Interleukin-1 Genetic Association With Periodontitis in Clinical Practice

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Background: Periodontitis is a bacterial disease modified by multiple risk factors. The pro-inflammatory cytokine interleukin-1 (IL-1) is a key regulator of the host responses to microbial infection and a major modulator of extracellular matrix catabolism and bone resorption. It has been reported that variations in the IL-1 gene cluster on chromosome 2 are associated with increased susceptibility to severe adult periodontitis.

Methods: The present study evaluated the association between a composite IL-1 genotype, including allele 2 at each of two loci (IL-1A +4845 plus IL-1B +3954), and a broad spectrum of periodontally healthy to diseased patients in a population that is typically encountered in a dental practice setting. Ninety patients, non-smokers or former smokers with less than 10 pack-year (pk/yr) history, were recruited from a private dental practice. The major outcome variable was bone loss determined by computerized linear measurements of radiographs. Genotypes were analyzed from finger-stick blood samples using previously reported methods.

Results: Multivariate logistic regression models demonstrated that patient age, former smoking history, and the IL-1 genotype were significantly associated with severity of adult periodontitis. For non-smokers or former light smokers (<5 pk/yr), IL-1 genotype positives were at increased odds ratio of having moderate to severe periodontal disease of 3.75 (95% CI: 1.04-13.50) to 5.27 (95% CI: 1.23-22.70), depending on ethnicity, compared to IL-1 genotype negatives. Former moderate smokers (>5 pk/yr and <10 pk/yr) who were IL-1 genotype negative were at increased odds ratio of having moderate to severe periodontal disease of 7.43 (95% CI: 1.20-46.20) compared to non-smokers or former light smokers who were IL-1 genotype negative. In addition, past smoking history was also a significant effect modifier as demonstrated by the statistically significant interaction between past smoking history status and IL-1 genotype status.

Conclusions: This study demonstrates that the composite IL-1 genotype is significantly associated with the severity of adult periodontitis. It also confirmed that both IL-1 genotyping and smoking history provide objective risk factors for periodontal disease in a private practice environment. J Periodontol 2000;71:156-163.

KEY WORDS
Interleukin-1; genotype; smoking; risk factors; periodontitis/epidemiology.

Although bacteria are the clear initiators and perpetuators of periodontitis, there is increasing evidence to suggest that host factors, such as diabetes, smoking, and genetics contribute to the exact clinical appearance, distribution of lesions, and severity of destruction.1,2 These observations indicate that periodontitis behaves clinically in a manner that is similar to many common diseases involving multiple factors that together determine the clinical presentation of the disease in a specific individual.3 Thus, the widespread, more advanced disease usually occurs primarily in a subset of the population.4

Studies of twins and epidemiological investigations on the natural history of periodontitis have suggested that genetic factors, not just the amount or type of plaque, play a major role in determining the actual clinical presentation of adult periodontitis.5-7 It has been estimated by some investigators that less than 20% of the variability in periodontal disease expression can be explained by the quantity of specific bacteria found in disease associated plaque.8 These findings provide at least a partial explanation of the clinical observations that some patients with a little plaque may have a lot of disease while other patients with a lot of plaque may have only minor involvement. A key role for a genetic influence...
in adult periodontitis was recently suggested by investigators who described an association between a specific genetic marker and severity of periodontitis in non-smoking adults. The marker includes 2 genetic polymorphisms in the interleukin-1 (IL-1) gene cluster on chromosome 2, one of which is in the IL-1B gene and is associated with up to 4-fold increases in IL-1 production. A recent study by Gore et al. also reported that allele 2 of IL-1B (+3954) genotype was significantly increased among patients with adult periodontitis compared to those with early and moderate disease. Investigators using a commercially available test to identify the genotype of periodontitis patients in a long-term periodontal maintenance program, investigators have also reported a significant correlation between the genotype and tooth loss.

The present study evaluated the association between the IL-1 genetic polymorphisms and a broad spectrum of periodontally healthy to diseased patients in a population that is typically encountered in a dental practice setting.

MATERIALS AND METHODS
The study employed a case control design involving 2 groups of patients in a private dental practice in Atlanta, Georgia. The protocol was approved by an Institutional Review Board and complied with Good Clinical Practices as defined in the relevant sections of Title 21 of the Code of Federal Regulations. Patients signed an informed consent to participate in the study.

Demographic and personal data were collected from patients, including medical history, birth date, gender, smoking status, and family history. In addition, self-reported family origin was included. Patients were asked to classify themselves, their mother, father, and grandparents into 1 of the 9 categories: Northern European, Southern European, Eastern European, Asian, Middle Eastern, African-American, Hispanic, Native American, and unknown.

One hundred three adults were initially recruited based on the following general selection criteria: 1) at least 35 years of age and were in good general health at the time radiographs were taken; and 2) had never smoked, or had quit smoking at least 5 years prior to the date radiographs were taken and had a pack-year history of less than or equal to 10 (pack-years were calculated by multiplying the number of years smoked by the average number of cigarette packs smoked per day). Control group subjects were either periodontally healthy or had mild periodontitis. In addition, control subjects had: 1) at least 24 teeth at the time radiographs were taken; and 2) no more than 2 sites with radiographic bone loss ≥3.0 mm (see radiographic methods below). Test group subjects had generalized moderate to severe periodontitis and 1) were <55 years of age at the time the radiographs were taken; 2) had at least 12 teeth at the time radiographs were taken; and 3) had a total mouth mean interproximal radiographic bone loss of ≥3.0 mm or at least 10 teeth with at least 1 site on each tooth with ≥3.0 mm bone loss and at least 2 sites in each quadrant with ≥3.0 mm bone loss (see radiographic methods below).

Subjects were excluded from the study prior to the time radiographs were taken for the following reasons: pregnancy or lactation; diabetes; HIV infection; bleeding disorders; immunosuppressive chemotherapy; or severely compromised immune function. In addition, patients in the control group were exited from the study for any condition requiring antibiotic premedication for dental appointments, chronic usage of >325 mg aspirin or non-steroidal anti-inflammatory per day for any condition within 3 years immediately preceding the date radiographs were taken, or heavy or continual antibiotic usage.

Radiographic Methods
Radiographs for control patients were either full mouth periapical radiographs or posterior vertical bite-wing radiographs or anterior and posterior vertical bite-wing radiographs. All radiographs were of sufficient quality and appropriate geometry to expose up to 2 mm of bone supporting the teeth from both the mandibular and maxillary arch. Only full mouth periapical radiographs were acceptable for patients in the test group.

Assessment for bone loss was measured at interproximal sites on radiographs of diagnostic quality in the practice office. Bone loss assessments were made from the midpoint of the second molar or the distal interproximal bone level of the most distal tooth in the quadrant to the mesial of the central incisor in each quadrant.

Radiographs were coded and submitted to an independent contract organization for analysis of bone loss on a masked basis with no knowledge of the genotype status of the subject. Radiographs were digitized and the distance from the cemento-enamel junction to the crest of the alveolar bone was measured using calibrated protocols and customized software.

Genotyping Methods
Finger stick blood samples were obtained using an automatic lancet processed and analyzed according to previous methods. The subject’s index or middle finger was cleaned with antiseptic wipes and then punctured with the lancet to produce approximately 2 full drops of blood. The blood was placed onto a blotting paper card which was free of DNAase or
RNAase, and the cards were coded for identification purposes. The cards were allowed to air-dry at room temperature for approximately 2 to 16 hours and then placed in individual envelopes. The envelopes were sealed with adhesive tape and stored in a refrigerator at 4°C for later analysis. The cards were sent to the Department of Molecular Genetics and Medicine at the University of Sheffield, United Kingdom, for genotyping of IL-1A (+4845) and IL-1B (+3954) polymorphisms. Genotyping was performed employing published polymerase chain reaction (PCR) and restriction fragment length product (RFLP) techniques. Laboratory personnel responsible for the genotyping did not know the clinical status or identity of the blood donors.

For subjects to be positive for the composite IL-1 genotype they must have at least one copy of allele 2 at each of 2 specific polymorphisms in the cluster of IL-1 genes found on chromosome 2. In a previous report, the composite IL-1 genotype was described as allele 2 at the IL-1A (−889) locus plus allele 2 at the IL-1B (+3954) locus. It has recently been determined that another polymorphism at IL-1A (+4845) is essentially 100% concordant with the IL-1A (−889) locus. Since the IL-1A (+4845) polymorphism is more easily assayed, it may be substituted for the IL-1A (−889) locus. In addition, the IL-1B polymorphism was previously referred to as IL-1B (+3953). We have renumbered this polymorphism to IL-1B (+3954) according to the convention, now widely accepted, that numbering from the start site of transcription begins at +1 instead of zero. Details of the PCR-RFLP methods are summarized below.

**IL-1A (+4845).** Forward primer: 5’-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA - 3’. Reverse primer: 5’-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT - 3’. Cycling was carried out for 1 cycle at 95°C for 1 minute, 35 cycles each at 94°C for 1 minute, 56°C for 1 minute, 72°C for 2 minutes, and 1 cycle at 72°C for 5 minutes. Digestion of PCR products with **Fnu4H I** yielded 29 + 124 bp fragments (allele 1[G]) and a single 153 bp fragment (allele 2[T]). In both cases, a constant 76 bp band was also produced which served as a restriction control site.

**IL-1B (+3954).** Forward primer: 5’-CTC AGG TGT CCT CTA AAG CAA - 3’. Reverse primer: 5’-GCT TTT TTT GGG TTA TAC TGA CCG - 3’. Cycling was carried out for 1 cycle at 95°C for 2 minutes, 38 cycles for 1 minute each at 95°C, 67.5°C, and 74°C; and 1 cycle at 72°C for 8 minutes. Digestion of PCR products with **Taq I** yielded 85 + 97 bp fragments (allele 1[C]) and a single 182 bp fragment (allele 2[T]). In both cases a constant 12 bp band was also produced which served as a restriction control site.

**Data Management**

Data collection consisted of obtaining informed consent, recording the subject’s medical and medication history, smoking history, number of missing teeth at the time radiographs were taken, and family origin data.

**Biometrics and Data Analysis**

As described above, periodontal bone loss was determined using radiographic measurements. The mean alveolar bone loss for each patient was obtained by averaging the bone loss at each measured site. Disease classification was based on each individual’s mean bone loss as well as site bone loss, as described for the test and control group.

Smoking history is considered an important risk factor for periodontal disease with dose dependent effect. Previous investigations have shown that individuals smoking >10 cigarettes per day (one-half pack) have a increased disease severity and a less favorable response to therapy. In the present study, all of the former smokers who smoked more than one-half pack/day had a smoking history of at least 5 pack-years. In order to adjust for the effect of smoking history on periodontal disease, subjects were classified into 2 groups: Group 1 consisted of subjects who had never smoked (referred to as non-smokers) or who had <5 pack-year history (referred to as former light smokers). Group 2 consisted of subjects who were former smokers for ≥5 pack years but ≤10 pack years (referred to as former moderate smokers).

Data were analyzed according to both disease classification and mean bone loss. Multivariate logistic regression was utilized to assess the relationship of genotype to disease status while adjusting for potential confounders. Confounders considered for this analysis included age, gender, and smoking history. Odds ratios were calculated with 95% confidence intervals.

**RESULTS**

A total of 103 patients were initially enrolled in the study. Thirteen patients were exited because they did not meet the radiographic criteria. A total of 90 patients completed the study. Forty-four (44) patients met the criteria for the test group and were categorized as having moderate to severe periodontitis with mean bone loss of 3.80 mm ± 0.80 mm (range = 2.61 mm to 5.67 mm). Forty-six (46) patients met the criteria for the control group and were classified as being periodontally healthy or having mild periodontitis. These patients had a mean bone loss of 1.50 mm ± 0.27 mm (range = 0.93 mm to 2.17 mm) (Table 1). The mean bone loss (average whole mouth score) was verified as a proper index for patient classification. Despite the highly skewed nature of bone loss values for the moderate to severe patients, the average whole mouth scores approximated a normal distribution, as verified by the Shapiro-Wilk test (P >0.1). No significant differences were observed when the average whole mouth

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scores were compared using repeated measures analysis of variance with average scores computed for molars, pre-molars, and anterior teeth separately \((P > 0.2)\) (Table 1). The results of these tests permit the use of average whole mouth scores (mean bone loss) as suitable for quantifying periodontal bone loss for each patient.

The initial data analysis focused on the correlation between disease status and each individual risk factor, without adjusting for other potential confounding factors. Because of the multifactorial nature of adult periodontitis, the data were further analyzed with a multivariate logistic regression model. Genotype effects on adult periodontitis were determined after adjusting for other potential confounding factors, such as smoking and age. In order to compare to previous findings, the logistic regression was also used to analyze data limited to patients of European heritage.

**Correlation Between Severity of Adult Periodontitis and a Single Risk Factor (unadjusted for other risk factors)**

Genotype status and periodontal disease severity are shown in Table 2. Although 41% of patients in the moderate to severe group were IL-1 genotype positive compared to 28% of patients in the healthy to mild group, there was no statistically significant association between genotype status and periodontal disease.

Smoking has been considered an important risk factor for progression and prognosis of periodontal disease\(^ {17-24}\) with dose dependent effect. Because the effect of periodontal bone loss from smoking is irreversible, the past smoking history for former smokers may also affect the periodontal disease outcome. The smoking history for each disease group is shown in Table 3. In this currently non-smoking population, 28/90 patients (31%) were former smokers. Eleven of 46 or 24% of the patients in the healthy to mild periodontitis group were former smokers, whereas 17/44 or 39% of the moderate to severe periodontitis were former smokers. Previous investigators\(^ {17,20,24}\) have shown that those individuals smoking >10 cigarettes per day have an increased risk of attachment loss and a less favorable response to intervention. In the present study all of the former smokers who smoked more than one-half pack/day had a smoking history of at least 5 pack-years. In the control group, 5/46 (11%) of the patients had a smoking history of more than 5 pack-years, compared to 15/44 (34%) in the test group (Table 4). The association of former moderate smoking (i.e., \(\geq 5\) and \(\leq 10\) pack-year history) and periodontal disease severity in this population was significant (odds ratio = 4.24; 95% CI: 1.39 to 12.98; \(P = 0.008\)).

Besides smoking and genotype, other factors were considered for analysis, including gender and age. Gender was not significantly associated with periodontal disease severity (odds ratio = 2.04 for males compared to females; 95% CI: 0.86 to 4.84; \(P = 0.106\)). However, age was significantly associated with periodontal disease severity (odds ratio = 1.26 for each additional year of age; 95% CI: 1.15 to 1.38; \(P < 0.001\)).

**Multivariate Logistic Regression Models**

In order to evaluate the association of the IL-1 genotype to periodontal disease severity while adjusting for significant confounders, a logistic regression model was fit. The final model is given in Table 5. As can be seen in Table 5, age and past smoking history
were significant confounders. In addition, past smoking history was also a significant effect modifier as demonstrated by the statistically significant interaction between past smoking history status and IL-1 genotype status. For non-smokers or former light smokers, IL-1 genotype positives were at increased odds of having moderate to severe periodontal disease of 3.75 compared to IL-1 genotype negatives ($P = 0.043$). Former moderate smokers who were IL-1 genotype negative were at increased odds of having moderate to severe periodontal disease of 7.43 compared to non-smokers or former light smokers who were IL-1 genotype negative ($P = 0.031$). Former moderate smokers who were IL-1 genotype positive had an increased odds of having moderate to severe periodontal disease of 1.68 compared to non-smokers or former light smokers who were IL-1 genotype negative ($P = 0.045$).

**Analysis of Northern European Origin Patients**

Because the data of genotype distribution for other ethnic groups were limited and also in order to compare to a previously reported genotyped population by Kornman et al.,9 the next analysis evaluated only patients of European heritage ($n = 74$, or 82% of the study population) (Table 6). In this subset of patients, the IL-1 genotype was again significantly associated with periodontitis ($P = 0.026$). Similar to the analysis of all patients described above, smoking and age were both significant confounders ($\alpha = 0.10$) along with smoking being an effect modifier. In this subset, IL-1 genotype positive non-smokers or former light smokers were at increased odds of having moderate to severe periodontal disease of 5.27 compared to IL-1 genotype negative non-smokers or former light smokers ($P = 0.026$). Borderline significance was noted between smoking and periodontal disease severity ($P = 0.088$) after being adjusted for other factors. IL-1 genotype positive former moderate smokers had an increased odds of having moderate to severe periodontitis of 1.09 compared to IL-1 genotype negative non-smokers or former light smokers ($P = 0.031$).

**DISCUSSION**

Clinical examination of patients provides information about the cumulative disease history of the patient; i.e., current disease severity, current signs of bacterial levels, and inflammation.26 The experienced clinician makes an explicit or an implicit assessment of the patient’s likelihood for future disease and expectation of response to therapy. The treatment plan is then devised based on those expectations. Of course, there are no facts about the future, only predictions based on what is currently known by the clinician. As new information becomes available, estimates of the future course of the patient’s status changes. For example, one would be more likely to assign a worse prognosis for a 40-year old smoker with mild periodontitis than for a non-smoking patient of comparable age and disease.

A true disease susceptibility test provides information about a patient’s potential for future disease. Of
course, a risk factor is not intended to measure current disease, so a positive susceptibility test is not synonymous with a diagnosis of disease, but rather an indication of future risk. In addition, since periodontitis is a multifactorial disease, one should not expect that a single risk factor correlates directly with all cases of disease. For example, not all severe periodontitis patients would be expected to be diabetic or heavy smokers. However, it is well established that patients who are heavy smokers or who are poorly controlled diabetics have a significantly increased risk for more severe periodontal disease. The IL-1 genotype appears to be another significant risk factor for more severe adult periodontitis.

High levels of IL-1 have been found in gingival crevicular fluid and periodontal tissue samples associated with severe inflammation and destruction. It has been reported that IL-1 genotype is also associated with severe adult periodontitis. The present study reinforced the association of the IL-1 genotype as a risk factor for more severe adult periodontitis. Without considering other confounding variables, there was an apparent trend of a higher percentage of genotype positive patients in the diseased group than in the healthy group, even though no statistically significant association was found. After adjusting for other confounders, such as age and smoking, the multivariate logistic regression model clearly showed a strong correlation between genotype and periodontal disease. The results demonstrated that IL-1 genotype positive non-smokers or former light smokers were at increased odds of having moderate to severe periodontal disease of 3.75 compared to IL-1 genotype negative patients with comparable smoking status. Similar results were also obtained among patients of European heritage (odds ratio = 5.27).

The strength of the smoking influence on disease is also evident in the present study. Although all current smokers and former smokers with >10 pack-year history were excluded from the study, former smokers in this study population with a 5 to 10 pack-year history (i.e., former moderate smokers) appeared to be at increased risk for more severe disease. IL-1 genotype negative former moderate smokers are at increased odds of having moderate to severe periodontal disease of 7.43 compared to IL-1 genotype negative non-smokers or former light smokers. These findings explain the absence of statistical association between genotype and the disease when genotype was the only factor considered, and the possibility that smoking, even at this level (history of 5 to 10 pack-years), had already caused sufficient disease to mask the influence of other risk factors.

The effect of smoking in the development and prognosis of periodontal diseases has been studied extensively. Cumulative periodontal destruction shows a dose-response relationship to smoking. Other clinical observations have noted a significant smoking effect on outcomes in patients smoking >10 cigarettes per day. In a prospective longitudinal study of periodontal therapy, individuals who smoked >10 cigarettes per day had significantly less improvement in both probing depth and clinical attachment level than non-smokers. Since genotype contributes significantly to the development of periodontal disease, it would be interesting to investigate the interaction between smoking and genotype in their effects on periodontal disease.

A potential interaction of smoking and genotype was noted in the present study. According to the logistic regression model, past smoking history was shown to be a significant effect modifier to genotype as demonstrated by the statistically significant interaction between past smoking history and IL-1 genotype. In the statistical analysis of this specific population, the presence of both former moderate smoking history (≥5 to ≤10 pack/year) and positive genotype showed a lower likelihood of developing the disease when compared to those with presence of only one of the risk factors. Such interaction findings are not uncommon in studies with small sample sizes and there are a few possible explanations that relate to inadvertent sample bias. This might occur, for example, due to: 1) just random patient selection phenomena; i.e., another study would not find the same effect; 2) those who had both risk factors may have lost teeth at an earlier time and were not eligible for entry into this study; or 3) patients with positive genotype and a relatively heavy smoking habit may have experienced signs of periodontal disease or other systemic health problems at an earlier age, prompting them to quit smoking and change home care habits and life style. Since another study had found that individuals who are heavy smokers plus genotype positive are at a synergistic risk for future tooth loss, the potential interaction between smoking and genotype should be re-evaluated in studies specifically designed to investigate these relationships.

While smoking increases the risk of developing periodontal disease, non-smokers are not guaranteed of being periodontally healthy in the future. Thus, some individuals who have no signs of disease may have risk factors for future disease, whereas some patients with advanced disease may not have a specific individual risk factor. In addition to smoking, diabetes, and the IL-1 genotype, other factors that have been associated with increased risk for more severe adult disease include estrogen depletion after menopause and certain types of psychosocial stress. A genetic susceptibility test does not guarantee the future outcome, but it does indicate the probability of a particular outcome.

Patients who have the genetic susceptibility...
marker produce more IL-1 in response to plaque than genotype negative individuals. This is important because IL-1 is a powerful regulator of the inflammatory response and increased amounts of IL-1 have been shown to be strongly associated with the immuno-pathology of the progressive periodontitis lesion. IL-1 is also known to be a critical determinant of bone and connective tissue destruction. IL-1 levels are higher in GCF from sites with periodontitis and recent findings indicate that specifically blocking both IL-1 and TNFα significantly reduced periodontal bone destruction in ligature induced periodontitis, in spite of a heavy bacterial challenge.

It is, of course, well established that specific bacteria initiate periodontitis. Since the IL-1 genetic factor is involved in immuno-inflammatory processes, the clinical effects of the genetic factor depend on the presence of bacteria to initiate the inflammation. The patient with this genetic factor is, therefore, not automatically condemned to severe periodontitis. Although direct data are not yet available, one would expect that removing the bacteria would ameliorate the effect of risk factors such as the IL-1 genotype. In the present study 28% of the healthy or mild periodontitis patients were genotype positive (Table 2). This finding reinforces the fact that a risk factor, such as the IL-1 genotype, can be present without always being associated with clinical disease. This is consistent with risk factors in other multifactorial diseases. For example, although elevated cholesterol is a well-established risk factor for cardiovascular disease, not everyone with elevated cholesterol develops overt clinical disease. One may postulate that the genotype positive patients with no periodontitis are likely controlling other factors that are necessary for disease, such as bacterial load.

Removing plaque (and calculus) and keeping it at low levels is sufficient to maintain periodontal health in the vast majority of individuals at risk. It is an accepted principle of periodontal therapy that the clinician must assess the level of future disease risk for each individual patient and design the frequency and extent of professional support that is necessary to maintain the attachment level. Therefore, for the highly susceptible person, it is reasonable to assume that plaque must be controlled to a greater degree than for the less susceptible individual. Since the data indicate that patients who have the specific IL-1 risk factor are associated with more severe periodontitis, more frequent professional care may be an appropriate strategy to manage the increased risk.

Knowledge of factors, such as smoking and the IL-1 genotype that are significantly associated more severe periodontitis, should therefore enhance the clinician’s ability to estimate the future course of disease for a specific patient. In addition, such information may be used to modify the patient’s risk and to guide prevention and therapy.

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REFERENCES
14. Hausmann E, Allen K. Reproducibility of bone height


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