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Polymorphic Regions in the Interleukin-1 Gene and Susceptibility to Chronic Periodontitis: A Genetic Association Study

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Objective: The objectives of this study were to determine the association between single nucleotide polymorphisms (SNPs) in *IL1B* (−511, +3954), *IL1A* (−889, +4845), and the variable number of tandem repeats (VNTRs) polymorphism in the IL-1RN gene with chronic periodontitis susceptibility and to analyze gene–gene interactions in a hospital-based sample population from South India. *Subjects and Methods:* A total of 400 individuals were recruited for this study; 200 individuals with healthy gingiva and 200 chronic periodontitis patients. Genomic DNA was isolated from peripheral blood samples and genotyping was performed for the above-mentioned single nucleotide and VNTR polymorphisms by polymerase chain reaction, DNA sequencing, and agarose gel electrophoresis. *Results:* A higher proportion of the variant alleles were observed in the chronic periodontitis group for all the SNPs examined. The SNP at +3954 (C>T) in the *IL1B* gene was found to be significantly associated with chronic periodontitis ($p=0.007$). VNTR genotypes (χ^2 value: 5.163, df=1, $p=0.023$) and alleles (χ^2 value: 6.818, df=1, $p=0.009$) were found to have a significant association with chronic periodontitis susceptibility. *Conclusion:* In the study population examined, the SNP in the *IL1B* gene (+3954) and VNTR polymorphisms in the *IL1RN* gene were found to have a significant association with chronic periodontitis susceptibility.

Introduction

Chronic periodontitis is an inflammatory disease of the supporting structures of the tooth with a microbial etiology (Haffajee and Socransky, 1994). Its progression and severity have been determined to be influenced by several factors: environmental factors (smoking) (Haber *et al.*, 1993), genetic factors (candidate gene polymorphisms) (Loos *et al.*, 2005), and systemic disease (diabetes mellitus type II) (Chapple and Genco, 2013). An important cytokine that plays a role in the pathogenesis of chronic periodontitis is interleukin-1 (Birkedal-Hansen, 1993). The interleukin-1 gene cluster is found in chromosome 2 and encodes for the cytokines; interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), and interleukin-1 receptor antagonist (IL-1 ra) (Deng *et al.*, 2013). IL-1α and IL-1β have a proinflammatory role in periodontitis pathogenesis, whereas IL-1ra is antagonistic to these cytokines (Rawlinson *et al.*, 2003).

The usefulness of IL-1β, IL-1α levels in gingival crevicular fluid (GCF) as a biomarker for chronic periodontitis has been evaluated in several studies. Masada *et al.* (1990) evaluated IL-1β m-RNA (gingival tissue) and protein levels in GCF samples from chronic periodontitis patients. An elevated level of the IL-1β was observed and it was concluded that IL-1β was produced locally and contributed to bone loss observed in chronic periodontitis. A positive correlation of GCF IL-1β levels with probing pocket depth (Perozini *et al.*, 2010) and IL-1α, IL-1β, and IL-1 activity (IL-1/IL-1ra ratio) with alveolar bone loss (Ishihara *et al.*, 1997) has been reported among patients with chronic periodontitis.

A landmark study by Kornman *et al.* (1997), evaluated the association of single nucleotide polymorphisms (SNPs) in genes coding for interleukin-1 cytokines, tumor necrosis factor alpha (TNFα), and susceptibility to chronic periodontitis. The authors demonstrated a positive association between the composite genotype (*IL1A* −889 and *IL1B* +3954) and severe chronic periodontitis. Ever since, several studies have evaluated the role of these polymorphisms in periodontitis susceptibility with varied reports in different ethnic populations (Trevilatto *et al.*, 2011; Tawil *et al.*, 2012; Yücel *et al.*, 2013).

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Previous studies in an Indian population have given conflicting results with a few studies showing a positive association (Shete *et al.*, 2010; Archana *et al.*, 2012), and one study showing no association between SNP at +3954 in the *IL1B* gene and chronic periodontitis (Kaarthikeyan *et al.*, 2009).

India is inhabited by 5000 anthropologically well-defined caste and tribal populations; they are linguistically classified into Indo-European (northern part of the country), Austro-Asiatic (central and north eastern states), Dravidian (southern states), and Tibeto-Burman (northeastern states and the foothills of the Himalayas). In this study, we have analyzed the association between SNPs in *IL1B* (−511, +3954), *IL1A* (−889, +4845), and variable number of tandem repeat (VNTR) polymorphism in *IL1RN* gene with chronic periodontitis susceptibility and gene–gene interactions in a hospital-based sample population from South India.

Materials and Methods

Study population

The study population consisted, a total of 400 individuals (>18 years) recruited from the Out Patient Department of Periodontology, Faculty of Dental Sciences, Sri Ramachandra University, Chennai. Sri Ramachandra University is a tertiary care teaching hospital located on the outskirts of the city of Chennai and patients visiting the institute represent a fair cross section of urban, semiurban, and rural population. The study was approved by the Institutional Ethics Committee of Sri Ramachandra University and has followed the Helsinki guidelines on Ethics for Human Research (IEC-NI/10/APR/15/05).

Sample size calculation

The sample size was calculated based on the allele frequencies reported by Masamatti *et al.* (2012). The variant allele frequency among cases and controls was 44.2% and 33.3% respectively. Based on the true probability of exposure, inclusion of 200 cases and 200 controls will be able to reject the null hypothesis with type I error of 5% and power of 80%.

All study participants were recruited after obtaining informed consent. Out of the 400, 200 were control individuals (healthy gingiva) and 200 patients were diagnosed to have generalized chronic periodontitis. The inclusion criteria for individuals with healthy gingiva were as follows: probing pocket depth of ≤3 mm, no attachment loss, absence of gingival bleeding on probing, absence of any clinical signs of gingival inflammation, and no previous history of periodontal disease. Individuals were diagnosed to have generalized chronic periodontitis based on attachment loss of >1 mm in at least 30% of the sites examined (Armitage, 1999) and radiographic evidence of bone loss, and presence of at least 10 natural teeth. Current and former smokers, pregnant women, individuals who were on any medication, and individuals with any known systemic disease were excluded from the study.

Clinical examination

A full mouth periodontal examination was performed for the following clinical parameters: Gingival Index (Silness and Loe, 1964), Plaque Index (Loe, 1967), Oral Hygiene Index-Simplified (OHI-S) (Greene and Vermillion, 1964), Clinical Attachment Level (CAL), and probing pocket depth at six sites per tooth excluding third molars. All the clinical parameters were assessed by the same examiner (Vamsi Lavu) using a UNC 15 manual periodontal probe.

Sample collection

Peripheral blood (3 mL) was collected with aseptic precautions from the ante-cubital vein in a vacutainer containing 0.5 mL of 0.129 mM sodium citrate (BD Biosciences, San Jose, CA). DNA extraction was done in 200 μL of the blood using commercially available extraction kits (Nucleo-pore DNA Sure Blood Mini Kit; Shivaji Marg, New Delhi, India) as per manufacturer advised protocols. The quality of extracted DNA was checked using nanodrop (260/280 nm ratio).

Genotyping

VNTR polymorphism in the *IL1RN* gene was determined by polymerase chain reaction (PCR) and agarose gel (2%) electrophoresis. The other polymorphisms in the IL-1 gene cluster, *IL1A* (+4845), *IL1A* (−889), *IL1B* (−511), and *IL1B* (+3954) were determined by PCR and DNA sequencing. PCR was performed on 50–100 μg of DNA in a 10 μL reaction containing 5 μL PCR Master Mix (Merck PCR Master Mix Kit; Merck, Rahway, NJ), 2.8 μL sterile water, and 30 pM of forward and reverse primers using an Eppendorf thermocycler (Eppendorf, Hamburg, Germany). The primers used, PCR conditions, and the different number of repeats for the VNTR polymorphism were referenced from a previous study (Meisel *et al.*, 2002). The quality of the PCR products was assessed by electrophoresis using a 2% agarose gel.

DNA sequencing

Cycle sequencing, for the PCR products, to determine the polymorphisms was performed using an ABI PRISM Big Dye Terminator Ready Reaction Cycle Sequencing Kit (Life Technologies, Carlsbad, CA). Sequencing was done in a final volume of 10 μL containing 0.5 μL of ready reaction mix, 1.5 μL of sequencing dilution buffer, 1 μL of primer (forward/reverse), 6 μL of sterile water, and 1 μL of PCR product. The cycle sequenced products were then subjected to purification before the sequencing. Analysis of sequencing results was done using Applied Biosystems Seqscape v2.7 software (Life Technologies) to determine the base transition in the sites of interest.

Statistical analysis

The mean, frequency, and standard deviation for all the continuous variables were determined. The allele and genotype frequencies for all the SNPs were calculated and their association with chronic periodontitis was assessed by chi-square test. The association of the VNTR genotypes and alleles with chronic periodontitis was assessed by the CLUMP software package (Sham and Curtis, 1995). The association of the genotypes of SNPs with clinical parameters and indices of chronic periodontitis was done by one-way ANOVA. Hardy–Weinberg equilibrium (HWE) was determined for both cases and controls for all the SNPs and VNTR and significance of any deviation was assessed by chi-square test. Linkage disequilibrium (LD) was assessed between the loci of the SNPs of the IL-1 gene cluster in cases and controls and the standardized disequilibrium coefficient (D′) and squared correlation coefficient (r^2) were calculated by Haploview software. Multifactorial dimensionality reduction (MDR)

Table 1. Descriptive Data of the Study Population

Clinical indices	*Control (n = 200)*	*Chronic periodontitis (n = 200)*	p-*Value*
Age range (years)	20–55	22–60	
Mean age (mean ± SD)	29.64 ± 5.5	38.16 ± 8.4	< 0.001
Gender			
Males	95 (47.5%)	112 (56.0%)	
Females	105 (52.4%)	88 (44.0%)	
OHI Score (mean ± SD)	0.415 ± 0.251	2.810 ± 0.857	< 0.001
Gingival Index (mean ± SD)	0.227 ± 0.190	1.837 ± 0.290	< 0.001
Plaque Index (mean ± SD)	0.250 ± 0.228	1.902 ± 0.392	< 0.001
Probing pocket depth (mm) (mean ± SD)	1.099 ± 0.199	4.124 ± 0.445	< 0.001
CAL (mm) (mean ± SD)	0.00 ± 0.00	3.414 ± 1.238	

p-Value < 0.05, statistically significant.
CAL, Clinical Attachment Level; OHI, Oral Hygiene Index.

analysis was performed to analyze the gene–gene interactions among the SNPs analyzed. The results were assumed to be statistically significant at $p < 0.05$.

Results

The demographic data pertaining to study population are summarized in Table 1. The genotype frequencies for the *IL1A* −889 C > T (rs1800587), *IL1A* +4845G > T (rs17561), *IL1B* −511C > T (rs16944) and *IL1B* +3954C > T (rs1143634) polymorphisms are shown in Table 2. The genotype distribution of three SNPs was found to be in HWE in both cases and controls. A deviation of the HWE was observed in the case group for the SNP in *IL1B* +3954C > T. The *IL1A* +4845G > T, and *IL1B* −511C > T genotypes were not associated with chronic periodontitis (Table 2). Both

Table 2. Association Analysis of Genotypes of SNP of *IL-1* Gene with Chronic Periodontitis

SNP in IL-1 *gene*	*Controls (n = 200)*	*Chronic periodontitis (n = 200)*	*OR (95% CI)*	p-*Value*
(rs1800587) *IL1A* −889 C > T				
CC	107 (53.5)	104 (52.0)	Ref	
CT	79 (39.5)	79 (39.5)	1.029 (0.68–1.55)	0.892
TT	14 (7.0)	17 (8.5)	1.25 (0.59–2.66)	0.564
CT + TT	93 (46.5)	96 (48.0)	1.062 (0.72–1.57)	0.764
HWE (*p*-value)	0.911	0.717		
(rs17561) *IL1A* +4845 G > T				
GG	101 (50.5)	96 (48.0)	Ref	
GT	75 (37.5)	81 (40.5)	1.14 (0.75–1.73)	0.551
TT	24 (12.0)	23 (11.5)	1.01 (0.53–1.91)	0.980
GT + TT	99 (49.5)	104 (52.0)	1.11 (0.75–1.64)	0.617
HWE (*p*-value)	0.091	0.354		
(rs16944), *IL1B* −511C > T				
CC	97 (48.5)	83 (41.5)	Ref	
CT	82 (41.0)	86 (43.0)	1.23 (0.80–1.87)	0.344
TT	21 (10.5)	31 (15.5)	1.73 (0.92–3.23)	0.088
CT + TT	103 (51.5)	117 (58.5)	1.33 (0.89–1.97)	0.160
HWE (*p*-value)	0.556	0.272		
rs1143634, *IL1B* +3954C > T				
CC	130 (65.0)	103 (51.5)	Ref	
CT	66 (33.0)	92 (46.0)	1.76 (1.17–2.65)	**0.007***
TT	4 (2.0)	5 (2.5)	1.58 (0.41–6.03)	0.505
CT + TT	70 (35.0)	97 (48.5)	1.75 (1.17–2.61)	**0.006***
HWE (*p*-value)	0.182	0.003		
IL1RN VNTR				
a1a1	127 (63.5)	137 (68.5)	Ref	
Rest all	73 (36.5)	63 (31.5)	0.80 (0.524–1.21)	0.292
HWE (*p*-value)	< 0.001	< 0.001		

*Bold indicates *p*-value < 0.05, statistically significant.
95% CI, 95% confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; SNP, single nucleotide polymorphism; VNTR, variable number of tandem repeat.

Table 3. Allele Frequencies of SNPs in *IL1A* and *IL1B* Genes in Periodontitis Patients and Controls

SNP in IL-1 *gene*	*Controls*	*Cases*	*OR (unadjusted) 95% CI*	p-*Value*
(rs1800587) *IL1A* −889 C>T				
C	293	287	Ref	—
T	107	113	1.08 (0.79–1.47)	0.635
(rs17561) *IL1A* +4845 G>T				
G	277	273	Ref	—
T	123	127	1.05 (0.78–1.41)	0.760
(rs16944), *IL1B* −511 C>T				
C	276	252	Ref	—
T	124	148	1.31 (0.97–1.75)	0.073
(rs1143634), *IL1B* +3954C>T				
C	326	298	Ref	—
T	74	102	1.51 (1.07–2.11)	**0.017***

*Bold indicates *p*-value <0.05, statistically significant.

codominant and dominant models of *IL1B* −511C>T showed an increased risk for periodontitis, but the association was not found to be statistically significant (Table 2). An association of the CT genotype and dominant genotype model (CT+TT) in *IL1B* +3954C>T polymorphism with chronic periodontitis was observed ($p=0.007$, $p=0.006$). The *IL1B* +3954C>T locus also showed significant association with chronic periodontitis in the allelic model (Table 3). The distribution of *IL1RN* VNTR genotypes and alleles for both chronic periodontitis and control individuals are shown in Table 4. The allele A1 was the most frequent allele followed by A2 in this study population. The allele A5 was observed only in the periodontitis group with low frequency (Table 4). The *IL1RN* VNTR genotypes significantly deviated from HWE in both periodontitis ($p<0.001$) and control groups ($p<0.001$). CLUMP analysis revealed a statistically significant difference between periodontitis and control group for both genotype and alleles of *IL1RN* VNTR using chi-square clumped 2×2 table (genotype T4 $\chi^2=5.163$, df=1, $p=0.023$ and allele T3 $\chi^2=6.818$, df=1, $p=0.009$). One-way ANOVA revealed no significant association between the clinical parameters and genotype frequencies of any of the SNPs evaluated in the chronic periodontitis group (Table 5). The pairwise LD was not strong and significant between the SNPs examined (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/gtmb). Haplotypes constructed using *IL1A* −889C>T, IL-1A 4845G>T, *IL1B* −511C>T and 3954C>T revealed the presence of a total of 16 haplotypes. Of the total 16 haplotypes formed, only the CTCC and TGTT haplotypes were found to be associated with periodontitis group (Supplementary Table S2). MDR analysis between the SNPs of the IL-1 gene revealed two-, and three-factor genotype–genotype interaction models and none of the model/s had an average cross-validation consistency (CVC) of 9 out of 10 and *p*-value of <0.05 (Table 6).

Discussion

The association of polymorphic regions in the interleukin-1 gene with chronic periodontitis susceptibility was assessed in this study. The SNP at +3954 (C>T) and VNTR in the intron 2 of *IL1RN* region were found to be associated with chronic periodontitis susceptibility.

Interleukin-1 is a potent proinflammatory cytokine, which plays a key role in periodontitis pathogenesis (Birkedal-Hansen, 1993). SNPs have been identified at various loci of the interleukin-1 gene (Kinane *et al.*, 2005). The potential role of these SNPs in periodontitis susceptibility has been studied in different populations across the world. Trevilatto *et al.* (2011), reported genotypes 2/2 of *IL1RN* and T allele of *IL1B* (−511) were risk indicators for chronic periodontitis in an Afro-American segment of the Brazilian population. Among a diabetic African American population with periodontitis, an

Table 4. Association Analysis (CLUMP) of *ILRN* VNTR Polymorphism with Chronic Periodontitis

	Case (periodontitis)	*Control*	*T1*	*T2*	*T3*	*T4*
Genotype						
a1a1	137 (68.5)	127 (63.5)				
a1a2	21 (10.5)	23 (11.5)				
a2a2	7 (3.5)	18 (9)				
a1a3	5 (2.5)	4 (2)				
a3a3	7 (3.5)	7 (3.5)	χ^2 value: 7.841	χ^2 value: 5.567	χ^2value: 5.163	χ^2 value:5.163
a1a4	13 (6.5)	11 (5.5)	df=9	df=4	df=1	df=1
a2a4	1 (0.5)	1 (0.5)	$p=0.550$	$p=0.234$	$p=0.023$	$p=0.468$
a3a4	3 (1.5)	4 (2)				
a4a4	4 (2)	5 (2.5)				
a1a5	2 (1)	0 (0)				
HW *p*-value	<0.001	<0.001				
Alleles						
a1	315 (78.8)	292 (73.0)				
a2	36 (9.0)	60 (15.0)				
a3	22 (5.5)	22 (5.5)	χ^2 value: 8.891	χ^2 value: 6.978	χ^2 value: 6.818	χ^2 value: 6.818
a4	25 (6.3)	26 (6.5)	df=4	df=3	df=1	df=1
a5	2 (0.5)	0 (0.0)	$p=0.064$	$p=0.072$	$p=0.009$	$p=0.041$

T1, T2, T3, and T4 are Pearson's χ^2 respectively for raw, rare alleles grouped together, 2×2 tables that compare one column against the rest grouped, and 2×2 tables comparing any combination of columns against the rest. *p*-Value <0.05, statistically significant.

Table 5. Comparison of Clinical Indices with *IL-1* Genotypes for rs1800587, rs17561, rs16944, and rs1143634

	TT (n = *104*)	*CT* (n = *79*)	*CC* (n = *17*)	F-*value*	p-*Value*
IL1A −899					
OHI Score	2.78 + 0.90	2.92 + 0.82	2.50 + 0.69	1.826	0.164
Gingival Index	1.84 + 0.28	1.84 + 0.30	1.81 + 0.31	0.091	0.913
Plaque Index	1.87 + 0.41	1.93 + 0.37	1.92 + 0.36	0.569	0.567
PPD	4.12 + 0.43	4.14 + 0.49	4.10 + 0.32	0.071	0.931
CAL	3.32 + 1.28	3.58 + 1.13	3.22 + 1.42	1.196	0.305
	GG (n = *96*)	*GT* (n = *81*)	*TT* (n = *23*)	F-*value*	p-*Value*
IL1A +4845					
OHI Score	2.80 + 0.89	2.82 + 0.82	2.83 + 0.84	0.025	0.975
Gingival Index	1.87 + 0.31	1.80 + 0.26	1.86 + 0.30	1.272	0.283
Plaque Index	1.92 + 0.38	1.86 + 0.40	1.97 + 0.41	0.892	0.409
PPD	4.14 + 0.43	4.14 + 0.47	4.00 + 0.39	1.053	0.351
CAL	3.36 + 1.27	3.47 + 1.17	3.44 + 1.35	0.189	0.829
	CC (n = *83*)	*CT* (n = *86*)	*TT* (n = *31*)	F-*value*	p-*Value*
IL1B −511					
OHI Score	2.72 + 0.92	2.89 + 0.82	2.85 + 0.76	0.873	0.419
Gingival Index	1.82 + 0.29	1.86 + 0.29	1.83 + 0.29	0.511	0.601
Plaque Index	1.82 + 0.44	1.94 + 0.36	2.01 + 0.33	3.31	0.039
PPD	4.13 + 0.47	4.15 + 0.47	4.04 + 0.28	0.63	0.534
CAL	3.37 + 1.32	3.58 + 1.21	3.09 + 1.04	1.908	0.151
	CC (n = *103*)	*CT* (n = *92*)	*TT* (n = *5*)	F-*value*	p-*Value*
IL1B +3954					
OHI Score	2.85 + 0.83	2.74 + 0.90	3.28 + 0.47	1.200	0.303
Gingival Index	1.81 + 0.29	1.85 + 0.29	2.08 + 0.26	2.220	0.111
Plaque Index	1.88 + 0.40	1.91 + 0.38	2.19 + 0.45	1.639	0.197
PPD	4.13 + 0.42	4.12 + 0.48	4.21 + 0.25	0.124	0.884
CAL	3.37 + 1.17	3.42 + 1.31	4.12 + 1.39	0.867	0.422

SNP's among chronic periodontitis subjects. *p*-Value < 0.05, statistically significant.

over-representation of allele 1 at *IL1B* (−511) and *IL1B* (+3954) was observed (Guzman *et al.*, 2003). Cullinan *et al.* (2001), in a prospective study in Queensland reported IL-1 genotype-positive subjects demonstrated attachment loss and increased mean pocket depth in nonsmokers. SNPs in the IL-1 gene were found not to contribute to chronic periodontitis susceptibility in Greek (Sakellari *et al.*, 2006), Jordanian (Karasneh *et al.*, 2011), Chinese (Armitage *et al.*, 2000), and Thai (Anusaksathien *et al.*, 2003) populations. The wide variation in this association may be due to the ethnicity of the target population. A recent systematic review and meta-analysis by Karimbux *et al.* (2012), reported a significant association between IL-1 genotype and chronic periodontitis in a white population only.

Table 6. Interaction Models by MDR Analysis

Best model	*TA*	*CVC*	χ^2	p-*Value*
rs16944, rs1143634	0.528	8/10	0.124	0.725
rs17561, rs16944, rs1143634	0.48	7/10	0.067	0.796

p-Value < 0.05, statistically significant.
CVC, cross-validation consistency; MDR, multifactorial dimensionality reduction; TA, testing accuracy.

In this study, a statistically significant association could be established between SNP at +3954 in the *IL1B* gene and chronic periodontitis susceptibility in the dominant and allelic models. This result gains significance as an important confounding variable; smoking/use of tobacco in any form was excluded from the study during subject selection. The SNP at *IL1B* +3954 position has been identified as a potential risk marker for chronic periodontitis in a similar ethnic population in recent studies (Shete *et al.*, 2010; Archana *et al.*, 2012; Masamatti *et al.*, 2012). The findings of this study are in agreement with the above-mentioned studies. In contrast, a previous study by Kaarthikeyan *et al.* (2009), reported a lack of association between the SNP at +3954 in the *IL1B* gene and chronic periodontitis susceptibility in a similar population. The observed difference in the results may be attributed to the small sample size used in the study by Kaarthikeyan *et al.* (2009), which may not have been adequate to detect the difference in genotypic or allelic variation between the groups.

A considerable variation in the variant (T) allele frequency has been observed in different studies in a similar demographic population. The variant (T) allele frequency observed in the chronic periodontitis subjects of this study population was 25.5% as compared to 38.5% (Masamatti *et al.*, 2012), 46.7% (among severe periodontitis group) (Archana *et al.*,

2012), 14% (Shete *et al.*, 2010) as reported in studies involving subjects of a similar demographic population.

IL-1ra is negative regulator of the interleukin-1 function and is coded for by the *IL1RN* gene (Rawlinson *et al.*, 2003). A VNTR polymorphism in the intron 2 of the *IL1RN* gene was evaluated and showed an association with chronic periodontitis susceptibility in this study. The results of our study are in agreement with previous studies, which have reported a significant over-representation of *IL1RN* gene among early onset (Tai *et al.*, 2002) and chronic periodontitis patients in an Asian population (Zhong *et al.*, 2003). A recent systematic review and meta-analysis (Ding *et al.*, 2012) also reported; *IL1RN* VNTR polymorphism could contribute to an increased risk for chronic periodontitis.

In our study, the haplotype analysis was performed for four SNPs of the IL-1 gene. A total of 16 different haplotypes were obtained as no LD was present between the SNPs examined. The CTCC haplotype was found to be significantly over-represented in subjects with healthy gingiva and TGTT, haplotype was over-represented in chronic periodontitis patients as compared to individuals with healthy gingiva. The haplotype analysis revealed CTCC to have a protective effect with respect to periodontitis susceptibility and the TGTT haplotype to be more susceptible for chronic periodontitis. A recent study on *IL1B* (-31, -511, and $+3954$) SNP haplotype distribution in a similar population (Shete *et al.*, 2010) reported a significant difference in the distribution of the rare allele haplotype (T-T) among controls and chronic periodontitis groups.

Strong LD between *IL1B* -31 and -511 in control, aggressive periodontitis and chronic periodontitis groups have been reported in a similar population by Shete *et al.* (2010). In the same study the authors reported, the T allele of *IL1B* -31 was in linkage with *IL1B* $+3954$ in the control group. In this study the SNP at *IL1B* -31 was not examined and no LD could be detected between the SNPs (*IL1B* -511, $+3954$, *IL1A* -889, and *IL1A* $+4845$) examined in this study.

A positive relationship between the rare alleles of the SNPs in IL-1 gene and severity of periodontitis has been reported in a previous study (Papapanou *et al.*, 2001). However in this study, the variant genotype frequencies of the SNPs in interleukin-1 gene were found to have no association with clinical periodontal parameters, and other clinical indices assessed.

Engebretson *et al.* (1999) reported a corelation between GCF levels of *IL1B* and periodontitis-associated genotype (PAG). The authors demonstrated that the *IL1β* in GCF was 2.5 times higher in the PAG-positive group before treatment and 2.2 times higher in the same group even after periodontal therapy. One of the limitations of this study is that the IL-1 protein levels in GCF were not assessed and corelated with the SNP prevalence in the individuals.

In conclusion, in the population examined, the individuals with SNP (C > T) in *IL1B* $+3954$ gene and variant alleles of the VNTR in *IL1RN* gene demonstrated an increased susceptibility for chronic periodontitis.

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Author Disclosure Statement

No competing financial interests exist.

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