Overview of the Interleukin-1 Genetic Test as a Risk Factor for Guiding Effective and Efficient Prevention and Treatment of Periodontitis

**Kenneth S. Kornman DDS, PhD, Lynn Doucette-Stamm PhD, Gordon W. Duff MD, PhD**

Chronic periodontitis is a common bacterially-induced inflammatory disease that is preventable, treatable by focusing on bacterial control, and varies based on host response.

1. Periodontitis is a bacterially induced chronic inflammatory disease that destroys bone and connective tissues that support the teeth (Williams, 1990).

2. Chronic Periodontitis is highly prevalent in the adult population and is found in approximately 47% of adults in the U.S. (Eke et al., 2012). Chronic periodontitis is the most common form of periodontitis throughout the world. It should be noted that there are some uncommon forms of periodontitis, grouped together under the name "Aggressive Periodontitis" that are characterized by severe destruction at an early age (childhood in some forms, prior to age 35 in others) and are mostly attributable to major deficiencies in host protective mechanisms, such as neutrophil defects. This overview only concerns Chronic Periodontitis, the common form observed in adults throughout the world.

3. Regular bacterial removal from the teeth prevents and treats periodontitis in most patients (Axelsson and Lindhe, 1981) but not everyone responds equally to the same level of bacterial removal. Among adults, 8-13% develop severe generalized periodontitis regardless of personal oral hygiene or access to professional dental care (Albandar et al., 1999; Eke et al., 2012; Page, 1995).

4. Treatment focused on bacterial removal controlled periodontitis in the majority of patients, and most disease progression after treatment occurred in a subset of 20-25% of the treated population (Lindhe and Nyman, 1984).

5. Although bacteria are essential for initiation and progression of periodontitis, the presence of the causative bacteria is not sufficient to explain the tissue destruction severity and response to treatment (Graves et al., 2011).

6. Analysis of disease expression in twins indicates that approximately 50% of the variance in clinical phenotype is explained by genetics (Michalowicz et al, 2000;

Schenkein, 2002).

7. The currently accepted general model for pathogenesis of chronic periodontitis is shown in Figure 1 (Page & Kornman 1997), in which bacteria initiate the disease by activating host immuno-inflammatory mechanisms that are instrumental in regulating tissue matrix-degrading enzymes and osteoclastic bone resorption that account for the clinically observed phenotype.

Figure 1

Pathogenesis of Human Periodontitis: General Concepts — Page & Kornman 1997
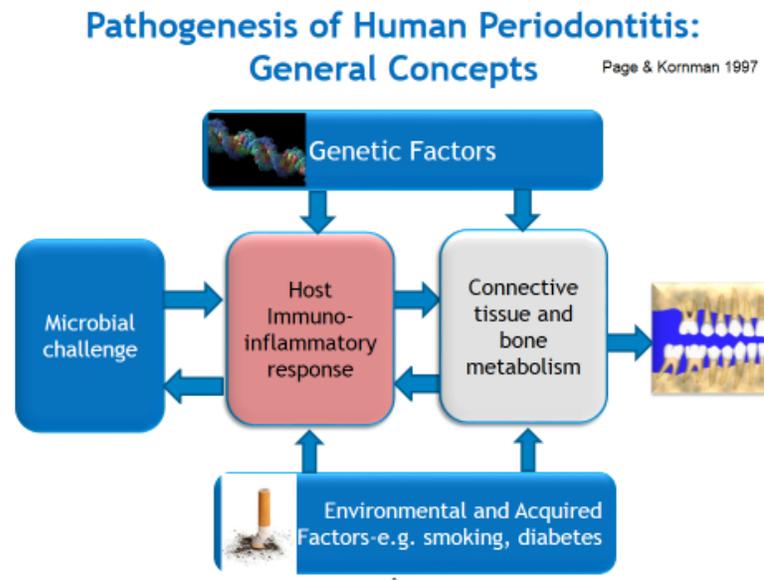
8. A small set of host factors, including most prominently smoking, diabetes, and IL-1 gene status, seem to confer a major portion of the risk of developing severe generalized chronic periodontitis in adults, as well as progression in previously treated cases.

IL-1β protein has been strongly implicated in periodontitis progression and severity and appears to be a key control point in the biologic mechanism leading to disease.

1. The IL-1 system is one of the earliest mechanisms that evolved for protection against infection, and it plays a central part in the control of human inflammation and immunity as well as many immuno-inflammatory diseases (Dinarello, 2011; Duff, 2007). IL-1β is one of the most well documented biomarkers associated

with periodontitis severity (Graves and Cochran, 2003; Offenbacher et al., 2007).

2. IL-1β is a primary driver of connective tissue destruction and bone loss in periodontitis through its stimulation of matrix metalloproteinases and activation of RANK ligand (RANKL) which stimulates bone-destroying osteoclasts (Pfeilschifter et al., 1989; Liu et al., 2003; Lefebvre et al., 1990; Ogata et al., 1992; Borden et al., 1997).

3. In animal models of periodontitis, IL-1 appears to be a key leverage point for controlling the disease.
    a. Animal models show that specific blockage of IL-1 activity reduces the majority of bone loss and connective tissue loss in periodontitis. In a model of periodontitis induction in monkeys, animals given a specific inhibitor of IL-1 activity had 70% less connective tissue loss and 60% less bone loss than animals with no treatment (Delima et al., 2002), supporting the critical role of IL-1 in progression and severity of periodontitis.
    b. In addition, exogenous application of recombinant human IL-1β accelerated periodontal bone loss and inflammation in an animal model (Koide et al., 1995; Nishihara et al., 1995), and animals that over-express IL-1 displayed a spontaneous type of periodontitis (Dayan et al., 2004).
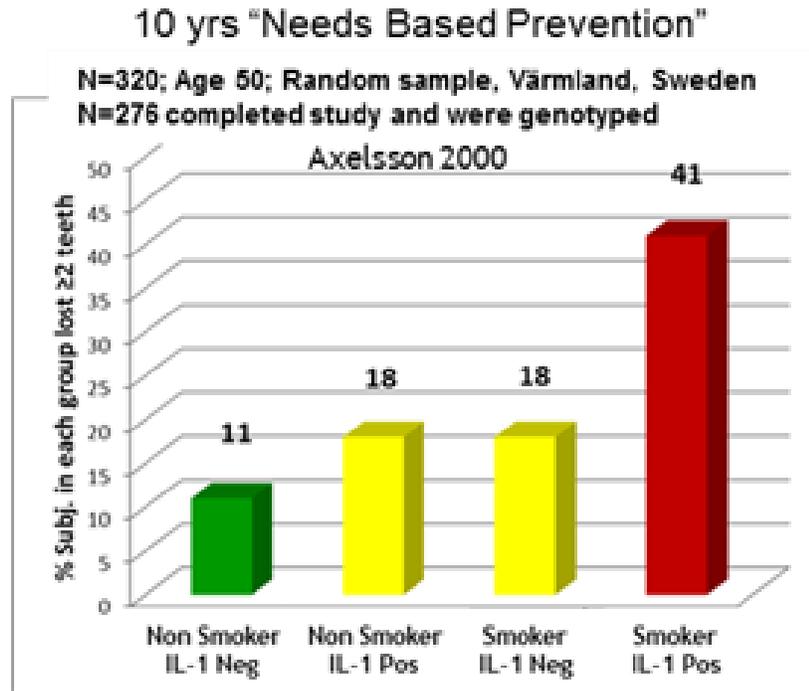
IL-1 gene variations are a major determinant of periodontitis severity and progression.

1. The role of specific genetic variants in chronic periodontitis in Caucasians has been evaluated and reported for approximately 37 candidate genes and in one genome-wide association study (GWAS) (Divaris et al, 2013). A small number of candidate gene studies in Asian populations (Suzuki et al, 2004; Tabeta et al, 2009) have also been reported in the literature. Although a few gene variations were very promising in the GWAS, statistical significance was not achieved for any of the markers studied (Divaris et al, 2013). Most candidate gene studies of chronic periodontitis have been underpowered, but by our analysis of the literature between 2003 and 2014, 7 candidate genes have been associated with chronic periodontitis in Caucasians in 3 or more studies.

2. Of all gene variations studied in chronic periodontitis, two interleukin-1 (IL-1) gene variations (IL1A (-889) rs1800587 or the concordant IL1A (+4845) rs17561; and IL1B (+3954) rs1143634) have been most consistently associated with severe or progressive periodontitis in Caucasians with significant associations reported for 19 of 27 studies and validated in two meta-analyses (Karimbux 2012; Nikolopoulos et al., 2008). However, these variants are infrequent and not

informative in Chinese (Armitage, 1999) and Japanese (Kobayashi, et al, 2009) and have uncertain value in other non-Caucasian races.

3. The PST test based on the markers described above has been associated with periodontitis severity or progression in 19 published studies in Caucasians, and meta-analyses of all the relevant literature report a significant association between IL-1 gene variations and periodontitis (Grigoriadou et al., 2010; Karimbux et al., 2012; Nikolopoulos et al., 2008).
    a. Most studies were small (<200) in size and employed a case-control design. All studies with >200 subjects (N=6) showed a statistically significant association between the genetic markers and disease.
    b. Of studies in which the primary outcome was tooth loss or radiographic bone loss, 5 of 8 and 10 of 15, respectively, reported a positive genetic association with the outcome.
    c. Caucasian studies are available with sufficient sample size for analysis given the relatively high minor allele frequencies (MAF) in Caucasians (*IL1A* rs1800587 MAF=0.25; *IL1B* rs1143634 MAF= 0.21) National Center for Biotechnology Information. Database of Single Nucleotide Polymorphisms (dbSNP). Available at: http;//www.ncbi.nlm.nih.gov/SNP/.dbSNPbuild ID: 134.
    d. Although a small number of studies of IL-1 genetics and chronic periodontitis have been reported in Asian population (Armitage et al; 2000; Anusaksathien et al, 2003; Kobayashi et al, 2007; Yoshie et al, 2007; Kobayashi et al, 2009; Duan et al, 2002; Zhong et al, 2002; Huang et al, 2004) the low allele frequencies in Asians (IL1A rs1800587 MAF=0.05-0.12; IL1B rs1143634 MAF= 0.01-0.06)(dbSNP) make conclusions unreliable given sample sizes.

4. Multiple studies in Caucasians have shown that the IL-1 genetic factors alone (as used in PST), and in combination with smoking, are strong predictors of long-term response to periodontal treatment (Eickholz et al., 2008; McGuire and Nunn, 1999; Persson et al., 2003), and have shown that IL-1 genotype positive patients required more treatment to maintain health than IL-1 genotype negative patients (Eickholz et al, 2008).

5. In a 10 year prospective periodontitis prevention study (Axelsson, 2002), IL-1 genotype (PST) and smoking were the dominant predictors of tooth loss due to periodontitis (Figure 2).

Figure 2

10 yrs "Needs Based Prevention"

N=320; Age 50; Random sample, Värmland, Sweden
N=276 completed study and were genotyped

Axelsson 2000

Axelsson 2002: Subjects age= 50 were randomly identified using postal codes and invited to participate in a 10-year prospective dental disease prevention program. Participants were seen regularly in a government periodontal clinic, and preventive care was assigned to each patient based on a predefined protocol using clinical assessment of need. Preventive care was adjusted during the 10 years as clinical findings changed. 283 patients completed the 10 year study and consented for genotyping.

6. In the University of Michigan "Prevention of Periodontal Disease Study" (PDPS), PST was included as one of three risk factors that together were shown to differentiate long-term tooth loss outcomes associated with different frequencies of preventive care (Giannobile et al, 2013).

IL-1 gene variations alter the levels of IL-1 protein expression

1. The same IL-1 variations associated with more severe periodontitis also have been associated with:
   a. higher gingival crevicular fluid (GCF) levels or monocyte expression of IL-1 alpha (IL-1α) (Shirodaria, et al, 2000).
   b. higher IL-1 beta (IL-1β) in most but not all studies (Gore et al, 1998; Galbraith et al, 1999; Trombelli, et al, 2010; Engebretson, et al, 1999).
   c. higher blood levels of C-reactive protein (CRP) (Berger et al, 2002).

2. We previously identified the IL-1 SNPs that are "causative" functional gene variations at the molecular level, that alter transcription levels of IL-1β, and showed that they function in haplotype context (Chen et al., 2006). The functional IL-1 SNPs (IL1B(-511), IL1B(-1464), IL1B(-3737)) are in the regulatory region for the IL-1β gene and are inherited in 4 specific haplotype patterns that are associated with significantly different levels of IL-1 protein in the gingiva and blood levels of CRP (Rogus et al., 2008).

PerioPredict Extends PST Coverage to All Major Ethnic Groups

1. All of the studies support that some specific IL-1 gene variations are involved in expression of protein and more severe disease. The first markers developed did not appear to be functional but effectively tagged the underlying functional SNPs that were responsible for disease. We know functional SNPS are more highly conserved in the world's population than tags that have no function hence we sought to discover the functional SNPs responsible for disease that would be related to IL-1 expression and be informative across all major ethnic groups.

2. Most validated gene variations that are found in >1% of a population and associated with a clinical characteristic are either the actual "causative" variant or are markers that tag a haplotype that contains the causative variants (Smith et al, 2010; McClellan and King, 2010). Haplotypes are combinations of DNA variations that are inherited together.

3. It is now well-established that IL-1 gene variations are associated with higher production of IL-1 protein and increased risk for severe, progressive periodontitis. PST includes two IL-1 SNPs (IL1A (+4845), IL1B(+3954)) that previously appeared non-functional and most likely tagged haplotypes for the IL-1 genetic differences that influence level of protein expression and severity of periodontitis.

4. The two PST markers are frequent in Caucasians, but the SNPs are in low frequency in Asian populations, therefore PST likely underestimates IL-1 genetic risk in Asians and perhaps other ethnic groups.

5. Different ethnic groups usually have different haplotype frequencies, for example, one of the IL-1 functional haplotypes associated with increased IL-1β represents 6% of the IL-1 haplotypes observed in Caucasians but 46% of those observed in African-Americans (Interleukin Genetics, unpublished data 2014).

6. The predominant haplotypes with the functional IL-1 SNPs include those which confer increased risk for severe periodontitis. We used the functional haplotypes

to define genotype patterns (PerioPredict) that are well represented in all major ethnic populations and are significantly associated with severe periodontitis in Caucasians, African Americans, Hispanics, and Asians (Wu et al., 2014).

7. In a predominately Caucasian population (University of Michigan Periodontal Disease Prevention Study) the PerioPredict test performed as well as the original PST test in differentiating long-term tooth loss outcomes associated with different frequencies of preventive care (Giannobile et al., 2013).

8. PerioPredict does not represent a different genetic finding, merely an extension of markers in the IL-1 genes to increase information content regardless of ethnicity.

References

Albandar JM, Brunelle JA, Kingman A (1999). Destructive periodontal disease in adults 30 years of age and older in the United States, 1988-1994. *J Periodontol* 70(1)*:*13-29.

Anusaksathien O, Sukboon A, Sitthiphong P, Teanpaisan R. Distribution of interleukin-1beta(+3954) and IL-1alpha(-889) genetic variations in a Thai population group. J Periodontol 2003;74:1796-1802.

Armitage GC, Wu Y, Wang HY, Sorrell J, di Giovine FS, Duff GW. Low prevalence of a periodontitis-associated interleukin-1 composite genotype in individuals of Chinese heritage. J Periodontol 2000;71:164-171.

Axelsson P, Lindhe J (1981). Effect of controlled oral hygiene procedures on caries and periodontal disease in adults. Results after 6 years. *J Clin Periodontol* 8(3)*:*239-48.

Axelsson P (2002). Diagnosis and Risk Prediction of Periodontal Diseases Carol Stream, IL: Quintessence.

Berger P, McConnell JP, Nunn M, Kornman KS, Sorrell J, Stephenson K, et al. (2002). C-reactive protein levels are influenced by common IL-1 gene variations. *Cytokine* 17(4)*:*171-4.

Borden, P. and Heller, R.A. (1997) Transcriptional control of matrix metalloproteinases and the tissue inhibitors of matrix metalloproteinases. Crit Rev Eukaryot Gene Expr, 7, 159-178.

Chen H, Wilkins LM, Aziz N, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum Mol Genet 2006;15:519-529.

Dayan S, Stashenko P, Niederman R, Kupper TS (2004). Oral epithelial overexpression of IL-1alpha causes periodontal disease. *J Dent Res* 83(10)*:*786-90.

Delima AJ, Karatzas S, Amar S, Graves DT (2002). Inflammation and tissue loss caused by periodontal pathogens is reduced by interleukin-1 antagonists. *J Infect Dis* 186(4)*:*511-6.

Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood 2011;117:3720-3732.

Divaris, K., Monda, K.L., North, K.E., Olshan, A.F., Reynolds, L.M., Hsueh, W.C., Lange, E.M., Moss, K., Barros, S.P., Weyant, R.J. et al. (2012) Exploring the genetic basis of chronic periodontitis: a genome-wide association study. Hum Mol Genet, 22, 2312-2324.

Duan H, Zhang J, Zhang Y. The association between IL-1 gene polymorphisms and susceptibility to severe periodontitis (Chinese). Hua Xi Kou Qiang Yi Xue Za Zhi 2002;20:48-51.

Duff, GW. Influence of genetics on disease susceptibility and progression. Nutr Rev 2007; 65(12 Pt2):S177-81.

Eickholz P, Kaltschmitt J, Berbig J, Reitmeir P, Pretzl B (2008). Tooth loss after active periodontal therapy. 1: patient-related factors for risk, prognosis, and quality of outcome. *J Clin Periodontol* 35(2)*:*165-74.

Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ (2012). Prevalence of Periodontitis in Adults in the United States: 2009 and 2010. *J Dent Res* 91(10)*:*914-920.

Galbraith GM, Hendley TM, Sanders JJ, Palesch Y, Pandey JP. Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. J Clin Periodontol 1999;26:705-709.

Genco RJ, and Borgnakke, W (2013). Risk factors for periodontal disease. Periodontol 2000 62:59-94.

Giannobile, W.V., Braun, T.M., Caplis, A.K., Doucette-Stamm, L., Duff, G.W. and Kornman, K.S. (2013) Patient stratification for preventive care in dentistry. *J Dent Res*, **92**, 694-701.

Gore EA, Sanders JJ, Pandey JP, Palesch Y, Galbraith GM. Interleukin-1beta+3953 allele 2: association with disease status in adult periodontitis. J Clin Periodontol 1998;25:781-785.

Graves DT, Cochran D (2003). The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J Periodontol* 74(3)*:*391-401.

Graves DT, Oates T, Garlet GP (2011). Review of osteoimmunology and the host response in endodontic and periodontal lesions. *J Oral Microbiol* 3:5304.

Huang HY, Zhang JC. Investigation on the association of interleukin-1 genotype polymorphism with chronic periodontitis (Chinese). Hua Xi Kou Qiang Yi Xue Za Zhi 2004;22:415-419.

Karimbux NY, Saraiya VM, Elangovan S, Allareddy V, Kinnunen T, Kornman KS, et al. (2012). Interleukin-1 Gene Polymorphisms and Chronic Periodontitis in Adult Caucasians: A Systematic Review and Meta-Analysis. *J Periodontol.*

Kobayashi T, Ito S, Kuroda T, et al. The interleukin-1 and Fcgamma receptor gene polymorphisms in Japanese patients with rheumatoid arthritis and periodontitis. J Periodontol 2007;78:2311-2318.

Kobayashi T, Murasawa A, Ito S, et al. Cytokine gene polymorphisms associated with rheumatoid arthritis and periodontitis in Japanese adults. J Periodontol 2009;80:792-799.

Koide M, Suda S, Saitoh S, Ofuji Y, Suzuki T, Yoshie H, et al. (1995). In vivo administration of IL-1 beta accelerates silk ligature-induced alveolar bone resorption in rats. *J Oral Pathol Med* 24(9)*:*420-34.

Lefebvre, V., Peeters-Joris, C. and Vaes, G. (1990) Modulation by interleukin 1 and tumor necrosis factor alpha of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim Biophys Acta, 1052, 366-378.

Lindhe J, Nyman S (1984). Long-term maintenance of patients treated for advanced periodontal disease. *J Clin Periodontol* 11(8)*:*504-14.

Liu, D., Xu, J.K., Figliomeni, L., Huang, L., Pavlos, N.J., Rogers, M., Tan, A., Price, P. and Zheng, M.H. (2003) Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. Int J Mol Med, 11, 17-21.

McGuire MK, Nunn ME (1999). Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival. *J Periodontol* 70(1)*:*49-56.
McClellan, J. and King, M.C. (2010) Genetic heterogeneity in human disease. *Cell*, **141**, 210-217.

Michalowicz BS, Diehl SR, Gunsolley JC, et al. Evidence of a substantial genetic basis for risk of adult periodontitis. J Periodontol 2000;71:1699-1707.

Nikolopoulos GK, Dimou NL, Hamodrakas SJ, Bagos PG (2008). Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls. *J Clin Periodontol* 35(9)*:*754-67.

Nishihara T, Ishihara Y, Koseki T, Boutsi E, Senpuku H, and Hanada N. (1995) Membrane-associated interleukin-1 on macrophages stimulated with Actinobacillus actinomycetemcomitans lipopolysaccharide induces osteoclastic bone resorption in vivo. *Cytobios* 81:229-37.

Offenbacher S, Barros SP, Singer RE, Moss K, Williams RC, Beck JD (2007). Periodontal disease at the biofilm-gingival interface. *J Periodontol* 78(10)*:*1911-25.

Ogata, Y., Pratta, M.A., Nagase, H. and Arner, E.C. (1992) Matrix metalloproteinase 9 (92-kDa gelatinase/type IV collagenase) is induced in rabbit articular chondrocytes by cotreatment with interleukin 1 beta and a protein kinase C activator. Exp Cell Res, 201, 245-249.

Page RC (1995). Critical issues in periodontal research. *J Dent Res* 74(4)*:*1118-28.

Page RC and Kornman KS. (1997) The pathogenesis of human periodontitis: an introduction. *Periodontol 2000* 14:9-11.

Persson GR, Matuliene G, Ramseier CA, Persson RE, Tonetti MS, Lang NP (2003). Influence of interleukin-1 gene polymorphism on the outcome of supportive periodontal therapy explored by a multi-factorial periodontal risk assessment model (PRA). *Oral Health Prev Dent* 1(1)*:*17-27.

Pfeilschifter, J., Chenu, C., Bird, A., Mundy, G.R. and Roodman, G.D. (1989) Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclastlike cells in vitro. J Bone Miner Res, 4, 113-118.

Rogus J, Beck JD, Offenbacher S, Huttner K, Iacoviello L, Latella MC, et al. (2008). IL1B gene promoter haplotype pairs predict clinical levels of interleukin-1beta and C-reactive protein. *Hum Genet* 123(4)*:*387-98

Schenkein, HA. Finding genetic risk factors for periodontal diseases: is the climb worth the view. Periodontol 2000 2002; 30:79-90.

Shirodaria, S., Smith, J., McKay, I.J., Kennett, C.N. and Hughes, F.J. (2000) Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease. *J Dent Res*, **79**, 1864-1869.

Smith, A.J., Palmen, J., Putt, W., Talmud, P.J., Humphries, S.E. and Drenos, F. (2010) Application of statistical and functional methodologies for the investigation of genetic determinants of coronary heart disease biomarkers: lipoprotein lipase genotype and plasma triglycerides as an exemplar. Hum Mol Genet, 19, 3936-3947.

Stashenko, P., Jandinski, J.J., Fujiyoshi, P., Rynar, J. and Socransky, S.S. (1991) Tissue levels of bone resorptive cytokines in periodontal disease. J Periodontol, 62, 504-509.

Suzuki, A., Ji, G., Numabe, Y., Ishii, K., Muramatsu, M. and Kamoi, K. (2004) Large-scale investigation of genomic markers for severe periodontitis. Odontology, 92, 43-47.

Tabeta, K., Shimada, Y., Tai, H., Ishihara, Y., Noguchi, T., Soga, Y., Takashiba, S., Suzuki, G., Kobayashi, T., Oka, A. et al. (2009) Assessment of chromosome 19 for genetic association in severe chronic periodontitis. J Periodontol, 80, 663-671.

Trombelli, L., Scapoli, C., Carrieri, A., Giovannini, G., Calura, G. and Farina, R. (2010) Interleukin-1 beta levels in gingival crevicular fluid and serum under naturally occurring and experimentally induced gingivitis. *J Clin Periodontol*, **37**, 697-704.

Williams, RC. Periodontal disease. N Engl J Med 1990; 322(6):373-82.

Wu X., Offenbacher S, Lopez MJ, et al. Association of interleukin-1 gene variations with moderate to severe chronic periodontitis in multiple ethnicities. J. Periondont Res 2014.

Yoshie H, Tai H, Kobayashi T, et al. Salivary enzyme levels after scaling and interleukin-1 genotypes in Japanese patients with chronic periodontitis. J Periodontol 2007;78:498-503.

Zhong L, Zhang Y, Zhang J, Yang A, Huang H. The association of interleukin-1 gene polymorphisms with the susceptibility to chronic periodontitis in Uighur (Chinese). Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2002;19:405-408.