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;
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.png)

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).
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


