Interleukin-1 Gene Cluster Polymorphisms Associated With Periodontal Disease in Type 2 Diabetes

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Background: Epidemiologic studies have shown an increased frequency, severity, and risk of periodontitis in patients with diabetes. Periodontitis is associated with certain interleukin (IL)-1 gene cluster polymorphisms. Diabetes is a proinflammatory state with increased levels of circulating cytokines suggesting a causal role for inflammation in its etiology. Common genetic factors may be involved in the susceptibility for diabetes and periodontitis. We evaluated the relationships among IL-1 gene polymorphisms, type 2 diabetes, and periodontitis.

Methods: One hundred twelve patients with diabetes and chronic periodontitis, 224 patients without diabetes but with chronic periodontitis, and 208 healthy subjects without periodontitis were studied. All received a clinical periodontal examination and assessment of standard periodontal parameters. IL-1A –889, -1B +3954, and -1B –511 polymorphisms were identified by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion and gel electrophoresis. Variable numbers of IL-1RN tandem repeats were detected by PCR amplification and fragment-size analysis.

Results: The severity and extent of periodontitis was significantly greater in patients with diabetes than in patients without diabetes. No significant differences in IL-1A –899, -1B +3954, or -1RN genotype frequencies were found between patients with diabetes and patients without diabetes. The IL-1A –889 TT genotype (odds ratio [OR] = 2.90; 95% confidence interval [CI] = 1.20 to 7.02), IL-1B +3954 TT genotype (OR = 3.54; 95% CI = 1.15 to 10.85), and IL-1B –511 CC genotype (OR = 2.10; 95% CI = 1.25 to 3.58) were significantly associated with periodontitis. The presence of an IL-1 positive genotype was significantly associated with periodontitis (OR = 1.61; 95% CI = 1.04 to 2.49). No interaction between smoking status and polymorphisms was found.

Conclusions: Periodontitis was significantly associated with some IL-1 gene polymorphisms. No association between diabetes and IL-1A and -1B gene polymorphisms was found. *J Periodontol 2009;80:1590-1598.*

KEY WORDS

Chronic periodontitis/etiology; cytokines; genetic markers; genetic predisposition to disease; interleukin-1; type 2 diabetes mellitus.

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Periodontal disease is a common, chronic, complex infectious disease with variable clinical presentation. The most prevalent form is chronic periodontitis, which commonly affects people 40 to 50 years of age. There are convincing data indicating that genetic factors play a significant role in the risk for periodontal disease.[1,2]

Type 2 diabetes mellitus is a heterogeneous disease that arises from an interaction among environmental factors such as obesity, sedentary lifestyle, high-calorie food intake, and genetic susceptibility that result in increased insulin resistance and the clinical manifestation of disease.[3]

Studies[4-7] have found an increased frequency and severity of periodontitis in patients with type 2 diabetes, and patients with diabetes have been shown to be at increased risk for periodontitis. Several pathogenic mechanisms have been proposed to explain the increased severity of periodontitis among patients with diabetes, including an altered inflammatory response to pathogens[8,9] and impaired immune cell function.[10]

The role of interleukin (IL)-1β and tumor necrosis factor (TNF)-α in periodontal pathogenesis has been extensively demonstrated, and local overproduction of IL-1β and TNF-α

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is a major contributor to periodontal tissue destruction.[11] There is molecular epidemiologic evidence that periodontitis reflects an excessive inflammatory response relative to the level of microbial burden presented by the oral biofilm.[12] Individuals who are highly susceptible to periodontitis show an overproduction of certain inflammatory mediators, and there is an exaggerated host inflammatory response in patients with more severe periodontitis.[12]

A subclinical inflammatory reaction precedes the onset of type 2 diabetes,[13] and hyperglycemia is considered a proinflammatory state that results in increased circulating cytokine levels, suggesting a causal role for hyperglycemia in the immune activation of diabetes.[14] Genes that code for proinflammatory cytokines are potential candidates for a susceptibility for diabetes[15,16] and periodontitis.[17] Patients with diabetes and periodontitis have significantly higher levels of proinflammatory mediators in gingival crevicular fluid compared to control subjects without diabetes.[18] Inflammation is a major determinant of clinical outcomes in periodontitis, and inflammatory mechanisms are involved in the development of diabetic complications. IL-1 plays a pivotal role in chronic inflammation and has been implicated in the pathology of both periodontitis[12] and diabetes.[19] The IL-1 gene complex codes for the three proteins IL-1α, -1β, and -1 receptor agonist (ra). IL-1α and -1β are strong inducers of inflammation, whereas IL-1ra binds to cellular IL-1 receptors without activating them, acting as a competitive inhibitor of IL-1.[20] Variations in the IL-1 gene cluster are associated with clinical phenotypes including in vivo levels of inflammatory markers and periodontitis[17] and type 2 diabetes mellitus.[19] The IL-1 gene cluster, particularly the T allele at both IL-1A –889 and -1B +3954 described by Kornman et al.,[17] has been extensively studied and is associated with periodontitis in different populations.[21-25] The relationship between periodontitis and allele 2 of the IL-1RN gene, which encodes IL-1ra, has not been extensively studied, although some studies[26,27] suggested a complex allele-dependent regulating effect of this gene on IL-1 secretion.

The association between IL-1 gene polymorphisms and periodontitis is attributed to allelic variations that may increase the secretion of IL-1.[17] In effect, monocytes from subjects carrying the T allele of the IL-1B +3954 produced a greater amount of IL-1β than subjects without this allele.[28] The IL-1A –889 T allele was associated with an almost four-fold increase in IL-1α levels in gingival crevicular fluid,[29] and the IL-1RN allele 2 is associated with enhanced IL-1β secretion in vitro.[28] Otherwise, the enhancing effect of IL-1RN allele 2 on IL-1ra plasma levels may require the presence of the T allele of IL-1B –511.[30]

Based on the data described above, it is biologically plausible that patients with diabetes who are carriers of proinflammatory polymorphisms might exhibit more severe periodontitis and that common genetic factors may be responsible for cross-susceptibility between periodontitis and diabetes. To test this hypothesis, an exploratory study was conducted to investigate the frequencies and the relationships among 1L-1 gene complex polymorphisms, type 2 diabetes mellitus, and chronic periodontitis.

MATERIALS AND METHODS

Study Patients

A total of 544 participants were recruited from patients who were receiving primary preventive care at a public health center in Santiago, Chile. Patients were selected from a homogeneous population with similar ethnic and socioeconomic status, level of education, and other demographic characteristics. Three groups of patients were selected: patients with diabetes and periodontitis, patients without diabetes but with periodontitis, and systemically healthy subjects without periodontitis. Potential participants were required to fulfill the following criteria: age >40 years, ≥14 teeth present, and no previous history of periodontal therapy. The diagnostic criteria for chronic periodontitis were the presence of ≥4 teeth with probing depths (PDs) ≥4 mm and concomitant clinical attachment level (CAL) ≥3 mm at interproximal sites and no evident clinical signs of aggressive periodontitis.

Exclusion criteria were as follows: rheumatoid arthritis, presence of other infections, systemic antibiotic treatment, or non-steroidal anti-inflammatory medication within 6 months prior to recruitment. Patients requiring antibiotic prophylaxis for periodontal probing were excluded. The recruitment of patients was done over an 18-month period from March of 2006 to September of 2007.

Four-hundred and eighty patients (aged 42 to 68 years) were screened for diabetes and periodontitis. One hundred twelve patients with type 2 diabetes and periodontitis were invited to participate. Of the 368 patients without diabetes, 224 patients with periodontitis were matched with patients with diabetes for age, gender, socioeconomic status, and educational level as well as smoking status. As a control group, 208 systemically healthy subjects without periodontitis were selected from those attending the same public health center. Controls were periodontally healthy subjects or those with mild gingivitis, with no sites with PD >3 mm except on distal surfaces of third molars, and no interproximal sites with CAL >2 mm.

A detailed medical and social history was taken for each participant. Age, gender, race/ethnicity, smoking status, level of education, family and individual

history of diabetes, and oral hypoglycemic drug intake or insulin administration were assessed by interview. To reduce genetic heterogeneity, patients with an Amerindian ethnic background were not selected. Smokers were those who had smoked ≥100 cigarettes in their lifetime; if they answered affirmatively to the question "Do you smoke now?," they were considered to be current smokers, and were considered to be former smokers if they answered negatively. The number of cigarettes smoked per day and years of smoking were also determined.

Diabetes was defined as serum glucose ≥126 mg/dl after fasting for ≥8 hours or self-reported current use of hypoglycemic medication or insulin.[31] Glycosylated hemoglobin (HbA1c)‡ from patients with diabetes was tested, and fasting glycemia from patients without diabetes but with periodontitis was tested when participants entered the study. The review committee for ethical norms of the School of Dentistry, University of Chile, approved the study. All subjects signed written consent forms before participating in the study.

Oral and Periodontal Examination

A full-mouth periodontal examination was performed by a calibrated examiner. All patients were examined by the same periodontist (NJL). Calibration sessions to measure intraexaminer reliability were conducted until satisfactory replication was achieved. The examiner was judged to be calibrated after meeting a percentage of agreement <2 mm between repeated measurements of ≥90%. The reproducibility was expressed as the proportion of agreement for clinical scores between different examinations performed on 10 patients over a 7-day interval. The agreement for PD and bleeding on probing (BOP) was 92%, and the agreement for CAL was 90%. All teeth present were examined and the following periodontal clinical parameters were assessed: PD, CAL, and BOP at six sites per tooth. Dichotomous measures of supragingival plaque accumulation were made by running a periodontal probe along the cervical area of four tooth surfaces.

PD was defined as the distance from the gingival margin to the most apical penetration of the University of North Carolina 15 probe. CAL was measured as the distance from the cemento-enamel junction to the most apical penetration of the probe. BOP was recorded during PD measurements and deemed positive if it occurred within 15 seconds after probing.

Analysis of Genetic Polymorphisms

Genomic DNA was extracted from peripheral blood lymphocytes. DNA samples were obtained according to the method described by Chomczynski and Sacchi.[32] A polymerase chain reaction (PCR)-restriction fragment length polymorphism assay was used to genotype IL-1A –889[33] (single nucleotide polymorphism identification [SNP ID] rs1800587), IL-1B +3954[28] (SNP ID rs1143634), and IL-1B –511[34] (SNP ID rs16944) variants. The IL-1RN variable-number tandem repeats were detected by PCR amplification and fragment-size analysis.[35]

Amplification reactions were carried out with 500 ng genomic DNA in a total volume of 50 μl containing 10 mM Tris-HCl, 50 nM KCl, 1 μM of each primer, 200 μM of each deoxyrinucleotide triphosphate, 1.5 nM $MgCl_2$, and 2.5 U Taq DNA polymerase.§

The thermal cycles were initiated for 5 minutes at 95°C, followed by 35 30-second cycles at 95°C, 45-second cycles at 56°C, a 1-minute cycle at 72°C, and a 7-minute final extension cycle at 72°C. Amplifications were carried out in a thermal cycler. PCR products were digested overnight with restriction enzymes‖ according to the manufacturer's protocols and analyzed by 3% agarose gel electrophoresis. Primers, restriction enzymes, and the length of the digested fragments are shown in Table 1. To determine the validity of the method, 20 samples were genotyped twice with identical results.

Data Analyses

Clinical periodontal parameters were computed for each patient and averaged across patients in the three groups. Significant differences among patients with diabetes, patients without diabetes, and healthy controls were determined using the two-sample *t* test for continuous variables and the χ^2 test for categoric variables. Hardy-Weinberg equilibrium was tested for genotype frequencies by the χ^2 test with 1 degree of freedom. Mean values of the clinical parameters were used as measures of periodontitis. The association between allele and genotype frequencies among the three groups of patients were analyzed by the χ^2 test. The strength of the association was determined using an odds ratio (OR) calculation and 95% confidence intervals (CIs). In the first analysis, genotypes and allele frequencies between patients with diabetes and patients without diabetes and between patients with diabetes and controls were compared without categorizing patients by smoking status. Periodontal clinical parameters were compared between IL-1 genotype-positive and -negative patients, categorizing both groups as smokers or non-smokers. The positive IL-1 genotype was defined as at least one T allele at both IL-1A –889 and -1B +3954.[17] To differentiate the extent of attachment loss, tertiles were calculated for patients with diabetes and patients without diabetes. The frequencies of polymorphisms in patients in the upper tertiles of

‡ oneHBA1c FS, DiaSys, Diagnostic Systems, Holzheim, Germany.
§ Amersham Pharmacia Biotech, Uppsala, Sweden.
‖ New England Biolabs, Ipswich, MA.

Table 1.

Genotyping of Polymorphisms Within the IL-1 Gene Cluster

Variant	Forward/Reverse Primer Sequence	PCR Product Size (bp)	AT (°C)	Restriction Enzymes
IL-1A –889	5′-GGTGTTCTACCACCTGAACTAGGC-3′ 5′-GGATTTTTACATATGAGCCTTCAATG-3′	107	61	*Nco*I; C = *Nco*I (+), T = *Nco*I (–)
IL-1B +3954	5′ –CTCAGGTGTCCTCGAAGAAATCAAA-3′ 5′ GCTTTTTTGCTGTGAGTCCCG- 3′	194	61	*Taq*I; C = *Taq*I (+), T = *Taq*I (–)
IL-B –511	5′-TGG CATTGATCTGGTTCATC-3′ 5′-GTTTAGGAATCTTCCCACTT-3′	305	53	*Ava*I; G = *Ava*I (+), A = *Ava*I (–)
IL-1RN VNTR	5′-CTCAGCAACACTCCTAT-3′ 5′-TCCTGGTCTGCAGGTAA-3′	240 = 2 repeats 326 = 3 repeats 412 = 4 repeats 498 = 5 repeats 584 = 6 repeats	52	–

bp = base pair; AT = annealing temperature; VNTR = variable-number tandem repeats.

Table 2.

Patient Characteristics (mean ± SD or n [%])

Characteristic	Patients With Diabetes and Periodontitis (n = 112)	Patients Without Diabetes but With Periodontitis (n = 224)	*P* Value*	Healthy Controls (n = 208)	*P* Value†
Mean age (years)	54.75 ± 9.34	55.05 ± 9.20	0.78	52.2 ± 7.80	0.001
Men	35 (31.25)	75 (33.48)	0.35	68 (32.69)	0.94
>12 years of education	34 (30.35)	76 (33.92)	0.16	74 (35.57)	0.80
Teeth lost (n)	8.64 ± 5.06	6.64 ± 4.87	0.001	6.40 ± 5.23	0.62
Mean PD (mm)	3.38 ± 0.95	3.07 ± 0.74	0.003	2.22 ± 0.32	0.001
Mean CAL (mm)	4.17 ± 1.57	3.50 ± 1.34	0.001	2.08 ± 0.78	0.001
Sites (%) with:					
BOP	53.59 ± 20.43	46.61 ± 21.02	0.004	18 ± 9.20	0.001
PD ≥4 mm	40.67 ± 28.02	33.87 ± 24.47	0.020	2.42 ± 0.97	0.001
PD ≥6 mm	6.82 ± 12.04	4.44 ± 8.25	0.034	0.00	0.001
CAL ≥3 mm	75.49 ± 24.81	67.76 ± 26.66	0.011	14.32 ± 8.56	0.001
CAL ≥5 mm	30.72 ± 29.56	19.74 ± 22.95	0.001	3.84 ± 2.89	0.001
Surfaces with plaque	87.15 ± 18.16	81.06 ± 21.85	0.012	46.92 ± 12	0.001
Family history of diabetes	79 (70.53)	82 (36.60)	0.001	78 (37.50)	0.022
Current smoker	38 (33.92)	74 (33.03)	0.87	65 (31.25)	0.77
Former smoker	42 (37.50)	63 (28.12)	0.081	54 (25.96)	0.69

* Between patients with diabetes and patients without diabetes.
† Among healthy controls and all subjects with periodontitis.

distribution (with major attachment loss) were compared to those in the lower tertiles (with minor attachment loss). Statistical significance was set at $P<0.05$. All analyses were performed using a statistical package.¶

RESULTS

The main characteristics of the study patients are shown in Table 2. No significant differences in gender

¶ SAS, version 10, SAS, Cary, NC.

and socioeconomic and educational levels among patients with diabetes, patients without diabetes, and healthy controls were found. Patients with periodontitis had a significantly higher mean age than the healthy controls.

Patients with diabetes had a significantly higher number of teeth lost. The duration of diabetes ranged from 2 to 12 years with a mean value of 5.8 years, and all patients with diabetes were receiving oral hypoglycemic medication and diet-control treatment.

All clinical periodontal parameters showed that patients with diabetes had significantly more severe and extensive periodontal disease than patients without diabetes (Table 2). There was a significantly higher proportion of patients with diabetes who had a family history of diabetes than patients with periodontitis and without diabetes (P= 0.0001) and healthy subjects (P= 0.0001). Among patients with diabetes, 53.8% had good metabolic control (HbA1c <8%). The means of all periodontal clinical parameters were higher in patients with diabetes with poor metabolic control than in patients with diabetes with good metabolic control, but only the difference between the number of sites with PD ≥4 mm was significant (47.22 ± 29.05 versus 36.44 ± 26.69; P= 0.046). There were no significant differences in the periodontal parameters of smokers versus non-smokers in patients with diabetes and patients without diabetes.

Frequency of Genotypes

The frequency of genotypes is shown in Tables 3 and 4. The frequency of the IL-1A –889 and -1B +3954 genotypes were in Hardy-Weinberg equilibrium in the healthy controls (P = 0.41 and P = 0.26, respectively) but not in patients with diabetes and patients without diabetes (P<0.05) due to an excessive number of TT homozygotes in both groups of patients. The IL-1RN genotypes were in Hardy-Weinberg equilibrium in patients with diabetes (P = 0.65) but not in patients without diabetes but with periodontitis (P = 0.002) or in the control group (P = 0.001). The cause of this deviation in genotype frequency is unknown, but it does not affect the results because the genotype frequencies of IL-1RN were very similar in the three groups

Table 3.

Distribution of IL-1A –889 and -1B +3954 Genotypes and Allele Frequencies in Patients With Diabetes, Patients Without Diabetes but With Periodontal Disease, and Healthy Controls

Genotype	Patients With Periodontitis				All With Periodontitis (n = 336)		Healthy Controls (n = 208)		*P* Value*
	Patients With Diabetes (n = 112)		Patients Without Diabetes (n = 224)						
IL-1A –889	n	%	n	%	n	%	n	%	
CC	63	56.25	111	49.55	174	51.78	119	57.21	0.25
CT	36	32.14	83	37.05	119	35.41	80	38.46	0.53
TT	13	11.60†	30	13.93‡	43	12.79	9	3.94†‡	0.001
CT or TT	49	43.75	113	50.44	162	48.21	89	42.78	0.25
Allele									
C	162	72.32	305	68.08	467	69.50	318	76.44	0.098
T	62	27.67	143	31.91	205	30.50	98	23.55	0.097
IL-1B +3954									
CC	76	67.85	134	59.82	210	62.50	161	77.40	0.0001
CT	27	24.0	69	30.80	96	28.57	42	20.19	0.037
TT	9	8.03§	21	9.37‖	30	8.92	5	2.40§‖	0.005
CT or TT	36	32.14	90	40.17¶	126	37.50	47	22.59¶	0.0001
Allele									
C	179	79.91	337	75.22	516	76.78	364	87.50	0.0001
T	45	20.08	111	24.77	156	23.22	52	12.50	0.0001
Positive genotype	29	25.89	69	30.80#	98	29.16	45	21.63#	0.066

* Among all patients with periodontitis versus healthy controls.
† P = 0.017.
‡ P <0.0001.
§ P = 0.039.
‖ P = 0.004.
¶ P = 0.0001.
# P = 0.040.

Table 4.

Distribution of IL-1RN and -1B –511 Genotypes and Allele Frequencies in Patients With Diabetes, Patients Without Diabetes but With Periodontal Disease, and Healthy Controls

Genotype	Subjects With Periodontitis				All With Periodontitis (n = 336)		Healthy Controls (n = 208)		*P* Value*
	Patients With Diabetes (n = 112)		Patients Without Diabetes (n = 224)						
IL-RN	n	%	n	%	n	%	n	%	
1-1	61	54.46	128	57.14	189	56.25	126	61.57	0.25
1-2	36	32.14	61	27.32	97	28.86	49	23.55	0.20
2-2	13	11.60	28	12.5	41	12.20	29	13.94	0.64
1-2 or 2-2	49	43.75	89	39.73	138	41.07	78	37.50	0.46
Others	2	1.78	7	3.12	9	2.67	7	3.36	0.84
Allele									
1	158	70.53	317	70.75	475	70.68	301	72.35	0.63
2	62	27.67	117	26.11	179	26.63	107	25.72	0.63
IL-1B –511									
CC	24	21.43	58	25.89	81	24.10	29	13.94	0.006
CT	48	42.86	117	52.23	165	49.10	107	51.44	0.65
TT	40	35.71‡	49	21.87†‡	89	26.49	72	34.61†	0.055
CT or TT	88	78.57	166	74.10§	254	75.60	179	86.05§	0.006
Allele									
C	96	42.85	233	52.00‖	329	48.95	165	39.66‖	0.043
T	128	57.14	215	47.99¶	343	51.04	251	60.34¶	0.043

* Among all subjects with periodontitis and healthy controls.
† $P = 0.005$.
‡ $P = 0.010$.
§ $P = 0.003$.
‖ $P = 0.013$.
¶ $P = 0.013$.

of patients. The IL-1B –511 genotypes were in Hardy-Weinberg equilibrium in the three groups of patients ($P > 0.05$).

No significant differences were found in the distribution of the IL-1A –899 and -1B +3954 genotypes in patients with diabetes compared to patients without diabetes (Table 3). A significantly higher frequency of the IL-1A –889 TT genotype was found in patients with periodontitis, patients with diabetes ($P = 0.017$), and patients without diabetes ($P < 0.0001$) than in healthy controls. Those with the IL-1A –899 TT genotype were at higher risk for periodontitis than those with CC or CT genotypes (OR = 2.90; 95% CI = 1.20 to 7.02; $P = 0.001$).

Those with IL-1B +3954 CT or TT genotypes were at higher risk for periodontitis (OR = 2.05; 95% CI = 1.38 to 3.04; $P = 0.001$), and the TT genotype had a stronger association with periodontitis (OR = 3.54; 95% CI = 1.15 to 10.84; $P = 0.006$). The prevalence of the IL-1 positive genotype among the 544 patients studied was 26.28% and significantly higher in patients without diabetes but with periodontitis (30.80%) than in controls (21.63%) (OR = 1.61; 95% CI = 1.04 to 2.49; $P = 0.040$) (Table 3). The CC/CC composite genotype at IL-1A –889 and -1B +3954 appeared to be protective for periodontitis (OR = 0.50; 95% CI = 0.34 to 0.73; $P = 0.002$). Patients with diabetes and patients without diabetes with the IL-1 positive genotype had a higher mean value for all periodontal parameters than patients with diabetes and patients without diabetes with the IL-1 negative genotype, but the difference was not significant.

The distribution of the IL-1RN genotypes was similar in the three groups (Table 4). No association between IL-1RN genotype and periodontitis or diabetes was found. Because the enhancing effect of the IL-1RN allele 2 on IL-1ra levels may require the presence of the IL-1B –511 T allele,[30] a combined analysis of the two polymorphisms was performed to assess the risk for both diabetes and periodontitis. No difference in risk association with the combined effect of the two polymorphisms was found.

The IL-1B –511 CC genotype was significantly associated with periodontitis in patients with diabetes and patients without diabetes but with periodontitis (OR = 2.10; 95% CI = 1.25 to 3.58; $P = 0.006$) (Table 4). The frequency of the IL-1B –511 TT genotype was significantly higher in patients with diabetes than in patients without diabetes ($P = 0.010$) and similar to

that of controls. This was the only significant difference in the distribution of all the genotypes studied between patients with diabetes and patients without diabetes but with periodontitis (Table 4). When the frequency of the IL-1B –511 TT genotypes in patients with periodontitis was compared to healthy controls, it appeared to be a protective factor for periodontitis (OR = 0.68; 95% CI = 0.46 to 0.98; $P = 0.044$). The distribution of the IL-1B –511 CT genotype was not significantly different in the three groups.

When patients with periodontitis in the upper tertiles of distribution with major attachment loss were compared to those in the lower tertile with minor attachment loss, no significant differences were found in the frequency of genotypes among patients with diabetes and patients without diabetes. Patients with diabetes and patients without diabetes who smoked had a higher mean for all periodontal parameters compared to nonsmokers, but the differences were not significant. No association between smoking status, IL-1 positive genotype status, and periodontitis was found in either patients with diabetes or patients without diabetes.

DISCUSSION

We evaluated the potential role of polymorphisms in the IL-1A, -1B, and -1RN genes on the risk of periodontitis in patients with diabetes and patients without diabetes. With the exception of the higher frequency of the IL-1B –511 TT genotype in patients with diabetes, the distribution of all the other genotypes was not significantly different in patients with diabetes or patients without diabetes. The IL-1B –511 CC genotype was strongly associated with periodontitis, whereas the IL-1B –511 TT genotype appeared to be a protective factor for periodontitis. Nevertheless, patients with diabetes had a similar frequency of TT genotype as controls. It is possible that the IL-1B –511 polymorphisms are in linkage disequilibrium with other unidentified polymorphisms that are more frequent in people with diabetes or that the protective effect of the TT genotype on periodontitis is neutralized in combination with other polymorphisms present in people with diabetes.

The association between the variants of the IL-1B –511 genotypes and periodontitis found in our study agrees, in part, with the results of a meta-analysis[36] that found that whites with IL-1B –511 CT and CC genotypes had a modestly increased risk for periodontitis, but an even higher association was present in Asians. However, in our study, the CT genotype was not associated with periodontitis. A recent study of 219 Danish subjects[37] also found that the IL-1B –511 C allele was associated with higher alveolar bone loss, and the T allele was associated with lower alveolar bone loss. The biologic basis for the association between IL-1 polymorphisms and periodontitis is hypothesized to be that these polymorphisms may modulate the secretion of IL-1β and -1α. The association between the IL-1B –511 CC genotype and periodontitis found in the our study is congruent with the finding that this genotype was associated with the highest in vivo levels of IL-1β.[38]

Only two studies[39,40] addressing a possible genetic association between periodontitis and type 2 diabetes have been published. One study[39] found that the IL-1B –511 C allele was significantly associated with periodontitis in a subset of African American patients with diabetes (n = 29). However, the study did not indicate the type of diabetes, and the stratification of genotype associations by ethnicity resulted in small groups of patients, making it difficult to interpret the results. Another study,[40] conducted in a German population, found that the IL-1 –511 CT or TT genotypes were significantly associated with periodontitis in patients with diabetes but not in patients without diabetes. This result is at variance with our results, suggesting that the TT genotype is a protective factor for periodontitis. There was a significant difference in the frequency of the CT and TT genotypes in a German population in the study by Struch et al.[40] (56.17%) and in the Chilean population in the current study (79.77%) ($P = 0.001$). Nevertheless, the frequency of CT and TT genotypes in the Danish population studied by Geismar et al.[37] (52.96%) was similar to that of Germans. The association between the IL-1B –511 C allele with severe alveolar bone loss in Danish patients agrees with the association between the CC homozygotes and periodontitis found in our study. It was reported that IL-1B gene transcription is influenced by four promoter polymorphisms, and that the individual polymorphism function in vitro is governed by the haplotype context.[38] Therefore, to determine the exact role of the IL-1B –511 CC genotype on periodontitis, it is necessary to identify other SNPs at the IL-1B gene that may influence the proinflammatory activity of the CC genotype.[38] Therefore, the disagreement between our results and those of Struch et al.[40] may be caused by the influence of other polymorphims that were not determined in either of these studies.

Our results support the notion that the IL-1A –889 TT or -1B +3954 TT genotypes increase the risk for periodontitis in patients with diabetes and patients without diabetes and that the IL-1 positive genotype is associated with periodontitis. These results agree with those of others that reported a significant association of the T alleles of IL-1A –889 and -1B +3954 with chronic periodontitis in Chileans[25] and other populations.[17,21,22,24,25,36,37,41-43]

The frequency of the IL-1 positive genotype in the current study (26.28%) is in the range described for the European population[22] and the white population in the United States,[42,43] although the contemporary

mixed population of Santiago, Chile, which stems from the admixture of aboriginal Amerindian, Spanish, and other European ethnic groups, has an average Amerindian admixture of 37%.[44]

The frequencies of the IL-1A –889 and -1B +3954 genotypes deviated from the Hardy-Weinberg expectation in patients with diabetes and in patients without diabetes but with periodontitis because there were an excess number of patients with periodontitis who were homozygous for the T allele, but not in the group of healthy patients. These data agree with the finding that those who are homozygous for the T allele at IL-1A –889 or -1B +3954 have an increased probability of having periodontitis.[17,25,36]

In the current study, patients with diabetes had more severe and more extensive periodontitis than patients without diabetes but with periodontitis, confirming previous studies[10,45,46] that showed that diabetes is a risk factor for periodontitis. Poor metabolic control that occurs in type 2 diabetes increases gingival inflammation,[46] but there is no clear evidence that it increases the severity of periodontitis.[47] In our patients, only the extent of periodontitis, measured by the number of sites with PD ≥4 mm, was significantly higher in patients with diabetes with poor metabolic control. In our study, patients with diabetes and patients without diabetes who were smokers had worse periodontal status than non-smokers. Although the difference in the periodontal parameters were not significant, this may have been because the majority of smokers were not heavy smokers.

The lack of association between IL-1RN gene polymorphisms and periodontitis or diabetes found in the current study is in concordance with other studies[48] in whites in which no association was found. We also evaluated the combined effect of the IL-1RN allele 2 and -1B –511 T allele and found no difference in risks for periodontitis or diabetes.

The IL-1RN genotypes were in Hardy-Weinberg equilibrium in patients with diabetes but not in patients without diabetes or in healthy patients because of a moderately higher number of participants who were homozygous for allele 1 in these last two groups. This suggests that patients with diabetes who are homozygous or heterozygous for IL-1RN have similar probabilities of having periodontitis.

Patients with diabetes and patients without diabetes who had an IL-1 positive genotype showed higher means values for all periodontal parameters than those who had an IL-1 negative genotype, but the differences were not significant.

CONCLUSIONS

The IL-1A –889 or -1B +3954 TT genotypes and the IL-1B –511 CC genotype was associated with periodontitis in patients with diabetes and patients without diabetes. The IL-1B –511 T allele appeared to be a protective factor for periodontitis. Data from the current study do not support a cross-susceptibility between periodontitis and diabetes associated with IL-1A –889, -1B +3954, and -1RN polymorphisms.

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