analysis, bead flow cytometry was used.	Sample analysis was blind (in the laboratory).	IL-8 concentrations were elevated. Study has confirmed the IL-8 association with periodontal inflammation and tissue destruction.	16
Silva et al.	2020	Brazilian individuals n=874.	SNP 251(T>A) rs4073, +396(T>G) rs227307 and +781(C>T) rs227306 genotyping by TaqMan.	None.	SNPs in the IL-8/CXCL8 gene were related to the diseased phenotypes, and individuals carrying the haplotype formed by the homozygous TTC/TTC were approximately twice as susceptible to developing T2DM and Periodontitis as comorbidities or to developing severe Periodontitis in Brazilian population.	17
Linhardtova et al.	2018	Unrelated individuals: n=153.	qPCR used for the gene determination and ELISA for IL-8 measurements of plasma levels.	Genotyping was performed by investigators unaware of the phenotype	The IL-8 plasma levels differed significantly between non-periodontitis HC and T1DM+CP/T2DM+CP patients ($P < 0.01$). CP does not influence the circulating IL-8 levels. Patients with T1DM+CP/T2DM+CP had higher circulating IL-8 levels than HC+CP/non-periodontitis HC	18
Sajadi et al.	2018	n=12 human subjects.	Neutrophils isolation by venepuncture, blood samples, 2 times, half a year apart. Genes were expressed by RT-qPCR.	None.	The association is positive, regarding the distribution of IL-8 – 845 T/C alleles and risk of periodontitis disease.	19
Xiao-Bing et al.	2017	Systematic literature review of n=1938 patients and n=1569 controls.	Analysis of 12 polymorphisms in association with IL-8 polymorphisms and periodontitis susceptibility.	None.	IL-8 C1633T and rs1126580 polymorphisms associated with periodontitis. IL-8 rs4073, A2767T, T11722T2, rs2234671, rs2230054, rs1126579, rs2227306, rs2227307, rs2227532, and T-738A polymorphisms not associated with periodontitis.	12
Houshmand et al.	2012	n=107 periodontitis affected patients. n=99 healthy individuals.	Genotyping for the IL-8 polymorphisms, polymerase chain reaction with sequence specific primers. Interleukin-8 genotype and allele frequencies in the disease group.	Interleukin-8 genotype and allele frequencies in the control group.	A2767T, T(1)1722T(2), and C781T polymorphism of IL-8 gene – no statistically significant differences between periodontitis patients and the control group. IL-8 A251T ($P < 0.0001$), G396T ($P < 0.0001$), and C1633T ($P < 0.0001$) – significant differences in these genotype frequencies between periodontitis and control group patients.	20
Linhardtova et al.	2013	n=492 patients. Groups: n=278 chronic periodontitis patients. n=58 aggressive periodontitis patients. n=158 control group subjects.	Genotyping with 5' nuclease TaqMan assay for IL-8 gene polymorphisms. Bacterial colonization (subgingival) investigation by DNA-microarray detection kit.	None.	No statistically significant differences between CP and AgP and control group subjects of IL-8 polymorphisms. Haplotypes A(-251)/T(+396)/T(+781) and T(-251)/G(+396)/C(+781) were statistically significantly less frequent in CP affected patients in comparison to control group subjects.	21

Table 2. (Continued.)

Study	Year	Population/Problem/ Sample size	Intervention	Comparison	Outcome	Ref.
Chen et al.	2014	n=12 studies meta-analysis. n=2233 cases and n=2655 control group subjects.	IL-8 -251A/T (rs4073) and -845T/C (rs227532) polymorphisms association with periodontitis affected patients.	None.	IL-8 -251A/T and -845T/C polymorphisms in Brazilian mixed population may be involved in periodontitis development. -251A/T allele T - in Asians appeared to be a periodontitis risk factor.	22
Khosropanah et al.	2013	n=227 chronic periodontitis patients (non-smokers). n=40 healthy control subjects	IL-8 (-251 A/T) gene polymorphisms detection by polymerase chain reaction. PPD, CAL, BL measurements for patients.	None.	No correlation that would be statistically significant was found between different genotypes of IL-8 and periodontal condition severity. No association was found between different IL-8 genotypes and PPD, CAL, BL, PI, BOP and avg. teeth number. No correlation was found between chronic periodontitis and 251 (A/T) IL-8 gene polymorphism.	23
Kavrikova et al.	2019	n=500 subjects. n=329 chronic periodontitis patients and n=171 healthy control subjects.	CXCR2 gene variability assessment in CP patients and relation between CXCR2 gene variants and presence of periodontal bacteria. Polymerase chain reaction techniques for three single-nucleotide polymorphisms (SNPs)	None.	None of the investigated SNPs (+785C/T (rs2230054), +1208T/C (rs1126579), and +1440A/G (rs1126580)) in the CXCR2 gene was associated with CP. CXCR2 gene variants can be associated with subgingival colonization of G- bacteria in men with CP in the Czech population.	24
Li et al.	2012	n=122 patients with chronic periodontitis in Chinese population.	Serum levels of MMP-1, MMP-3, MMP- 9, IL-2, IL-8 and COX-2 measured with ELISA for the investigation of association with periodontitis.	None.	MMP-1-1067, MMP-3-1171, MMP-9-1562 and IL-8-251 polymorphisms were associated with susceptibility to CP. MMP-1-1067 2G, MMP-3-1171 6A, MMP-9-1562 T and IL-8- 251 A allele were associated with decreased susceptibility to CP in Chinese population.	25
Scarel et al.	2011	n=493 individuals. n=223 affected by periodontitis. n=270 controls.	SNPs rs2227307 (+396) and rs2227306 (+781) and rs4073 (-251) Association with CP susceptibility. Clinical periodontal examination and DNA sample collection.	None.	+396TT genotype and the haplotypes ATC/TTC and AGT/ TGC were associated significantly with CP susceptibility in Brazilians.	26
Sippert et al.	2013	n=124 affected by CP. n=187 controls.	DNA extraction by salting out method. For Duffy genotypes and IL-8 gene promoter polymorphisms PCR-RFLP was used.	None.	Erythroid DARC plus IL-8 -353T>A SNPs were associated with chronic periodontitis in Brazilian individuals. The FY*02N.01 with IL-8 -353A SNP was associated with CP protection in Afro-Brazilians patients.	27
Andia et al.	2011	n=289 CP affected patients and healthy controls.	RT-PCR for IL-8 mRNA levels detection.	None.	A allele had an increased frequency in the disease group. IL-8 mRNA levels were higher in the periodontitis group, presented with TA genotype. SNP rs4073 associated with the CP group in non-smoker Brazilian subjects.	28

Table 2. (Continued.)

Study	Year	Population/Problem/ Sample size	Intervention	Comparison	Outcome	Ref.
Li et al.	2013	n=458 controls, n=576 periodontitis patients.	Polymorphisms (-607A > C and -137G>C) assessment in meta-analysis.	None.	C variant of IL-18 -607A>C polymorphism was associated with increased periodontitis risk. C variant of IL-18 -607A>C polymorphism was associated with increased periodontitis risk. IL-18 Plasma levels of periodontitis patients was higher than in the control group.	29
Zhang et al.	2014	n=400 cases and n=750 controls from Han Chinese population.	23 SNPs selected from 219 SNPs in the NCBI dbSNP and HapMap.	None.	rs4073 and rs2227307 associated with periodontitis. rs4073-rs2227307-rs2227306 associated with chronic periodontitis. no significant gender-specific associations in IL-8 were observed.	30
Kim et al.	2009	n=224 controls and N=276 periodontitis affected patients.	DNA extraction from buccal epithelial cells. Genotyping SNP rs4073 (sequence-specific primer polymerase chain reaction).	None.	SNP (rs4073) in the IL-8 gene is not associated with susceptibility to periodontitis in Brazilian individuals, even after controlling for covariates.	31
Corbi et al.	2012	n=38 controls and n=41 chronic periodontitis affected patients.	ELISA for IL-8 level determination in gingival crevicular fluid.	None.	No significant differences were found between IL-8 concentration in gingival crevicular fluid of both periodontal conditions with the presence or absence of the IL-8 ATC/TTC haplotype. The IL-8 haplotype of susceptibility to CP was associated neither with IL-8 cytokine levels nor with clinical periodontal parameters	32
Amaya et al.	2012	n=120 total subjects, out of which n=63 with suppurative apical periodontitis and n=57 with chronic nonsuppurative apical periodontitis.	Genotyping for IL1B +3954 (rs1143634), IL-8 / CXCL8 -251 (rs4073), IL12B +1188 (rs3212227) and TNFA -308 (rs1800629). Method: PCR-restriction fragment length polymorphisms.	None.	IL-8 / CXCL8 -251 T allele, which is associated with higher production of IL-8/CXCL8, is also associated with a higher risk of developing acute suppurative form of AP. IL-8 / CXCL8 -251 A allele, which is associated with lower production of IL-8/CXCL8, is associated with chronic nonsuppurative form of AP.	33
Da Silva et al.	2017	n=71531 participants in meta-analysis.	25 polymorphisms in seven interleukins (IL-1A, IL-1B, IL-4, IL-6, IL-8, IL-10, and IL-18), three cellular receptors (Fcγ receptors: FCGR2A, FCGR3A, and FCGR3B), and five inflammatory mediators (COX-2, MMP-2, MMP-3, MMP-8, and MMP-9).	None.	IL-8 polymorphisms had no significant association with risk of developing periodontitis.	34

Table 2. (Continued.)

Study	Year	Population/Problem/ Sample size	Intervention	Comparison	Outcome	Ref.
Rusyanti et al.	2019	n=32 aggressive periodontitis and n=29 non-aggressive periodontitis patients.	Polymorphism was identified using polymerase chain reaction (PCR) technique. IL-8 in the gingival crevicular fluid was identified using the ELISA test.	None.	Presence of the polymorphism of c576 T > C > G of FPR1 gene caused 5.04 times higher occurrence of aggressive periodontitis. Low level of IL-8 (below 0.064 pg/ μ L) showed 34.5 times higher occurrence of aggressive periodontitis. Polymorphism of c576 T > C > G correlated significantly with the IL-8.	35
Viana et al.	2010	n=487 individuals, n=215 controls, and n=272 periodontitis affected patients.	Investigation and association with periodontitis of +785(C/T), +1208(T/C), and +1440(G/A) single-nucleotide polymorphisms (SNPs) in the CXCR2 gene. SNPs were investigated using the sequence-specific primer-polymerase chain reaction method	None.	Findings were the first to indicate an association between the +1440 SNP and haplotypes in the CXCR2 gene with susceptibility to or protection against periodontitis in Brazilian individuals.	36
Corbi et al.	2014	n=41 individuals (n=20 susceptible to CP; n=21 not susceptible to CP).	IL-8 levels were determined by an ELISA on 1-st and 45-th days of periodontal therapy.	None.	Outcome of nonsurgical periodontal therapy nor the IL-8 levels were influenced by the IL-8 ATC/TTC CP-susceptibility haplotype.	37
Cirelli et al.	2017	n=104 patients (n=596 subgingival sites). 4 groups: susceptibility to CP by IL-8 haplotype ATC/TTC (IL-8+); non-susceptible to CP by the IL-8 AGT/TTC (IL-8-); susceptible to CP by the IL4 TCI/CCI (IL4+); protection against CP by the IL4 TTD/CTI (IL4-).	Biofilm samples (subgingival) obtained from CP and healthy sites. Before periodontal treatment and 45 days after periodontal treatment.	None.	Haplotypes in the IL-4 gene (but not the IL-8 haplotype) influenced the levels of <i>A. actinomycetemcomitans</i> in subgingival sites before and after the non-surgical periodontal treatment. Significant correlations between genetic, microbiological and immunological factors, with IL-8 and IL-4 haplotypes.	38
Scarel et al.	2011	n=395 Brazilian subjects (periodontitis free and affected by chronic periodontitis).	SNP rs2234671 was identified using the sequence-specific primer-polymerase chain reaction method (SSP-PCR)	None.	rs2234671 SNP in the CXCR1 gene was not useful as a genetic risk factor for chronic periodontitis affected patients in the studied Brazilian population.	39
Finoti et al.	2013	n=30 patients, groups according to IL-8 ATC/TTC or AGT/TTC haplotypes.	Non-surgical periodontal treatment. Subgingival plaque sample analysis by qPCR.	None.	Non-surgical therapy was equally effective in improving clinical parameters and decreasing the levels of periodontopathogens, independent of the genotype groups produced by the IL-8 haplotype.	40

Table 2. (Continued.)

Study	Year	Population/Problem/ Sample size	Intervention	Comparison	Outcome	Ref.
Kim et al.	2010	n=400 Brazilian subjects (n=182 control and n=218 periodontitis affected patients).	-845(T/C) and -738(T/A) single nucleotide polymorphisms (SNPs) genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.	None.	No SNPs showed different distributions between the control and chronic periodontitis groups. Non-smokers carrying the TTA/CAT haplotypes were genetically susceptible to chronic periodontitis. TTT/TAA haplotype was associated with protection against the development of periodontitis.	41
Sato et al.	2012	n=12 controls and n=100 screened for rs11536889 genotypes. All of the population were Japanese.	C/C subjects and their controls were enrolled in the enzyme-linked immunosorbent assay (ELISA) and quantitative reverse transcription PCR (qRT-PCR) study.	None.	PBMCs from the C/C and G/C subjects secreted significantly higher levels of IL-8 in response to LPS than PBMCs from the G/G subjects. This SNP regulates the expression of TLR4 and has some influence on the response to LPS.	42
Yang et al.	2016	n=1811 periodontitis cases and n=2043 controls.	Systemic literature search and meta-analysis of IL-8 -251A/T polymorphism and its association with periodontitis.	None.	No significant associations were found between the IL-8 -251A/T polymorphism and the risk of periodontitis. IL-8 -251A/T polymorphism may increase the risk of periodontitis in Asian and mixed populations.	43
Pigossi et al.	2019	n=12,6 patients each haplotype (ATC/TTC (Hap-1) or of the ATT/TTC (Hap-2) haplotypes in the IL-8 gene).	Primary neutrophil isolation and gene expression by RT-qPCR.	None.	The presence of the ATC/TTC IL-8 haplotype was associated with an enhanced response of neutrophils and leukocytes to Gram-negative bacteria associated with periodontitis. Moreover, the presence of the T allele (rs4073) in the IL-8 proximal promoter region was the major feature associated with increased promoter activity.	44
Andia et al.	2013	n=184 unrelated subjects (Brazilian individuals).	PCR-RFLP in 13 nuclear families and 184 unrelated subjects (transmission pattern of the IL-8 rs4073 risk allele A and its association with susceptibility to aggressive periodontitis).	None.	SNP (rs4073) was not associated with AgP in unrelated individuals and there is no evidence of over transmission of the alleles in families with AgP, from Brazilian individuals.	45

Article inclusion criteria

- Articles, in which patients were affected with periodontitis.
- Research on patients who were affected by periodontitis and had their gene polymorphisms analysed.
- Articles on IL-8 polymorphisms and their association with periodontitis.
- Research regarding IL-8 salivary concentrations in patients affected by periodontitis.
- Meta-analysis and systematic reviews regarding IL-8 polymorphisms association with periodontal pathologies.
- Articles written in English and not older than 15 years.

Article exclusion criteria

- Articles analyse IL-8 polymorphisms but patient's periodontal status is not considered.
- Articles include patients with periodontitis but no IL-8 polymorphisms are analysed.
- Articles in other language than English and older than 15 years.
- Articles where research was done on experimental animals.

Focused question of the research (PICO)

For the development of the question focus, PICO (population (P), intervention (I), control (C), and outcome (O)) study design protocol was used, and the following question was formed: Are IL-8 polymorphisms associated with periodontitis pathogenesis?

Synthesis of results

The findings on genes and their polymorphisms associated with periodontitis are shown in Table 1 and Table 2.

RESULTS**Selection of studies for the research**

Number of records that were identified through database analysis: 2422.

No additional records were identified through other sources.

Filters that were applied for publications: a) not older than 15 years, b) articles written in English. 703 records were found.

The records were screened for eligibility. During screening, titles and abstracts were analysed, and the inclusion/exclusion criteria were applied.

In total, 37 full-text articles were assessed for eligibility. Six studies were excluded, due to not matching the aim of our research and the criteria: researches not covering IL-8 polymorphisms association with periodontal diseases; researches which did not include patients affected by periodontitis; researches covering taste receptor topics; IL-1 studies; IL-17 studies.

31 articles were included in qualitative synthesis.

The search sequence (PRISMA flow diagram) is shown in Fig. 1.

Characteristics of the studies

Two studies were done with periodontitis affected patients, analysing IL-8 polymorphisms association with the disease^{16,19}. Two studies analysed periodontitis and type 1 or 2 diabetes mellitus association with IL-8 polymorphisms along with patients affected by periodontal pathologies^{17,18}. Seven studies reviewed IL-8-251 polymorphism^{17,18,22,23,25,33,43}. One study summarized MMP gene polymorphisms²⁵. CXCR1 and CXCR2 genes and their polymorphisms were reported by four studies^{18,24,36,39}. Twenty-two studies focused on IL-8 polymorphisms and their different variants findings^{12,17-23,25-28,30-33,37,40,41,43-45}.

Synthesis of results

This literature analysis revealed that IL-8 polymorphisms and concentrations are associated with periodontal diseases. A study done by Liukkone et al. included 455 participants. A clinical examination was performed by two periodontologists, and 3 groups were formed: BOP (bleeding on probing), pocket depth, alveolar bone loss, and each group had flow cytometry done for the IL-8 concentration measurements¹⁶. Research results have shown elevated results for patients who had BOP >25%, a higher number of teeth, or deeper gingiva pockets, which concludes that IL-8 concentration has association with periodontal tissues inflammation and tissue destruction¹⁶.

A study done by Silva et al. analysed 874 Brazilian individuals who were affected by type 2 diabetes mellitus (T2DM) and periodontitis. Three different groups were formed: healthy individuals (n=307), periodontitis group

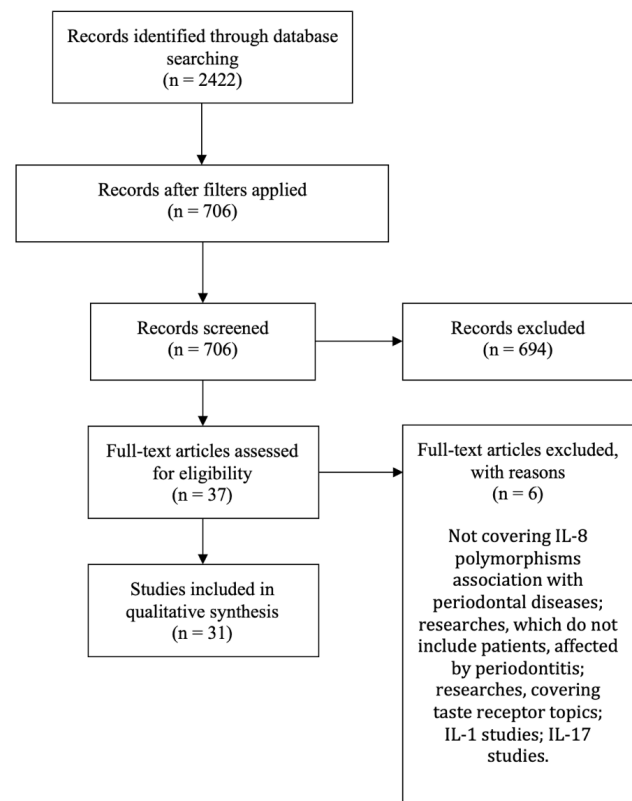


Fig. 1. Articles search sequence (PRISMA flow diagram).

(n=334), and T2DM group (n=233). Genotyping of SNP 251(T>A) rs4073, +396(T>G) rs2227307 and +781(C>T) rs2227306 was done by TaqMan (ref.¹⁷). Research conclusions have demonstrated that heterozygous genotypes of the 251 (rs4073), 396 (rs2227307) and 781 (rs2227306) SNPs in the IL-8/CXCL8 gene were related to the diseased phenotypes, and patients who were carrying this haplotype formed by homozygous TTC/TTC were around twice as susceptible to periodontitis and T2DM development, or severe periodontitis development, as non-carriers in Brazilian population¹⁷.

Additional research covered unrelated individuals. Scientists Linhartova et al. split 153 patients into 4 groups: patients affected with type 1 diabetes mellitus (T1DM) + chronic periodontitis (CP) (n=34), patients affected by T2DM+CP (n=44), healthy individuals (HC) affected with CP (n=32) and non-periodontitis HC (n=41) (ref.¹⁸). For the determination of genes, qPCR was used, and for IL-8 plasma levels measurements, ELISA was the first-choice option, whereas the investigators were unaware of the phenotype when genotyping¹⁸. The research showed that IL-8 plasma levels were scientifically significantly different between non periodontitis HC group and T1DM+CP/T2DM+CP patients ($P<0.01$), and no significant associations between IL-8 plasma levels and IL-8 and CXCR2 polymorphisms were found ($P>0.05$) (ref.¹⁸).

Another research done by Sajadi et al. included 65 patients from Iran affected by periodontitis (18 with chronic form, and 47 with aggressive form) and 55 healthy controls whose DNA extraction was done for IL-8 +781 C/T and -845 T/C polymorphisms assessment¹⁹. The results showed a significantly positive association between the IL-8 -845 alleles distribution and the risk of periodontal disease; C allele of IL-8-845 increased the risk of periodontal disease 9.08-fold (9.08 (95% CI 1.14–72.12, $P=0.03$) (ref.¹⁹). As for +781 C/T locus, no statistically significant correlation was found between patients and controls, but there was a statistically significant difference between the TT vs. CC + CT genotypes that had a role of defending against periodontal disease with value of 0.38 (95% CI 0.16–0.90, $P=0.02$) (ref.¹⁹).

Study done by Xiao-Bing et al., in which information was systemically gathered from multiple scientific sources, consisting of over 1938 patients affected by periodontitis, stated that IL-8 C1633T and rs1126580 polymorphisms are associated with this disease¹². On the other hand, the same review found that IL-8 rs4073, A2767T, T11722T2, rs2234671, rs2230054, rs1126579, rs2227306, rs2227307, rs2227532, and T-738A are not associated with periodontal pathology¹².

Scientists Houshman et al. genotyped 107 patients affected by periodontitis and compared the results with 99 healthy subjects and found significant differences in IL-8 A251T ($P<0.0001$), G396T ($P<0.0001$), and C1633T ($P<0.0001$) genotype frequencies²⁰. In 2017, in a study done by Linhartova et al. where 492 patients were divided into chronic, aggressive and control group subjects, no statistically significant differences were found when analyzing IL-8 polymorphisms²¹. However, haplotypes A(-251)/T(+396)/T(+781) and T(-251)/G(+396)/C(+781)

were less frequently found in chronic periodontitis affected patients than in the control group²¹.

In 2014, a meta-analysis was conducted by scientists Chen et al., which included 2233 periodontitis cases, and it concluded that in Brazilian mixed population, IL-8 -251A/T and -845T/C polymorphisms might be associated with the development of periodontitis⁹. Also, -251A/T allele T was also included as a risk factor for the development of periodontitis⁹. On the other hand, Khosropanah et al. composed a research in 2013, which compared 227 chronic periodontitis affected patients with 40 healthy subjects and found that there was no statistically significant correlation between different IL-8 genotypes and the severity of periodontal condition²³. Kavrikova et al. conducted a study, in which 329 chronic periodontitis affected patients were investigated for SNP in CXCR2 gene, and it concluded that polymorphisms +785C/T (rs2230054), +1208T/C (rs1126579), and +1440A/G (rs1126580) in the CXCR2 gene are not associated with chronic periodontitis²⁴.

Scientists Li et al. examined 122 chronic periodontitis affected Chinese patients, and found, that the MMP-1-1067, MMP-3-1171, MMP-9-1562 and IL-8-251 polymorphisms are related to the susceptibility to chronic periodontitis²⁵. Scarel et al., who investigated SNPs rs2227307 (+396) and rs2227306 (+781) and rs4073 (-251) polymorphisms relation with chronic periodontitis, found that +396TT genotype and haplotypes ATC/TTC and AGT/TGC meant a significant risk of this disease's susceptibility in Brazilian patients²⁶. Also, Sippert et al. stated that in Brazilian individuals, Erythroid DARC plus IL-8 -353T>A single nucleotide polymorphisms were associated with chronic periodontitis disease²⁷. In the same study, a FY*02N.01 with IL-8 -353A nucleotide polymorphism was found to be associated with protection from chronic periodontitis in Afro-Brazilians²⁷.

Andia et al. investigation revealed, that in chronic periodontitis affected patients, A allele had an impact of increasing the disease frequency²⁸. Furthermore, SNP rs4073 was associated with chronic periodontitis group in Brazilian subjects (non-smokers) (ref.²⁸). In a study done by Li et al., IL-18 plasma levels were found to be higher in periodontitis patients, and C variant of IL-18 -607A>C polymorphism was shown to have an association with increased risk of periodontitis²⁹. Zhang et al. reported relation between rs4073, rs2227307 and rs2227306 SNP's and periodontitis in a study done on Han Chinese population³⁰. However, in a study done by Kim et al. on 276 periodontitis affected Brazilian patients, it was found that SNP (rs4073) in the IL-8 gene had no relation to susceptibility to periodontitis³¹. In a study by Corbi et al., the results showed that susceptibility to chronic periodontitis had no association with IL-8 cytokine levels or periodontal clinical parameters³². In a study by Amaya et al., where individuals with suppurative and non-suppurative forms of periodontitis were analyzed, IL-8 / CXCL8 -251 T allele was associated with higher production of IL-8/CXCL8 and higher risk of aggressive periodontitis acute suppurative form development³³. Also, Scarrel et al. in their 2011 study done on Brazilian population stated that rs2234671

SNP in the *CXCR1* gene was not useful as a genetic risk factor for chronic periodontitis affected patients³⁹.

Scientists Da Silva et al. also conducted a meta-analysis with 71531 individuals, in which 25 polymorphisms were analyzed, and a conclusion was approached that IL-8 polymorphism had no significant association with the risk of periodontitis development³⁴.

Scientists Rusyanti et al. reported that low levels of IL-8 (below 0.064 pg/ μ L) showed 34.5 times higher occurrence of aggressive periodontitis³⁵. Another factor increasing the prevalence of this disease was polymorphism c576 T>C>G of FPR1 gene, which also correlated significantly with IL-8 (ref.³⁵). Andia et al. assessed 184 unrelated subjects, and their findings showed that SNP (rs4073) was not associated with aggressive periodontitis in unrelated individuals, and there was no evidence of over-transmission of the alleles in families with aggressive periodontitis of Brazilian individuals⁴⁵. A study done on Brazilian individuals by Viana et al. indicated an association between +1440 SNP and haplotypes in the *CXCR2* gene with susceptibility to or protection against periodontitis³⁶. Researchers Kim. Et al. in their work concluded that SNPs did not show different distributions between the control and chronic periodontitis groups, and that the TTT/TAA haplotype was associated with protection against the development of periodontitis⁴¹. Although in Yang et al. study done in 2016 it was noted that there were no significant associations between IL-8 -251A/T polymorphism and the risk of periodontitis, nevertheless, it may have an impact of increasing the risk of developing periodontitis in Asian and mixed populations⁴³.

Corbi et al. studied 41 individuals and analyzed their IL-8 levels before and after periodontal therapy and found that nor the outcome of the periodontal therapy (non-surgical), nor the levels of IL-8 were influenced by the ATC/TTC CP-susceptibility haplotype³⁷. In 2017, Cirelli et al. conducted a research, in which they stated that haplotypes in the IL-4 gene, but not in IL-8, influenced levels of *A. actinomycetemcomitans* in sub-gingival regions, measured before and after non-surgical periodontal treatment³⁸. It was also noted that there were significant correlations, when assessing microbiological, genetic and immunological factors, in IL-8 and IL-4 haplotypes³⁸. Nevertheless, in Pigossi et al. research, it was concluded that the presence of the ATC/TTC IL-8 haplotype was associated with an enhanced response of neutrophils and leukocytes to Gram-negative bacteria associated with periodontitis⁴⁴. Also, Finoti et al. in their work stated that non-surgical therapy is equally effective in improving clinical parameters and decreasing the levels of periodontopathogens, independent of the genotype groups produced by the IL-8 haplotype⁴⁰.

DISCUSSION

Multiple researchers have analysed association of IL-8 gene polymorphisms with periodontal diseases, although studies regarding this topic are not limited to the aforementioned polymorphisms only. One of the most

researched gene families, regarding periodontal pathologies, is the IL-1. In a study done by Papathanasiou et al., IL-1 family members were associated with periodontal inflammation, and therapeutic blockade was assessed, although research in this topic is quite limited⁴⁶. Also, IL-1B was found to influence bone resorption and continuity of bone loss in patients, which was stated in the work done by scientists Cheng et al. They have suggested IL-1B blockage using the antibodies, inhibitors, and even plant-derived substances for reducing IL-1B (ref.⁴⁷) but stated that more investigations were needed for further analysis of these methods in periodontal treatment. The aforementioned therapeutic blockades were not analysed with IL-8 polymorphisms in our research and could be of interest in later studies.

The same gene IL1B polymorphisms were assessed in a research done by Brodzikowska et al., noting the rs1800587 and rs1143634 association with periodontal inflammation; however, geographical and ethical factors were mentioned as having a role in prevalence of specific polymorphisms in different populations⁴⁸. On the other hand, Lopez et al. research analysed Chilean subjects to determine the prevalence of the IL-1A-889 and IL-1B+3954 polymorphisms and concluded that individuals who carry the positive genotype have a significantly higher risk for periodontitis development⁴⁹.

Another factor found in the research, which increases the prevalence of periodontitis, is diabetes. A study done by Struch et al., in which they genotyped 1515 subjects for IL-1, has shown that diabetes patients had an increased risk of periodontal disease, which was aggravated when combined with IL-1A/1B genotype⁵⁰. Study by Kashiwagi et al. also stated that proinflammatory cytokines are expressed when a patient has high glucose levels, which also explains TLR activation and inflammatory response provoking, although hyperglycaemia did not affect the IL-8 expression⁵¹. It contradicts the findings of Lihartova et al., explaining that statistically significant levels of IL-8 were found in patients affected by periodontitis and T1DM (ref.¹⁸). Nevertheless, more research is needed for additional analysis of IL-8 expression in diabetes affected patients.

Regarding clinical symptoms, a study done by Engrebetson et al. showed a positive gingival crevicular fluid IL-8 correlation with the probing depth of patients affected by periodontitis⁵², although other measurements – clinical attachment level, bleeding on probing or plaque index – did not correlate with the aforementioned levels⁵². IL-8, according to a study done by Lagdive et al., is highly associated with the status of periodontal tissues, and the level of the interleukin-8, found in the gingival crevicular fluid changes according to the severity of the periodontal disease⁵³. Some research has been done to engineer the IL-8 gene haplotypes, and to affect the susceptibility of the disease. One such study was done by scientists Benakanakere et al., which provided evidence that ATC/TTC haplotype may increase neutrophil levels in some cases of inflammatory lesions and could affect the disease susceptibility⁵⁴.

As found in the research, IL-8 polymorphisms are highly associated with periodontal pathologies. In the future, further studies must be done to fully analyse and understand the pathological mechanisms that associate IL-8 with periodontitis.

FUTURE PERSPECTIVES OF CYTOGENETIC MARKERS COMPLEXES DETECTION

In the future, novel molecular marker complexes should be determined in patients with periodontitis. Such markers might suggest additional linking mechanisms in the disease pathogenesis. Considering that the exact etiological agents responsible for the initiation of periodontitis remain unknown, it seems that multifactorial interaction might play a role here. Once significant sets of cytogenetic markers have been identified, we will be able to monitor their changes over time, adjust lifestyle, and initiate early treatment.

Scientific discoveries around the world will allow to develop methods of total analysis of periodontitis development and to select cytogenetic marker complexes required for understanding prognosis and treatment of periodontitis. If these marker complexes could be applied in practice in future, the costs of treatment would be significantly reduced.

CONCLUSIONS

After analysing the articles, the following conclusions can be drawn:

1. IL-8 and its polymorphisms are associated with an increased risk of periodontal diseases.
2. Patients carrying IL-8 gene are prone to severe forms of periodontitis.
3. Patients with periodontitis do not have increased levels of circulating IL-8.
4. Deeper analysis and search of new cytogenetic markers of periodontitis is necessary in future.

Search strategy and selection criteria

PRISMA protocol was used for the analysis of literature. ScienceDirect, Pubmed (MEDLINE) and Wiley Online Library electronic literature databases were used for the literature analysis. The time period of literature search was between 2021 February 10th and March 30th. The articles were not older than 15 years (2006-2021).

Additional scientific literature search was done manually. All of the related and similar articles were reviewed, including the reference lists of the selected articles.

Keywords (included in MESH) "periodontitis, IL-8, gene, polymorphisms" were used along with their variations to ensure the highest number of results possible. The references of the papers were also analysed, in order to identify any potential additional results.

Articles were analysed and investigated by each author independently. Investigators discussed and compared their selections and matched the differences through dis-

cussion. The last stage of the research started with the screening of the articles. Exclusion of articles was done after investigating their titles and abstracts. Whether to include the publication or not, was discussed only after analysis of the full text, according to the inclusion and exclusion criteria.

Author contribution: All authors contributed equally to preparing the manuscript.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Kaur G, Grover V, Bhaskar N, Kaur RK, Jain A. Periodontal Infectogenomics. *Inflamm Regen* 2018;38:8.
2. Gasner NS, Schure RS. Periodontal Disease. [Updated 2020 May 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK554590/>
3. Data and statistics [Internet]. Euro.who.int. 2021. Accessed: 18 May 2021: <https://www.euro.who.int/en/health-topics/disease-prevention/oral-health/data-and-statistics>
4. Loos BG, Van Dyke TE. The role of inflammation and genetics in periodontal disease. *Periodontol* 2000;83(1):26-39.
5. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, Taylor GW, Page RC, Beck JD, Genco RJ. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. *J Periodontol* 2015;86(5):611-22.
6. Gerritsen AE, Allen PF, Witter DJ, Bronkhorst EM, Creugers NH. Tooth loss and oral health-related quality of life: a systematic review and meta-analysis. *Health Qual Life Outcomes* 2010;5:8:126.
7. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol* 2018;89:S159-S172.
8. Mehrotra N, Singh S. Periodontitis. [Updated 2020 Jul 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK541126/>
9. Michaud DS, Fu Z, Shi J, Chung M. Periodontal Disease, Tooth Loss, and Cancer Risk. *Epidemiol Rev* 2017;39(1):49-58.
10. Shi Q, Cai C, Xu J, Liu J, Liu H, Huo N. Is there an association between IFN- γ +874A/T polymorphism and periodontitis susceptibility?: A meta-analysis. *Medicine (Baltimore)* 2017;96(25):e7288.
11. Jin SH, Guan XY, Liang WH, Bai GH, Liu JG. TLR4 polymorphism and periodontitis susceptibility: A meta-analysis. *Medicine (Baltimore)* 2016;95(36):e4845.
12. Ni XB, Jia C, Yu HD, Li YQ, Zeng XT, Leng WD. Comprehensive analysis of interleukin-8 gene polymorphisms and periodontitis susceptibility. *Oncotarget* 2017;8(30):48996-49004.
13. Yang ZJ, Tang XP, Lai QG, Ci JB, Yuan KF. Interleukin-8 -251A/T polymorphism and periodontitis susceptibility: a meta-analysis. *Genet Mol Res* 2016;21;15(4).
14. Mlachkova A, Popova C, Doseva V. Presence of IL-8 Gene Polymorphism and IL-8 Serum Levels in Patients with Chronic Periodontitis - Literature Review. *Folia Med (Plovdiv)* 2020;30;62(2):253-57.
15. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* 2009;62(10):1006-12.
16. Liukkonen J, Gürsoy UK, Könönen E, Gürsoy M, Metso J, Salminen A, Kopra E, Jauhiainen M, Mäntylä P, Buhlin K, Paju S, Sorsa T, Nieminen MS, Lokki ML, Sinisalo J, Pussinen PJ. Salivary biomarkers in association with periodontal parameters and the periodontitis risk haplotype. *Innate Immun* 2018;24(7):439-447.
17. Silva BRD, Cirelli T, Nepomuceno R, Theodoro LH, Orrico SRP, Cirelli JA, Barros SP, Scarel-Caminaga RM. Functional haplotype in the Interleukin8 (CXCL8) gene is associated with type 2 Diabetes

- Mellitus and Periodontitis in Brazilian population. *Diabetes Metab Syndr* 2020;14(6):1665-72.
18. Borilova LP, Kavrikova D, Tomandlova M, Poskerova H, Rehka V, Dušek L, Izakovicova HL. Differences in Interleukin-8 Plasma Levels between Diabetic Patients and Healthy Individuals Independently on Their Periodontal Status. *Int J Mol Sci* 2018;19(10):3214.
 19. Sajadi M, Shahmohammadi A, Mahmazi S, Bashiri H, Bavandpour M, Yari K. Study of association between interleukin-8 -845 T/C and +781 C/T polymorphisms with periodontitis disease among population from Western Iran. *Mol Biol Rep* 2018;45(5):1263-68.
 20. Houshmand B, Hajilooi M, Rafiei A, Bidgoli M, Soheilifar S. Evaluation of IL-8 gene polymorphisms in patients with periodontitis in Hamedan, Iran. *Dent Res J (Isfahan)* 2012;9(4):427-32.
 21. Borilova Linhartova P, Vokurka J, Poskerova H, Fassmann A, Izakovicova Holla L. Haplotype analysis of interleukin-8 gene polymorphisms in chronic and aggressive periodontitis. *Mediators Inflamm* 2013;2013:342351.
 22. Chen X, Huang J, Zhong L, Ding C. Quantitative assessment of the associations between interleukin-8 polymorphisms and periodontitis susceptibility. *J Periodontol* 2015;86(2):292-300.
 23. Khosropanah H, Sarvestani EK, Mahmoodi A, Golshah M. Association of IL-8 (-251 a/t) gene polymorphism with clinical parameters and chronic periodontitis. *J Dent (Tehran)* 2013;10(4):312-8.
 24. Kavrikova D, Borilova Linhartova P, Lucanova S, Poskerova H, Fassmann A, Izakovicova Holla L. Chemokine Receptor 2 (CXCR2) Gene Variants and Their Association with Periodontal Bacteria in Patients with Chronic Periodontitis. *Mediators Inflamm* 2019;4;2019:2061868.
 25. Li G, Yue Y, Tian Y, Li JL, Wang M, Liang H, Liao P, Loo WT, Cheung MN, Chow LW. Association of matrix metalloproteinase (MMP)-1, 3, 9, interleukin (IL)-2, 8 and cyclooxygenase (COX)-2 gene polymorphisms with chronic periodontitis in a Chinese population. *Cytokine* 2012;60(2):552-60.
 26. Scarel-Caminaga RM, Kim YJ, Viana AC, Curtis KM, Corbi SC, Sogumo PM, Orrico SR, Cirelli JA. Haplotypes in the interleukin 8 gene and their association with chronic periodontitis susceptibility. *Biochem Genet* 2011;49(5-6):292-302.
 27. Sippert EA, de Oliveira e Silva C, Visentainer JE, Sell AM. Association of duffy blood group gene polymorphisms with IL8 gene in chronic periodontitis. *PLoS One* 2013;26;8(12):e83286.
 28. Andia DC, de Oliveira NF, Letra AM, Nociti FH Jr, Line SR, de Souza AP. Interleukin-8 gene promoter polymorphism (rs4073) may contribute to chronic periodontitis. *J Periodontol* 2011;82(6):893-9.
 29. Li ZG, Li JJ, Sun CA, Jin Y, Wu WW. Interleukin-18 promoter polymorphisms and plasma levels are associated with increased risk of periodontitis: a meta-analysis. *Inflamm Res* 2014;63(1):45-52.
 30. Zhang N, Xu Y, Zhang B, Zhang T, Yang H, Zhang B, Feng Z, Zhong D. Analysis of interleukin-8 gene variants reveals their relative importance as genetic susceptibility factors for chronic periodontitis in the Han population. *PLoS One* 2014;7;9(8):e104436.
 31. Kim YJ, Viana AC, Curtis KM, Orrico SR, Cirelli JA, Scarel-Caminaga RM. Lack of association of a functional polymorphism in the interleukin 8 gene with susceptibility to periodontitis. *DNA Cell Biol* 2009;28(4):185-90.
 32. Corbi SC, Anovazzi G, Finoti LS, Kim YJ, Capela MV, Secolin R, Marcaccini AM, Gerlach RF, Orrico SR, Cirelli JA, Scarel-Caminaga RM. Haplotypes of susceptibility to chronic periodontitis in the Interleukin 8 gene do not influence protein level in the gingival crevicular fluid. *Arch Oral Biol* 2012;57(10):1355-61.
 33. Amaya MP, Criado L, Blanco B, Gómez M, Torres O, Flórez L, González CI, Flórez O. Polymorphisms of pro-inflammatory cytokine genes and the risk for acute suppurative or chronic nonsuppurative apical periodontitis in a Colombian population. *Int Endod J* 2013;46(1):71-8.
 34. da Silva MK, de Carvalho ACG, Alves EHP, da Silva FRP, Pessoa LDS, Vasconcelos DFP. Genetic Factors and the Risk of Periodontitis Development: Findings from a Systematic Review Composed of 13 Studies of Meta-Analysis with 71,531 Participants. *Int J Dent* 2017;2017:1914073.
 35. Rusyanti Y, Widyaputra S, Maskoen AM. Periodontal tissue destruction in aggressive periodontitis: Determination of gene or environmental factors. *Saudi Dent J* 2019;31(2):290-99.
 36. Viana AC, Kim YJ, Curtis KM, Renzi R, Orrico SR, Cirelli JA, Scarel-Caminaga RM. Association of haplotypes in the CXCR2 gene with periodontitis in a Brazilian population. *DNA Cell Biol* 2010;29(4):191-200.
 37. Corbi SC, Finoti LS, Anovazzi G, Tanaka MH, Kim YJ, Secolin R, Marcaccini AM, Gerlach RF, Orrico SR, Cirelli JA, Scarel-Caminaga RM. Clinical outcomes of periodontal therapy are not influenced by the ATC/TTC haplotype in the IL8 gene. *J Periodontol Res* 2014;49(4):489-98.
 38. Cirelli T, Finoti LS, Corbi SCT, Anovazzi G, Nepomuceno R, Orrico SRP, Cirelli JA, Mayer MPA, Scarel-Caminaga RM. Absolute quantification of *Aggregatibacter actinomycetemcomitans* in patients carrying haplotypes associated with susceptibility to chronic periodontitis: multifaceted evaluation with periodontitis covariants. *Pathog Dis* 2017;75(7). doi: 10.1093/femspd/ftx092
 39. Scarel-Caminaga RM, Curtis KM, Renzi R, Sogumo PM, Anovazzi G, Viana AC, Kim YJ, Orrico SR, Cirelli JA. Variation in the CXCR1 gene (IL8RA) is not associated with susceptibility to chronic periodontitis. *J Negat Results Biomed* 2011;10:14.
 40. Finoti LS, Corbi SC, Anovazzi G, Teixeira SR, Capela MV, Tanaka MH, Kim YJ, Orrico SR, Cirelli JA, Mayer MP, Scarel-Caminaga RM. Pathogen levels and clinical response to periodontal treatment in patients with Interleukin 8 haplotypes. *Pathog Dis* 2013;69(1):21-8.
 41. Kim YJ, Viana AC, Curtis KM, Orrico SR, Cirelli JA, Mendes-Junior CT, Scarel-Caminaga RM. Association of haplotypes in the IL8 gene with susceptibility to chronic periodontitis in a Brazilian population. *Clin Chim Acta* 2010;411(17-18):1264-8.
 42. Sato K, Yoshimura A, Kaneko T, Ukai T, Ozaki Y, Nakamura H, Li X, Matsumura H, Hara Y, Ogata Y. A single nucleotide polymorphism in 3'-untranslated region contributes to the regulation of Toll-like receptor 4 translation. *J Biol Chem* 2012;287(30):25163-72.
 43. Yang ZJ, Tang XP, Lai QG, Ci JB, Yuan KF. Interleukin-8 -251A/T polymorphism and periodontitis susceptibility: a meta-analysis. *Genet Mol Res* 2016;21;15(4).
 44. Pigossi SC, Anovazzi G, Finoti LS, de Medeiros MC, de Souza-Moreira TM, Mayer MPA, Zanelli CF, Valentini SR, Rossa Junior C, Scarel-Caminaga RM. The ATC/TTC haplotype in the Interleukin 8 gene in response to Gram-negative bacteria: A pilot study. *Arch Oral Biol* 2019;107:104508.
 45. Andia DC, Letra A, Casarin RC, Casati MZ, Line SR, de Souza AP. Genetic analysis of the IL8 gene polymorphism (rs4073) in generalized aggressive periodontitis. *Arch Oral Biol* 2013;58(2):211-7.
 46. Papathanasiou E, Conti P, Carinci F, Lauritano D, Theoharides TC. IL-1 Superfamily Members and Periodontal Diseases. *J Dent Res* 2020;99(13):1425-34.
 47. Cheng R, Wu Z, Li M, Shao M, Hu T. Interleukin-1 β is a potential therapeutic target for periodontitis: a narrative review. *Int J Oral Sci* 2020;12(1):2.
 48. Brodzikowska A, Górka R, Kowalski J. Interleukin-1 Genotype in Periodontitis. *Arch Immunol Ther Exp* 2019;67:367-73.
 49. López NJ, Jara L, Valenzuela CY. Association of interleukin-1 polymorphisms with periodontal disease. *J Periodontol* 2005;76(2):234-43.
 50. Struch F, Dau M, Schwahn C, Biffar R, Kocher T, Meisel P. Interleukin-1 gene polymorphism, diabetes, and periodontitis: results from the Study of Health in Pomerania (SHIP). *J Periodontol* 2008;79(3):501-7.
 51. Kashiwagi Y, Takedachi M, Mori K, Kubota M, Yamada S, Kitamura M, Murakami S. High glucose-induced oxidative stress increases IL-8 production in human gingival epithelial cells. *Oral Dis* 2016;22(6):578-84.
 52. Engebretson SP, Vossughi F, Hey-Hadavi J, Emingil G, Grbic JT. The influence of diabetes on gingival crevicular fluid beta-glucuronidase and interleukin-8. *J Clin Periodontol* 2006;33(11):784-90.
 53. Lagdive SS, Marawar PP, Byakod G, Lagdive SB. Evaluation and comparison of interleukin-8 (IL-8) level in gingival crevicular fluid in health and severity of periodontal disease: a clinico-biochemical study. *Indian J Dent Res* 2013;24(2):188-92.
 54. Benakanakere M, Finoti L, Tanaka U, Grant GR, Scarel-Caminaga RM, Kinane DF. Investigation of the functional role of human Interleukin-8 gene haplotypes by CRISPR/Cas9 mediated genome editing. *Sci Rep* 2016;6:31180.