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IL8 Gene Promoter Polymorphism (rs4073) may Contributes to Chronic Periodontitis

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Abstract

Background—The proinflammatory chemokine interleukin-8 (IL8) is important in the regulation of the inflammatory response. Single nucleotide polymorphism (SNP) rs4073 have shown that the A allele up regulates the IL8 levels after stimulation with lipopolysaccharides. We investigated the association of the SNP rs4073 with chronic periodontitis.

Methods—Genotyping was performed by standard polymerase chain reaction-restriction fragment length polymorphism assay in 289 genomic DNA samples of healthy control and chronic periodontitis patients; analyses were adjusted by the multivariate logistic regression modeling. Real-time PCR performance was used to detect the levels of the IL8 mRNA.

Results—Analysis points to a statistical significant association of chronic periodontitis with the heterozygous TA genotype ($p = 0.001$); the results showed an increase in the frequency of the A allele in the diseased group (36% in the control $\times$ 48% in the periodontitis group). The higher levels of the IL8 mRNA were found in the periodontitis group, mainly in individuals who presented the TA genotype ($p=0.03$).

Conclusion—The SNP rs4073 is associated with chronic periodontitis, in non-smoker Brazilian subjects, since the frequency of A allele is higher in the disease than control group and the TA genotype was associated with increased levels of IL8 mRNA transcripts.

Keywords

periodontitis; polymorphism; single nucleotide; Interleukin-8; genotype; alleles

Periodontitis is a complex and multifactorial disease that results from the interaction of the host defense mechanisms with the plaque microorganisms. This interaction leads to the primary clinical features of periodontitis, which are gingival inflammation, attachment loss, periodontal pocketing and alveolar bone loss.¹ The bacterial activation in the junctional

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epithelium initiates vascular endothelial responses that involve, primarily, neutrophil migration to the sulcus.²

Interleukin-8 (IL8), the most potent known chemokine, is responsible for inducing chemotaxis, which is the directed migration of cells to a site of inflammation.³ This chemokine also mediates the activation and migration of neutrophils into tissue from peripheral blood.^{4,5} Multiple stimuli will induce the secretion of IL8, including lipopolysaccharide, live bacteria, early proinflammatory cytokines such as tumor necrosis factor (TNF) and Interleukin-1 (IL-1).^{3,6} IL8 is relatively unique, since it may be produced early in the inflammatory response, but will persist for a prolonged period of time, for days or even weeks,^{3,7} in contrast to most inflammatory cytokines, which are typically made and cleared during *in vivo* situations in a matter of a few hours.³

It is now evident that there is a genetic basis for most diseases, including periodontitis. Evidence from classical twin studies⁸ suggests that genetic determinants are significant modifiers of the periodontitis phenotype.⁸⁻¹⁰ Elucidation of the genetic basis of periodontitis should permit a better understanding of disease etio-pathogenesis, allowing improved classification, diagnosis, and treatment. Thus, enormous efforts have been expended to identify gene polymorphisms associated with the risk for the periodontitis.¹¹⁻¹⁶

A well-characterized *IL8* gene single-nucleotide polymorphism (SNP) at position -251T/A (rs4073 - reference sequence number from the NCBI's Entrez system) was associated with several diseases as gastric cancer, bronchiolitis, breast cancer, macular degeneration, oral squamous cell carcinoma and prostate cancer.¹⁷⁻²² Analyses have shown that the A allele up regulates IL8 levels after stimulation with lipopolysaccharide (LPS);²³ therefore, it could be interesting to investigate the functional relationship of -251T/A polymorphism with periodontitis. Thus, this study was designed to investigate the association of the SNP rs4073 with generalized chronic periodontitis, attending to the levels of IL8 mRNA in control and diseased tissues related with the respective genotypes.

MATERIAL AND METHODS

Study Population

Subjects from the Southeastern region of Brazil were recruited from the patient pool of the Dental Clinics of the School of Dentistry at Piracicaba, State University of Campinas, during the period of July 2008 to July 2009. This study was approved by the Ethical Committee for Research of the State University of Campinas (Protocol number 095/97), and informed consent was signed by each subject after explanations were provided. Two hundred and eight nine (289) non-smoking subjects (control group n=108 subjects and generalized chronic periodontitis n=181 subjects) were included in this study and the demographic characteristics of the studied population are presented in the Table 1. All patients received a complete intraoral and periodontal examination, including a full-mouth periodontal probing, tooth mobility, gingival bleeding on probing, radiographic examination, medical and dental anamnesis. The inclusion periodontal criteria were (i) diagnosis and classification of generalized chronic periodontitis were performed based on the 1999 Consensus Classification of Periodontal Diseases (Armitage 1999)²⁴; (ii) good general healthy; (iii) diagnosis of generalized chronic periodontitis was characterized by the presence of periodontal pockets with clinical attachment loss of ≥ 5 mm, bleeding on probing and radiographic bone loss; (iv) age over 35 years; (v) at least eight teeth with a probing pocket depth of ≥ 5 mm and bleeding after pocket probing and at least two of the eight qualifying teeth must have probing pocket depth ≥ 7 mm; and (vi) ≥ 20 teeth in both jaws (wisdom teeth excluded).

Measurements of probing pocket depth and clinical attachment loss were recorded at six points around each tooth. The individuals of the control group also were examined attending the same clinical parameters as used in the chronic periodontitis group, but the subjects did not show any sites with clinical attachment loss and probing pocket depth ≥ 3 mm and presented full-mouth bleeding score (FMBS) = 25%; tooth mobility was not found. The clinical parameters were obtained by one calibrated examiner (D.C.A.; intraclass correlation = 0.94).

Subjects did not have any of the following exclusion criteria: smoking, diseases of the oral hard or soft tissues (except caries and periodontitis), use of orthodontic appliances, need for pre-medication for dental treatment, chronic usage of anti-inflammatory drugs, a history of diabetes, hepatitis or HIV infection, immunosuppressive chemotherapy, history of any disease known to severely compromise immune function, presence of acute necrotizing ulcerative gingivitis, or current pregnancy or lactation.

Genetic Analysis

Isolation of genomic DNA—The sampling of buccal epithelial cells was performed as described by Trevilatto & Line (2000).²⁵ DNA was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation. DNA was dissolved in 70 ml TE buffer [10mM Tris (pH 7.8), 1mM EDTA] and its concentration estimated by measuring absorbance at 260 nm using a spectrophotometer⁸. DNA integrity was checked using agarose gel electrophoresis.

Polymerase chain reaction (PCR)—A fragment of 349 bp of the *IL8* gene promoter (GenBank M28130) was PCR amplified with specific primers: (forward) 5'-CATGATAGCATCTGTAATTAAGT 3' and (reverse) 5'-CTCATCTTTTCATTATGTCAGAG-3'. PCR was carried out in a total volume of 10 μ l, containing 1 μ l genomic DNA, 1.5 μ l of each primer and 5 μ l of Go Taq Green Master Mix^{||}. The solution was incubated for 5 min. at 95°C, followed by 35 cycles of 1 min. at 95°C, 1 min. at 51°C and 1min. at 72°C, with a final extension of 72°C for 7 min.

Genotyping SNP rs4073—The restriction endonuclease digestion was prepared using a 5 μ l aliquot of *IL8* PCR products mixed with a 5 μ l solution containing 1 μ l 10 X NE Buffer (50mM NaCl, 10mM Tris-HCl, 10mM MgCl₂, 1mM dithiothreitol, pH 7.9), 0.25 μ l *MfeI* (10,000 U/ml)^{||} and 3.75 μ l sterile deionized H₂O. The solution was incubated at 37°C overnight. The A allele completes a corresponding restriction endonuclease site, while the T allele does not. The length difference of the RFLP fragments was 147bp and 55bp (TT 349bp; TA 349bp, 202bp and 147bp; AA 202bp and 147bp). Aliquots of 5 μ l of the RFLP products were electrophoresed on 10% vertical non-denaturing polyacrylamide gel at 20 mA. The gel was stained by a nucleic acid gel stain[#]. All samples were genotyped in duplicate and the genotyping was performed blind with respect to clinical diagnosis.

RNA Extraction

Twelve control and twelve generalized chronic periodontitis gingival biopsies were obtained from single teeth from subjects undergoing periodontal surgery for periodontal disease-related (generalized chronic periodontitis group) and non-disease-related reasons (control group) at Dental Clinics of the School of Dentistry at Piracicaba, State University of Campinas. Following blocks anesthesia, tissues on the tooth surface where periodontal pockets were evaluated were incised to include the entire soft tissue walls of the pockets to be investigated. Entire pocket, junctional epithelium and connective tissues were removed and immediately stored^{**} at room temperature for 24 h. Then samples were frozen at -20°C for 24 h and after frozen at -70°C until analyses. The RNA extraction was carried out in a

hood dedicated to this work and sterile latex gloves were employed throughout. Each sample was mechanically homogenized for 5 min on dry ice until the still-frozen mixture appeared uniform.²⁶ Total RNA was purified using the TRIZOL reagent^{††} following the manufacturer's recommendations.

Reverse Transcription And Real Time PCR

One microgram of total RNA was DNase^{‡‡} treated and 500 ng was used for cDNA synthesis. The reaction was carried out using the first strand cDNA synthesis kit^{§§} following the manufacturer's recommendations. After, a fragment of 172 bp was amplified using specific primers (forward 5'gccaagagaatatccgaactttaat3'/reverse 5'ctggctagcagactagg3') by quantitative PCR^{||} using a kit.^{¶¶} The reaction product was quantified using the Relative Quantification tool, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene.

Statistical Analysis

The associations between periodontitis, SNP rs4073, gender, age and ethnic group in the control and generalized chronic periodontitis subjects were adjusted by a multivariate logistic regression modeling,^{##} which allows, simultaneously, a better control on the experimental variables. There were four ethnic groups enrolled in this study: Caucassoid, african-american, mulatto and japanese. Since only two individuals were japanese, the statistic program automatically dropped these individuals as reference to the regression analyses (data not shown). Similarly, the statistic program dropped the TT genotype, as can be observed in the *p* value and coefficient columns (table 2) and also for gender (data not shown). Analysis of Hardy-Weinberg equilibrium was used to test deviation of genotype distribution^{***}. With respect to gene expression, an intergroup analysis was performed by the Mann-Whitney test at a level of 5%. In order to verify statistical power of our sample, a statistical program was used^{†††27}. The input parameters were: moderate effect size = 0.3, based on genotype distribution data; statistical significance level $\alpha = 0.05$; one and two degrees of freedom for allelic and genotype analysis, respectively. Statistical power for our sample was higher than 90% for association detection.

RESULTS

The demographic characteristics of the studied population are summarized in Table 1 and the genotype distributions for the SNP rs4073 were in Hardy-Weinberg equilibrium ($P > 0.05$). Statistical analysis showed a significant association between TA genotype and generalized chronic periodontitis ($p = 0.005$; coefficient 1.3); in addition, the multivariate analysis also showed an association between age and generalized chronic periodontitis ($p < 0.001$; coefficient 0.07). The observed genotypes frequencies in the control group were TT = 35.2%, TA = 52.8% and AA = 12.0%, resulting in an A allele frequency of 36% and carrier frequency of at least one A allele of 62.8%. On the other hand, the frequencies of TT, TA and AA genotypes found in the generalized chronic periodontitis group were 13.8%, 74.6% and 11.6% respectively, resulting in an A allele frequency of 48% and carrier frequency of at least one A allele of 86.2%. Individuals with the TA genotype are approximately 2.6 times more likely to develop periodontitis ($p = 0.0002$; Odds ratio (Or) = 2.62; 95% confidence interval (CI) 1.58 – 4.34) (Table 2).

Data for the gene expression analysis demonstrated that the IL8 mRNA levels were higher in the generalized chronic periodontitis subjects, mainly in those who presented the TA genotype. The difference amongst the two groups was statistically significant ($p = 0.03$; Mann-Whitney Test) (Figure 1). None of the 24 samples submitted to the gene expression analysis presented the AA genotype.

DISCUSSION

The subjects under investigation were Brazilian individuals with generalized chronic periodontitis whose genotypes were compared to those from controls with adjustments made for age, gender and ethnicity. We analyzed the SNP 4073 that is transmitted at linkage disequilibrium with 5 other alleles in the coding sequences of the *IL8* gene, including the functional +781 T allele that influences *IL8* gene transcription levels.²⁸ Hacking et al.²⁸ showed that the SNP 4073 (position -251 in the *IL8* gene promoter) in response to TNF stimulation is not functional. However, they observed that this SNP is transmitted at linkage disequilibrium with a functional variant in the *IL8* gene, compounding haplotypes. The study shows that are two major haplotypes: Haplotype 1 (52%): T_{allele-251}/T_{allele+396}/C_{allele+781}/insA_{allele+1238}/C_{allele+1633}/A_{allele+2767} and Haplotype 2 (41%): A_{allele-251}/G_{allele+396}/T_{allele+781}/delA_{allele+1238}/T_{allele+1633}/T_{allele+2767}. The haplotype-2 which contains the -251A allele, up regulates the IL8 transcription and may be expressed more in IL8 haplotype-1/-2 heterozygous individuals. This suggestion could explain the association found in the study only for heterozygous individuals (TA). The homozygous AA genotype was not associated with periodontal disease, however, the odds ratio analysis demonstrated that the AA genotype also had a meaningful OR, showing that individuals carrying at least one A allele have an increased risk for periodontitis.

There are few reports associating IL8 expression and *IL8* genotypes in the literature, however Hull & Thomson & Kwiatkowski,¹⁸ investigating the association of respiratory syncytial virus bronchiolitis with the SNP rs4073, suggest that white blood cells from individuals carrying the A allele produce higher levels of IL8 in response to stimulus with LPS. One year later, in another study,²³ the authors described eight novel polymorphisms in the *IL8* gene, in addition to SNP rs4073. They observed that the -251 A allele at linkage disequilibrium with +781 T allele haplotype showed a significant association with susceptibility to bronchiolitis. A recent study, in a Mexican population, analyzed 78 patients with histologically-confirmed distal gastric cancer and 259 non-cancer controls and showed that the A allele is related to the development of distal gastric cancer.¹⁷ Furthermore, Kamali-Sarvestani & Aliparasti & Atefi¹⁹ observed an increased frequency in the production of IL8 in breast cancer patients carrying the AA genotype. With regard to aged-related macular degeneration (AMD), Goverdhan et al.²⁰ concluded that the AA genotype is an important risk factor for AMD; and Vairaktaris et al.²¹ reported that the A allele was observed in all DNA samples of 158 German and Greek patients with squamous cell carcinoma, in comparison to 156 healthy controls of equivalent sex, ethnicity and age.

In this study, logistic regression modeling demonstrated an association between the SNP rs4073 and generalized chronic periodontitis. The multivariate analysis was also used to assess important factors known to influence the pathogenesis of periodontitis, such as gender, age and ethnicity. Results demonstrated an association between age and generalized chronic periodontitis. In fact, both the prevalence and severity of periodontitis increase with age.^{1,29} However, the relationship between age and periodontitis is not straightforward and the literature on the effects of age on periodontitis needs to be interpreted with caution.²⁹ The TA genotype and age coefficients were 1.3 and 0.007 respectively and both were found to be associated with periodontitis; therefore, the impact of the association of the TA genotype is greater than age, in this particular study. Since this study was designed to investigate the association of the SNP rs4073 with generalized chronic periodontitis, discussing regarding association with age are not possible at this time. As such, we may suggest an association of the TA genotype with chronic periodontitis although we must not forget the importance of age. As regard ethnic groups, the logistic regression modeling demonstrated a statistical association of the TA genotype with an effect size (coefficient) of 1.3. Afterwards, this association was confirmed by means of analyzes in the Caucasian

individuals separately, with a small effect size (1.06), compared to the total population. False positive results should always be tested in studies with mixed ethnicities, as carried out in the present investigation. When the Caucasians were analyzed separately and the association was preserved, although with a small effect size, we can refute the potential for a false positive association, in population studied.

Results showed a decrease in the frequency of the TT genotype (35.2% in the control group versus 13.8% in the diseased group) and an increase in the frequency of the TA genotype (52.8% in the control group versus 74.6% in the diseased group). Since the A allele is considered as the risk allele and up regulates the IL8 levels after stimulation with LPS²³ and with TNF,²⁸ it is possible to infer that the T allele could be a protection allele against the disease. The presence of the heterozygous genotype (TA) in the subjects who presented generalized chronic periodontitis was about 75%. This percentage is higher than reported for African-American (37%) and European populations as well (~40%–46%); on the other hand, our findings are in agreement with the percentage of the A allele found in the Europeans (36%) (www.ncbi.nlm.nih.gov). In addition, 86.2% of the individuals who presented chronic periodontitis carried at least one A allele.

Although there is a substantial variability in the levels of IL8 mRNA transcripts in the gingival biopsies of the individuals analyzed, the highest levels of transcripts were found in the generalized chronic periodontitis group, mainly in individuals who presented the TA genotype and, therefore, the A allele. The statistical difference amongst the TA and TT genotypes, in the generalized chronic periodontitis group, was not significant. However, the difference in the IL8 transcript levels, amongst the TA genotypes between the two groups studied was statistically significant ($p=0.03$). Therefore, all the results obtained suggest an association of the A allele with periodontal disease, probably causing an increase in the production of the IL8 transcripts during the inflammation resulting from the periodontitis.

In contrast to the findings of the present paper, an investigation¹⁵ with the same SNP, also in a Brazilian population, fail to demonstrate a positive association with chronic periodontitis. Both studies showed almost the same percentage of individuals with the TA genotype, in the control group. However, Kim et al.¹⁵ did not find an increase in the number of individuals with the TA genotype, in the periodontitis group, as we did in our study. Another important point is the difference in the distribution of ethnics between both studies. The literature demonstrates that genetic associations may not be uniformly consistent across populations and that the frequency of occurrence of polymorphisms appears to vary extensively between ethnic groups.³⁰ Moreover, the literature shows that the subject samples involved in studies are generally of small size, the definitions of the outcome variable (periodontitis) vary considerably, and adjustments for other important covariates and risk factors have frequently not been performed.³⁰ In this particular case, some of these factors may be involved. In addition, the subjects included in the current study were strictly non-smokers inasmuch as the sample of the Kim et al.¹⁵ study was composed of smokers and non-smokers; according to Kornman et al.³¹ the risk related to smoking could obscure genetic risk factors. In addition, the authors did not associate the levels of the IL8 transcripts with the genotypes, as we did in this current study. The failure to replicate results is a common event in the search for genetic determinants of complex diseases, due either to genuine population heterogeneity or a different sort of bias.²⁹ Recently, in another investigation, Kim et. al.³² investigated the same SNP (rs4073) compounding a haplotype (–845TC, –738TA and –353AT) and demonstrated an association with susceptibility to chronic periodontitis in Brazilian individuals. It should be noted that the SNP (rs4073) has previously been referred to as –251¹⁸ and –353³³. The distribution of the haplotypes arranged as genotypes was significantly different between the control and chronic periodontitis groups and nonsmokers carrying the TAT/CTA genotype were six times more likely to develop chronic

periodontitis. Results revealed that, when analyzed independently, none of the three SNPs could be associated with susceptibility to periodontitis; however, the association with the disease was found when these SNPs were analyzed together, as haplotypes.

The results of the present investigation suggest that the SNP rs4073 is associated with generalized chronic periodontitis in non-smoking Brazilian subjects and may be used as a genetic marker for this disease, since the A allele is segregated together with the functional haplotype loci and associated with high levels of IL8 transcripts. Importantly, this significance was preserved even after correcting for factors such as age, gender and ethnicity. The enrichment of knowledge regarding factors, including genetic factors, involved in the periodontitis pathogenesis furthers our understanding of the disease and may improve the development of new approaches of treatments and therapies in the future.

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References

1. Flemmig TF. Periodontitis. *Ann Periodontol*. 1999; 4:32–37.
2. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman K. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontol* 2000. 1997; 14:216–248. [PubMed: 9567973]
3. Remick DG. Interleukin-8. *Crit Care Med*. 2005; 33:S466–467. [PubMed: 16340423]
4. Marshall JC. Neutrophils in the pathogenesis of sepsis. *Crit Care Med*. 2005; 33:S502–504. [PubMed: 16340434]
5. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, Cassatella MA. The neutrophils as a cellular source of chemokines. *Immunol Rev*. 2000; 177:195–203. [PubMed: 11138776]
6. Yucel-Lindberg T, Brunius G. Epidermal growth factor synergistically enhances interleukin-8 production in human gingival fibroblasts stimulated with interleukin-1 β . *Arch Oral Biol*. 2006; 51:891–898.
7. DeForge LE, Fantone JC, Kenney JS, Remick DJ. Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood. *J Clin Invest*. 1992; 90:2123–2129. [PubMed: 1331181]
8. Michalowicz BS. Genetic and heritable risk factors in periodontal disease. *J Periodontol*. 1994; 65:479–488. [PubMed: 8046564]
9. Hart TC, Kornman KS. Genetic factors in the pathogenesis of periodontitis. *Periodontol* 2000. 1997; 14:202–215. [PubMed: 9567972]
10. Schenkein HA. Finding genetic risk factors for periodontal diseases: is the climb worth the view? *Periodontol* 2000. 2002; 30:79–90. [PubMed: 12236898]
11. Astolfi CM, Shinohara AL, da Silva RA, Santos MC, Line SRP, de Souza AP. Genetic polymorphisms in the MMP-1 and MMP-3 gene may contribute to chronic periodontitis on a Brazilian population. *J Clin Periodontol*. 2006; 33:699–703. [PubMed: 16899023]
12. Ferreira SB Jr, Trombone AP, Repeke CE, et al. An interleukin-1beta (IL-1beta) single-nucleotide polymorphism at position 3954 and red complex periodontopathogens independently and

- additively modulate the levels of IL-1 β in diseased periodontal tissues. *Infect Immun*. 2008; 76:3725–3734. [PubMed: 18541658]
13. Louropoulou A, van der Velden U, Schoenmaker T, Catsburg A, Savelkoul PH, Loos BG. Mannose-binding lectin gene polymorphisms in relation to periodontitis. *J Clin Periodontol*. 2008; 35:923–930. [PubMed: 18823346]
 14. Park OJ, Shin SY, Choi Y, et al. The association of osteoprotegerin gene polymorphisms with periodontitis. *Oral Dis*. 2008; 14:440–444. [PubMed: 18938269]
 15. Kim YJ, Viana AC, Curtis KM, Orrico SR, Cirelli JA, Scarel-Caminaga RM. Lack of association of a functional polymorphism in the interleukin 8 gene with susceptibility to periodontitis. *DNA Cell Biol*. 2009; 28:185–190. [PubMed: 19364277]
 16. Nibali L, D'Aiuto F, Donos N, et al. Association between periodontitis and common variants in the promoter of the interleukin-6 gene. *Cytokine*. 2009; 45:50–54. [PubMed: 19084430]
 17. Garza-Gonzalez E, Bosques-Padilla FJ, Mendoza-Ibarra SI, Flores-Gutierrez JP, Maldonado-Garza HJ, Perez-Perez GI. Assessment of the toll-like receptor 4 Asp299Gly, Thr399Ile and interleukin-8 -251 polymorphisms in the risk for the development of distal gastric cancer. *BMC Cancer*. 2007; 7:70. [PubMed: 17462092]
 18. Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax*. 2000; 55:1023–1027. [PubMed: 11083887]
 19. Kamali-Sarvestani E, Aliparasti MR, Atefi S. Association of interleukin-8 (IL8 or CXCL8) -251T/A and CXCR2 +1208C/T gene polymorphisms with breast cancer. *Neoplasma*. 2007; 54:484–489. [PubMed: 17949231]
 20. Goverdhan V, Ennis S, Hannan SR, et al. Interleukin-8 promoter polymorphism -251A/T is a risk factor for age-related macular degeneration. *Br J Ophthalmol*. 2008; 92:537–540. [PubMed: 18310311]
 21. Vairaktaris E, Yapijakis C, Serefoglou Z, et al. The interleukin-8 (-251A/T) polymorphism is associated with increased risk for oral squamous cell carcinoma. *Eur J Surg Oncol*. 2007; 33:504–507. [PubMed: 17174061]
 22. McCarron SL, Edwards S, Evans PR, et al. Influence of cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res*. 2002; 62:3369–3372. [PubMed: 12067976]
 23. Hull J, Ackerman H, Isles K, et al. Unusual haplotypic structure of *IL8*, a susceptibility locus for a common respiratory virus. *Am J Hum Genet*. 2001; 69:413–419. [PubMed: 11431705]
 24. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999; 4:1–6. [PubMed: 10863370]
 25. Trevilatto PC, Line SRP. Use of buccal epithelial cells for PCR amplification of large DNA fragments. *J Forensic Odontostomatol*. 2000; 18:6–9. [PubMed: 11324090]
 26. Miller CL, Yolken RH. Methods to optimize the generation of cDNA from postmortem human brain tissue. *Brain Res Brain Res Protoc*. 2003; 10:156–167. [PubMed: 12565686]
 27. Faul F, Erdfelder E, Lang AG, Buchner AG. Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007; 39:175–191. [PubMed: 17695343]
 28. Hacking D, Knight1 JC, Rockett1 K, et al. Increased in vivo transcription of an IL-8 haplotype associated with respiratory syncytial virus disease-susceptibility. *Genes Immun*. 2004; 5:274–282. [PubMed: 15085176]
 29. Borrell LN, Papapanou PN. Analytical epidemiology of periodontitis. *J Clin Periodontol*. 2005; 32:S132–158.
 30. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet*. 2001; 29:306–309. [PubMed: 11600885]
 31. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol*. 1997; 24:72–77. [PubMed: 9049801]
 32. Kim YJ, Viana AC, Curtis KMC, et al. Association of haplotypes in the IL8 gene with susceptibility to chronic periodontitis in a Brazilian population. *Clin Chim Acta*. 2010; 411:1264–1268. [PubMed: 20488171]

33. Renzoni E, Lympay P, Sestini P, et al. Distribution of novel polymorphisms of the interleukin-8 and CXC receptor 1 and 2 genes in systemic sclerosis and cryptogenic fibrosing alveolitis. *Arthritis Rheum.* 2000; 43:1633–1640. [PubMed: 10902769]

Figure 1.

Dot Plot graphic showing the mRNA levels of the *IL8* gene in gingival tissues of patients from the control and generalized chronic periodontitis groups. *Indicates that there is a statistically significant difference between the groups ($p=0.03$, Mann-Whitney test). GAPDH, glyceraldehyde-3-phosphate dehydrogenase. IL8, interleukin-8.

Table 1

Demographic parameters of studied population

Studied population	Control <i>n</i> =108	Generalized chronic PD <i>n</i> =181	p value
<i>Age (years)</i>			
Mean ($\pm$ SD)	37.23 $\pm$ 12.82	44.06 $\pm$ 8.77	<0.01*
<i>Gender (%)</i>			
Female	66.33	67.15	0.97**
Male	33.67	32.85	
<i>Ethnic group (%)</i>			
Caucasoid	75.00	77.36	0.15**
Afro-American	12.96	06.62	
Mulatto	10.18	16.02	
Japanese	01.86	00.00	

* ANOVA;

** χ^2 test.

Table 2

Genotype, ethnic and age results adjusted by multivariate logistic regression modeling and alleles distribution of the *IL8* SNP (4073).

SNP (rs4073)	Control (N=108)	Generalized Chronic PD (N=181)	P value	Coefficient
<i>Genotype (All Ethnic)</i>				
<i>AA</i>	13 (12.0%)	21 (11.6%)	0.224	0.07
<i>TA</i>	57 (52.8%)	135 (74.6%)	0.005*	1.3
<i>TT</i>	38 (35.2%)	25 (13.8%)	reference	reference
<i>Genotype (Caucasian)</i>				
<i>AA</i>	8 (10%)	15 (14.60%)	0.225	0.58
<i>TA</i>	41 (51.25%)	73 (70.9%)	0.001*	1.06
<i>TT</i>	31 (38.75%)	15 (14.5%)	reference	reference
<i>Age</i>			0.000	0.07
<i>Alleles</i>				
<i>A</i>	36%	48%	0.08	
<i>T</i>	64%	52%		

SNP indicates single nucleotide polymorphism; PD indicates periodontal disease; N indicates number of informative individuals.

* Statistically significant difference was observed comparing control versus generalized chronic PD, adjusted by multivariate logistic regression modeling.

Table 3

Estimate of Odds ratio of the *IL8* SNP (rs4073).

SNP (rs4073)	Odds ratio	95% CI
<i>Genotype</i>		
(TA + AA) × TT	2.62	1.58–4.34
TT × (AA + TA)	0.29	0.16–0.52
TA × TT	3.6	1.00–6.5
AA × TT	2.45	1.04–5.78

SNP indicates single nucleotide polymorphism; CI indicates confidence interval.