Hittite Journal of Science and Engineering, 2018, 5 (4) 285-292
ISSN NUMBER: 2148-4171
DOI: 10.17350/HJSE19030000105

Combinations of Interleukin-10 Gene Promoter Polymorphisms with -1082A, -819T, -592A Minor Allele are Associated with Sinonasal Polyposis.

Sibel Ozdas
Adana Science and Technology University, Department of Bioengineering, Adana, TURKEY

ABSTRACT

Sinonasal polyposis (SP) is an inflammatory disease involving multiple etiologies and pathogenesis. The disarray of Interleukin (IL)-10 is associated with a raised immunopathological response during the progression of many autoimmune diseases as well as the response to infection. We studied the possible role of the single nucleotide polymorphisms (SNPs) in IL-10 gene and their genotypic combinations in the SP pathogenesis. The IL-10's promoter was genotyped in 200 participants (100 patients and 100 controls). The sites that were encompassed -1082, -819, and -592 SNP regions of extracted DNAs were analyzed by sequencing for polymorphisms. The IL-10 gene promoter polymorphisms with -1082A, -819T, -592A minor alleles, their heterozygotes and homozygotes mutant genotypes had significantly higher risks for SP ($P<0.05$). Also, haplotype analysis demonstrated that the GTC, ACC, and GCA haplotypes in IL-10 were high-risk of SP but ATA was only in controls ($P= 0.007$). Also, the multifactor dimensionality reduction (MDR) analysis indicated that the IL-10-1082_-819 and -1082_-819_-592 were the best predictive models for SP with 66.6% and 69% accuracy, respectively. IL-10 genotypic variations and their combinations are linked with a high-risk for the SP development in the Turkish population. The IL-10 genetic polymorphisms may lend to SP by altering the arrangement of the gene expression, affecting the severity of the inflammatory response.

Article History:
Received: 2018/02/13
Accepted: 2018/04/04
Online: 2018/04/27

***Correspondence to**: Sibel Özdaş,*
Adana Science and Technology University,
Department of Bioengineering, 01250,
Adana, TURKEY
E-Mail: sozdas@adanabtu.edu.tr
Phone: +90 505 511 89 47
Fax: +90 322 455 00 09

Keywords:
Sinonasal polyposis; IL-10 gene; Single nucleotide polymorphisms; Interaction; Multifactor dimensionality reduction.

INTRODUCTION

Sinonasal polyposis (SP) is a cronic inflammation of the sinonasal mucosa. Chronic sinusitis, odor loss headache and nasal obstruction are among the clinical symptoms of SP with low life quality among the patients. Sinonasal polyposis is often accompanied by allergic rhinitis, allergy, aspirin susceptibility and asthma [1]. SP has been implicated in many immunological pathways and is a localized manifestation of systemic disorders [2,3]. SP cases frequently accompanied by T helper (Th) type 1-, Th2-, or Th17-biased inflammatory processes regulated by increased expression levels of a number of cytokines with pro- and anti-inflammatory activities [4]. However, the underlying etiology of SP is multifactorial. Also the pathogenesis of SP is still unexplained.

Interleukin (IL)-10 is a crucial Th2 group anti-inflammatory cytokine. IL-10 plays an important role in defense against host pathogens, protection against excessive tissue damage, and development of immune response [5,6]. Since the increased IL-10 gene expression triggers local inflammatory response that result in increased tissue damage, it has been associated with many chronic inflammatory diseases. Various studies have demonstrated that the levels of IL-10 in serum [7], blood [8], nasal secretion [9] and tissue samples significantly increased [10]. In addition, Xu et al [11] had shown an increase in IL-10 mRNA level and had claimed that it could have played a role in SP pathogenesis.

The IL-10 gene has single nucleotide polymorphisms (SNPs), and the vast majority of these are found in the promoter region. The SNPs at sites -1082, -819 and -592 in the IL-10 gene show strong linkage disequ-

Table 1. The primer's designs

SNP positon	Direction	Primers	Fragment size
-1082	Forward Reverse	5′-ACACACACACAAATCCAAG-3′ 5′-TAGGAGGTCCCTTACTTTCCTC-3′	277 bp
-819	Forward Reverse	5′-CTCTAAGGCCAATTTAATCCAAGGTTT-3′ 5′-GAAGTCGTTCACGTCTATGATGAGAAGG-3′	215 bp
-1082	Forward Reverse	5′-AAATCGGGGTAAAGGAGC-3′ 5′-AGCAGCCCTTCCATTTTACT-3′	270 bp

SNP: Single-nucleotide polymorphism; bp: base pairs

ilibrium leading to variations in expression level and are associated with various disease pathogenesis [12,13].

We studied the IL-10 -1082, -819, and -592 SNPs and their genotypic combinations in SP patients and controls to investigate the role of IL-10 in pathogenesis of SP in this study.

METHODS

Subjects

The samples used in this study were obtained from the individuals who were admitted to otorhinolaryngology clinic. One hundred (mean age 45.5945 ± 10.031; range: 22-64 years) patients with SP were included. Patients were received to the clinic with complaints of nasal obstruction/congestion, loss or reduction of smell, facial pressure and poor quality of life. The patients were diagnosed with nasal endoscopic examination as SP [3]. In the last four weeks, The patients that had an acute upper respiratory tract infection, cystic fibrosis, inverted papillomas, fungal sinusitis, and antrochoanal polyps were not included in the study.

According to the Lund-Mackay system, computed tomography scanning of paranasal sinus were acquired and evaluated [14]. The size of the polyp was classified according to the Lidtholdt scale [15]. The presence of asthma and aspirin susceptibility were assessed in the clinical history and the allergy presence was assessed by prick skin test.

A 100 (mean age 44.62 ± 11.615; range 18-63 years) healthy volunteer subjects with no history of sinonasal disorder, inflammatory-related disorder or any other disease was included in the control group. In addition, absence of rhinosinusitis and SP were confirmed with the nasal endoscopic examination. The study has ethics committee approval.

Genotyping

The genomic DNA was extracted from blood sample with kit (Qiagen Inc.). The polymerase chain reaction (PCR) for direct sequencing was used to amplify specific regions of the IL-10 gene's promotor and primer's designs were summarized in Table 1.

The PCR reaction mixture (25 µL) constituted of 0.5 µL dNTP (10 µM concentration), 1 µL forward and revers primer (5 µM), 0.2 µL SuperHot Taq DNA polymerase (Bioron Inc.), 5 µL 10x PCR buffer (Bioron Inc.) and 2.5 µL template DNA (20-50 ng/µL). Thermal conditions of the PCR was followed by initial-denaturation at 95^{0}C for 10 min, denaturation at 95^{0}C for 45 sec, annealing at 60^{0}C for 45 sec, extension at 72^{0}C for 45 sec. Also the final extension was at 72^{0}C for 10 min. The amplified products were verified by 2% agorose gel

Table 2. Clinical characteristics of subjects

Clinical characteristics		Patients with SP (n: 100)	Controls (n: 100)	P value
Age, years		45.59 ± 10.031	44.62 ± 11.615	> 0.05
Gender, Male/Female		49/51	44/46	> 0.05
Computed tomography score		8,58 ± 3,994 (4-19)	-	-
Asthma presence, n (%)		34 (34)	0 (0)	< 0.001
Aspirin intolerance presence, n (%)		19 (19)	0 (0)	< 0.001
Allergy presence, n (%)		35 (35)	0 (0)	< 0.001
Polyp size, n (%)	1	40 (40)	0 (0)	< 0.001
	2	33 (33)	0 (0)	< 0.001
	3	27 (27)	0 (0)	< 0.001

SP: Sinonasal polyposis

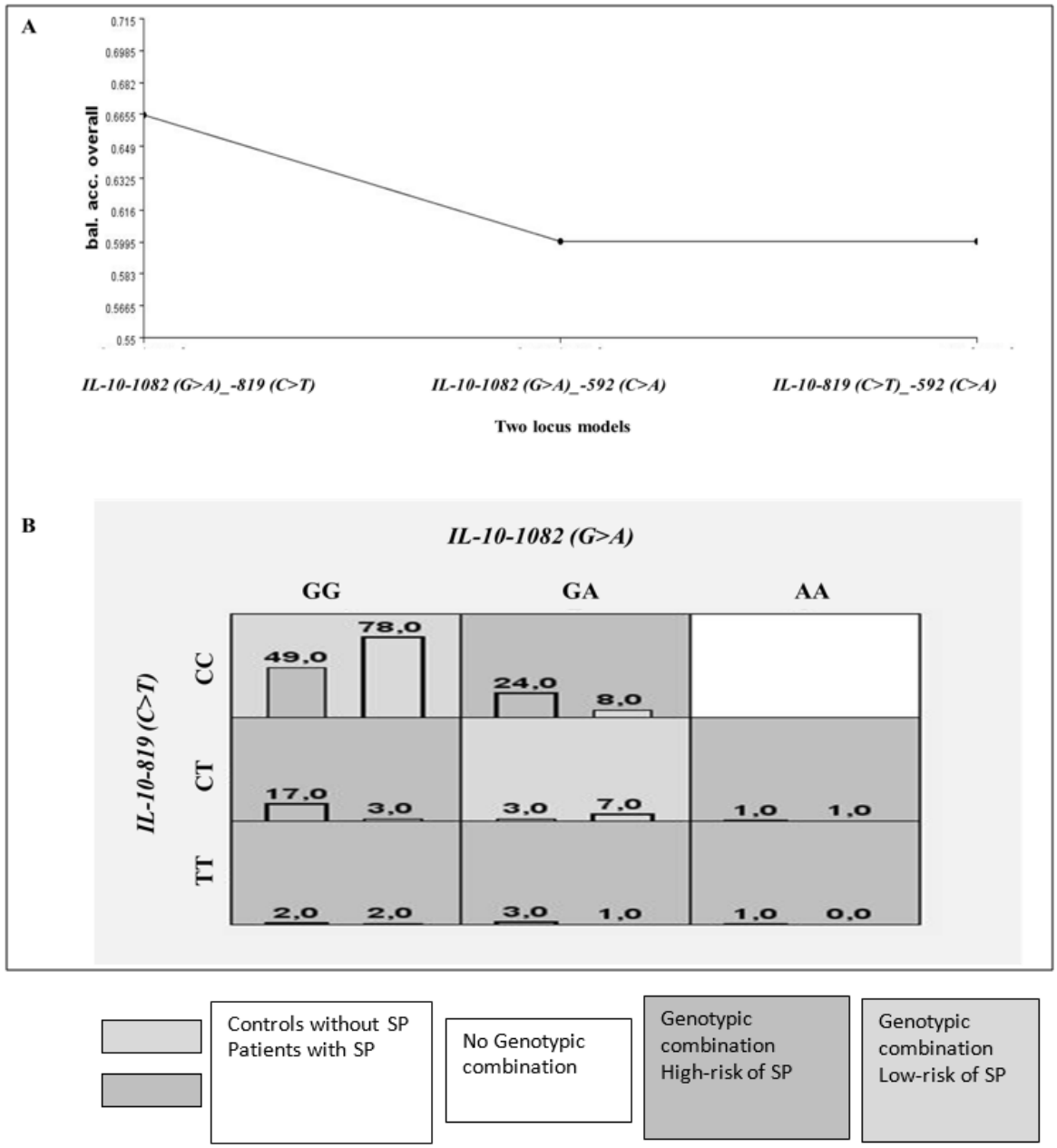

Figure 1. The best predictive models in application of MDR
A. The graphic shows the character of the interactions between the three SNPs in the IL-10. The strongest interacting pair is the IL-10-1082_-819 SNPs. B. The IL-10-1082_-819 genotypic combinations which are able to accurately predict SP presence. The IL-10 AA+TT, GG+CT, GA+CC, and GA+TT diplotypes had a ∞-, 5-, 3-, and 3-fold elevated risk for developing SP. However but only patients with SP but also controls had the AA+CC diplotype (TBA 0.66, CVC 10/10, OR 5.18, 95% CI 2.60-10.20, P= 0.001)

electrophoresis ethidium bromide was used to stain DNA fragments in the gel for visualization.

PCR products were purified and sequenced. The sequencing reaction mixture (20 µL) constituted of 2 µL purified PCR product, 1.2 µL forward and revers primer (5 µM), 2 µL BigDye 3.1 reaxtion mix (Applied Biosystems Inc.), 4 µL of 5x reaction buffer (Applied Biosystems Inc.). PCR product thermal conditions comprised of an at 95^{0}C for 20 seconds (50 cycles/minute), followed by 25 seconds at 50^{0}C (50 cycles/minute), and 2 minutes at 72^{0}C.

Capillary electrophoresis was performed using ABI 3130 (Applied Biosystems Inc.) capillary electrophoresis device and in accordance with the manufacturer's protocol. Electroforegrams were analyzed by using SeqScape 2.5.0 (Applied Biosystems Inc.) software and sequence variations were determined.

Statistical analysis

SPSS version 16.0 was used for statistical analysis. The genotypic distributions were checked consistency with Hardy-Weinberg-Equilibrium (HWE) [16]. The genotypic and haplotypic frequencies were evaluated using a software (http://bioinfo.iconcologia.net/SNPstats) [17]. P-value<0.05 was considered significant. The Multifactor Dimensionality Reduction (MDR) package software was performed all potential recognition of SNP-SNP interactions and that are fine identified as playing a significant role in insight complicated properties. MDR has the testing balance accuracy (TBA) and cross-validation consistency (CVC) [18].

RESULTS

SP and control groups were similar in age distribution and gender (P> 0.05). The demographic data of the subjects shown in Table 2.

The genotypic distributions in the controls found consistent with the HWE (for -1082 P= 0.47; for -819 P= 0.07; for -592 P= 1). In patients with SP, DNA sequence analysis of the IL-10 gene revealed that in all genetic models carrying the A allele for -1082 (G>A), the dominant and log-addictive genetic model carrying the T allele for -819 (C>T), the genotype frequencies in all other genetic models except the overdominant model bearing the A allele for -592 (C>A) were statistically significant (P < 0.05 for all). We also found that the minor allele frequencies for three SNPs were significantly elevated in patients with SP, and summarized in Table 3.

The IL-10 ACC, GTC, GCA haplotype frequencies were higher in patients with SP. Morever the ATA haplotype was only in patients, but the GTA only in the controls (-1082, -819, -592, respectively) (P< 0.05) (Table 4).

MDR analysis was evaluated all potantial combinations, and two models for SP's prediction were found. Binary SNP interplay between the best two-locus predictive model IL-10-1082_-819 was detected with a CVC 10/10 and a TBA 66.6% (Fig. 1A). According to this model, individuals with GG+CT and GA+CC, GA+TT diplotypes have 5-, 3- and 3-fold higher SP risks when compared to control group, respectively. On the contrary, GG+CC and GA+CT diplotypes were 1.5 and 2.5-fold higher in controls, respectively [Odds ratio (OR)= 5.18, 95% Confidence interval (CI)= 2.60-10.20, P= 0.001). In Fig. 1, high-risk diplotypes for SP are shown as dark, and protective diplotypes are shown as light gray boxes (Fig. 1B).

The triple SNP interaction between the best tree-locus predictive model IL-10-1082_-819_-592 was detected with a CVC 10/10 and a TBA 69%. Individuals carrying triplotypes of GG+CT+CA, GG+CT+CC, GA+CC+CC, and GA+CT+CA according to this model have 4-, 6-, 2.3- and 1.5-fold higher SP risks when compared to control group, respectively. In contrast, triplotypes GG+TT+AA, GA+TT+CA and AA+CT+CA were observed only in the controls (OR= 5.9, 95% CI = 3.10-11.40, P= 0.001).

DISCUSSION

SP is a complex mucosal inflammatory disorder involving pathogenesis and multiple etiologies. Despite many efforts, the sinonasal polyposis's pathogenesis is still unexplained. Most of the studies indicate cytokine balance leading to inflammation [5]. IL-10, which was previously reported to be up-regulated in inflammatory diseases, has recently been demonstrated to have increased expression levels in nasal fluid and SP mucosa compared to control groups [9-10]. The up-regulated activity of the immune response regulatory IL-10 gene in the inflammatory sinus mucosa has been considered as one of the key mechanisms in polypogenesis. However, there might be a relationship between the polymorphisms that may lead to regulatory defects in the level of IL-10 gene expression and polyp formation that may affect SP susceptibility.

The IL-10 has remarkable genetic polymorphisms, environmental factors together with variations in the promoter region may cause the emergence of a variety of disease phenotypes [12]. IL-10 gene promoter SNPs affect the affinity of a nuclear protein to binding site on promoter, and cause change in the level of gene expression. IL-10 gene -1082, -819, and -592 positions of the proximal promoter are the most studied polymorphisms. The presence of common allelic combinations in these three SNPs, their linkage disequilibrium and varying IL-10 serum levels that depend on their combinations has been confirmed in several studies [12,13].

Malagutti and colleagues found that the IL-10 serum levels in sinonasal polyposis patients were significantly higher, and examined the exonic and intronic regions of the IL-10 by DNA sequencing. In that study, it was reported that some intronic genotypes of the IL-10 were found to be highly frequent in the patients (30%) and that these genotypes might cause high serum levels of IL-10. In advanced studies, it has been reported that the genetic polymorphisms of promoter and regulatory region in the IL-10 may be critical in the polyp genesis of sinonasal polyposis patients [19,20]. However, far as we know, there is no study investigating the relationship between the IL-10 gene SNPs -1082, -819, -592 and SNP-SNP interactions SP pathogenesis in the literature. In this study, the IL-10 gene SNPs -1082, -819, -592 and their genotypic combinations in 100 SP patients and 100 volunteer-healthy controls in the Turkish population were

Table 3. The genotyping distributions for promotor SNPs in IL-10 gene

SNPs	Genotype_ Allele	Patients with SP (n: 100) n (%)	Controls (n: 100) n (%)	Genetic Models	Odds ratio (%95 CI)	Minor allele frequencies	P value	P value for HWE
-1082 (G>A)	GG	68 (68)	83 (83)	Codominant	1.00 (Reference)	0.33-0.97	0.037	0.47
	GA	30 (30)	16 (16)		0.43 (0.19-0.87)			
	AA	2 (2)	1 (1)		0.50 (0.15-6.57)			
	GG	68 (68)	83 (83)	Dominant	1.00 (Reference)		0.017	
	GA+AA	32 (32)	17 (17)		0.43 (0.21-0.89)			
	GG+GA	98 (98)	99 (99)	Recessive	1.00 (Reference)		0.72	
	AA	2 (2)	1 (1)		0.56 (0.16-6.99)			
	GG+AA	70 (70)	85 (85)	Overdominant	1.00 (Reference)		0.019	
	GA	30 (30)	15 (15)		0.41 (0.21-0.89)			
	---	---	---	Log-addictive	0.44 (0.21-0.99)		0.022	
	A‡	0.17	0.09		1.256 (1.189-1.331)		0.028	
-819 (C>T)	CC	73 (73)	86 (86)	Codominant	1.00 (Reference)	0.16-0.70	0.19	> 0.05
	CT	21 (21)	11 (11)		0.41 (0.19-1.00)			> 0.05
	TT	6 (6)	3 (3)		0.35 (0.03-1.90)			> 0.05
	CC	73 (73)	86 (86)	Dominant	1.00 (Reference)		0.031	< 0.001
	CT+TT	27 (27)	14 (14)		0.41 (0.19-0.94)			> 0.05
	CC+CT	94 (94)	97 (97)	Recessive	1.00 (Reference)		0.27	> 0.05
	TT	6 (6)	3 (3)		0.41 (0.07-2.09)			> 0.05
	CC+TT	98 (98)	88 (88)	Overdominant	1.00 (Reference)		0.081	< 0.001
	CT	21 (21)	12 (12)		0.49 (0.21-1.13)			> 0.05
	---	---	---	Log-addictive	0.51 (0.27-1.00)		0.036	> 0.05
	T‡	0.16	0.08		1.257 (1.175-1.334)		0.023	> 0.05
-592 (C>A)	CC	81 (81)	92 (92)	Codominant	1.00 (Reference)	0.26-1	0.016	< 0.001
	CA	14 (14)	8 (8)		0.45 (0.15-1.26)			> 0.05
	AA	5 (5)	0 (0)		0.00 (0.00-NA)			> 0.05
	CC	81 (81)	92 (92)	Dominant	1.00 (Reference)		0.022	> 0.05
	CA+AA	19 (19)	8 (8)		0.33 (0.15-0.93)			< 0.001
	CC+CA	95 (95)	100 (100)	Recessive	1.00 (Reference)		0.016	> 0.05
	AA	5 (5)	0 (0)		0.00 (0.00-NA)			> 0.05
	CC+AA	86 (86)	92 (92)	Overdominant	1.00 (Reference)		0.15	> 0.05
	CA	14 (14)	8 (8)		0.49 (0.18-1.34)			< 0.001
	---	---	---	Log-addictive	0.34 (0.16-0.86)		0.0082	< 0.001
	A‡	0.12	0.04		1.159 (1.101-1.216)		0.048	< 0.001

SNP: Single-nucleotide polymorphism; n (%): Frequency; CI: Confidence interval; HWE: Hardy-Weinberg Equilibrium
‡ *Minor risk allele*

investigated.

In this study, DNA sequence analysis of the IL-10 gene's promoter in patients with SP revealed 32% of position -1082 guanine into adenine (c.-1082 G>A), 27% of position -819 cytosine into thymine (c.-819 C>T) and 19% of position -592 cytosine into adenine conversions (c.-592 C> A). The allele frequencies of -1082A, -819T, -592A in patients with SP

Table 4. The haplotypes distributions for promotor SNPs in IL-10 gene

Haplotipler IL-10 (-1082_-819_-592)	*Haplotype frequencies*		*Odds ratio (95% CI)*	*P value**
	Patients with SP	*Controls*		
GCC	*0.6125*	*0.7902*	*1.00 (Reference)*	*0.20*
ACC	*0.1142*	*0.0907*	*0.62 (0.29 - 1.30)*	*0.18*
GTC	*0.0988*	*0.0724*	*0.59 (0.27 - 1.25)*	*0.010*
GCA	*0.1184*	*0.0277*	*0.24 (0.08 - 0.71)*	*<0.0001*
GTA	*0*	*0.0127*	-	*<0.0001*
ATA	*0.0619*	*0*	-	---

SP: Sinonasal polyposis; CI: Confidence interval;
**P-value= 0.0071*

were 0.17, 0.16, 0.12, while the allele frequencies in control were 0.09, 0.08 and 0.04, respectively. Minor allele frequencies are high and significant in the patients according to databases (www.ncbi.nlm.nih.govsnp; www.hapmap.org). Wide variation has been also recorded in the frequencies of IL-10 gene's promoter SNPs among distinct populations. Despite the fact that the global prevalence notified of the allele frequencies of -1082, -819, and -592 SNPs in IL-10 gene in control groups vary, and they are same to last studies in Turkish society [13, 21, 22].

In addition, the current study, the effect of IL-10 gene promoter SNPs on clinical heterogeneity of the disease was investigated by separating the patient group into subgroups according to clinical characteristics such as CT score, asthma presence, aspirin susceptibility, allergy presence and polyp phase, and no connection was observed.

The frequencies of IL10-1082GA, AA, GA-AA and AA genotypes containing the minor allele were found to be higher and statistically significant compared to IL-10-1082GG, GG-GA and GG-AA genotypes in SP patients. Furthermore, the frequency of IL10-819CT, TT genotypes containing the minor allele was found to be higher and statistically significant compared to IL-10-819CC, CC-CT and CC-TT genotypes in patients with SP. The IL-10-592CA, AA, CA-AA genotypes's frequency containing the minor allele were higher and statistically significant compared to IL-10-592CC, CC-CA genotypes in SP patients. MDR showed that the individual with the IL-10-1082_-819 GG+CT, GA+CC, GA+TT diplotypes had 5-, 3- and 3-fold risk of developing SP, respectively.

The estimated frequency of haplotypes formed by the investigated SNPs was calculated in the control and patient groups. The haplotypes GCC, ACC and GCA were commonly observed whereas ATA and GTA haplotypes were less frequent (< 0.01). The distribution of haplotypes was significantly different between patients and controls. Based on the most common haplotype (GCC) in the control group, the probability rate of other haplotypes was assessed. When compared, a significant difference was observed between the patient and the control group for ACC, GTC and GCA haplotypes. This data show that those carrying the ACC, GTC and GCA haplotypes are at a greater risk of developing SP than those carrying the GCC haplotype. The disease is not associated with a single mutation or allelic variation, but may occur with the common effect of multiple SNPs or mutations. When rare alleles come together, they can prepare the ground for disease. These rare alleles with the contribution of environmental factors may also be critical in the pathogenesis of the disorder [23]. However, considering these haplotypes and their frequencies, further research is needed to reach a definite conclusion. According to the tree-locus model MDR analysis, GG+CT+CC, GG+CT+CA, GA+CC+CC and GA+CT+CA triplotype had 6-, 4-, 2.3- and 1.5-fold risk of developing SP.

The haplotypes of the IL-10 gene SNPs also show ethnic differences. These polymorphisms may constitute a variety of haplotypes, but generally only three haplotypes (ATA, ACC, GCC) with GCC (50-52%) as the most common have been identified [24,25]. In the previous studies, haplotypes were shown to be the combinations carrying GCC, ACC, or ATA alleles at IL-10-1082, -592 junctions [7, 8, 12]. The haplotypes and their frequencies found in our study are consistent with the literature.

We have analyzed the best dual IL-10-(-1082_-819) and triple IL-10-(-1082_-819_-592) locus models in MDR analysis and p value was found to be 0.001 in both. Among the dual locus models, the IL-10-1082_-819 model showed a high (66%) predictive value in SP diagnosis compared to other SNP-SNP interactions. In addition, in dual and triple locus models, for individuals carrying diplotypes and/or triplotypes involving the minor allele of SNPs, the SP risk was found to be significantly higher than control group. MDR data is consistent with genotype and haplotype analysis results. However, the observation of higher frequency GG+CC and GA+CT diplotypes in SP in the dual locus

model, and the presence of GG+TT+AA triplotype only in the control group in the triple locus model emphasize the protective effect of the -1082G allele. Previous studies have reported a significant decrease in the amount of serum IL-10 in individuals carrying the IL-10-1082A allele compared to those carrying IL-10-1082G alleles and that the -1082 allele has a predictive value in determining IL-10 secretion levels [7, 8, 12].

In studies related to SP pathogenesis it was reported that there was an unbalance between Th1 and 2 cytokines including IL-10 (Th1/Th2) ratio, and IL-10's levels in serum were especially high in eosinophilic sinonasal polyposis and allergic or asthmatic sinonasal polyposis [7,19]. In our study, although the level of IL-10 serum was not investigated, 34% of patients with SP were asthmatic, 19% were aspirin sensitive and 35% were allergic.

Understanding the clinical characteristics of SP patients with IL-10 gene promoter SNPs may provide important clinical data for follow-up of the patient's disease progression and treatment. Although the literature on the IL-10 gene is quite extensive, there has been no published study investigating the sinonasal polyp-linkage of promoter genotypic variations and their interactions with each other yet. Although our study was the first community-based study of the IL-10 gene promoter SP patients, our results were limited due to the low sample number and financial problems. The number of samples in community-based studies is an important constraint, and further studies involving more subjects may increase the reliability of the data or the results may change. For this reason, our results carry preliminary meaning for future studies.

CONCLUSION

In conclusion, the IL-10's genotypic variations in SP patients were examined. The genotypes and haplotypes including the -1082A, -819T and -592A minor alleles that were associated with SP susceptibility, affecting the level of genetic expression, altering the severity of inflammation and contributing to SP development were identified in our study. Further work including functional studies evaluating the effect of hereby reported IL-10 gene promoter nucleotide variations on the amount of protein and including higher number of subjects to investigate the relationship between this variation and the pathogenesis of inflammatory diseases are required.

ACKNOWLEDGMENT

This work was supported by The Turkey Ministry of Health through a research Grant No16103018 (2015/SAGEM/0005)]

References

1. Cingi C, Demirbas D, Ural A. Nasal polyposis: an overview of differential diagnosis and treatment. Recent Patents Inflammation Allergy Drug Discovery 5 (2011) 241-252.
2. Hulse KE, Stevens WW, Tan BK, Schleimer RP. Pathogenesis of nasal polyposis. Clinical and Experimental Allergy 45 (2015) 328-346.
3. Riechelmann H, Europäischen Akademie für Allergie und Klinische Immunologie (EAACI) und der European Rhinologic Society (ERS). [Chronic Rhinosinusitis - EPOS 2012 Part I].Laryngorhinootologie 92 (2013) 193-201; quiz 202-3.
4. Kato A. Immunopathology of chronic rhinosinusitis. Allergology International 64 (2015) 121-130.
5. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Critical Reviews In Immunology 32 (2012) 23-63.
6. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annual Review of Immunology 29 (2011) 71-109.
7. Ozkara S, Keles E, Ilhan N, Gungor H, Kaygusuz I, Alpay HC. The relationship between Th1/Th2 balance and 1alpha,25-dihydroxyvitamin D(3) in patients with nasal polyposis. European Archives of Otorinolaryngology 269 (2012) 2519-2524.
8. Shen Y, Hu GH, Kang HY, Tang XY, Hong SL. Allergen induced Treg response in the peripheral blood mononuclear cells (PBMCs) of patients with nasal polyposis. Asian Pacific Journal of Allergy and Immunology 32 (2014) 300-307.
9. Kim DW, Eun KM, Jin HR, Cho SH, Kim DK. Prolonged allergen exposure is associated with increased thymic stromal lymphopoietin expression and Th2-skewing in mouse models of chronic rhinosinusitis. Laryngoscope 126 (2016) E265-72.
10. Okano M, Fujiwara T, Kariya S, Higaki T, Haruna T, Matsushita O, Noda Y, Makihara S, Kanai K, Noyama Y, Taniguchi M, Nishizaki K. Cellular responses to Staphylococcus aureus alpha-toxin in chronic rhinosinusitis with nasal polyps. Allergology International 63 (2014) 563-573.
11. Xu J, Han R, Kim DW, Mo JH, Jin Y, Rha KS, Kim YM. Correction: Role of Interleukin-10 on Nasal Polypogenesis in Patients with Chronic Rhinosinusitis with Nasal Polyps. PLoS One 11 (2016) e0161013.
12. Trifunović J, Miller L, Debeljak Ž, Horvat V. Pathologic patterns of interleukin 10 expression- a review. Zagre: Croatian Society for Medical Biochemistry and Laboratory Medicine 25 (2015) 36-48.
13. Özdaş S, Özdaş T, Acar M, Erbek SS, Köseoğlu S, Göktürk G, Izbirak A. Association of Interleukin-10 gene promoter polymorphisms with obstructive sleep apnea. Sleep Breath 20 (2016) 855-866.
14. Lund VJ, Mackay IS. Staging in rhinosinusitus. Rhinology 31 (1993) 183–184.
15. Lildholdt T, Rundcrantz H, Lindqvist N. Efficacy of topical corticosteroid powder for nasal polyps: a double-blind, placebo-controlled study of budesonide. Clin Clinical Otolaryngology and Allied Sciences 20 (1995) 26-30.
16. Lee WC. Testing for Sufficient-Cause Gene-Environment Interactions Under the Assumptions of Independence and Hardy-Weinberg Equilibrium. American Journal of Epidemiology 182 (2015) 9-16.
17. Moradi MT, Khazaei M, Khazaei M. The effect of catalase C262T gene polymorphism in susceptibility to ovarian cancer in Kermanshah province, Western Iran. Journal of Cases in Obstetrics & Gynecology 8 (2018) 1-5.

18. Moore JH, Andrews PC. Epistasis analysis using multifactor dimensionality reduction. Methods in Molecular Biology 1253 (2015) 301-314.
19. Malagutti N, Stomeo F, Pelucchi S, Ronchin R, Ceccon M, Malacrida G, Ciorba A, Pastore A, Borin M, Rizzo R. Analysis of Il-10 gene sequence in patients with sinonasal polyposis. International Journal of Immunopathology and Pharmacology 28 (2015) 434-439.
20. Zhang ML, Ni PH, Cai CP, Chen NJ, Wang SL. Association of susceptibility to chronic rhinosinusitis with genetic polymorphisms of IL-4 and IL-10. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 47 (2012) 212-217.
21. Fidancı ID, Zülfikar B, Kavaklı K, Ar MC, Kılınç Y, Başlar Z, Cağlayan SH A polymorphism in the IL-5 gene is associated with inhibitor development in severe hemophilia A patients. Turkish Journal of Hematology 31 (2014) 17–24.
22. Nursal AF, Pehlivan M, Sahin HH, Pehlivan S. The Associations of IL-6, IFN-γ, TNF-α, IL-10, and TGF-β1 Functional Variants with Acute Myeloid Leukemia in Turkish Patients. Genetic Testing and Molecular Biomarkers 20 (2016) 544-551.
23. DiStefano JK, Kingsley CB. Identification of Disease Susceptibility Alleles in the Next Generation Sequencing Era. Methods in Molecular Biology 1706 (2018) 3-16.
24. Reynard MP, Turner D, Navarrete CV. Allele frequencies of polymorphisms of the tumour necrosis factor-alpha, interleukin-10, interferon-gamma and interleukin-2 genes in a North European Caucasoid group from the UK. European Journal of Immunogenetics 27 (2000) 241-249.
25. Kalish RB, Vardhana S, Gupta M, Perni SC, Witkin SS. Interleukin-4 and -10 gene polymorphisms and spontaneous preterm birth in multifetal gestations. American Journal of Obstetrics and Gynecology 190 (2004) 702-706.