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Single Nucleotide Polymorphisms of Salivary Antimicrobial Peptides in Periodontitis

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ABSTRACT
The present study reviews salivary antimicrobial peptides and their single nucleotide polymorphisms in periodontitis. Periodontitis is an important inflammatory condition of the oral cavity where there is destruction of periodontal ligament and adjacent supporting alveolar bone resulting in tooth loss. MEDLINE, PubMed and Google Scholar were searched for relevant literature using the combination of following search terms: "Periodontitis", "Single Nucleotide Polymorphism", "Salivary Antimicrobial Peptides", "Defensin", "Lactoferrin", "Cathelicidin". Periodontal disease is initiated by bacterial invasion and colonization and this is not sufficient for the predisposition and progression of the disease. Besides this, several genetic factors influence disease susceptibility and severity. Many studies have reported the role of genetic polymorphisms for the difference in the host inflammatory responses against periodontal pathogens. Our review focuses on the salivary antimicrobial peptides which are important constituents of the innate immune system secreted mainly by epithelial cells and neutrophils and form the first line of defense against the pathogenic microorganisms in the oral cavity. Investigation of genetic polymorphisms in salivary antimicrobial peptides will aid in identifying an individual's genetic susceptibility and might be valuable for developing prognostic markers in periodontitis.

Keywords: *Single Nucleotide Polymorphism, Periodontitis, Defensin, Lactoferrin, Cathelicidin*

INTRODUCTION

Periodontal diseases are broadly classified into inflammatory conditions - gingivitis and periodontitis. Both are type of gum diseases: in the former inflammation is restricted to gingiva characterized by gingival redness, edema and is usually reversible by good oral hygiene and in the later inflammation deepens to form periodontal pockets and results in the destruction of supporting tissues including alveolar bone loss [1] [2]. According to the 2017 system of classification, there are three forms of periodontitis: necrotizing periodontitis, periodontitis as a manifestation of systemic disease, and periodontitis which comprises "chronic" or "aggressive", now grouped under a single category, "periodontitis" [3]. Periodontitis is further classified based on staging and grading system as described in Tonetti *et al*., 2018 [4], Papapanou *et al*. 2018 [5]. Oral microflora is home to several bacterial species but only a few have been implicated in the pathogenesis and progression of periodontal disease. Major species that are linked with periodontitis are *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Bacteroides forsythus, Prevotella intermedia, Peptostreptococcus micros* and *Fusobacterium nucleatum* [6]. The presence of periodontal pathogens is not a sufficient factor for the development and progression of

periodontitis [7]. It has been shown that other risk factors make few individuals susceptible to periodontitis that include lifestyle habits (smoking, and alcohol), diabetes, obesity, stress and inadequate dietary consumption of calcium and vitamin D [7]. An individual's genetic makeup is also considered a major factor in the susceptibility to periodontitis [8].

CONTRIBUTION OF GENETICS TO PERIODONTITIS

Heritability play a pivotal role in the predisposition to periodontitis [9] and evidence for the role of genetics in periodontitis can be gained from the population, family, and twin studies [10]. A twin study conducted by Michalowicz *et al.*,1991 examined the relative contribution of host genetic factors to the clinical measures of periodontal disease such as probing depth, clinical attachment loss, gingivitis, and plaque and a significant genetic contribution to the development of above measures was identified [11]. Another population-based study on Swedish twins demonstrated the role of genetic factors in periodontitis and potential gene-environment interactions [12]. Studies indicate that approximately half of the variance in disease in the population is attributed to genetic variance [13]. The genetic component of different individuals can alter the host's reaction to the pathogens and in particular can have different inflammatory responses to the oral pathogens. Single nucleotide polymorphisms (SNPs) are genetic variations between individuals and are becoming increasingly popular markers in molecular genetics for the identification of susceptibility to many diseases [14]. Some individuals are prone to certain diseases and SNPs are known to play a role in the difference in the susceptibility of individuals to diseases. Identification of polymorphisms that play a major role in the difference in immune responses against the periodontopathogens can help in the development of diagnostic markers or better therapeutic interventions [15]. Due to rapid and severe infection and inflammation of periodontium, people with periodontitis are at an increased risk of tooth and alveolar bone loss. So early genetic diagnosis especially SNPs of the genes that contribute to oral diseases can help in maintaining healthy gums and prevent tooth loss. In addition to the significant economic burden and negative impact of the disease on the quality of life, periodontal infections have also been implicated as potential risk factors for several systemic diseases [16].

SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS IN PERIODONTITIS IN INDIAN POPULATION

According to the 1999 classification, periodontal disease was broadly classified into chronic and aggressive periodontitis with sub-classification as localized and generalized periodontitis. The new classification scheme of 2017 has removed this demarcation and has classified periodontitis into (i) different stages (Stage 1-4), (ii) extent and distribution and (iii) grades. The genetic composition that is polymorphisms in the genes between individuals is an important considering factor for the susceptibility to periodontitis. With SNP studies, several genes have been reported to be associated with periodontitis. Research has been carried out at the cellular and molecular level, especially on IL-1, IL-4, IL-6, TNF-α, Vitamin-D receptor, Fc-gamma receptor (FCGR), IL-10, and matrix metalloproteinase [17] and few studies conducted in India population are mentioned below. In a small case-control study with 91 Tamil speaking Dravidian race subjects, it was shown that a positive association existed between the - 1087 IL-10 promoter polymorphism and severe chronic periodontitis which also indicated that A allele at -1087 loci as a risk factor for the development of severe periodontitis in subjects with poor oral hygiene [18]. In a study conducted in North-Indian subpopulation, the genotypic and allelic distributions of IL-1β + 3954 revealed no association with chronic periodontitis [19] but in sample of Brazilian individuals, the polymorphism found in the same locus +3954 of IL-1β gene was a risk factor for chronic periodontitis [20].. Studies of TNF-α gene promoter polymorphisms in the eastern Indian population suggested that rs1800629 (-308G/A) polymorphism of TNF-α gene was associated with both aggressive and chronic periodontitis but rs1799724 (-857C/T) and rs1799964 (-1031T/C) polymorphisms were associated only with the increased susceptibility to chronic periodontitis [21]. In another study conducted in the eastern region of India with matrix metalloproteinases (MMPs), it was concluded that there was a significant association of MMP8 -799C-T polymorphism with chronic periodontitis while MMP1 -519A-G and MMP7 -181A-G polymorphisms showed a combinatorial synergistic effect on chronic periodontitis risk [22]. In a study conducted between chronic periodontitis and FCGR2A 131His/Arg (rs1801274), FCGR2B 232Ile/Thr (rs1050501), TNF α -1031T/C (rs1799964), and -863C/A (rs1800630) polymorphisms in South Indian population revealed no association [23]. Cyclooxygenase (COX) polymorphism in association with chronic

periodontitis in North Indian subpopulations suggested that SNPs COX2 -1195G>A and COX2 8473T>C were not individually associated with chronic periodontitis, however, haplotype AT significantly increased the risk of chronic periodontitis [24]. This difference in the susceptibility of individuals to the disease could be because of the difference in genetic makeup between individuals and SNPs are population specific.

Salivary antimicrobial peptides (AMPs) are important components of the innate immune system and are involved in fighting against bacterial invasion. AMPs are found to be expressed in the periodontium in both healthy state and disease conditions like periodontitis and help maintain homeostasis of the oral environment [25]. The study of genetic expression in periodontal disease and their association between SNPs in antimicrobial peptides to the susceptibility of periodontitis will open new avenues for better disease management.

ANTIMICROBIAL PEPTIDES IN ORAL CAVITY

The oral cavity is constantly exposed to various microorganisms which in favorable circumstances can colonize and lead to various diseases. Epithelial cells of the oral cavity in response to microbial invasion alert immune cells by secreting various interleukins and other chemokines and cytokines and attract neutrophils and other immune cells. Salivary AMPs are one such effector molecules produced mainly by epithelial cells and immune cells that fight against the invaders like periodontal pathogens. Natural AMPs are produced constitutively and are also induced in response to microbial exposure. AMPs provide the first line of defense and act against a wide variety of pathogens- bacteria, yeasts, fungi, viruses and these molecules have been found in many species from microorganisms to human beings. Many of the AMPs are cationic and amphipathic [26]. Based on the secondary structures, cationic AMPs are broadly classified into three groups namely α-helical (cathelicidin peptide LL-37, lactoferrin), β-sheet (defensin) and peptides with coil structure rich in certain amino acids such as Trp, Pro, Arg, His and Gly [27] [28]. Cationic AMPs such as defensin, cathelicidin LL-37, lactoferrin possess positively charged and hydrophobic amino acid residues that enable them to adopt an amphipathic conformation and this structure allows increased electrostatic interaction with negatively charged surfaces (lipopolysaccharide of gram-negative bacteria and teichoic acid of gram-positive bacteria) of bacterial membranes [29]. This interaction disrupts the phospholipid layer and results in the formation of transmembrane pores and causes leakage of small molecules from the bacterial cell eventually leading to its death [30]. Some AMPs kill bacteria by inhibiting intracellular functions such as blocking enzyme activity, protein folding or inhibiting protein and nucleic acid synthesis [30]. AMPs cannot interact with eukaryotic membranes as the outer monolayers are composed of zwitterionic (overall neutral) lipids like sphingomyelin and therefore mainly target prokaryotic cells [29]. Apart from antimicrobial activity, AMPs perform immuno-modulatory activities such as inhibition of pro-inflammatory responses induced by LPS, recruitment of leukocytes and chemokine production [31]. Due to the overuse of antibiotics, microbes are becoming increasingly resistant to antimicrobial agents and some patients do not respond to conventional therapies necessitating new strategies to be developed. So, there is an urgent need to design novel antimicrobial agents that can target the microbes effectively. AMPs are now being considered as a new strategy for fighting against infections although some mechanisms of resistance to natural AMPs have been noted for example through upregulation of efflux pumps, membrane and cell envelope alterations, proteolytic degradation of the peptides and biofilm formation [32]. The alternative use of AMPs as an antimicrobials is found to be a promising strategy for combating antibiotic-resistant bacteria [33]. The major AMPs of the salivary secretions include cystatins, histatins, lysozyme, lactoferrin, lactoperoxidase, defensins, cathelicidin, and calprotectin [34]. The role of major salivary AMPs defensin, lactoferrin and cathelicidin and their association with susceptibility to periodontitis are discussed.

Defensins

One major subgroup of mammalian salivary AMPs is defensin and was first described in the 1980s. Defensins have been found to possess the ability to strengthen the innate immune system by directly targeting the pathogens and can also enhance the adaptive immune system by chemotaxis of monocytes, dendritic cells, mast cells, and T-lymphocytes to the infection site [35]. The mammalian defensins can be subdivided into three main classes based on their structural differences: α-defensins, β-defensins and θ-defensins. Only α-defensins.and β-defensins are expressed in humans. Both α- and β-defensins consist of a triple-stranded β-sheet structure with a molecular weight ranging between 3 and 6 kDa. They mainly differ in the pairing of

cystine-disulphide bridges with cysteine residues in α-defensins linked between 1-6/2-4/3-5 and in β-defensins 1-5/2-4/3-6 cys-cys patterns [36]. α-defensins 1-4 are also known as human neutrophil peptides (HNPs). HNPs1-3 are numerous in the oronasal cavity and are found in neutrophil granules, monocytes, and natural killer cell, HNP4 is found in primary neutrophil azurophil granules and human α-defensins 5 and 6 are found in mucosal Paneth cells associated with the gut. HNPs 1-3 differ from each other by a single amino acid residue at the N-terminus and HNP1 has an N-terminal alanine residue whereas HNP3 has an N-terminal aspartic acid residue [37].

β-defensins

Human β-defensins (hBDs) are produced by epithelial cells of many organs including skin, lung, kidney, pancreas, uterus, eye, nasal and oral mucosa [38]. Out of several hBDs known, only the first four hBDs (hBD1-4) have been characterized in detail with hBD-1 being constitutively expressed in the epithelium, hBD-2 and hBD-3 secretions dependent on the stimulation from bacteria and proinflammatory cytokines [38]. In gingival tissues, hBD-1 and hBD-2 are localized in sulcular epithelium but not in junctional epithelium and hBD- 3 in the basal layer of gingival epithelium [39]. β-defensins are broad-spectrum antimicrobial peptides, however,their effect is considerably salt-dependent [40]. α- and β-defensins have reduced antimicrobial activities in the presence of physiological concentration of salts with hBD-3 having the lowest sensitivity to salts and hBD-1 having the highest sensitivity [40, 41]. Defensins are likely to exhibit their direct antimicrobial effect on the surface of the skin and mucosal epithelia and *in vivo* in the phagocytic vacuoles [41]. The mRNA and protein levels of hBD-2 were found to be higher in the mouse human gingival graft model stimulated with *P. gingivalis* than in unstimulated grafts which suggested that the higher expression of hBD-2 might be induced by bacterial infection [42].

β-defensin 1 polymorphism studies in periodontitis

β-defensin 1 gene (*DEFB1* gene) polymorphisms g. -20G>A (rs11362) and g. -44 C>G (rs1800972) were significantly associated with severe periodontitis susceptibility in Italian population when compared with g.-52G>A (rs1799946), c*5G>A (rs1047031), c*87A>G (rs1800971) [43]. Several polymorphism studies to check the association of β-defensin 1 in periodontitis have been reported - Ikuta *et al.*, 2015 [44], Tian Y *et al*., 2013 [45], Loo WT *et al.*,2012 [46], Schaefer *et al.*, 2010 [47], Ozturk *et al.*, 2010 [48], Boniotto *et al.*, 2004 [49] . In a study conducted by Ikuta *et al.*, 2015 in Japanese population in 5'-UTR of *DEFB1*: -52 G/A (rs1799946), -44 C/G (rs1800972) and -20 G/A (rs11362) reported that only the genotype rs1800972 was found to be associated with susceptibility to chronic periodontitis [44]. In contrast, other studies showed that SNPs rs1800972 and rs11362 were not associated with early-onset periodontitis [49] and chronic periodontitis [50], respectively. Schaefer *et al.*, 2010 showed that 3'UTR SNP rs1047031 was associated with chronic and aggressive periodontitis [47].

Lactoferrin

Lactoferrin, an 80kDa iron-binding glycoprotein is present in external secretions such as milk, tears, saliva, urine and secondary granules of neutrophils. The antibacterial activity of lactoferrin is mainly attributed to its ability to bind and sequester environmental iron thereby depriving pathogens of this essential nutrient [51]. Lactoferrin's activity against bacteria can be bacteriostatic or bactericidal. Iron deprivation caused by lactoferrin is bacteriostatic and cause a delay in microbial growth. Similar to other cationic AMPs, lactoferrin is a polycationic molecule with a maximal density of surface positive charge located in the N-terminal region and this positive cluster bind to the lipid A part of lipopolysaccharide molecules present on the outer membrane of gram-negative bacteria causing membrane disruption and this mechanism is bactericidal [52]. Lactoferrin inhibited the biofilm of *P. gingivalis* and *P. intermedia in vitro* [53]. It is released from neutrophils in response to periodontitis thereby it could be a potential marker of periodontal disease [54].

Lactoferrin polymorphism studies in periodontitis

SNPs in lactoferrin play a role in the susceptibility of individuals in periodontitis. The association of lactoferrin missense polymorphisms (rs1126477 and rs1126478) showed that rs1126478 polymorphism was correlated with a higher risk of chronic periodontitis [43]. Wu *et al.*,2009 studied the association of polymorphism rs1126478 with aggressive periodontitis in Taiwanese

patients and found that this polymorphism was significantly associated with the disease [55]. But Ikuta *et al.*, 2015 showed that there was no significant association of lactoferrin rs1126478 polymorphism with aggressive and chronic periodontitis [44]. The polymorphism in rs1126477 was associated with aggressive periodontitis in African-American but not in the Caucasian population [56]. β-defensin 1 and lactoferrin polymorphism studies reported in periodontitis are summarized in Table 1.

Cathelicidin

Cathelicidin is expressed in neutrophils and mucosal epithelia such as airways, buccal mucosa, tongue, esophagus, cervix, vagina, and salivary glands [57]. Humans express only one cathelicidin known as hCAP-18 (human cathelicidin antimicrobial peptide of 18kDa). The cathelicidin hCAP-18 is characterized by a highly conserved cathepsin-L-inhibitor (cathelin)-like domain which is flanked by a signal peptide domain on its N-terminus and by an antimicrobial peptide region on its C-terminus LL-37 [58]. LL-37 exerts direct bactericidal effect by inserting into the bacterial membrane and triggering cell rupture and leakage of cytoplasm [59]. The production of LL-37 was up-regulated in the inflamed gingival tissues compared to healthy gingival tissues [60] and possessed antibacterial activity against *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* [61].

Cathelicidin mutation study in periodontitis

A novel missense mutation in cathelicidin antimicrobial peptide (CAMP) gene, p.S34N (serine to asparagine substitution), was reported by Turkoglu *et al.*, 2011 [62]. It was reported that generalized aggressive periodontitis was significantly associated with p.S34N mutation but not chronic periodontitis and suggested that p.S34N mutation was a contributing factor for developing generalized aggressive periodontitis.

Various polymorphism studies have been carried out with some studies showing associations and others contradictory results. This difference could be attributed to different ethnic origins and sample size used in the studies.

CONCLUSION:

Analysis of variations in the genetic sequences could help in determining individual's susceptibility to the disease. Identification of salivary antimicrobial peptide polymorphisms in the various stages of the periodontal disease could help in early detection, facilitate tailored personalized treatment and predict responsiveness to treatment therapies.

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REFERENCES

1. Trombelli L, Farina R, Silva CO, Tatakis DN. Plaque-induced gingivitis: Case definition and diagnostic considerations. *J Clin Periodontol*. 2018;45:S44-S67.

2. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Prim*. 2017;3:17038.

3. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS et al. A new classification scheme for periodontal and peri-implant diseases and conditions–Introduction and key changes from the 1999 classification. *J Periodontol*. 2018;89:S1-S8.

4. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Clin Periodontol*. 2018;45:S149-S161.

5. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH et al. Periodontitis: Consensus

report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89:S173-S182.

6. AlJehani YA. Risk factors of periodontal disease: review of the literature. *Int J Dent*. 2014;2014.

7. Genco RJ, Borgnakke WS. Risk factors for periodontal disease. *Periodontol 2000*. 2013;62(1):59-94.

8. da Silva MK, de Carvalho ACG, Alves EHP, da Silva FRP, Pessoa L dos S, 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.

9. Nibali L, Di Iorio A, Tu Y, Vieira AR. Host genetics role in the pathogenesis of periodontal disease and caries. *J Clin Periodontol*. 2017;44:S52-S78.

10. Loos BG, Papantonopoulos G, Jepsen S, Laine ML. What is the contribution of genetics to periodontal risk? *Dent Clin*. 2015;59(4):761-780.

11. Michalowicz BS, Aeppli D, Virag JG, Klump D, Hinrichs E, Segal N et al. Periodontal findings in adult twins. *J Periodontol*. 1991;62(5):293-299.

12. Mucci LA, Björkman L, Douglass CW, Pedersen NL. Environmental and heritable factors in the etiology of oral diseases—a population-based study of Swedish twins. *J Dent Res*. 2005;84(9):800-805.

13. Michalowicz BS, Diehl SR, Gunsolley JC, Sparks B, Brooks C, Koertge T et al. Evidence of a substantial genetic basis for risk of adult periodontitis. *J Periodontol*. 2000;71(11):1699-1707.

14. Teama S. DNA Polymorphisms: DNA-Based Molecular Markers and Their Application in Medicine. In: *Genetic Diversity and Disease Susceptibility*. IntechOpen; 2018.

15. Toy VE, Uslu MO. Do genetic polymorphisms affect susceptibility to periodontal disease? A literature review. *Niger J Clin Pract*. 2019;22(4):445.

16. Colombo APV, MagalhŃes CB, Hartenbach FARR, do Souto RM, da Silva-Boghossian CM. Periodontal-disease-associated biofilm: A reservoir for pathogens of medical importance. *Microb Pathog*. 2016;94:27-34.

17. Wankhede AN, Wankhede SA, Wasu SP. Role of genetic in periodontal disease. *J Int Clin Dent Res Organ*. 2017;9(2):53.

18. Crena J, Subramanian S, Victor DJ, Gnana PPS, Ramanathan A. Single nucleotide polymorphism at−1087 locus of interleukin-10 gene promoter is associated with severe chronic periodontitis in nonsmoking patients. *Eur J Dent*. 2015;9(3):387.

19. Daing A, Singh SV, Saimbi CS, Khan MA, Rath SK. Single nucleotide polymorphisms at interleukin (IL)-1β+ 3954 and vitamin D receptor (VDR) TaqI in chronic periodontitis patients: A pilot study in North Indian population. *J Int Clin Dent Res Organ*. 2015;7(1):18.

20. Moreira PR, De Sá AR, Xavier GM, Costa J, Gomez R, Gollob K et al. A functional interleukin-1β gene polymorphism is associated with chronic periodontitis in a sample of Brazilian individuals. *J Periodontal Res*. 2005;40(4):306-311.

21. Majumder P, Thou K, Bhattacharya M, Nair V, Ghosh S, Dey SK. Association of tumor necrosis factor-α (TNF-α) gene promoter polymorphisms with aggressive and chronic periodontitis in the eastern Indian population. *Biosci Rep*. 2018;38(4):BSR20171212.

22. Majumder P, Ghosh S, Dey SK. Matrix metalloproteinase gene polymorphisms in chronic periodontitis: a case–control study in the Indian population. *J Genet*. 2019;98(1):32.

23. Lavu V, Venkatesan V, Bhaskar LVKS, Priyanka, V , Kumarasamy P, Paul SF et al. Polymorphic Regions in Fc Gamma Receptor and Tumor Necrosis Factor-α Genes and Susceptibility to Chronic Periodontitis in a Cohort From South India. *J Periodontol*. 2016;87(8):914-922.

24. Daing A, Singh SV, Saimbi CS, Khan MA, Rath SK. Cyclooxygenase 2 gene polymorphisms and chronic periodontitis in a North Indian population: a pilot study. *J Periodontal Implant Sci*. 2012;42(5):151-157.

25. Mallapragada S, Wadhwa A, Agrawal P. Antimicrobial peptides: The miraculous biological molecules. *J Indian Soc Periodontol*. 2017;21(6):434.

26. Teixeira V, Feio MJ, Bastos M. Role of lipids in the interaction of antimicrobial peptides with membranes. *Prog Lipid Res*. 2012;51(2):149-177.

27. Takahashi D, Shukla SK, Prakash O, Zhang G. Structural determinants of host defense peptides for antimicrobial activity and target cell selectivity. *Biochimie*. 2010;92(9):1236-1241.

28. Mahlapuu M, Håkansson J, Ringstad L, Björn C. Antimicrobial peptides: an emerging category of therapeutic agents. *Front Cell Infect Microbiol*. 2016;6:194.

29. Bechinger B, Gorr S-U. Antimicrobial Peptides: Mechanisms of Action and Resistance. *J Dent Res*. 2017;96(3):254-260. doi:10.1177/0022034516679973

30. Le C-F, Fang C-M, Sekaran SD. Intracellular targeting mechanisms by antimicrobial peptides. *Antimicrob Agents Chemother*. 2017;61(4):e02340-16.

31. Pasupuleti M, Schmidtchen A, Malmsten M. Antimicrobial peptides: key components of the innate immune system. *Crit Rev Biotechnol*. 2012;32(2):143-171.

32. Hollmann A, Martinez M, Maturana P, Semorile LC, Maffia PC. Antimicrobial peptides: interaction with model and biological membranes and synergism with chemical antibiotics. *Front Chem*. 2018;6.

33. Zharkova MS, Orlov DS, Golubeva OY, Chakchir OB, Eliseev IE, Grinchuk TM et al. Application of antimicrobial peptides of the innate immune system in combination with conventional antibiotics–a novel way to combat antibiotic resistance? *Front Cell Infect Microbiol*. 2019;9:128.

34. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. *Immunol Lett*. 2014;162(2):22-38.

35. Bedi T, Mahendra J, Ambalavanan N. Defensins in periodontal health. *Indian J Dent Res*. 2015;26(4):340.

36. Winter J, Wenghoefer M. Human defensins: potential tools for clinical applications. *Polymers (Basel)*. 2012;4(1):691-709.

37. Kohlgraf KG, Pingel LC, Dietrich DE, Brogden KA. Defensins as anti-inflammatory compounds and mucosal adjuvants. *Future Microbiol*. 2010;5(1):99-113.

38. Abiko Y, Saitoh M, Nishimura M, Yamazaki M, Sawamura D, Kaku T. Role of β-defensins in oral epithelial health and disease. *Med Mol Morphol*. 2007;40(4):179-184.

39. Bhagawati SB, Bhagawati BT, Mittal S. Role of defensins in oral diseases: an overview. *J Indian Acad Oral Med Radiol*. 2012;24(2):142.

40. Gursoy UK, Könönen E. Understanding the roles of gingival beta-defensins. *J Oral Microbiol*. 2012;4(1):15127.

41. Yang D, Biragyn A, Kwak LW, Oppenheim JJ. Mammalian defensins in immunity: more than just

microbicidal. *Trends Immunol*. 2002;23(6):291-296.

42. To M, Kamata Y, Saruta J, Shimizu T, Sato T , Kondo Yu et al. Induction of β-defensin expression by porphyromonas gingivalis-infected human gingival graft transplanted in nu/nu mouse subdermis. *Acta Histochem Cytochem*. 2013;46(1):25-34.

43. Zupin L, Robino A, Navarra CO, Pirastu N, Di Lenarda R, Gasparini P et al. LTF and DEFB 1 polymorphisms are associated with susceptibility toward chronic periodontitis development. *Oral Dis*. 2017;23(7):1001-1008.

44. Ikuta T, Inagaki Y, Tanaka K, Saito,T, Nakajima Y, Bando M et al. Gene polymorphism of β-defensin-1 is associated with susceptibility to periodontitis in Japanese. *Odontology*. 2015;103(1):66-74.

45. Tian Y, Li J, Hao L, Yue Y, Wang M, Loo W, et al. Association of cytokines, high sensitive C-reactive protein, VEGF and beta-defensin-1 gene polymorphisms and their protein expressions with chronic periodontitis in the Chinese population. *Int J Biol Markers*. 2013;28(1):100-107.

46. Loo WTY, Bai L, Fan C, Yue Y, Dou Y, Wang M et al. Clinical application of human β-defensin and CD14 gene polymorphism in evaluating the status of chronic inflammation. In: *Journal of Translational Medicine*. Vol 10. BioMed Central; 2012:S9.

47. Schaefer AS, Richter GM, Nothnagel M, Laine M, Rühling A, Schäfer C et al. A 3′ UTR transition within DEFB1 is associated with chronic and aggressive periodontitis. *Genes Immun*. 2010;11(1):45.

48. Ozturk A, Famili P, Vieira AR. The antimicrobial peptide DEFB1 is associated with caries. *J Dent Res*. 2010;89(6):631-636.

49. Boniotto M, Hazbón MH, Jordan WJ, Lennon GP, Eskdale J, Alland D et al. Novel hairpin-shaped primer assay to study the association of the− 44 single-nucleotide polymorphism of the DEFB1 gene with early-onset periodontal disease. *Clin Diagn Lab Immunol*. 2004;11(4):766-769.

50. Wohlfahrt JC, Wu T, Hodges JS, Hinrichs JE, Michalowicz BS. No association between selected candidate gene polymorphisms and severe chronic periodontitis. *J Periodontol*. 2006;77(3):426-436.

51. El-Loly MM, Mahfouz MB. Lactoferrin in relation to biological functions and applications: a review. *Int J Dairy Sci*. 2011;6(2):79-111.

52. Valenti P, Antonini G. Lactoferrin. *Cell Mol Life Sci*. 2005;62(22):2576.

53. Wakabayashi H, Yamauchi K, Kobayashi T, Yaeshima T, Iwatsuki K, Yoshie H. Inhibitory effects of lactoferrin on growth and biofilm formation of Porphyromonas gingivalis and Prevotella intermedia. *Antimicrob Agents Chemother*. 2009;53(8):3308-3316.

54. Berlutti F, Pilloni A, Pietropaoli M, Polimeni A, Valenti P. Lactoferrin and oral diseases: current status and perspective in periodontitis. *Ann Stomatol (Roma)*. 2011;2(3-4):10.

55. Wu Y, Juo S, Ho Y, Ho K, Yang Y, Tsai C. Association between lactoferrin gene polymorphisms and aggressive periodontitis among Taiwanese patients. *J Periodontal Res*. 2009;44(3):418-424.

56. Jordan WJ, Eskdale J, Lennon GP, Pestoff R, Wu L, Fine D H, et al. A nonconservative, coding single-nucleotide polymorphism in the N-terminal region of lactoferrin is associated with aggressive periodontitis in an African-American, but not a Caucasian population. *Genes Immun*. 2005;6(7):632.

57. Braff MH, Mi'i AH, Di Nardo A, Lopez-Garcia B, Howell MD, Wong C et al. Structure-function relationships among human cathelicidin peptides: dissociation of antimicrobial properties from host immunostimulatory activities. *J Immunol*. 2005;174(7):4271-4278.

58. Dürr UHN, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin

family of antimicrobial peptides. *Biochim Biophys Acta (BBA)-Biomembranes*. 2006;1758(9):1408-1425.

59. Vallabhan CG, Sivarajan S, Ahamed M, Chandramohan S, Thomas BR, Geetha S. A comprehensive review of LL-37 in periodontal disease. *J Int Oral Heal*. 2016;8(1):147.

60. Hosokawa I, Hosokawa Y, Komatsuzawa H, Goncalves R B, Karimbux N, Napimoga M H et al. Innate immune peptide LL-37 displays distinct expression pattern from beta-defensins in inflamed gingival tissue. *Clin Exp Immunol*. 2006;146(2):218-225.

61. Ouhara K, Komatsuzawa H, Yamada S, Shiba H, Fujiwara T, Ohara M et al. Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, β-defensins and LL37, produced by human epithelial cells. *J Antimicrob Chemother*. 2005;55(6):888-896.

62. Türkoğlu O, Berdeli A, Emingil G, Atilla G. A novel p. S34N mutation of CAMP gene in patients with periodontal disease. *Arch Oral Biol*. 2011;56(6):573-579.

Table 1: Summary of β-defensin 1 and lactoferrin polymorphism studies performed in periodontitis

Gene	SNP Analyzed	SNP Associated	Associated Disease	References
β-defensin 1	rs1799946, rs1800972, rs11362, rs1047031, rs1800971	rs1800972, rs11362	Chronic periodontitis in Italian population	43
β-defensin 1	rs1799946, rs1800972, rs11362;	rs1800972	Chronic periodontitis in Japanese population	44
β-defensin 1	rs2738047	rs2738047	Chronic periodontitis in Chinese population	45
β-defensin 1	rs2738047	rs2738047	Chronic periodontitis in Chinese population	46
β-defensin 1	rs11362, rs1800972, rs1799946	-	No association with periodontal disease in Caucasians and African-American	48
β-defensin 1	rs1800972	-	Not associated with early-onset periodontitis in Caucasians and African-American	49
β-defensin 1	rs11362	-	Not associated with chronic periodontitis non-Hispanic whites	50
Lactoferrin	rs1126477,	rs1126478	Chronic	43

	rs1126478		periodontitis in Italian population	
Lactoferrin	rs1126478	-	No association with aggressive and chronic periodontitis in Japanese population	44
Lactoferrin	rs1126478	rs1126478	Aggressive periodontitis in Taiwanese patients	55
Lactoferrin	rs1126477	rs1126477	Associated with aggressive periodontitis in African-American but not in the Caucasian population	56