Association of Interleukin-1 Polymorphisms With Aggressive Periodontitis

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Background: Genetic polymorphisms for interleukin (IL)-1α and -1β have been proposed as potential genetic markers for periodontal diseases. Since the prevalence of these polymorphisms could be race-related, and no data exist about the frequency of these polymorphisms in the Chilean population, the aim of the current study was to investigate the association of the interleukin-1 gene polymorphisms with aggressive periodontitis (AgP).

Methods: Thirty-six patients with AgP, 75 healthy controls, and 75 subjects of unknown periodontal status (reference population) were genotyped for the IL-1A –889 and IL-1B +3954 loci, by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion and gel electrophoresis. Data were analyzed using the chi-square test, calculating odds ratios (OR) and confidence intervals (CI).

Results: The prevalence of the positive composite IL-1 genotype was higher in patients (25%) than in healthy controls (12%), but the difference was not significant ($P = 0.14$). The IL-1B +3954 homozygous for allele 1 frequency was higher in controls than in patients suggesting a protective factor for AgP. The heterozygous for allele 2 of the IL-1B showed a significant association with AgP (OR = 2.86, 95% CI 1.06 to 7.71, $P = 0.030$). No association was observed in localized AgP and generalized AgP between the extent of disease and the presence of the composite positive genotype. Because the number of smokers was too small in patients and in controls, no other analyses were performed.

Conclusion: The results of the present study support a positive association between AgP and the presence of the IL-1B +3954 allele 2 polymorphism. *J Periodontol 2004;75:1509-1515.*

KEY WORDS

Chileans; genetic markers; interleukin-1; periodontal diseases/diagnosis; periodontitis, aggressive/diagnosis; polymorphism, genetic; racial stocks.

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Aggressive periodontitis (AgP) is a group of periodontal diseases characterized by localized or generalized loss of alveolar bone usually affecting individuals under 30 years of age.[1] The primary causal factors for periodontitis are bacteria, but the extent and severity of periodontal lesions can be influenced by environmental factors, acquired diseases, and genetic predisposition.[2,3] The results of family studies indicate that genetic factors seem to have a strong influence on susceptibility to AgP.[4,5]

Several proinflammatory cytokines, such as interleukin-1α and interleukin 1-β, have been implicated in the immunopathology of periodontitis.[6] The role of inflammatory mediators in the pathogenesis of periodontal disease is well established.[7-9] IL-1 has been particularly studied as a critical determinant of bone and connective tissue destruction[7,10] and, therefore, playing a significant role in the pathogenesis of periodontal diseases. Furthermore, some studies have reported that increased levels of both IL-1α and IL-1β in gingival crevicular fluid correlate with the severity of periodontal disease.[11,12] Variation in cytokine levels among individuals is well documented[13-15] and may contribute to disease susceptibility. Such differences may be attributed in part to the alleles of polymorphic cytokine genes, since particular alleles have been associated with increased cytokine levels.[16]

There are three genes that regulate the production of IL-1, located in a 415 kb region on the long arm of the chromosome 2q13.[17,18] The IL-1 gene cluster includes IL-1A, IL-1B, and IL-1RN genes that code for IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra), respectively. Polymorphisms

of the IL-1 gene cluster have been implicated with the susceptibility and severity of various chronic inflammatory diseases[16,19-22] including marginal periodontitis.[23]

Several studies[23-26] have indicated a role for interleukin-1 gene cluster polymorphism in the risk assessment for adult periodontitis, specifically for individuals carrying the allele 2 of the biallelic restriction fragment length polymorphism of the IL-1B +3954 and the IL-1A –889 loci in either the heterozygous or homozygous state at both loci. These subjects were found to have a significantly greater risk for developing severe periodontitis when compared to a mild periodontitis group or to periodontally healthy subjects.[23-26]

The possible role of the IL-1A and IL-1B genetic polymorphisms were evaluated in early-onset periodontitis (EOP), now defined as aggressive periodontitis, in 28 African American and seven Caucasian families using the transmission disequilibrium test.[27] Allele 1 of the IL-1A –889 polymorphism and allele 1 of allele 1B +3954 polymorphism were transmitted significantly more often than allele 2 in generalized EOP cases, now defined as generalized aggressive periodontitis (GAgP). A similar trend was reported for localized juvenile periodontitis (LJP), now defined as localized aggressive periodontitis (LAgP), although the difference was not statistically significant. Evidence for linkage disequilibrium for allele 1 of the IL-1B +3954 locus was even stronger for the GAgP. Allele 1 at IL-1B +3954 was transmitted more often than allele 2 for LAgP cases, but the difference was not statistically significant. Based on these results and on the observation of the predominant form of IL-1 in periodontal tissue is IL-1β,[6,7] it has been suggested that the IL-1B +3954 polymorphism may be more important than the IL-1A –889 polymorphism for the association with the AgP phenotype. Walker et al.[28] found that 8% of African American patients with LAgP and 14.5% of control subjects were genotype positive, and that the prevalence of the IL-1B +3954 allele 1 polymorphism was higher than 99% in control individuals and 100% in LAgP patients. Hodge et al.[29] did not find an association between IL-1A and IL-1B genetic polymorphisms with GAgP in unrelated European Caucasian patients. However, another study[30] found that an IL-1B genotype in combination with smoking are risk factors for AgP.

Since genetic polymorphisms vary in different ethnic populations, conclusions about disease association cannot be extended to other populations. The contemporary mixed population of Santiago, Chile, stems from the admixture of aboriginal Amerindians and Spanish, with an average Amerindian admixture of 37%.[31]

The aim of the present study was to evaluate the prevalence of IL-1 genotypes in the Chilean population and the association of IL-1A –889 and IL-1B +3954 polymorphisms with AgP.

MATERIALS AND METHODS

Study Populations

AgP patients (N = 36) aged 17 to 42 years (mean age 35 ± 4.3) attending a public health center and a private dental clinic in Santiago, Chile, were recruited over a 2-year period. The clinical diagnosis of the patients was based on American Academy of Periodontology criteria.[32]

For the diagnosis of LAgP: 1) the patient should be 30 years or younger at diagnosis and systemically healthy; 2) interproximal attachment loss of ≥4 mm should be present on at least two permanent teeth, one of which had to be a first molar, and involving no more than two teeth other than first molars and incisors.

For the diagnosis of GAgP: 1) the patient should be 30 years or younger at diagnosis and clinically healthy; 2) generalized interproximal attachment loss of ≥5 mm affecting at least eight teeth, three of which should not be first molars or incisors.

Clinical diagnosis was confirmed by evidence of interproximal bone loss on full-mouth periapical radiographs. Fourteen patients were <35 years old when they were recruited to the study. However, they were <30 years at the time of original diagnosis and available radiographs and data in their clinical records showed a clear history of clinical symptoms prior to the age of 30.

Subjects without periodontal disease (N = 75) were included as healthy controls. The healthy subjects were between 35 to 42 years old (mean age 38 ± 3.2), in good general health, with no history of periodontal therapy, and no proximal sites with clinical attachment level ≥1 mm and probing depth ≤3 mm.

Patients and healthy controls received a full-mouth periodontal examination which included clinical measurement of probing depth (PD) and clinical attachment loss (CAL) at six sites on each tooth using a calibrated manual probe. Patients with AgP were categorized according to the extent of periodontal disease, which was defined as the percentage of sites with clinical attachment loss ≥4 mm. The median of the percentage of affected sites with CAL ≥4 mm was determined, and the patients were assigned to one of two categories according to the percentage of affected sites under or over the median.

Another 75 subjects used as a reference control population were recruited. These individuals were anonymous healthy blood donors, aged 18 to 80 years old (mean age 40 ± 8), with unknown periodontal status and representing a random sample of the Chilean population. The inclusion of this group of subjects was done to determine if subjects recruited in this way, as has been done in previous studies,[29,33] is an appropriate control group.

Since periodontal disease is strongly influenced by cigarette smoking,[34] controlling for the confounding effects of smoking is mandatory in any identification

of risk factors for periodontal disease. Patients and healthy controls were classified as smokers or non-smokers according to their smoking habits. Former smokers were classified as non-smokers if they had stopped smoking >5 years before periodontitis was diagnosed.

Purification of Genomic DNA

Informed written consents were obtained from all study participants, and the study protocol was approved by the institutional review board. Venous blood (7 ml) was taken by standard venipuncture and mixed with 1 mg/ml EDTA. Genomic DNA was extracted from peripheral blood lymphocytes. Samples were obtained according to the method described by Chomczynsky and Sacchi.[35] The resulting DNA samples were stored at –20°C in TE buffer (10 mM Tris, 1 mM EDTA) until required. DNA integrity was checked and DNA was quantified using agarose gel electrophoresis.

Analysis of Genetic Polymorphisms

IL-1A –889. Polymerase chain reaction (PCR) amplifications of the IL-1A –889 were carried out according to Kornman et al.[23] PCR amplification was performed in 50 µl volume containing 100 ng of genomic DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM of MgCl2, 0.2 mM of each dNTP, 0.8 µM of each primers (5′-AAG CTT GTT CTA CCA CCT GAA CTA GGC-3′ and 5′-TTA CAT ATG AGC CTT CCA TG-3′) and 1.5 U of Taq DNA polymerase. The thermal cycles were initiated for 2 minutes at 96°C, followed by 45 cycles of 1 minute at 94°C, 1 minute at 50°C, and 1 minute at 72°C, and a final extension at 72°C for 10 minutes. The PCR products were digested with 5 U of NcoI for 4 hours at 37°C using the conditions described by the supplier,§ and products were dissolved on 3% agarose gel. This gave products of 83bp + 16bp (allele1) and 99pb (allele 2).

IL-1B +3954. PCR amplifications were carried out to detect the IL-1B polymorphism as described by Kornman et al.[23] PCR amplifications were performed in 50 µl volume containing 100 ng of genomic DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM of MgCl2, 0.2 mM of each dNTP, 2 µM of each primer (5′CTC AGG TGT CCT CGA AGA AAT CAA A-3′ and 5′-GCT TTT TTG CTG TGA GTC CCG-3′) and 1.5 U of Taq DNA polymerase. The thermal cycles were initiated for 2 minutes at 95°C, followed by 67.5°C for 1 minute, 74°C for 1 minute cycled 2×, 35 cycles of 95°C for 1 minute, 67.5°C for 1 minute, 74°C for 1 minute 35×, 3× at 95°C for 1 minute, 67.5°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR products were digested with 5 U Taq I at 65°C for 3 hours. The resulting products of 12bp + 85bp + 97bp (allele 1) and 12bp + 182bp (allele 2) were dissolved on 3% agarose gel and ethidium bromide staining.

Table 1.

Characteristics of AgP Patients and Healthy Controls (mean ± SD)

Characteristic	Patients (N = 36)	Healthy Controls (N = 75)
Age	35 ± 4.3	38 ± 3.2
Probing depth	3.51 ± 1.8	1.82 ± 0.29
Clinical attachment level	3.02 ± 1.3	0.47 ± 0.34
Bleeding on probing (%)	63 ± 16	19.32 ± 10.5
Smokers (%)	33.33	58.66

In order to determine the validity of the method, 20 samples were genotyped twice and the results were identical.

Data Analysis

The associations of allele and genotype frequencies in patients, reference population, and healthy controls were analyzed by the chi-square test. The strength of the associations was determined using an odds ratio (OR) calculation and 95% confidence intervals (CI). Statistical significance was set at a $P<0.05$. All analyses were performed using a software program.‖

RESULTS

The clinical characteristics of patients and healthy controls are described in Table 1. As expected, all the clinical characteristics of patients were significantly higher than those of the healthy controls. Of the 36 AgP patients, seven (19.44%) had LAgP and 29 (80.55%) GAgP. The IL-1A and the IL-1B genotype and allele frequencies were essentially similar between the patients and the reference population, without significant differences between the groups ($P>0.05$) (Table 2). Conversely, there were significant differences in distribution of the IL-1A and IL-1B genotypes, and in the distribution of IL-1B alleles 1 and 2 between the reference population and healthy controls. Since the reference population of unknown periodontal status was not equivalent to the healthy controls, for all the following analyses only the healthy subjects were used as the control group.

Prevalence of IL-1A –889 Genotypes and Alleles

A higher frequency of homozygous of the allele 1 of the IL-1A (60% versus 55.50%) and of heterozygous (37.33% versus 33.33%) was observed in the control group than in patients (Table 2); for the IL-1A –889 site gene, 11.11% of patients and 2.66% of controls were

§ New England Biolabs, Beverly, MA.
‖ Version 10, SAS, Cary, NC.

Table 2.

IL-1A –889 and IL-1B +3954 Genotype and Allele Frequencies in AgP Patients, Reference Population, and Healthy Controls

		Patients (N = 36)		Reference Population (N = 75)		Healthy Controls (N = 75)	
	Genotype	N	%	N	%	N	%
IL-1A –889	1/1	20	55.50	42	56	45	60
	1/2	12	33.33	22	29.33	28	37.33
	2/2	4	11.11	11	14.66*	2	2.66*
Allele	1	52	72.22	106	70.6	118	78.66
	2	20	27.77	44	29.4	32	21.33
IL-1B +3954	1/1	25	69.44	49	65.33†	63	84†
	1/2	11	30.55§	24	32‡	10	13.33‡§
	2/2	0	0	2	2.66	2	2.66
Allele	1	61	84.72	122	73.49‡	136	90.66‡
	2	11	15.27	28	26.51‡	14	9.33‡

* $P = 0.020$.
† $P = 0.015$.
‡ $P = 0.011$.
§ $P = 0.030$.

Table 3.

IL-1A –889 and IL-1B +3954 Composite Genotypes in AgP Patients and Healthy Controls

Composite genotype	Patients (N = 36)		Healthy Controls (N = 75)	
IL-1A/IL-1B	N	%	N	%
1-1/1-1	18	50.0	42	56.0
1-2/1-1 or 1-1/1-2	9	25.0	23	30.66
1-2/1-2	5	13.8	6	8.0
1-2/2-2 or 2-2/1-2	4	11.1	3	4.0
2-2/1-1	0	0	1	1.33
Positive genotype	9	25.0	9	12.0

homozygous for the allele 2 (2/2); a higher frequency of IL-1A allele 2 was found in patients than in controls (27.77% versus 21.33%); none of these differences reached statistical significance.

Prevalence of IL-1B +3954 Genotypes and Alleles

For the IL-1B gene site +3954, a higher percentage of healthy controls (84%) than patients (69.44%) was homozygous for allele 1 (1/1); however, the difference was not statistically significant ($P = 0.056$) (Table 2). A significantly higher percentage of patients (30.55%) than healthy controls (13.33%) was heterozygous for the IL-1B ($P = 0.030$), and the heterozygous status for this genotype was significantly associated with AgP (OR = 2.86, 95% CI 1.06 to 7.71).

The prevalence of the IL-1B allele 2 was more elevated in patients (15.27%) than in controls (9.33%), but the difference was not significant ($P = 0.27$).

The total frequency of the composite positive genotype, defined as at least one allele 2 present at each locus, was 20.96%. The frequency of the composite positive genotype was more than two times higher in patients (25%) than in healthy subjects (12%), but the difference was not statistically significant (Table 3). The composite positive genotype was significantly higher in reference controls of unknown periodontal status (28%) than in healthy subjects ($P = 0.025$). Because the number of smokers was so small, statiscal analyses were not performed. The frequency of the composite positive genotype was similar in patients with LAgP (28.57%) and in patients with GAgP (24.13%).

DISCUSSION

Approximately half of the clinical variability in chronic periodontitis has been attributed to genetic factors.[36] There is substantial evidence to support the existence of an association between diseases which involve an inflammatory pathogenesis, such as occurs in periodontal disease, and certain cytokine gene polymorphisms.[23] Several researchers have studied the polymorphic variation in the IL-1 genes in relation to periodontal diseases, but the data do not agree in supporting a positive association between susceptibility to periodontitis and the IL-1 gene polymorphisms.[37]

The presence of at least one allele 2 at each locus of interleukin-1 gene genotype has been associated with increased severity of chronic periodontitis in Caucasian non-smokers,[23,26,38] and in Caucasians with AgP.[30] However, other studies have found no association between IL-1 gene polymorphisms and AgP in Caucasians[33] or in African-Americans.[28] In a Chinese population study[39] it was not possible to associate these polymorphisms with periodontal disease due to the low prevalence of IL-1 composite positive genotype in that population. The frequency of specific IL-1 alleles and positive genotype has been reported to vary in

different ethnic populations. A frequency of positive genotype in the range between 29.1% to 46% has been described in the European population,[26,29,30,33] a range between 29% to 38% of genotype-positive subjects has been reported in the U.S. Caucasian population,[23,24,38,40,41] and 26% genotype-positive subjects was found in a Mexican population.[42] The lowest frequencies of positive genotype have been found in Chinese (2.3%)[39] and in African Americans (14%).[28] The total prevalence of 20.96% of genotype-positive subjects found in the present study is slightly lower than that of Mexican[42] and that of European populations.[33,37,43-47]

In the current study, the homozygous for allele 1 for both the IL-1A and IL-1B was the most frequent genotype detected both in the patient and both control groups. The frequencies for the IL-1A –889 polymorphisms found in the present study agree with reported data in Caucasians.[33,37,43] However, no association was found between AgP and IL-1A –889 genotype. The frequency of the IL-1A allele 2 carriage in patients (27.7%) and in healthy controls (21.3%) in the present study is lower than other reports where carriage frequencies ranging from 39.7% to 48.5% were found.[25,30,43-46] Otherwise, the prevalence of the genotype homozygous for allele 1 for the IL-1B found in healthy controls in the present study (84%) is higher than that found in Caucasian control subjects (range 38.9% to 62.5%),[26,30,33,44] in AgP patients (range 50% to 71%),[29,30,43] or in chronic periodontitis patients (range 51% to 62.5%).[29,38,44]

Our results agree with Diehl and coworkers[27] who suggested that the IL-1B +3954 polymorphism was more significantly associated with AgP than the IL-1A –889 polymorphism. However, our data do not show an association of allele 1 of the IL-1B with AgP, as was found by Diehl and coworkers[27] and Parkhill and coworkers.[30] Conversely, the results of our study show that the condition of homozygous for allele 1 of the IL-1B may be protective for AgP given the higher frequency of this genotype in healthy controls than in patients. Our results show a significant association of carriage of allele 2 of IL-1B at +3954 site with AgP (OR = 2.83, 95% CI 1.02 to 7.84, $P = 0.036$) as has been found in AgP[43] and in chronic periodontitis.[25]

The frequency of the homozygous for allele 2 of the IL-1B found in the present study was 2.15%, and was similar to that of Mexicans (2%)[42] but lower than rates ranging from 5.63%[30] to 12.5%[29] reported for Caucasians. The large disparity in the frequencies of the homozygous for allele 2 of the IL-1B probably reflects ethnic and racial differences in gene polymorphisms. For example, in African-Americans,[28] the frequency of homozygous for allele 2 of the IL-1B reported was 0.7%, and in Chinese[39] and Japanese[48] populations it was absent. There are no other studies concerning the prevalence of IL-1 polymorphisms in the Chilean population to compare with our results.

The association between severity of periodontitis and the IL-1 composite positive genotype has been found when smokers were excluded.[23,24] However, other studies[30,47] found an increased risk of periodontal disease for genotype-positive smokers. Because the number of smokers in the present study was so small, statistical analyses were not performed.

Since the association between the IL-1 genotype and chronic periodontitis was reported,[23] several other studies[24-26] have confirmed this relationship. However, other studies reported contradictory results, disputing a role of IL-1 gene polymorphisms in AgP[29] or in chronic periodontitis.[33]

Direct comparisons of the results of studies investigating the association of the IL-1 genotype polymorphisms with periodontal diseases are difficult because of the different diagnosis criteria used, the low number of subjects studied (as is also the case in the present study), and the type of subjects used as controls. Some studies have used subjects of unknown periodontal status as reference controls,[28,33] which involves the possibility of chance deviations in the sample studied, especially when there are no available data of the prevalence of IL-1 polymorphisms in the population studied. In the present study, no significant differences in the distribution of IL-1 genotypes were found between patients and the reference population, but significant differences were detected between healthy controls and the reference population, showing that the latter two groups were not equivalent. When a random sample of subjects of unknown periodontal status is used as a reference control, it is highly probable that the sample includes a percentage of subjects with periodontal disease similar to the proportion of affected subjects that exists in the study populations. If we had used only subjects of unknown periodontal status as a control group, the conclusion of no association at all between IL-1 genotype polymorphisms and AgP would have to be accepted. Thus, the use of subjects of unknown periodontal status as reference controls may favor the null hypothesis of no association.

In the present study, patients and healthy controls were recruited from the same public dental center and the same private dental clinic, in order to obtain equivalence between the groups. The composite positive genotype was more than two times higher in patients than in healthy subjects in the present study, even though the difference did not attain statistical significance. This was the result of the lack of power of the statistical test used to detect whether the difference was significant, due to the sample size studied.

The present study demonstrated a positive association between the presence of the heterozygous genotype of the IL-1B +3954 and AgP, as has been found by Rogers et al.[43] To explain the association of allele 2 of the IL-1B +3954 with several chronic inflammatory diseases, it has been proposed that cytokine genotypes

influence the disease pathogenesis by an increased secretion of cytokines,[49] and these findings have been extrapolated to chronic periodontitis.[23] The predominant form of IL-1 produced in tissues affected by periodontitis is IL-1β,[6,7] and the amount of IL-1 produced in LAgP is greater than in chronic periodontitis.[50] Pociot et al.[49] found that monocytes obtained from individuals possessing IL-1B +3954 allele 2 produced a greater amount of IL-1β than patients without this allele. However, other studies[25,26] evaluated the relationship between allele 2 of the IL-1B +3954 polymorphism and IL-1β production, and the results conflicted with those of Pociot et al.[49]

Additional studies using larger samples of both patients and healthy controls are needed to clarify the association of AgP with the IL-1 composite positive genotype. Periodontal diseases are complex disorders, and IL-1 genetic variations may be one of several genetic factors that contribute to the susceptibility of the disease. The genetic susceptibility to periodontal diseases is probably under the control of several other genes involved in the molecular etiopathogenesis of the disease.[4] Additional studies exploring extended haplotypes are necessary to better clarify the genetic factors involved in the etiopathogeny of periodontal diseases.

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