Original Article

IL-13 Gene Polymorphisms (-1112 C/T and -1512 A/C) in Patients with Chronic and Aggressive Periodontitis: Effects on GCF and Outcome of Periodontal Therapy

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ABSTRACT

Background: IL-13 is the key cytokine in the regulation of inflammatory with an autoimmune disease and has an anti-inflammatory effect. **Aims:** This study aimed to compare IL-13 (-1112 C/T and -1512 A/C) gene polymorphisms in patients with aggressive periodontitis (AgP), chronic periodontitis (CP), and periodontally healthy group (C) and evaluate the effect of nonsurgical periodontal therapy on gingival crevicular fluid (GCF) IL-13 levels in patients. **Materials and Methods:** One hundred thirty patients with AgP, 120 patients with CP, and 70 periodontally healthy subjects were included in this study. Clinical parameters were recorded (plaque and gingival index, probing pocket depth, clinical attachment level), and GCF and blood samples were taken at baseline and 6-week. Nonsurgical periodontal therapy was performed in patients with periodontitis. Gene analyses (IL-13 − 1112C/T (rs1800925) and − 1512 A/C (rs1881457) were performed with real-time polymerase chain reaction (PCR) and cytokine levels were determined by an enzyme-linked immunosorbent assay method. **Results:** AgP and CP patients showed significant improvement in clinical parameters after periodontal therapy ($P < 0.05$). According to results, genotype distributions and allele frequencies in IL-13 variants − 1112C/T and − 1512 A/C were found similarly in all groups ($P > 0.05$). In the AgP group, GCF IL-13 cytokine level is statistically significant and increased in 6 weeks; however, in the CP group, there is no statistically significant difference between baseline and 6 week. In the AgP group, baseline GCF IL-13 cytokine level is lower than those of the CP group and C group ($P < 0.05$). **Conclusion:** Within the limits of this study, IL-13 -1112 and -1512 gene polymorphisms have not been associated with AgP and CP, and GCF IL-13 cytokine level is increased after treatment in the AgP group.

KEYWORDS: *Aggressive periodontitis, gene polymorphism, IL-13, periodontal therapy*

INTRODUCTION

Periodontitis is a chronic inflammatory disease characterized by irreversible damage to the cementum and periodontal ligament, periodontal pocket formation, clinical attachment loss, gingival recession, and alveolar bone destruction.[1] The rate of progression of the disease varies in different types of periodontitis. Aggressive periodontitis (AgP) is a rare periodontal disease with a low prevalence in the general population. It usually affects systemically healthy individuals under the age of 35 years. It differs from chronic periodontitis (CP) due to the age of onset of the disease,

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rapid progression, the severity of disease, structure of subgingival flora, differences in host response, changes in response to treatment, and familial transmission characteristics of the disease.[2,3]

Many recent studies showed the effects of genes leading to individual differences at the onset and/or progression of periodontal disease.[4,5] Genetic polymorphisms are important parameters in the classification of diseases, in describing the etiology of the disease and the individual's susceptibility to disease, in diagnosing diseases and planning treatment.[4]

Cytokines, soluble proteins that play an important role in immune response regulations produced by a number of T cells, are characterized by their pleiotropism and pluripotential.[6] Among these cytokines, IL-4 and IL-13 are produced by T-helper type-2 (Th2) lymphocytes.[7] IL-13 is capable of suppressing the production of proinflammatory cytokines by monocytes/macrophages.[8] Also, IL-13 may play a role in regulating collagen homeostasis in gingival fibroblasts and the cultured gingival fibroblasts with IL-13 produced more TGF-β than unstimulated cells.[9] The IL-13 expression has also been found in periodontitis lesions.[10,11] IL-13 mRNA was detected in 21% of periodontitis K samples of patients with CP but not detected in none of the biopsies from healthy tissue.[12] Conversely, another study suggested that IL-13 and TGF-β were only detected in healthy/gingivitis group and IL-4 was not detected in any of the aggressive or healthy/gingivitis individuals.[13] However, mRNA for interferon-γ and IL-13 was upregulated, whereas IL-4 and IL-10 were downregulated following *porphyromonas gingivalis* stimulation.[14]

Recently, several different single-nucleotide polymorphisms in the IL-13 promoter region have been described. The -1112 C/T and -1512 A/C polymorphisms have been analyzed in patients AgP in the North European population. The results showed that the genotype and allele frequencies did not differ between AgP and healthy control groups.[15] However, in another study conducted in the Chinese population, the relationship between IL-13 -1112 gene polymorphism and AgP and CP patients was investigated. The CC genotype and C allele were found to be associated with the risk of disease development in nonsmoking AgP patients. However, there was no relationship between IL-13 gene polymorphism and CP.[7] Based on these findings, there is no consensus about IL-13 gene polymorphism, which affects different periodontal diseases. Therefore, we evaluate the IL-13- 1112 C/T and IL-13- 1512 A/C polymorphisms and their effects on GCF IL-13 levels in aggression and CP.

Material and Methods

Patient population

Subjects were recruited to this study collecting whole mouth clinical periodontal data, gingival crevicular fluid (GCF), and blood samples. The study was designed as a case-control study. The study protocol of the present study was approved by the Medical Research Ethics Committee of Cumhuriyet University according to the Helsinki declarations (2013-03/021), and signed informed consent was achieved from all individuals. The total number of subjects in this study was 320, comprising 130 with AgP (90 women, 40 men), 120 with CP (75 women, 45 men), and 70 who were control (40 women and 30 men).

Clinical examinations (probing depth and attachment loss) and panoramic radiographs were taken and evaluated for interproximal bone loss measurements from the cemento–enamel junction of the tooth to the bone crest for diagnosing GAgP or GCP or being periodontally healthy. The diagnostic criteria for GAgP and GCP were defined in accordance with the classification agreed at the World Workshop for Periodontics and The American Academy of Periodontology (1999).[16]

Briefly, subjects older than 35 years of age, with attachment loss ≥5 mm at more than one tooth site and with more than three sites of probing depth >6 mm involving more than one tooth distributed in each quadrant, were diagnosed as having CP. Subjects who had more than eight teeth with attachment loss of ≥5 mm and probing the depth of ≥6 mm, and at least three affected teeth that were not first molars or incisors, were diagnosed as having AgP.

Subjects with no evidence of attachment loss at more than one site or pocket depth of ≥3 mm were diagnosed as periodontally healthy and used as controls. Exclusion criteria were receipt of antibiotics or periodontal treatment 3 months before the study, conservative or prosthetic restorations at the anterior region, periodontal destruction caused by poor restorations, pregnancy and lactation, a history of systemic disease or medication that may affect the periodontal condition, and patients who did not have the capacity to consent for themselves. Also, smoking can be a confounding factor in genetic analyses, thus smokers were excluded from the study.

Clinical measurement and periodontal treatment

Full-mouth measurements of probing pocket depth (PPD) and clinical attachment levels (CAL) were obtained at six points per tooth. The presence of supragingival plaque was scored using plaque index (PI).[17] Gingival inflammation was scored using gingival index (GI).[17] Bleeding on probing was also recorded. PD and CAL

measures were obtained using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). A single examiner collected clinical data and samples (EPG) Emine Pirim Görgün. Intraexaminer reproducibility of PPD was assessed and the intraexaminer reliability was high (≥98%)

GCF collection and analysis

Before GCF collection, the area around the tooth was isolated with cotton rolls, dried, and the supragingival plaque was removed with a sterile curette. GCF samples were collected with Periopaper® strips and stored at − 80°C until analyzed. 100 µL of phosphate buffer solution was added to the Eppendorf tubes for the extraction of the collected GCF samples by impregnating the periopapers. The vial was mixed with the vortex mixer for 1 min to allow passage of the GCF liquid into the solution. The obtained contents were used in the IL-13 analysis. IL-13 Abs levels were measured using an enzyme-linked immunosorbent assay kit (YH Bioresearch, Shangai, China) according to the manufacturer's instructions.

GCF cytokine levels were calculated from the standard curves and defined as picogram/site for the total amount of cytokine levels. Sites with cytokine levels below the limits of the assay's detectability were scored as 0.

Blood samples

From each patient, 10 ml of blood from the antecubital vein was collected. The blood samples were collected in sodium EDTA vacutainers and stored at − 80°C until genetic analysis was performed.

Analysis of the IL-13 genotype

IL-13 promoter region (rs1800925, rs1881457) polymorphism was determined by using SNP real-time Genotyping kit (QIAGEN, Hilden, Germany, Pyromark PCR Kits) with the kit is provided in a convenient master mix format consisting of HotStarTaq DNA Polymerase and optimized PyroMark Reaction Buffer for highly specific amplification. Real-time PCR condition was initial denaturation (95°C, 15 min), 10 cycles of denaturation (95°C, 30 s), and first extension (60°C, 30 s) followed by 45 cycles of the second denaturation (94°C, 30 s) and second extension steps (72°C, 30 s). We genotyped an amplified PCR product for IL-13 promoter region polymorphism by allelic discrimination assay according to the manufacturer's instruction.

Periodontal Treatment

AgP and CP patients received full-mouth nonsurgical periodontal therapy. The treatment procedure was performed quadrant per quadrant under local anesthesia in four visits using specific Gracey curettes and scalers (Hu-Friedy, Chicago, IL, USA). Treatment was completed within 10 days. No antibiotics or anti-inflammatory drugs were prescribed during the treatment. All patients were treated by a single periodontist (EPG).

Statistical analyses

After creating a dataset for the genotype information (AGP, CP, and control populations) for two promoter regions of IL-13, Hardy–Weinberg exact test, which in open access Genepop (genepop.curtin.edu.au/) program, was carried out. The power of the analysis was defined by utilizing data from a previous publication.[7] The sample size was calculated under a 5% error giving a required sample size of 65 in each group, with a statistical power of 80%.

Clinical and biochemical analyses were performed using SPSS 22 (IBM, Chicago, IL, USA) program. The Kolmogorov–Smirnov test was applied for the distribution of the data. Chi-square test was used to determine the differences in terms of IL-13 (-1112) and (-1512) gene polymorphisms between the groups. Comparisons between groups were assessed using the Mann–Whitney *U* test. Differences between baseline and sixth-week values in patients with AgP were assessed by the Wilcoxon test. The relationship between disease genotypes and AgP was assessed by logistic regression analysis taking into account factors contributing to potential diseases, such as age and sex. The significance level was taken as $P < 0.05$.

Results

A total of 130 patients with GAgP, 120 patients with CP, and 70 healthy controls took part in the study. The demographic characteristics of the study groups are summarized in Table 1. When comparing the groups with the healthy controls, no statistically significant differences in gender could be detected. The mean age was significantly lower in the GAgP group than those of the other groups ($P < 0.05$).

After the periodontal treatment, GAgP and CP groups

Table 1: Demographic characteristics of study groups

Variable	AgP (*n*=130)	CP (*n*=120)	Healthy controls (*n*=70)
Age (years)			
Mean±SD	29.2±4.5*,¥	34.7±5.9	32.4±6.7
Range	22-41	24-49	21-58
Gender			
Male *n* (%)	40 (30.8)	45 (37.5)	30 (57.1)
Female *n* (%)	90 (69.2)	75 (62.5)	40 (42.9)

SD: standard deviation; *n*=number. *P<0.05 different from healthy controls. ¥P<0.05 different from chronic periodontitis group

Table 2: Clinical parameters and GCF cytokine levels at baseline and 6 weeks for study groups (pg/30 s, mean±SD)

Parameters	Sample times	AgP	CP	Healthy controls
Plaque index	Baseline	1.3±0.7[b]	1.5±0.6	0.6±0.4
	6-week	0.3±0.4[a,b]	0.5±0.5[a]	
gingival index	Baseline	1.6±0.6[b]	1.8±0.4	0.4±0.5
	6-week	0.3±0.5[a]	0.5±0.5[a]	
Pocket depth	Baseline	5.6±1.2[b]	5.5±1.1	0.4±0.5
	6-week	3.6±1.3[a,b]	3.4±1.9[a]	
Clinical attachment level	Baseline	9.1±2.6	9.1±2.3	NA
	6-week	7.6±2.4[a]	7.8±2[a]	
GCF IL-13	Baseline	58.61±14.6[c,b]	79.14±16.34	84.52±29.1
	6-week	75.26±14.3[a]	81.10±29.08	

[a]$P<0.05$ different from baseline, [b]$P<0.05$ different from healthy controls, [c]$P<0.05$ different from CP, NA, not applicable

Table 3: Distribution of the IL-13 genotypes and allele frequencies for study groups *n* (%)

Genotypes	AgP *n*=120	CP *n*=130	Healthy controls *n*=70	*P*
IL-13 (-1512)				
A/A	70	64	39	$P=0.967$
A/C	49	43	25	
C/C	11	13	6	
IL-13 (-1112)				
C/C	81	65	41	$P=0.737$
C/T	42	45	24	
T/T	7	10	5	
Allele frequencies				
IL-13 (-1512)				
A	189 (72.6)	171 (71.2)	103 (73.5)	$P=0.158$
C	71 (27.4))	69 (28.8)	37 (26.5)	
IL-13 (-1112)				
C	204 (78.4)	175 (72.9)	106 (75.7)	$P=0.213$
T	56 (21.6)	65 (27.1)	34 (24.3)	

$P<0.05$

showed clinical improvement comparing those of the baseline ($P < 0.05$) [Table 2]. Also, there was a significant difference between the GAgP and C groups regarding PI and GI values in 6 weeks ($P < 0.05$).

In the GAgP group, GCF IL-13 levels were significantly increased after periodontal treatment [$P < 0.05$, Table 2]. Also, in the GAgP group, GCF IL-13 cytokine levels at baseline were found significantly lower compared to the CP and C groups ($P < 0.05$). Also in the CP group, GCF IL-13 cytokine levels at baseline and 6 weeks are similarly compared to those of the C group. After periodontal treatment, GCF IL-13 levels were similar in all groups.

The genotype distribution fulfilled Hardy–Weinberg's criteria. The distribution of all genotypes for study genes among the study groups is presented in Table 3. The distribution of the genotypes for two variants within groups was not significantly different ($P > 0.05$). Also, allele frequencies did not differ among the study groups ($P > 0.05$).

In GAgP and CP groups, individuals were categorized according to the IL-13-1112 and IL-13-1512 genotype distribution and then we evaluated whether IL-13 genotype distributions affect the response to nonsurgical periodontal therapy. When comparing the CC, CT, and TT genotypes for the IL-13 (-1112) region in the GAgP group, all genotypes are similar to initial PD, CAL, and GCF cytokine levels, however, PD and CAL levels were decreased ($P > 0.05$), also IL-13 levels were increased ($P > 0.05$) in CC and CT genotypes at 6 weeks [Table 4]. However, there were no significantly different genotypes in terms of GCF IL-13 level.

In the GAgP group, there was no significant decrease in PI values of CC genotype for IL-13 (-1512) region after treatment ($P > 0.05$) and the significant decrease in other clinical parameters ($P < 0.05$). However, GCF IL-13 level was increased after periodontal treatment in all genotypes ($P < 0.05$).

In GCP patients when genotypic comparison (-1112 region) was performed, no significant changes were observed in PI, PD, and CAL levels in patients with TT genotypes [$P > 0.05$, Table 5]; however, significant clinical improvements were observed in other genotypes (CC and CT) in 6 weeks ($P < 0.05$). GCF IL-13 levels did not change after treatment for all genotypes.

When the distributions of the genotypes belonging to the other gene region (-1512) were analyzed in the GCP group, not only CD and CAL values but also GCF IL-13 level were not significantly improved in 6 weeks in the CC genotype. There was no significant change in genotypic comparisons for clinical parameters and GCF IL-13 levels in intergroup or intragroup comparisons.

Table 4: Clinical parameters and GCF cytokine levels in aggressive periodontitis group distributed by IL-13 (-1112) ve (-1512) genotypes (mean±SD)

Parameter	IL-13-1112			IL-13-1512		
	CC *n*=14	CT *n*=14	TT *n*=2	CC *n*=5	AC *n*=13	AA *n*=12
PI						
Baseline	1.2±0.8	1.4±0.7	1±0	0.8±0.4	1.4±0.7	1.3±0.8
6-week	0.2±0.4	0.3±0.4	0±0	0.2±0.4	0.3±0.5	0.2±0.4
P	*P*=0.000	*P*=0.000		*P*=0.070	*P*=0.000	*P*=0.000
GI						
Baseline	1.6±0.7	1.7±0.6	1±0	1.4±0.8	1.7±0.5	1.5±0.6
6-week	0.2±0.6	0.4±0.5	0±0	0.0±0.0	0.4±0.5	0.3±0.6
P	*P*=0.000	*P*=0.000		*P*=0.025	*P*=0.000	*P*=0.000
PD						
Baseline	5.5±1.3	5.7±1.1	6.5±2.1	5.8±2	5.8±1	5.4±1.1
6-week	3.2±0.7	3.7±1.5	5±2.8	3.8±1.9	3.7±1.4	3.3±0.8
P	*P*=0.000	*P*=0.000	*P*=0.205	*P*=0.022	*P*=0.000	*P*=0.000
CAL						
Baseline	9.5±3.1	8.7±2.2	8.5±0.7	7.4±1.5	9.07±2.06	9.9±3.2
6-week	7.7±2.8	7.7±2.3	6.5±0.7	5.8±1.0	7.8±2.2	8.2±2.8
P	*P*=0.000	*P*=0.000		*P*=0.003	*P*=0.000	*P*=0.000
IL-13 GCF						
Baseline	60.5±9.7	56.8±18.4	57.84±21.2	64.3±13.7	53.9±18.4	61.2±8.8
6-week	75.1±17.8	75.7±9.8	72.9±24.3	76.72±14.6	74.8±9.82	75.05±19
P	*P*=0.018	*P*=0.008	*P*=0.091	*P*=0.023	*P*=0.008	*P*=0.041

Table 5: Clinical parameters and GCF cytokine levels in chronic periodontitis group distributed by IL-13 (-1112) ve (-1512) genotypes (mean±SD)

Clinical parameters	IL-13-1112			IL-13-1512		
	CC *n*=12	CT *n*=13	TT *n*=3	CC *n*=7	AC *n*=13	AA *n*=10
PI						
Baseline	1.5±0.6	1.6±0.5	1.0±0.7	1.4±0.9	1.5±0.5	1.5±0.5
6-week	0.4±0.5	0.6±0.5	0.6±0.5	0.4±0.5	0.6±0.5	0.5±0.5
P	*P*=0.001	*P*=0.000	*P*=0.178	*P*=0.038	*P*=0.000	*P*=0.001
GI						
Baseline	1.9±0.5	1.7±0.4	2±0	1.8±0.3	1.9±0.4	1.8±0.4
6-week	0.8±0.5	0.3±0.4	0.4±0.5	0.4±0.5	0.4±0.6	0.7±0.4
P	*P*=0.000	*P*=0.000	*P*=0.003	*P*=0.000	*P*=0.000	*P*=0.000
PD						
Baseline	5.5±1.1	5.6±1.1	5.2±1.3	5.1±1.2	5.8±1.0	5.3±1.1
6 week	3.1±0.7	3.0±0.4	4.8±4.6	4.1±3.9	3.1±0.5	3.2±0.6
P	*P*=0.000	*P*=0.000	*P*=0.818	*P*=0.435	*P*=0.000	*P*=0.000
CAL						
Baseline	9.7±1.2	9.1±2.5	7.8±3.4	8±2.8	9.3±2.5	9.8±1.2
6-week	8.0±1.5	7.3±2.4	8.4±2.3	7.8±2.3	7.3±2.4	8.4±1.2
P	*P*=0.000	*P*=0.001	*P*=0.795	*P*=0.093	*P*=0.000	*P*=0.003
IL-13 GCF						
Baseline	79.7±19.4	77.9±12.0	80.7±21.3	79.4±16.62	76.9±14.0	81.9±19.9
6-week	84.5±28.6	75.4±34.5	87.3±10.3	87.47±8.7	69.7±40.6	91.4±11.8
P	*P*=0.603	*P*=0.820	*P*=0.464	*P*=0.168	*P*=0.543	*P*=0.298

Periodontal disease susceptibility in AgP and CP group was evaluated with logistic regression analysis of both IL-13 gene regions after adjustment of age and gender. Genotype diversity was not affected by either age or gender.

Discussion

Several genetic-related studies have been published about the distribution of single-nucleotide polymorphisms addressing cytokines in periodontitis patients. In this study, we evaluated the role of anti-inflammatory cytokines, IL-13, in CP and AgP patients and their effects on GCF levels and periodontal treatment. We found that there were significant improvements after periodontal treatment in clinical parameters, and the GCF IL-13 levels were markedly decreased at baseline in the GAgP group and after treatment; similar GCF IL-13 levels were observed in all groups. Also, our results indicated that the IL-13 -1112 C/T and -1512 A/C gene polymorphisms were not associated with GCP and GAgP.

Various laboratory-based diagnostic methods are used in the diagnosis of periodontal diseases in addition to the clinical parameters. These diagnostic methods are mostly based on the examination of saliva, bacterial plaque, GCF, peripheral blood polymorphonuclear leukocytes, and blood serum. The most popular of these is the GCF content, which is regarded as an exudative origin.[18] GCF is a fluid that releases important information about the development of periodontal disease of both the components it contains, and at the same time, it is an important step in the host defense mechanism.[19,20] In our study, GCF was collected by paper strips along 30 s to avoid serum contamination and cytokine secretion induced by the mechanical irritation provoked by a longer collection period. Furthermore, we evaluated the total amount of the cytokines in the GCF samples, because the total amount of cytokines in GCF sample per sampling time has been suggested as a better indicator of relative GCF constituent activity rather than the GCF volume that might result in the decrease of the cytokine concentration.[21,22]

IL-13 is a multifunctional Th2 cytokine that can play inflammatory responses and the role of IL-13 in periodontitis pathogenesis was still controversial. mRNA expression of IL-13 was determined in gingival biopsies from patients with AgP comparing to gingivitis and healthy individuals by Suárez *et al*.[13] They reported that IL-13 and TGF-β were only detected in healthy/gingivitis patients and IL-4 was not detected in any of the individuals. Furthermore, in a study that was investigated to analyze the expression and production of IL-2, IFN-γ, IL-4, and IL-13 in CD4+ cells from the peripheral blood of patients with AgP and periodontally healthy controls, they found that IL-4 and IL-13 expressions were higher in activated CD4+ cells in AgP patients, but were not significant compared to controls.[23] A recent study, Zein Elabdeen *et al*.[24] studied the content of cytokines in GCF as well as in plasma patients with AgP and healthy controls using 27-multiplex fluorescent bead-based immunoassays. They found that interferon-γ was the only cytokine found in significantly lower levels in GCF of AgP patients. Also, levels of IL-10, IL-13, IL-1Ra, and monocyte chemoattractant protein-1, regulated on activation normal T-cell expressed and secreted, granulocyte-colony-stimulating factor and granulocyte-macrophage colony stimulating factor were lower in plasma of AgP compared to healthy controls. They suggested that the lower ratio of Th1:Th2 cytokines in GCF samples of AgP patients suggests a role for Th2 at the local site of disease. In the present study, while no significant differences were found between the baseline and 6 weeks in GCF IL-13 levels in patients with CP, GCF IL-13 levels were lower at baseline in AgP patients compared to CP and controls. Overall, these findings are in accordance with the findings reported by Zein Elabdeen *et al*.[24] Also, we found that GCF IL-13 levels were increased especially in -1112 CC and CT genotypes at 6-week.

IL-13 has two C/T polymorphisms (-1112 and -1512 positions) in the promoter region. These two single-nucleotide polymorphisms have been associated with several autoimmune diseases, such as rheumatoid arthritis, autoimmune hepatitis, systemic sclerosis, and autoimmune thyroid diseases.[25] In a study that investigated the association between the polymorphism in the IL-3 and IL-13 genes in rheumatoid arthritis, they found that IL-13 -1112 C/T polymorphism was not associated with rheumatoid arthritis, but may be associated with increased risk of rheumatoid arthritis in erythrocyte sedimentation <25.00 patients.[26] Pavkova Goldbergova examined the relation between polymorphisms and serum levels of selected cytokines (IL-6, IL-13, and IL-15), production of autoantibodies, and factors describing rheumatoid arthritis, such as DAS28 and total sharp score. They reported that the frequency of the T allele of the IL-13 polymorphism -1112 C/T was higher in the subgroup with faster progression of the disease, but there was no association between this promoter polymorphism and serum IL-13 levels.[27] Although it has been proposed that similar pathogenic mechanisms may be observed in periodontitis, there was no correlation found between IL-13 -1112 C/T and -1512 A/C genotype distributions and periodontitis in our study. However, similar to our results, Marinou *et al*.[28] suggested that IL-13 rs1800925 (-1112) C/T did not significantly contribute to rheumatoid arthritis susceptibility in an English Caucasian population.

Gonzales *et al*.[15] investigated possible associations between the IL-4 –590 C/T, IL-4 –34 C/T, IL-13 – 1112 C/T and IL-13 –1512 A/C promoter

polymorphisms, and 58 GAgP patients. Similar to our results, they found no associations betweeen IL-13 – 1112 C/T and IL-13 –1512 A/C polymorphism in GAgP patients, however, IL-4 –590 C/T and IL-4–34 C/T polymorphisms were detected in GAgP patients. The authors did not investigate the effects of different genotypes on periodontal treatment, effects of the level. In another study, IL-13 -1112 C/T gene polymorphism was investigated in the Taiwanese (Chinese) population with different periodontal diseases. The study performed participants 60 with AgP, 204 with CP, and 95 who were healthy controls. In contrast to our results, CC genotype and C allele distribution were significantly different between GAgP and healthy control groups. However, when comparing the genotype distributions of IL-13 gene in CP and healthy controls group, results were found to be similar. Also, they found that a significant difference in the IL-13 -1112 CC allele between AgP and healthy controls was only found in the nonsmoking group.[7] Also, in our study, we excluded smokers, which can be confounding factor for genetic analysis. Conversely, in another study, with similar population of the same race, the relationship between CP and IL-13 gene polymorphisms in Chinese Han population was investigated. The study included 440 patients with moderate or severe CP and 324 healthy subjects. The area studied for IL-13 was rs 1800925 and similar to our results, there was no correlation found associated with CP.[8] However, a recent meta-analysis found that the IL-13 -1112 gene polymorphism may be associated with susceptibility to CP but not to AgP and these findings must be confirmed on large-scale, multiethnic case-control trials.[29]

This study has several limitations, such as analyses of a single SNP locus may not provide us with a comprehensive understanding of the genetic effects of these candidate genes and our sample size was not large enough. Therefore, our results must be interpreted with caution and further studies must be conducted to confirm our results.

Within the limitations of this study, we conclude that the GCF IL-13 levels were lower at baseline in the GAgP group, and after treatment, similar GCF IL-13 levels were observed in all groups. Also, there was no association between IL-13 gene polymorphisms and GCP and GAgP and IL-13 genotype distributions did not affect the response to nonsurgical periodontal therapy and its GCF levels.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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