Association of Interleukin-1 Polymorphisms With Periodontal Disease

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Background: Several studies have investigated genetic polymorphisms for cytokines as potential genetic markers for periodontitis. Some studies have found that interleukin (IL)-1A and IL-1B polymorphisms are associated with a higher severity of periodontitis, while others found no association. The aims of this study were to determine the prevalence of the IL-1A–889 and IL-1B+3954 (previously described as +3953) polymorphisms in Chileans and their association with periodontitis.

Methods: Subjects aged 20 to 48 were selected from people requesting dental treatment at a public health center in Santiago, Chile. A case-control study of 330 cases of periodontitis patients and 101 healthy controls was performed. A full-mouth periodontal examination was performed on each subject and a structured questionnaire was conducted to determine smoking habits. Cases were categorized as having initial, moderate, or severe periodontitis according to the percentage of sites with clinical attachment loss ≥3 mm. Genomic DNA was analyzed for polymorphism in the IL-1A gene at site –889 and IL-1B gene at site +3954 by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion and gel electrophoresis. Data were analyzed by chi square test, analysis of variance (ANOVA), and by calculating odds ratio (OR) and 95% confidence intervals (CI).

Results: Demographic and socio-economic characteristics of subjects were similar in cases and in controls. A higher frequency of heterozygous of the IL-1A–889 was found in cases than in controls, but the difference was not significant. The heterozygous of the IL-1B+3954 was significantly higher in cases than in controls and was associated with periodontitis (OR 3.12, 95% CI 1.59 to 6.09, $P = 0.001$). The homozygous for allele 1 of the IL-1B+3954 was a protective factor for periodontitis (OR 0.35, 95% CI 0.19 to 0.66, $P = 0.001$). The prevalence of positive genotype (at least one allele 2 present at each locus) was significantly higher in cases (26.06%) than in controls (9.9%) and was significantly associated with periodontitis (OR 3.21, 95% CI 1.60 to 6.44, $P = 0.001$), irrespective of the smoking status and periodontitis severity. Sensitivity of positive genotype was 26%, the specificity 90%, and the positive predictive value 89%.

Conclusion: Within the limits of this study, the results show that individuals carrying the positive genotype have significantly greater risk for developing periodontitis. *J Periodontol 2005;76:234-243.*

KEY WORDS

Chileans; cytokines; genetic markers; periodontitis/etiology; risk factors.

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There are convincing data indicating that genetic factors may play a significant role in the risk of periodontal diseases.[1,2] The role of cytokines in the pathogenesis of periodontal diseases has been the focus of many studies, particularly of interleukin-1, because it is implicated in many important pathogenic mechanisms of periodontitis.[3,4] Active periodontal disease increases the amount of both α and β interleukins.[5,6] The predominant form of IL-1 in the periodontal tissues is IL-1β and, because of its multiple proinflammatory properties, it has an important role in the pathogenesis of periodontitis. The level of cytokine secretion in response to bacterial challenge may explain the individual differences in susceptibility and severity of periodontal diseases.[7,8] Some reports indicate that polymorphisms in the IL-1 gene cluster may influence the variations in the synthesis of cytokines, and thus modify the individual responses to bacterial stimuli.[9,10]

Several studies have investigated genetic polymorphisms for cytokines as potential genetic markers for periodontitis, but the role for interleukin-1 gene cluster polymorphisms in the risk assessment for periodontal disease is controversial. Some studies have found that IL-1A and IL-1B polymorphisms are associated with a higher severity of periodontal diseases,[11-14] while others found no association between these polymorphisms and incidence, onset, or disease severity.[15-17] These conflicting results may be explained by variations in the frequency of genetic

polymorphisms according to ethnicity or different diagnosis criteria.[18,19] The majority of the studies[11-13,15-17] have used a convenience sample of a small number of subjects and some studies have used subjects of unknown periodontal status or from dental or hospital staffs as reference controls.[18,19]

The aim of the present study was to determine the prevalence of the IL-1A–889 and IL-1B+3954 polymorphisms in the Chilean population and the association of these polymorphisms with periodontal disease. The Chilean mixed population of Santiago, Chile, stemming from the admixture of aboriginal Amerindian, Spanish, and other European ethnic groups, has an average Amerindian admixture of 40%.[20]

MATERIALS AND METHODS

Study Population

Five hundred fifty subjects, aged 20 to 48, were recruited from people requesting dental treatment at a public health center in Santiago, Chile. All subjects were interviewed with respect to systemic diseases, current medication, history of periodontal therapy or of preventive measures for periodontitis, and smoking habits. Potential participants were required to fulfill the following criteria: 1) over 20 years old; 2) free of systemic diseases; 3) no previous history of periodontal therapy or professional treatment to prevent periodontitis; and 4) no evident clinical signs of the local form of aggressive periodontitis. All subjects had both parents and grandparents of Chilean Caucasian heritage. Subjects with an Amerindian ethnic background were not selected in order to reduce genetic heterogeneity. There were no restrictions regarding levels of plaque, gingival inflammation, severity of periodontitis, or number of natural teeth present. Smoking habits were recorded through a structured questionnaire and subjects were classified as: smokers, subjects who were current smokers or had stopped smoking ≤5 years before being recruited to the study, or non-smokers, subjects who had never smoked or had stopped smoking >5 years previously. Cigarette consumption was calculated in packs per year.

Of the 550 subjects interviewed, 462 fulfilled the inclusion criteria. Of these, 441 subjects agreed to participate in the study. The study protocol was approved by the Institutional Committee of Research. The nature of the investigation was explained to all the participants and written informed consent was obtained.

Measurement of Periodontal Status

Upon entering the study all subjects received a full-mouth periodontal examination and the following variables were determined: oral hygiene status, gingival inflammation, probing depth (PD), and clinical attachment level (CAL). Oral hygiene status was assessed as the percentage of surfaces demonstrating plaque. Dichotomous measures of supragingival plaque accumulation were made by running a periodontal probe at the cervical surface of each tooth. The presence of plaque was positive when a continuous band of plaque was found in contact with the gingival tissue on the cervical portion of mesial, buccal, distal, and lingual tooth surfaces. Plaque scores were calculated as the percentage of surfaces examined demonstrating plaque.

Probing depth and attachment level measurements were made at the mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual positions of every tooth with the exception of third molars. The CAL was measured using the cemento-enamel junction as a reference point. Bleeding on probing (BOP) was assessed on the six sites at which probing depth was done and deemed positive if it occurred within 15 seconds after probing. Bleeding on probing was expressed as the percentage of sites showing bleeding.

Clinical measurements were recorded to the nearest millimeter using a North Carolina calibrated periodontal probe by a single calibrated examiner.

DIAGNOSES OF PERIODONTAL STATUS

Subjects were classified as cases or controls according to their periodontal status. Controls were periodontally healthy subjects or with gingivitis, with a mean loss of clinical attachment <1 mm, and no interproximal sites with clinical attachment loss ≥2 mm. Cases were subjects who were diagnosed as having periodontitis. Cases were classified into three levels of severity according to the percentage of sites with ≥3 mm CAL and tertiles were calculated. Cases in the upper tertile were classified with severe periodontitis; in the medium with moderate disease; and in the lower with mild periodontitis.

Blood Collection and Isolation of Genomic DNA

Genomic DNA was isolated from venous blood according to the method of Chomczynsky and Sacchi.[21] The DNA samples were stored at –20°C in TE buffer (10 mM Tris, 1 mM EDTA) until required. DNA integrity was checked and DNA quantified using agarose gel electrophoresis. Genomic DNA was analyzed for polymorphism in the IL-1A gene at position –889 and IL-1B gene at position +3954 by polymerase chain reaction (PCR).

Genotyping

IL-1A–889. PCR amplifications of the IL-1A–889 were carried out according to McDowell et al.[22] 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, and 0.8 uM of each oligonucleotide primer (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.‡ Cycling was performed as follows: 96°C for 2 minutes, 45 cycles at 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute. PCR product was digested overnight at 37°C with six units per 30 µl reaction of *Ncol*, using conditions described by the manufacturer. The resulting products of 83bp+16bp (allele 1) and 99bp (allele 2) were sized on 3% agarose gel and ethidium bromide staining.

IL-1B+3954. 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 NM of each dNTP, and 2 uM of each oligonucleotide primers 5′CTC AGG TGT CCT CGA AGA AAT CAA A-3′ and 5′-GCT TTT TTG CTG TGA GTC CCG-3′. Cycling was performed as follows: 95°C for 2 minutes; 35 cycles at 94°C for 1 minute; 53°C for 1 minute; and 72°C for 1 minute. Twenty-three µl of amplicon was digested with two to five U Taq restriction endonuclease at 65°C for 2 hours. The resulting products of 12bp+85bp+97 bp (allele1) and 12bp+182bp (allele 2) were sized on 3% agarose gel and ethidium bromide staining.

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

Table 1.

Study Population Clinical Characteristics

Characteristic	Cases (N = 330)	Controls (N = 101)	*P* Value
Age (years)	30.23 ± 5.25	29.33 ± 5.47	0.13
Female (%)	69.00	72.00	
Subjects with <12 years of education (%)	83.42	77.82	0.24
Smokers (%)	42.12	52.47	0.08
N teeth	23.60 ± 3.88	24.29 ± 3.47	0.058
Sites (%) with			
Plaque	81.59	63.91	0.001
BOP	41.05	19.00	0.001
PD ≥4 mm	7.70	0.68	0.001
CAL ≥2 mm	62.31	14.94	0.001
CAL ≥3 mm	32.05	0.85	0.001
Mean PD	2.33 ± 1.56	1.83 ± 0.30	0.001
Mean CAL	1.93 ± 0.93	0.48 ± 0.33	0.001

Data Analysis

Hardy-Weinberg equilibrium in both control and case groups was tested for genotype frequencies by a chi-square test with 1 degree of freedom. In all analyses the individual subject was the computational unit. Mean values for all clinical parameters were calculated for each subject. Mean values of the clinical parameters BOP, PD, and CAL were used as measures of periodontitis. The association of allele and genotype frequencies in cases and controls was analyzed by the chi square test. The strength of the associations was determined using an OR calculation and 95% CI. In the first analysis, IL-1 genotype and allele frequencies between cases and controls were compared without categorizing subjects by smoking status. Periodontal clinical parameters were compared between genotype positive and genotype negative cases, categorizing both groups in smokers and non-smokers. In separate analyses, IL-1 genotype and allele frequencies were compared between smoker and non-smoker cases and controls. A composite positive genotype was defined as at least one allele 2 present at each locus.[11] Comparisons of means of clinical parameters were performed by Student *t* test. The periodontal characteristics of cases and the frequency of positive genotype of subjects with severe periodontitis were compared with those with moderate and initial periodontitis by analysis of variance. The frequencies of positive genotype of the subgroups of cases with severe, moderate, and initial periodontitis were compared with controls using the chi square test. The impact of the IL-1 polymorphisms on clinical parameters was studied by means of simple regression models. Statistical significance was set at a $P < 0.05$. All the analyses were performed using a statistical package.§

RESULTS

No differences were found between observed and expected distributions of genotypes for the control group for IL-1A ($P = 0.30$) and for IL-1B ($P > 0.05$) or for the case group for IL-1B ($P > 0.05$); therefore, it was considered to be in Hardy-Weinberg equilibrium. However, the genotype frequencies of each variant of the IL-1A–889 (1-1,1-2, and 2-2) in the case group deviated from those predicted by the Hardy-Weinberg law ($\chi^2 = 0.67, 4.68, 8.10$, respectively; $P < 0.0001$).

Of the 441 subjects enrolled, 330 were diagnosed with periodontitis (cases) and 101 subjects as periodontally healthy (controls). All the subjects were of low or middle low socio-economic status, and the percentage of subjects with less than 12 years of education was similar in both groups (Table 1). The frequency of smokers was higher in controls (52.47%) than in cases (42.12%; $P = 0.08$) but the difference was not significant. Smoker cases smoked an average of 94 packs per year and smoker controls smoked an average of 75.5 packs. Of the smoker cases, 9.16%

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

Table 2.

Characteristics of Smokers and Non-Smoker Cases and All Controls

	Smoker Cases (N = 139)	Non-Smoker Cases (N = 191)	Controls (N = 101)
Age	30.07 ± 5.55	30.97 ± 5.03	29.33 ± 5.47
<12 years of education (%)	78.34	86.00	77.82
N teeth	23.46 ± 4.11	23.52 ± 3.70	24.29 ± 3.47
Sites (%) with			
Plaque	82.56	80.72	63.91
BOP	41.05	40.95	19.00
PD ≤4 mm	8.28	9.57	0.68
CAL ≤2 mm	67.00	60.85	14.94
CAL ≤3 mm	34.41	30.53	0.85
Mean PD	2.46 ± 2.31	2.42 ± 1.98	1.83 ± 0.30
Mean CAL	1.99 ± 0.84	1.97 ± 0.89	0.48 ± 0.33

Table 3.

Distribution of Genotype and Allele Frequencies of IL-1A–899 and IL-1B+3954

	Cases (N = 330)		Controls (N = 101)	
Genotype	N	%	N	%
IL-1A–899				
1-1	186	56.36	65	64.35
1-2	140	42.40	34	33.66
2-2	4	1.51	2	1.98
Allele				
1	512	77.57	164	81.18
2	148	22.42	38	18.81
IL-1B+3954				
1-1	232	70.30*	88	87.12*
1-2	91	27.57*	11	10.89*
2-2	7	2.12	2	1.98
Allele				
1	555	84.09	187	92.57
2	105	15.90†	15	7.43†

* $P = 0.001$.
† $P = 0.003$.

smoked >10 cigarettes a day, 25% smoked five to nine cigarettes a day, and 65.73% smoked less than five cigarettes a day.

Cases had a mean age slightly higher and a lower mean of teeth than controls, but the differences were not significant. All the teeth lost from cases and controls had been extracted as a result of extensive decay or extra-oral trauma. As was expected, all the periodontal parameters were significantly higher in cases than in controls. Smoker cases showed slightly higher values of periodontal parameters than non-smokers cases, but no significant differences were detected in any of the periodontal characteristics between smoker cases and non-smoker cases (Table 2).

Prevalence of IL-1A–889 Genotypes and Alleles

Table 3 shows the IL-1A–889 and IL-1B+3954 genotype and allele frequencies in all cases and controls. A higher frequency of homozygous for allele 1 of the IL-1A was found in controls (64.35%) than in cases (56.36%), while a higher frequency of heterozygous of the IL-1A was found in cases (42.40%) than in controls (33.66%), but none of these differences reached statistical significance ($P > 0.05$). The frequency of the rare homozygous for allele 2 of the IL-1A was similar in cases and controls. The prevalence of allele 2 of the IL-1A–889 was higher in cases than in controls, but the difference was not statistically significant.

Prevalence of IL-1B+3954 Genotypes and Alleles

The homozygous for the allele 1 of the IL-1B+3954 was the most frequent genotype in cases and controls, and a significantly higher percentage of controls (87.12%) than cases (70.30%) was homozygous for allele 1 ($P = 0.001$), while a significantly higher percentage of cases (27.57%) than controls (10.89%) was heterozygous ($P = 0.001$) (Table 3). The homozygous for the allele 1 of the IL-1B showed to be a protective factor for periodontitis (OR = 0.35, 95% CI 0.19 to 0.66, $P = 0.001$), while the heterozygous of the IL-1B showed a positive association with periodontitis (OR = 3.12, 95% CI 1.59 to 6.09, $P = 0.001$). The frequency of the rare homozygous for allele 2 of the IL-1B was very low and similar in cases and controls.

The prevalence of the allele 2 of the IL-1B was significantly higher in cases (15.90%) than in controls (7.43%, $P = 0.003$) and the carriage of allele 2 showed a positive association with periodontitis (OR 2.36, 95% CI 1.33 to 4.15, $P = 0.003$). Conversely, the carriage of allele 1 showed to be a protective factor for periodontitis (OR 0.42, 95% CI 0.24 to 0.74, $P = 0.003$).

Distribution of IL-1A and IL-1B Composite Genotypes

The most frequent IL-1A and IL-1B composite genotype was the homozygous for the allele 1 at both of the loci studied (1-1/1-1); it was more frequent in controls (61.38%) than in cases (52.72%), but the difference was not significant ($P = 0.14$) (Table 4). The prevalence of the composite heterozygous genotype for the IL-1A and IL-1B genotypes (1-2/1-2) was significantly higher in

Table 4.

Distribution of the IL-1A–889 and IL-1B+3954 Composite Genotypes

	All Cases (N = 330)		All Controls (N = 101)	
Composite Genotype	N	%	N	%
1-1/1-1	174	53.72	62	61.38
1-2/1-1 or 1-1/1-2	69	20.90	28	27.72
1-2/1-2	77	23.63*	7	6.93*
1-2/2-2 or 2-2/1-2	7	2.12	3	2.97
2-2/1-1	1	0.30	1	0.99
2-2/2-2	1	0.30	0	0
Positive genotype	86	26.06*	10	9.90*

* $P = 0.001$.

cases (23.63%) than in controls (6.93%, $P = 0.001$), and the carriage of this genotype was strongly associated with periodontitis (OR = 4.09, 95% CI 1.82 to 9.18; $P = 0.001$). The distribution of the other composite genotypes showed no significant differences between cases and controls.

The prevalence of the denominated positive composite genotype (at least one allele 2 present at each locus)[11] among the 441 subjects studied was 21.76%, and was significantly higher in cases (26.06%) than in controls (9.90%, $P = 0.001$). The carriage of the positive genotype showed a positive association with periodontitis (OR = 3.21, 95% CI 1.60 to 6.44, $P = 0.001$).

Distribution of IL-1A and IL-1B Genotypes and Smoking Status

Table 5 shows the IL-1A and IL-1B genotype and allele frequencies in smoker and non-smoker cases and in controls. The distribution of IL-1A genotypes in smoker and non-smoker cases and controls showed the same tendency found in the analysis of all cases. The frequency of the heterozygous of the IL-1A was higher in smoker and non-smoker cases than in controls, but the differences did not reach statistical significance.

The prevalence of the IL-1B genotypes and alleles showed significant differences between smoker and non-smoker cases and controls. The prevalence of the homozygous for allele 1 of the IL-1B was significantly higher in controls (87.12%) than in smoker (69.78%) and non-smoker cases (70.68%, $P = 0.003$), and showed to be a protective factor for periodontitis in smokers (OR = 0.34, 95% CI 0.17 to 0.68, $P = 0.003$) and in non-smokers (OR = 0.36, 95% CI 0.18 to 0.69,

Table 5.

Distribution of IL-1A and IL-1B Genotype and Allele Frequencies in Smoker and Non-Smoker Cases and All Controls

	Smoker Cases (N = 139)		Non-Smoker Cases (N = 191)		All Controls (N = 101)	
Genotype	N	%	N	%	N	%
IL-1A–899						
1-1	84	60.43	102	53.92	65	64.35
1-2	54	38.84	85	43.97	34	33.66
2-2	1	0.72	4	2.09	2	1.98
Allele						
1	222	79.85	290	75.91	164	81.18
2	56	20.14	92	24.09	38	18.81
IL-1B+3954						
1-1	97	69.78*	135	70.68*	88	87.12*
1-2	41	29.49†	50	26.17‡	11	10.89†‡
2-2	1	0.72	6	3.14	2	1.98
Allele						
1	235	69.78	320	83.76	187	92.57
2	43	30.93†	62	16.23‡	15	7.42†‡

* $P = 0.003$.
† $P = 0.001$.
‡ $P = 0.004$.

$P = 0.003$). The carriage rate of the heterozygous genotype for allele 2 of IL-1B was significantly higher in smoker (29.49%) and non-smoker cases (26.17%) than in controls (10.89%, $P = 0.004$), and showed a positive association with periodontitis in smokers (OR = 3.34, 95% CI 1.62 to 6.91, $P = 0.001$) and non-smokers (OR = 2.91, 95% CI 1.44 to 5.87, $P = 0.004$). The frequency of allele 2 of the IL-1B genotype was significantly higher in smoker (30.93%) and non-smoker cases (16.23%) than in controls (7.42%, $P = 0.001$ and 0.004, respectively). Carriage of the allele 2 was associated with periodontitis in smokers (OR = 2.28, 95% CI 1.22 to 4.23, $P = 0.012$) and non-smokers (OR = 2.42, 95% CI 1.34 to 4.37, $P = 0.004$). Conversely, carriage of allele 1 at the IL-1B+3954 locus was found to be a protective factor for periodontitis both for smokers (OR = 0.43, 95% CI 0.23 to 0.81, $P = 0.012$) and non-smokers (OR = 0.41, 95% CI 0.23 to 0.75, $P = 0.004$).

The heterozygous composite genotype of IL1-A and IL1-B (1/2-1/2) was significantly higher in smoker (23.2%) and non-smoker cases (23.56%) than in controls (6.93%, $P = 0.001$), and also showed a strong association with periodontitis in smokers (OR = 3.97, 95% CI 1.68 to 9.43, $P = 0.002$) and in non-smokers (OR = 4.14, 95% CI 1.79 to 9.56, $P = 0.001$) (Table 6). The carriage of positive genotype was also significantly more frequent in smoker (24.46%) and non-smoker

Table 6.

Distribution of Composite Genotype of IL-1A and IL-1B in Smoker and Non-Smoker Cases and All Controls

Composite Genotype	Smoker Cases (N = 139)		Non-Smoker Cases (N = 191)		All Controls (N = 101)	
	N	%	N	%	N	%
1-1/1-1	77	55.39	97	50.78	62	61.38
1-2/1-1 or 1-1/1-2	28	20.14	41	21.46	28	27.72
1-2/1-2	32	23.02*	45	23.56†	7	6.93*†
1-2/2-2 or 2-2/1-2	2	1.43	6	3.14	3	2.97
2-2/1-1	0	0	1	0.52	1	0.99
2-2/2-2	0	0	1	0.52	0	0
Positive genotype	34	24.46‡	52	27.22†	10	9.90‡†

* $P = 0.002$.
† $P = 0.001$.
‡ $P = 0.007$.

Table 7.

Clinical Characteristics and Distribution of Positive Genotype in Cases With Initial, Moderate, and Severe Periodontitis

Characteristic	Periodontitis			*P* Value
	Initial (N = 110)	Moderate (N = 110)	Severe (N = 110)	
Age	28.84 ± 5.19	30.33 ± 5.00	31.31 ± 5.21	0.002
Smokers (%)	32.72	46.80	44.54	0.050
N teeth	24.14 ± 3.19	23.72 ± 3.67	22.66 ± 4.56	0.014
Sites (%) with				
Plaque	76.14 ± 17.13	81.20 ± 14.85	88.68 ± 13.12	0.001
BOP	29.56 ± 16.14	39.00 ± 12.80	56.98 ± 14.56	0.001
PD ≥4 mm	2.76 ± 4.50	6.94 ± 10.60	16.35 ± 14.32	0.001
CAL ≥3 mm	8.38 ± 4.23	26.95 ± 7.01	62.55 ± 15.06	0.001
Mean PD	2.01 ± 0.39	2.41 ± 2.39	2.69 ± 0.53	0.002
Mean CAL	1.37 ± 2.27	1.82 ± 0.33	2.88 ± 0.73	0.001
Positive genotype (%)	26.36	23.63	28.18	0.730

cases (27.22%) than in controls (9.90%, $P = 0.001$), and was significantly associated with periodontitis in smokers (OR = 2.94, 95% CI 1.38 to 6.29, $P = 0.007$) as well as in non-smokers (OR = 3.31, 95% CI 1.64 to 6.99, $P = 0.001$).

Table 8.

Odds Ratios of Positive Genotype in Cases With Initial, Moderate, and Severe Periodontitis

Periodontitis	Positive Genotype		Odds Ratio	95% CI	*P* Value
	N	%			
Initial (N = 110)	29	26.36	3.26	1.50-7.10	0.004
Moderate (N = 110)	26	23.63	2.82	1.28-6.14	0.001
Severe (N = 110)	31	28.18	3.57	1.65-7.74	0.001

The results showed that the strength of the association between positive genotype and periodontitis was maintained irrespective of smoking status.

IL-1 Polymorphisms and Severity of Periodontitis

The percentage of sites with CAL ≥3 mm used as a criterion to categorize patients according to the severity of periodontitis and the determination of tertiles distinguished the different levels of periodontitis severity very well. All the differences between the clinical characteristics of cases in the three subgroups of severity were statistically significant (Table 7). The distribution of smokers as well as the prevalence of positive genotype was similar in cases with initial, moderate, and severe periodontitis. The prevalence of positive genotype was significantly higher in the subgroups of cases with initial (26.36%, $P = 0.004$), moderate (23.63%, $P = 0.014$), and severe periodontitis (28.18%, $P = 0.001$) than in controls (9.9%). The carriage of positive genotype consistently appeared associated with periodontitis, regardless of the severity of periodontitis, with statistically significant odds ratios ranging from 2.82 for moderate periodontitis to 3.57 for severe periodontitis (Table 8).

A significantly lower number of teeth ($P = 0.048$) and a higher percentage of sites with ≥2 mm attachment loss ($P = 0.023$) was found in positive genotype carriers, cases and controls taken together, compared to all the negative genotype carriers.

The predictive ability of the positive genotype to identify patients who do have periodontitis (sensitivity) was 0.26 (95% CI 0.21 to 0.31). The ability of the positive genotype to identify patients who do not have the disease (specificity) was 0.90 (95% CI 0.82

to 0.94). The positive predictive value for positive genotype was 89% and the negative predictive value was 27%.

DISCUSSION

The results of the present study strongly support an association between variation at polymorphic sites in the IL-1 genes and risk of periodontal disease, irrespective of the smoking status. Since Korman et al.[11] reported an association between the IL-1 polymorphisms and adult periodontitis, several other studies have provided variable evidence that has confirmed[12-14] or rejected[15-17] this relationship. Differences in the results between studies may be ascribed to several factors. Changes of the frequency of the IL-1 polymorphism in the populations studied due to ethnic variations may explain some differences. For example, Armitage et al.[23] found that only 2.3% of subjects of Chinese heritage carry the positive composite IL-1 genotype, and Walker et al.[24] studied an African-American population and reported that the prevalence of the positive genotype was 14% in a control group and 8% in the localized form of aggressive periodontitis. Given the high frequency of the IL-1B+3954 allele 1 (99% of controls and 100% of patients), with most subjects being homozygous for this allele, the IL-1B+3954 polymorphism provides little information for aggressive periodontitis in African-Americans.

The contradictory results found in several studies may also be explained by differences in the diagnostic criteria used to define periodontal disease, the low number of subjects studied, and the characteristics of the controls.

According to Kinane and Hart,[19] the number of cases and controls of some studies were too small to obtain robust conclusions of the association between the IL-1 polymorphisms and periodontal disease. The majority of studies,[15-17,24-26] with the exception of four,[13,14,23,30] used samples of less than 200 subjects, including both patients and controls. All the studies, except for two,[14,30] used a convenience sample of patients with periodontal disease who were enrolled while or after receiving periodontal therapy or while in maintenance therapy. Other studies did not use healthy subjects as a control group,[11-13] and several studies have used subjects of unknown periodontal status[15,17,24] or subjects of the dental school or hospital staffs as reference controls.[16,17] The use of subjects of unknown periodontal status as controls involves the possibility of chance deviations, especially when there are no available data of the prevalence of IL-1 polymorphisms in the population studied. Otherwise, subjects belonging to dental hospital staffs used as controls may not be equivalent to the patients of the study.

Periodontal diseases behave clinically in a way that is similar to many common diseases involving microbiological, genetic, and behavioral factors that together determine the clinical characteristics of the disease in a specific subject. Although periodontal pathogens are the clear initiators and perpetuators of the disease, there is increasing evidence that the host characteristics, including genetic factors, are important contributors to the clinical appearance or phenotype of the periodontitis.[1,2]

On the other hand, there is evidence that environmental, cultural, and socio-economic factors may enhance the initiation of periodontitis and also that the initiation of the disease may be halted or delayed by personal and professional preventive measures. Since there are many factors that influence the initiation, progression, and severity of periodontal diseases, ideally subjects used to study the association between periodontal diseases and genetic factors have to be subjects who have not undergone periodontal treatment, dental prophylaxis, or oral hygiene instructions.[19] An important characteristic of the present study is that subjects were selected from a homogeneous population with similar ethnic, socio-economic status, level of education, and other demographic data. Additionally, none had received periodontal therapy or individual or personal instructions on how to prevent periodontal disease, and all the known factors that may influence the initiation and severity of periodontitis were similarly distributed in cases and controls. Thus, the population of the current study is very suitable for the investigation of the association between periodontal disease and genetic factors. A frequency of positive genotype ranging from 29.1% to 46% has been described in European populations,[14,16,17,25,26] and a range between 29% to 38% of genotype-positive subjects has been reported in a U.S. Caucasian population.[11,12,27,28] However, the frequencies found in many of these studies refer to patients from dental clinics and not to that of the respective general population. Even though the prevalence of positive genotype in the Chilean population was lower than the prevalence reported in Caucasians[11,12,27,28] and in a Mexican population (26%),[29] the positive genotype showed a significant association with periodontitis.

The most frequent genotype of the IL-1A gene at site –889 found in the present study was the homozygous for allele 1 and was more frequent in controls than in cases, as has been found in several other studies in Caucasian,[14,17] African-American,[24] and Chinese[23] populations. In the present study, the carriage of the allele 2 of the IL-1A amounted to 41.76%, lower than the rate described in Caucasians (range 40.6% to 54%).[11,17,32] IL-1A carriage rate of allele 2 by the case group in our study tended to be higher than that for the control group, but this relationship was not statiscally significant. Additionally, the frequencies of the IL-1A genotypes deviated from the Hardy-Weinberg

expectation due to an excessive number of heterozygous in the case group, but not in the control group. These data suggest that the heterozygous of the IL-1A is associated with periodontitis. Our results of the association between the heterozygous of the IL-1A with periodontitis are in concordance with Kornman et al.[11] who found that allele 2 of the IL-1A tended to be more frequent in cases than in controls. Our results also agree with Shirodaria et al.[34] who found a higher risk of severe periodontitis in carriers of the allele 2 of the IL-1A and with Laine et al.[32] who found a higher prevalence of heterozygous of the IL-1A in patients of Dutch Caucasian heritage. However, other studies in Caucasians[16,17] and Japanese[33] have not found an association between the allele 2 of the IL-1A and periodontitis.

The frequency of the homozygous for allele 2 of the IL-1A found in the present study (1.39%) was lower than most studies in Caucasians that reported rates from 4.4%[14] to 18%.[32] However, it should be expected that such rates vary greatly between ethnically distinct populations. For example, in African-Americans[24] the frequency of homozygotes for allele 2 of the IL-1A was 1.41%; in Chinese,[23] it was 0.66%; in Japanese, homozygotes for the allele 2 of both the IL-1 A and IL-1B were not found.[33] According to Diehl et al.,[31] the homozygous for allele 2 of both the IL-1A and IL-1B is quite rare (<3%). To our knowledge, there are no other studies concerning the prevalence of IL-1 polymorphisms in the Chilean population to compare with our results.

In the present study, the most frequent genotype of the IL-1B was the homozygous for allele 1, which was more frequent in controls than in cases, as has been found in other studies.[13-17,23-26,33,35,36] Parkhill et al.[25] studied subjects of Caucasian heritage and found that the homozygous for allele 1 of the IL-1B was the most frequent genotype in 70 patients with aggressive periodontitis, while the heterozygous was the most frequent genotype in the 72 healthy controls. Diehl et al.[31] also found that allele 1 of the IL-1B is likely to be more important for the risk of aggressive periodontitis in a pooled population of African-Americans and Caucasians. The findings of the Parkhill et al.[25] and Diehl et al.[31] studies are at odds with the present study to the extent that we found that the carriage of homozygous for allele 1 of the IL-1B appeared to be a protective factor for periodontitis, and the heterozygous genotype of the IL-1B showed a strong positive association with periodontitis, regardless of smoking status. The positive association between allele 1 of the IL-1B and aggressive periodontitis found both in Caucasians[25,31] and in African-Americans[24] and the negative association of this allele with chronic periodontitis found in the present study may suggest different genetic profiles of the two forms of periodontitis.

The association of allele 2 of the IL-1B with periodontitis found in the present study agrees with other authors who reported a significantly higher prevalence of this allele in advanced chronic periodontitis[11,13,14,36] and in aggressive periodontitis.[15]

The results of our study are in contrast to some studies in which the IL-1 positive genotype increased the risk for periodontitis only after smokers were excluded from the analysis.[11,13,14, 26,30]

In our study, the periodontal characteristics of smoker cases were slightly higher than those of non-smoker cases. This seems to be in contrast with other studies that have shown that smokers have more severe and extensive periodontitis than non-smokers.[37-39] It has been shown that the effect of smoking on the prevalence and extent of periodontal destruction is dose dependent,[40,41] and increases with smoking duration and age.[42-44] There is a threshold period for tobacco smoking after which the accumulated effect of smoking becomes clinically observable.[45] The mean age (30 ± 5.36) and the range of age (20 to 48 years) of the present study smokers are lower than those of other studies that have investigated the effect of smoking on periodontal health, in which subjects with an age range between 25 and 64 were selected.[37-41] The time of exposure to tobacco in the smokers in our study was probably not long enough to produce significant clinically negative effects on periodontal tissues.

The subjects included in the current study were over 20 years old. It is reasonable to expect that some of the control subjects in their twenties might develop periodontitis in their forties, meaning some current controls would become cases. There were 39 controls (38.61%) and 155 cases (47%) who were in their twenties. The high proportion of subjects younger than 30 in the case group demonstrated that the population studied showed evidence of chronic periodontitis at an earlier age. Subjects of the present study were selected from a very homogeneous population and all of them were exposed to a high risk (i.e., low socioeconomic status, poor oral hygiene, lack of dental care) for periodontitis. It is reasonable to speculate that subjects in their twenties not affected by periodontitis, and identified as control, were less susceptible to periodontitis than subjects of the same age in the case group. However, the possibility that some subjects identified as controls in their twenties may develop the disease in their forties, cannot be excluded.

The biological mechanisms behind the association between positive genotype and periodontal disease lie in the hypothesis that these polymorphisms may modulate the secretion of IL-1. However, this relationship has not been completely demonstrated. In effect, Pociot et al.[46] found that monocytes from patients carrying the allele 2 of the IL-1B gene at site +3954 produced

a greater amount of IL-1β than patients without this allele. Engebretson el al.[47] also found elevated levels of IL-1β in the gingival crevicular fluid in shallow sites in patients carrying the positive genotype, but no statistical differences were found for deeper pockets. In another study[48] no significant differences were found in IL-1β production from monocytes obtained from positive-genotype and negative-genotype patients. Gore et al.[35] found that neutrophils from patients with advanced periodontitis who were carriers of the IL-1B+3954 allele 2 produced increased IL-1β levels compared to patients without this allele; however, the differences were not significant due to the large interindividual variability. Galbraith et al.[36] found no significant relationship between the genotype status and the level of cytokine production, and another study[49] found that allele 2 of the IL-1B+3954 polymorphism was associated with decreased IL-1β secretion. Thus, the association between IL-1B polymorphism and levels of IL-1β is not fully clarified. In relation to the influence of allele 2 of IL-1A–889, the carriage of this allele was associated with an almost 4-fold increase in IL-1α protein levels.[34]

According to the sensitivity and specificity of the positive genotype calculated in the present study, 26% of the subjects with periodontitis are correctly identified as diseased if they carried the positive genotype, and 90% of the subjects without periodontitis are correctly identified as healthy if they were not carriers of the positive genotype. Thus, the percentage of time that the positive genotype helps to correctly identify patients with periodontitis or who may develop it is rather low. However, the positive predictive value of the positive genotype, that is, the probability that a subject who carries the positive genotype really has periodontitis or will develop periodontitis, is 89%. Otherwise, the negative predictive value of positive genotype, i.e., the proportion of all patients who do not carry the positive genotype and do not have periodontitis, is 90%.

CONCLUSIONS

In the present study, IL-1 genotype-positive subjects were found to be three times more likely to have periodontitis than genotype-negative subjects. The association between positive genotype and periodontitis was independent of smoking status and the strength of the association was maintained in patients with initial, moderate, or severe periodontitis. The results of the present population-based study showed that the IL-1 positive polymorphism significantly increased the risk for periodontitis in this Chilean population.

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