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Inflammation and Genetic Risk Indicators for Early Periodontitis in Adults

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Abstract

OBJECTIVE—This report is a further analysis of a study designed to determine clinical and microbial risk indicators for progressing periodontitis.

MATERIAL AND METHODS—One hundred and ninety subjects periodontally healthy or adults with early signs of periodontitis (20–40 years) were monitored clinically at 6-month intervals followed by supragingival cleaning. At each visit, GCF and blood were collected for determination of IL-1 β content (GCF) and IL-1 genotype (blood). Inter-proximal sites with >1.5 mm increase in clinical attachment over 18 months were considered disease active. Characteristics were compared between active and inactive subjects.

RESULTS—IL-1 β levels in GCF increased with severity of disease and correlated well with clinical signs of incipient disease. However, IL-1 genotype did not show any significant associations with disease or extent of disease.

CONCLUSIONS—Indicators of inflammation may be important clinical determinants of future periodontal disease progression, but IL-1 genotype was not a risk indicator for early (slight) periodontitis as defined in this subject population.

Keywords

genotype; gingival crevicular fluid; health; Periodontitis; race/ethnicity smoking

Introduction

Periodontal diseases are widely prevalent in the United States with over half of the adults in the nation affected by some level of disease (1). Moderate to severe periodontitis is an important public health problem impacting quality of life by causing discomfort, masticatory problems, abscesses and tooth loss. Diseases of the oral cavity are also known to impact systemic health. Periodontitis has been identified as a risk factor for cardiovascular disease

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and low birth weight and premature infants, and it is a known complication of diabetes affecting metabolic control and other complications (2–10).

The general consensus of clinicians and investigators is that bacteria associated with periodontal diseases are the primary etiologic agents of these diseases (11, 12). Yet, it has been known for some time that pathology only occurs in susceptible individuals (13, 14), and more recent evidence has suggested that tissue damage in periodontitis is the result of inflammation, not the direct action of bacteria on tissues (for a recent review, see Van Dyke (14)). Interleukin-1 (IL-1) is a central mediator of innate immunity and inflammation, which is induced by microbial infections. IL-1 exerts certain host protective actions, in particular, the priming of neutrophils and macrophages for enhanced phagocytic and bactericidal activity, in the short term. The expression of IL-1, however, has also been correlated with destruction of periodontal tissues in the chronic lesion by stimulating matrix metalloproteinase secretion, connective tissue degradation, and inflammatory bone resorption (15).

The major isoforms of IL-1, IL-1 α and IL-1 β , are highly potent bone resorptive cytokines that are elevated in periodontal diseases (16, 17). Both isoforms are present in increased concentrations in gingival crevicular fluid (GCF) from individuals with periodontitis, and furthermore, gingival tissue extracts from sites undergoing active attachment loss show elevated IL-1 β levels (17, 18). In non-human primates, blockade of IL-1 with soluble IL-1 Type 1 receptors significantly inhibits periodontal, inflammation, connective tissue and bone loss (19). A non-synonymous polymorphism in the coding region of the gene for IL-1 α (IL1A) and a synonymous polymorphism in the coding region of the gene for IL-1 β (IL1B) have been reported to show linkage disequilibrium in people with severe periodontitis, when smokers are excluded (20, 21). The IL-1 genetic associations with chronic adult periodontitis are supported by meta-analysis of all studies on chronic periodontitis in Caucasians (22) but not by a meta-analysis focused on a small number of prospective studies (23). Current data suggest these findings apply to Caucasians whereas generalizability in different populations is questionable (23–25). Data such as these strongly support a central role for inflammation, and particularly IL-1, in the pathogenesis of human periodontitis.

The identification of predictors of future attachment loss in periodontitis remains elusive. The best predictor of future periodontal attachment loss in adults is still the extent of existing attachment loss (26–28). In earlier work, we studied early periodontitis in adults to determine clinical and other risk indicators for periodontitis before the onset of disease. Interestingly, the presence of, or absolute numbers of specific microorganisms did not predict future attachment loss; however, the levels of inflammation (gingivitis) did (29, 30).

The aim of this study was to examine the relationships between inflammation and periodontitis focusing on IL-1 β levels in GCF of healthy subjects and individuals with early signs of periodontitis. We also examined the associations between IL-1 gene polymorphisms and early signs of disease and IL-1 levels. Our findings indicate that GCF levels of IL-1 β correlate with inflammation and early attachment loss, but IL-1 composite genotype had no predictive value in this study population.

Materials and Methods

Subject Population

Healthy and ‘early’ (incipient) periodontitis subjects were recruited to three dental clinics in Boston: The Forsyth Institute, Brigham and Women’s Hospital, and the Clinical Research Center at Boston University School of Dental Medicine. Baseline clinical data were collected for 225 subjects and has been previously reported (29, 30). A subset of 190

subjects from whom usable samples were obtained was evaluated in this study. Subjects enrolled were between 20 and 40 years of age, medically healthy by medical history, not pregnant or lactating, had not taken antibiotics or had a dental cleaning in the previous 6 months and had no history of previous periodontal therapy. Subjects had a mean periodontal attachment loss of less than 2.0 mm; subjects with moderate or advanced periodontitis were excluded. Study participants signed an informed consent approved by the Institutional Review Boards of the participating institutions. Subject enrollment and evaluation was performed between 1998 and 2004.

Clinical Measurements and Longitudinal Monitoring

On entry into the study, subjects completed a medical and dental history and a questionnaire that included their racial/ethnic background and smoking history (non-, previous or current tobacco use). Gingival crevice fluid (GCF) was collected from the mesial subgingival sites of the 1st molar in each quadrant. If the tooth was missing, the samples were obtained from the 2nd molar. Samples were collected onto filter paper strips, and the volume measured with a calibrated PeriotronTM. Strips were placed into an elution buffer containing 100 µg/ml bovine serum albumin [#], 100 µg/ml Zwittergent-12 ^{**}, 10 µl HEPES buffer, 1 µg/ml aprotinin [#], 1 µg/ml leupeptin [#] and 0.1 ml EDTA in RPMI-1640 medium ^{††}, as described (31), and eluted by centrifugation. IL-1β content was measured using a sandwich ELISA (32). Blood was obtained for IL-1 genotyping by finger stab and collected on DNase-free blotting paper and air dried. IL-1 genotyping was performed by Interleukin Genetics as previously described ^{§§} (20). Subjects were determined to be genotype positive if they had at least one copy of allele 2 of either of the two specific polymorphisms, in IL-1α at +4845 and in IL-1β at +3854 (20).

Clinical measurements included measurements of probing depth (PD), clinical attachment level (AL, estimated to the nearest 0.5 mm), and Plaque Index (33), Gingival Index (34) and bleeding on probing at 6 sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) on all teeth except third molars, as previously described (29, 30). Radiographs were not taken. Subjects were recalled at 6 month intervals over 18 months. If any site demonstrated >1.5 mm of clinical attachment loss during the monitoring period, the site was treated by subgingival scaling.

Clinicians making clinical measurements (LM, NM, MC) participated in pre-study training by the clinician (LM) at the Forsyth Institute (the primary examiner) and calibration exercises as described previously (29), which were repeated at 6-month intervals during the study. The intra-class correlations (35) for duplicate measurements between examiners were 0.87 for PD and 0.85 for CAL (29). For each subject, longitudinal clinical measurements were made by the same examiner, except early in the study when there were changes in examiners. The new examiners (MM, LN) were calibrated to the primary examiner.

Statistical Analysis

Subjects were divided into three disease groups according the following criteria: Healthy Subjects had no sites with over 2 mm clinical attachment loss, and had a mean attachment loss of 1.5 mm or less. Early Periodontitis Subjects had one or more sites with at least 2 mm clinical attachment loss. Early Periodontitis subjects were further subdivided into subjects with mean attachment loss of < 1.5 mm (Early Periodontitis 1) and those with mean clinical

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attachment loss > 1.5 mm (Early Periodontitis 2). The mean clinical measurements and GCF IL-1 β levels were calculated for each subject.

Non-parametric analysis of variance was used to evaluate associations between subject disease categories and mean GCF IL-1 β levels, age and clinical measurements. Contingency tables were used to evaluate associations between disease category, IL-1 gene polymorphism, gender, smoking history, and race/ethnicity. The significance of the associations was evaluated by Chi-square analysis.

The impact of smoking on GCF IL-1 β was assessed taking subject means of GCF IL-1 β , Gingival Index, percent sites bleeding on probing and pocket depth, and comparing among subjects according to smoking history (never smokers, former smokers, and current smokers) using analysis of variance (ANOVA). Associations between subject smoking category and Gingival Index, percent sites bleeding on probing and pocket depth were examined by calculating Pearson and Spearman correlation coefficients. Subject mean GCF IL-1 β levels were also compared between IL-1 genotype positive and negative subjects by smoking history.

The association between IL1 genotype and early periodontitis was evaluated by combining the EP1 and EP2 categories and estimating odds ratios and relative risks from the resulting 2 $\times$ 2 tables. For Caucasians only (the largest ethnic group in the study), associations were obtained from 2 $\times$ 2 tables (separately for all subjects and for nonsmokers) by standard point estimates and confidence intervals of relative odds and relative risks and chi square tests. Similar summary estimates of relative risks and relative odds adjusted for ethnicity were obtained from 2 $\times$ 2 tables stratified over the four largest ethnic groups (Asian, African-American, Hispanic, Caucasian non-Hispanic) and evaluated by Mantel-Haenszel analyses, again done separately for all subjects and all nonsmoking subjects.

For associations with IL-1 genotype, GCF IL-1 β levels were compared among IL-1 α and IL-1 β genotype categories. Subjects with sites that increased in attachment loss > 1.5 mm over the 18 month observation period were considered to be disease active. The proportions of disease active and inactive subjects by IL-1 genotype were compared. GCF IL-1 β levels were compared between the ethnic (Hispanic) and racial groups (Asian, African American, and Caucasian), by IL-1 composite genotype.

Results

Of the 190 subjects examined in the study, 42 were periodontally healthy (AL < 1.5 mm, no site > 2.0 mm), 103 were categorized as Early Periodontitis 1 (EP1, mean AL < 1.5 mm, >1 site more than 2 mm AL), and 45 subjects were categorized as Early Periodontitis 2 (EP2, mean AL > 1.5 mm, > 1 site more than 2 mm AL). The majority of subjects were never smokers with a lower proportion of never smokers with early periodontitis compared to periodontal health (not significant, NS), but a higher proportion of current smokers with early periodontitis compared with periodontal health (NS). There were significant differences in extent of disease by race/ethnicity ($p = 0.002$, Chi-square). Higher proportions of African American and Hispanic subjects were in the Early Periodontitis 2 category. In contrast, higher proportions of Caucasian subjects were in the periodontal health category compared with early Periodontitis (Table 1).

Subjects in the EP2 category had significantly higher Gingival Index, percent sites BOP, probing depth and clinical attachment loss compared with subjects in the other clinical categories that exhibited less attachment loss (Table 2). The levels of IL-1 β in GCF samples (mean of four samples per subject) were evaluated, and were correlated with clinical factors. As shown in Table 2, GCF IL-1 β was significantly elevated in Early Periodontitis, with

elevated levels in EP1 compared to health, and further elevated levels in EP2. The elevated levels of IL-1 β in GCF correlated closely with clinical parameters (Table 3). Taken together, these data indicate that GCF IL-1 β levels correlate well with all clinical manifestations of incipient periodontitis.

Subjects positive for the periodontitis susceptibility trait (IL-1 genotype), defined as possession of at least one copy of allele 2 of both the IL-1 α and IL-1 β gene polymorphisms (20) were identified by analysis of blood samples. As shown in Table 4, 58 of 190 (30.4%) of subjects were composite IL-1 genetic test positive, a frequency that is comparable to that reported previously in a northern European population (20). However, there was an inverse association between IL-1 genotype positivity and Early Periodontitis, with healthy subjects exhibiting the highest frequency of the IL-1 genotype (Table 1). This association did not change significantly when only Caucasian never-smokers were evaluated. Hispanics had the highest frequency of IL-1 genotype positivity (6/14, 43%), followed by Caucasians 42/120, 35%), Asians (3/16, 19%) and African Americans (4/30, 13%, Table 5).

The association between IL1 genotype and early periodontitis was further evaluated by combining the EP1 and EP2 categories and estimating odds ratios and relative risks from the resulting 2 $\times$ 2 tables. For Caucasians only, the odds ratio for early periodontitis in IL1 genotype positive subjects was 0.81 with 95% confidence limits (0.35, 1.88) and the relative risk was 0.94 (0.75, 1.19). For 86 nonsmoking Caucasians only the odds ratio was 0.69 (0.26 to 1.80) and the relative risk was 0.88 (0.63 to 1.23). Similar values were obtained for all subjects in the four largest ethnic groups (Asian, African-American, Hispanic, Caucasian non-Hispanic) from 2 $\times$ 2 tables stratified by ethnicity using Mantel-Haenszel methods. The summary odds ratio adjusted for ethnicity was 0.73 (0.35, 1.53) and the summary relative risk was 0.92 (0.76, 1.12). For all nonsmoking subjects summary estimates adjusted for ethnicity were Rel. Odds = 0.68 (0.29, 1.61) and Rel. Risk = 0.89 (0.68, 1.17). There was not association between genotype and disease activity. None of the odds ratios or relative risks was significantly different from 1.0. All point estimates for relative odds and relative risks were <1.0 with confidence intervals including 1.0, providing no meaningful evidence in this subject group for positive associations between IL1 genotype and early periodontitis. Sample sizes in the ethnic groups other than Caucasians were too small for separate evaluations.

The relationship of genotype to disease was further explored by examining GCF IL-1 β levels in subjects with different composite IL-1 genotypes. As shown in Table 4, 45/58 subjects had 2 positive IL-1 alleles (IL-1 α 1.2 and IL-1 β 1.2), 7/58 had three (IL-1 α 1.2/IL-1 β 2.2 or IL-1 α 2.2/IL-1 β 1.2) and 6/58 had four (IL-1 α 2.2/IL-1 β 2.2) positive alleles, respectively. There was no clear gene dose effect for these groups between the polymorphisms and GCF IL-1 β levels (18.13, 22.13, and 16.07 pg/site, respectively). The original study on this genotype reported that the correlation between composite IL-1 genetic test positivity and periodontitis is manifested in Caucasian non-smokers. We therefore explored the effect of ethnicity (Table 5) and smoking (Table 6) on various parameters. As shown in Table 5, there was no significant relationship between composite IL-1 genetic test positivity, GCF IL-1 β and the clinical parameters GI, %BOP and pocket depth. Table 6 shows data for all subjects and, for comparison, results for Caucasian subjects only. Although never smokers had the highest mean GCF IL-1 β levels relative to former and current smokers, differences were not significant. There were also no significant relationships between composite IL-1 genetic test positivity, GCF IL-1 β levels and smoking status. Not surprisingly since they comprised over 60% of the sample, results for Caucasian subjects closely tracked results for all subjects.

Discussion

In this study, we addressed the question of the role of inflammation in the onset of early periodontitis. The goal was to investigate longitudinally the first stages of periodontal disease initiation. Our study population included subjects with periodontal health and early periodontitis, but our definition of early periodontitis would probably be classified as periodontally healthy in some other investigations (36–38). Despite the modest amount of periodontal disease progression observed, we were able to make several significant cross-sectional and longitudinal observations relating to inflammation and the association of other known risk indicators in the onset of periodontitis. There was a significant positive correlation between the inflammatory marker, IL-1 β in GCF and the onset of disease. GCF IL-1 β levels positively correlated with clinical parameters of early periodontitis (attachment level, pocket depth, GI and BOP), but not with IL-1 genotype. There were a significantly higher proportion of current smokers in the early periodontitis group and more non-smokers in the periodontal health group.

In previous reports (29, 30), we examined the relationship between specific bacteria, and gingival inflammation and disease progression in early periodontitis in the same study cohort as examined here. Those results revealed that no specific organism or group of organisms of those assessed predicted future attachment loss. Interestingly, the absolute numbers or proportions of different microorganisms were poor predictors as well, but plaque index was positively correlated with disease progression. These findings were surprising in light of the overwhelming cross-sectional associative data implicating specific microorganisms in the pathogenesis of periodontitis (11, 12). Clearly, the longitudinal aspect of this study, which asks the question: “does colonization with specific organisms precede attachment loss”, added an important element in our understanding of disease initiation and progression. In the same prior study (29, 30), the microbiota of progressing lesions was analyzed cross-sectionally to assess the quantity and ecology of the microbiota. The results revealed an increased frequency of detection suggesting overgrowth of the specific pathogens *Porphyromonas gingivalis* and *Tannerella forsythia*, replicating the findings of previous cross-sectional studies (12, 36, 38). Gingival inflammation, as measured by GI also proved to be a statistically significant predictor of future attachment loss.

In the context of the findings reported here, the data suggest that measureable inflammation, presumably induced by early plaque colonizers, creates a local environment conducive to the overgrowth of periodontal pathogens. The consequences of overgrowth of *P. gingivalis*, *T. forsythia* and other periodontal pathogens on disease is well known clinically and the virulence of these organisms has been well documented experimentally (for recent reviews, see (39–41). These observations may raise more questions than they answer. For instance, is the initiation of inflammation mediated by specific microorganisms or their products, or is the degree of inflammation predominantly under the genetic control of the host? To further investigate the role of the host, a well-known genetic test for IL-1 polymorphisms was used to determine a specific genotype that has been reported to be predictive of periodontitis in certain populations known as the periodontitis susceptibility trait (composite IL-1 genetic test) (20), which is a composite polymorphism in both the IL-1 α and IL-1 β gene loci. In the present study, we examined possible relationships between composite IL-1 genetic test and GCF IL-1 β levels in adult subjects with early periodontitis and also directly evaluated relative odds and relative risks between composite IL-1 genetic test positivity and early periodontitis. Our findings demonstrate a lack of significant correlations among these parameters, and suggest that the composite IL-1 genetic test genotype is not useful for identifying individuals at the earliest stages of periodontal disease.

There are several possible reasons for this that relate to population specificity. Using composite IL-1 genetic test in an African American cohort, an inverse relationship of disease to genotype was reported (25). Likewise, in an Asian cohort, Armitage *et al.* were unable to demonstrate an association between genotype and disease (24). Given the mixed racial and ethnic background of our cohort, even within the Caucasian group, it is unlikely that our sample size was sufficient to uncover any trends. Another proviso is that subjects with moderate or severe periodontitis were excluded from this study. Thus, individuals who manifest frank periodontitis in the third and fourth decades of life, variously estimated to comprise 5–20% of human populations (42, 43), and who might be considered to be the most susceptible group, were not evaluated for clinical associations with the composite IL-1 genetic test.

The goal of longitudinal studies of early disease is to identify targets for prevention or therapy, or to identify a population at risk, so that appropriate preventive or treatment measures can be undertaken. Once the risk indicators are identified, the findings must be verified in randomized, controlled, longitudinal intervention studies. The results of this and other recent studies (44, 45) implicate the inflammatory response as a target for intervention in the prevention and treatment of periodontitis. It is also interesting, albeit preliminary, that data from this and other studies suggest that the degree of inflammation may influence the composition of the microflora in the biofilm and contribute to the overgrowth of periodontal pathogens (29, 30, 46). These findings have significant implications for the development of pharmacologic strategies for the control of periodontitis that include direct management of inflammation.

Summary and Conclusions

We performed a cross-sectional and longitudinal evaluation of adult subjects with early periodontitis aged 20–40 years. We found that GCF IL-1 β levels positively correlated with attachment level, Pocket depth, GI, BOP and smoking. The composite IL-1 genetic test genotype did not correlate with disease or disease progression. We conclude that indicators of inflammation may be important clinical determinants of future periodontal disease progression, but that IL-1 genotype is not a risk indicator for early periodontitis as defined in this subject population.

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Table 1

General and Demographic Characteristics of Study Population at Baseline

	Health ^a n= 42	Early Periodontitis 1 ^b n=103	Early Periodontitis 2 ^c n=45
Mean Age (in yrs) $\pm$ SEM ^{de}	26.8 $\pm$ 0.8	29.2 $\pm$ 0.6	31.8 $\pm$ 1.0
Gender n (% male) ^f	12 (29%)	46 (45%)	24 (53%)
Smoking history: n (% clinical group) ^{gh}			
Never smoker n (%)	35 (83%)	67 (66%)	27 (60%)
Former smoker n (%)	4 (10%)	19 (19%)	7 (16%)
Current smokers n (%)	3 (7%)	16 (16%)	11 (24%)
Race/Ethnicity: n (% clinical group) ⁱ			
Asian n (%)	3 (7%)	10 (11%)	3 (7%)
African American n (%)	2 (5%)	15 (15%)	13 (29%)
Hispanic n (%)	5 (12%)	2 (2%)	7 (15%)
Caucasian n (%)	31 (74%)	68 (66%)	21 (47%)
IL-1 gene polymorphism			
All subjects (%) ^j	17 (40%)	31 (30%)	10 (22%)
Caucasian never-smoker only			
n/total (%) by clinical group	10/27 (37%)	13/44 (30%)	4/15 (27%)

^aHealth: mean AL $\leq$ 1.5 mm, no sites 2 mm AL loss^bEP1: mean AL $\leq$ 1.5 mm, $\geq$ 1 site 2 mm AL loss^cEP2: mean AL $>$ 1.5 mm^dSEM = Standard Error of the Mean^ep = 0.0007 (1 way analysis of variances of means across clinical categories)^fp = 0.02 Mantel-Haenszel Chi Square^gOne subject without smoking history in EP1 category^hp = 0.013 Mantel-Haenszel Chi Square of association between smoking and disease categoriesⁱp = 0.002 Chi Square test between 4 major race/ethnic groups.

Subjects in "Other" racial groups are not shown and included one subject in the Health category, eight subjects in the EP1 category, and one subject in the EP2 category.

Table 2Gingival Crevice Fluid IL-1 β Levels and Clinical Characteristics of Sampled Sites

	Health n= 42	Early Periodontitis 1 n=103	Early Periodontitis 2 n=45
GCF IL-1β (pg) (mean $\pm$ SEM) ^a	13.95 $\pm$ 2.69	16.79 $\pm$ 1.75	23.07 $\pm$ 3.16
GCF IL-1β Never smokers (n=129) (pg)(mean $\pm$ SEM) ^b	13.54 $\pm$ 2.88	19.60 $\pm$ 2.48	25.4 $\pm$ 4.65
Gingival Index (mean $\pm$ SEM) ^c	0.60 $\pm$ 0.06	0.90 $\pm$ 0.06	1.33 $\pm$ 0.11
Sites BOP (percent, mean $\pm$ SEM) ^c	8.9 $\pm$ 2.6	23.8 $\pm$ 2.9	45.6 $\pm$ 5.4
Probing Depth (mm) (mean $\pm$ SEM) ^c	2.42 $\pm$ 0.05	2.56 $\pm$ 0.03	3.11 $\pm$ 0.07
Attachment Level (mm) (mean $\pm$ SEM) ^c	1.42 $\pm$ 0.04	1.62 $\pm$ 0.03	2.11 $\pm$ 0.06

^a p = 0.001 regression of gcf on clinical categories, adjusted for smoking^b p < 0.076 (1 way analysis of variances across means of clinical categories)^c p < 0.0001 (1 way analysis of variances across means of clinical categories)

Table 3Correlations Between GCF IL-1 β and Clinical Parameters

	Pearson Correlation (Parametric)	Spearman Rank (Non-Parametric)
All subjects (n=190)		
Mean GI	0.17 (p=0.016)	0.25 (p<0.001)
Mean PD	0.23 (p=0.001)	0.18 (p=0.014)
Mean % BOP	0.26 (p<0.001)	0.20 (p=0.006)
Non smokers (n=129)		
Mean GI	0.14 (p=0.107)	0.21 (p=0.019)
Mean PD	0.19 (p=0.027)	0.07 (p=0.410)
Mean % BOP	0.23 (p=0.008)	0.15 (p=0.094)
Former smokers (n=30)		
Mean GI	0.39 (p=0.034)	0.31 (p=0.097)
Mean PD	0.40 (p=0.030)	0.48 (p=0.008)
Mean % BOP	0.26 (p=0.170)	0.13 (p=0.495)
Current smokers (n=30)		
Mean GI	0.33 (p=0.079)	0.38 (p=0.037)
Mean PD	0.43 (p=0.018)	0.49 (p=0.006)
Mean % BOP	0.54 (p=0.002)	0.47 (p=0.008)

Table 4Gingival Crevice Fluid IL-1 β levels by IL-1 α and IL-1 β Genotype Groups

IL-1 α	IL-1 β	n ^a	GCF IL-1 β (pg) $\pm$ SEM ^b
1.1	1.1	99	17.82 $\pm$ 1.82
	1.2	14	16.81 $\pm$ 3.89
1.2	1.1	16	16.04 $\pm$ 3.58
	1.2	45	18.13 $\pm$ 3.61
	2.2	2	14.89 $\pm$ 7.49
2.2	1.1	2	11.20 $\pm$ 0.55
	1.2	5	25.02 $\pm$ 6.81
	2.2	6	16.07 $\pm$ 4.88

■ = IL-1 Composite Genotype positive

^a n = 189, one subject with no IL1 α classification.

^b SEM = Standard Error of the Mean

No differences were seen in COMPOSITE IL-1 GENETIC TEST positive and COMPOSITE IL-1 GENETIC TEST negative GCF-IL-1 levels

Table 5

Gingival Crevice Fluid IL-1 β and Associations with Site Clinical Measures by Subject IL-1 β Genotype and Race/Ethnicity

	GCF IL-1 β (pg) $\pm$ SEM	Mean GI	Mean % BOP	Mean PD
Genotype negative				
Asian (n = 13)	22.4 $\pm$ 5.9	0.63 $\pm$ 0.13	17.3 $\pm$ 5.9	2.6 $\pm$ 0.1
African American (n = 26)	15.3 $\pm$ 2.8	0.96 $\pm$ 0.13	33.7 $\pm$ 6.5	2.8 $\pm$ 0.1
Hispanic (n = 8)	16.4 $\pm$ 4.4	1.38 $\pm$ 0.27	28.1 $\pm$ 8.8	2.9 $\pm$ 0.2
Caucasian (n = 78)	16.8 $\pm$ 2.0	0.90 $\pm$ 0.06	23.1 $\pm$ 3.1	2.6 $\pm$ 0.04
Other (n = 7)	20.0 $\pm$ 7.4	1.07 $\pm$ 0.20	21.4 $\pm$ 11.5	2.8 $\pm$ 0.2
Caucasian non-smokers (n=59)	18.3 $\pm$ 2.4	0.86 $\pm$ 0.07	22.9 $\pm$ 3.4	2.6 $\pm$ 0.1
All subjects n = 132	17.3 $\pm$ 1.5	0.92 $\pm$ 0.05	25.0 $\pm$ 2.5	2.7 $\pm$ 0.04
Genotype Positive				
Asian (n = 3)	42.0 $\pm$ 25.4	1.08 $\pm$ 0.17	66.7 $\pm$ 33.3	2.7 $\pm$ 0.3
African American (n = 4)	13.2 $\pm$ 3.8	1.31 $\pm$ 0.31	25.0 $\pm$ 17.7	2.7 $\pm$ 0.2
Hispanic (n = 6)	10.9 $\pm$ 3.9	1.29 $\pm$ 0.58	29.2 $\pm$ 18.7	2.6 $\pm$ 0.3
Caucasian (n = 42)	16.5 $\pm$ 2.6	0.90 $\pm$ 0.10	24.4 $\pm$ 5.5	2.7 $\pm$ 0.1
Other (n=3)	42.8 $\pm$ 36.3	0.58 $\pm$ 0.22	25.0 $\pm$ 25.0	2.6 $\pm$ 0.2
Caucasian non-smokers (n=27)	19.5 $\pm$ 3.71	0.88 $\pm$ 0.13	27.8 $\pm$ 7.3	2.7 $\pm$ 0.1
All subjects (n = 58)	18.4 $\pm$ 2.9	0.96 $\pm$ 0.10	27.2 $\pm$ 5.0	2.7 $\pm$ 0.06

No differences in GCF IL-1 β in IL-1 genotype positive and negative subjects in the 4 ethnic/racial groups (Asian, African American, Hispanic, Caucasian). Subjects in other race/ethnicity categories not included in comparisons.

Table 6
Gingival Crevice Fluid IL-1 β levels in all subjects and by IL-1 Genotype and Smoking History

	All Subjects (Caucasian Subjects only)		IL-1 Genotype Negative		IL-1 Genotype Positive	
	GCF IL-1 β (pg) $\pm$ SEM	N = 132 (N=78)	GCF IL-1 β (pg) $\pm$ SEM*	N = 57 (N=41)	GCF IL-1 β (pg) $\pm$ SEM	
Never Smokers						
All (Caucasian)	19.2 $\pm$ 1.8 18.7 $\pm$ 2.0	91 59	18.1 $\pm$ 1.9 18.3 $\pm$ 2.4	38 27	21.8 $\pm$ 4.2 19.5 $\pm$ 3.7	
Former smokers						
All (Caucasian)	14.3 $\pm$ 2.8 11.0 $\pm$ 2.8	20 12	16.6 $\pm$ 4.1 12.4 $\pm$ 4.6	10 8	9.6 $\pm$ 2.3 8.8 $\pm$ 1.3	
Current smokers						
All (Caucasian)	15.0 $\pm$ 2.0 14.9 $\pm$ 3.8	21 7	14.8 $\pm$ 2.6 14.4 $\pm$ 6.3	9 6	15.4 $\pm$ 3.3 15.5 $\pm$ 4.6	

*SEM = Standard Error of the Mean

All subjects in boldface.

No significant differences by ANOVA between IL-1 genotypes within smoking groups for either all subjects or Caucasian subjects only.

One subject with no smoking history data.