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Original Article

Evaluating the Distribution of (+ 2044G / A, R130Q) Rs20541 and (-1112 C / T) Rs1800925 Polymorphism in IL-13 Gene: An Association-Based Study with Asthma in Ahvaz, Iran

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Background and Aims: Asthma is a complicated chronic inflammatory disease associated with pulmonary inflammation, severe immune responses of the respiratory tract, and change or destruction in the respiratory tract structure. Several factors, especially genetic and environmental factors, contribute to the pathogenesis of asthma. Interlukin (IL)-13 is considered the most important inflammatory mediator of asthma that play an effective role in the different stages of B cell maturation and differentiation. It also increases the expression of major histocompatibility complex II and CD23 and is effective in IgE isotypes switching. Single nucleotide polymorphisms (SNPs) cause the wide genetic diversity in the genome, and SNP analysis helps evaluate and diagnose disease-related genes. The present study was conducted to investigate the association of -1112C / T and + 2044 G /A polymorphisms in the IL-13 gene with asthma.

Material and Methods: Blood samples (5 ml) from asthma patients (167) and controls (172) after spirometry test were collected into tubes, and then DNA was extracted to determine the genotype of asthma patients. Real-time polymerase chain reaction was performed by the Taq Man method, and the results were analyzed using SPSS software version 22.

Results: There was a significant difference between the genotype of IL-13 -1112C / T polymorphism in the patient group and the control group ($p = 0.028$). The IL13 + 2044G / A polymorphism results showed no significant difference between the two groups ($p = 0.319$).

Conclusions: The present study showed that IL-13 -1112C / T polymorphism positively correlates with the induction of asthma, but there was no significant association between the polymorphism + 2044G / A IL-13 and the risk of asthma.

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Introduction

Asthma is a chronic inflammatory disorder of the airways system, and upon the Global Initiative for asthma (GINA) guidelines, uncontrolled cells and their mediators have the main role in inflammation, hyperresponsiveness, wheezing and breathlessness, coughing, chest tightness, and lung obstruction [1, 2]. It has also shown that the interaction of genetics and environmental factors led to ashma and some aspects of asthma are strongly followed by a hereditary pattern but not Mandli's pattern [3-5]. In asthma patients, due to the dis-regulation of immune responses, especially the imbalance of inflammatory/anti-inflammatory cytokines, clinical signs of asthma are induced and developed [6, 7].

Cytokines are small glycoproteins that interact with specific receptors and stimulate or regulate the immune responses. The existence of polymorphisms in promoter region or encoding region of the cytokine or cytokine receptor genes could change their structure and function. Therefore, any polymorphism in these regions positively or negatively affects disease outcomes [8]. Interlukin (IL)-13 and IL-4 are the greatest Thelper (Th)2- secreted cytokine and have determinative roles in asthma disease pathogenesis [9]. IL-13 acts by affecting maturation and differentiation, increasing the expression of major histocompatibility complex II (MHCII) and CD23, and IgM to IgE isotype switching of the B-lymphocytes [10]. This cytokine could modulate macrophage activity and inhibit the production of pro-inflammatory cytokines/ chemokines such as IL-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α. Also, IL-13 leads to an increase in the differentiation of goblet cells, activation of fibroblasts, increased mucosal secretion, and hyperresponsiveness in bronchial tubes [11].

Several single nucleotide polymorphisms (SNPs) in association with allergic diseases such as asthma have been reported in the IL-13 gene. Therefore, the IL-13 gene can be considered a candidate for the development, progression, or clinical signs of asthma [12]. Currently, two SNP with biological function has been identified in the IL13 gene. One of SNP is in the promoter of the gene in the (-112C / T) region and seems to play a role in regulating the gene expression. The second SNP is located in the fourth exon of the IL-13 gene and causes the conversion of arginine to glutamine amino acid in codon 130, which is likely to play a role in the affinity of receptor binding [13]. We have investigated (+2044G/A, R130Q) rs20541 and (-1112 C/ T) rs1800925 polymorphisms in the IL-13 gene in this projectability of their SNPs on asthma.

Materials and Methods

Patients and healthy controls

Three hundred thirty-nine people enrolled in this study and divided into two groups. One hundred sixty-seven diagnosed asthmatic patients (84 women and 83 men), according to the GINA criteria in 2011, and 172 controls (161 women and 11 men). All patients enrolled in this study lived in Southwest of Iran (same race, same mean age) and were recruited from educational hospitals of Jundishapur University of Medical Sciences (Ahvaz, Iran). Upon symptoms and

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diagnostic criteria of GINA, asthma patients selected by specialist physicians and healthy people without any history or signs of asthma and other respiratory and allergic diseases were considered the control group. Informed consent was obtained from the patients and healthy controls. The Ethical Committee of the Jundishapur University of Medical Sciences approved this study (IR.AJUMS.REC.1392. 308).

Spirometry test

Circuit-close and circuit-open spirometry method was performed for all precipitations. The spirometry report sheet was adjusted according to the date and time of the spirometry, reason of test, family asthma history, smoking history, and room temperature, age, height, and sex. Besides, we considered as exclusion criteria: the usage of any allergy drugs and any history of autoimmune diseases also IgE titer antibodies in the patient group as well as no history of any allergies, asthma, IgE deficiency, or autoimmune diseases in the control group.

Sampling and DNA extraction

Blood samples (5 ml) from each patient and healthy control were collected into ethylenediamine tetraacetic acid (EDTA) tubes. DNA was extracted using a blood genomic DNA extraction mini kit (Yekta tajhiz azma, Iran) according to the manufacturer's instructions (DNA Yield: 4~8 µg/ 200-µl whole blood). Nanodrop (America, Thermo Scientific) confirmed the purity of extracted DNA, and the ratio of relative absorbance at 260:280 OD was measured; after that, extracted DNA was stored at -20˚C to perform the genotyping test.

Genotyping Taqman real-time polymerase chain reaction (PCR)

The genotype of DNA samples of asthma patients and healthy control subjects was done by Taqman real-time PCR genotyping method, through specific probes of C-1112T IL-13 and G +2044A IL-13 (TaqMan™ SNP Genotyping Assay, human kit, USA) (Table 1) and using step one real-time PCR device (ABI, USA). In a sterile tube, 1 µl of diluted DNA (10 µg), 5 µl of Taqman Genotyping Master Mix, 0.14 µl of SNP IL-13 Probes (separately), and 3.86 µl of RNase-free water was added and adjusted in 10 µl total volume. After that, tubes underwent cycling conditions: 95˚C for 30 s, followed by 35 cycles at 95˚C for 5 s, and annealing and extension at 60˚C for 30 s.

Statistical analysis

Statistical analysis of the allelic distribution and genotype of the two polymorphisms in the patient and control groups was performed using the Chi-square test and SPSS-22 software. A P-value of less than 0.05 was considered significant.

Results

The information of sex and gender of the patient and healthy control groups and the information of forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio of the patient group were presented in Table 2. The results of determining the genotype of each polymorphism for wild homozygous, heterozygote, and mutants homozygote and

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allelic distribution were measured in patient and control groups using Chi-square test, and the risk ratio for allele distribution was calculated, and $p < 0.05$ were considered to be significant. The results of allelic and genotypic frequency for rs1800925 and rs20541 polymorphisms are presented in Table 3. In the present study, the frequency of wild homozygote genotypes for rs1800925 and rs20541 was 53.2% and 61% in the patient group and 66.2% and 53.4% control group, respectively. The frequency of mutant homozygous genotype for rs1800925 in the patient group was 10.7% and in the control group was 5.2%, and the frequency distribution of mutant homozygote genotype for rs20541 was observed 7.78% and 11% in the patient and control groups, respectively. In the patient group, the heterozygote genotype frequency of rs1800925 was 35.9% and 35.1% for rs20541, respectively, and in the healthy control group, the heterozygote genotype frequency of rs1800925 and rs20541 was 28.4% and 35.5%, respectively. Our results showed that rs1800925 polymorphism was associated with a significant increase of AA mutant homozygote genotype in the patient group compared to control (p=0.005), but is not significant for rs20541 polymorphism, and the frequency of mutant genotype in the healthy group was more than the patient group (p= -0.108). The genotypic distribution results in both males and females in the patients' group are shown in Table 4. The distribution of genotypes in both sexes was approximately the same, and no difference was observed (p= -0.608) (Table 4).

Table 1. The Information of TaqMan probes for IL-13 -1112C/T and IL-13 +2044G/A polymorphisms

SNP ID	**rs1800925**
Gene name	Interleukin 13
Chromosome location	Chr.5: 131992809
Polymorphism	C/T, transition substitution
Context sequence [VIC/FAM]	
GGTTTCTGGAGGACTTCTAGGAAAA[C/T]GAGGGAAGAGCAGGAAAAGGCGACA	
SNP ID	**rs20541**
Gene name	Interleukin 13
Chromosome location	Chr.5: 131995964
Polymorphism	A/G, transition substitution
Context sequence [VIC/FAM]	
TTAAAGAAACTTTTTCGCGAGGGAC[A/G]GTTCAACTGAAACTTCGAAAGCATC	

Table 2. The information of the precipitated patient and healthy control subjects in the study

		Case	**Control**
Sex/ Age	**Male/ Female**	83/84	11/161
	1-20	6/5	3/16
	21-40	50/47	6/82
	41-60	22/29	2/54
	61-80	5/3	0/9
FEV1 (Mean)		67.1%	
FVC (Mean)		72.3%	
FEV1/FVC (Mean)		74.7%	

FEV1= Forced expiratory volume in one second; FVC= Forced vital capacity

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Table 3. The results of allelic and genotypic frequency for IL-13 -1112C/T polymorphisms (A) and IL-13 +2044G/A polymorphisms (B)

(A) Genotype	Control		Asthma		p-value
IL-13 -1112C/T	N	%	N	%	
Homozygus CC	114	66.27	89	53.2	
Heterozygus CT	49	28.4	60	35.9	0.028
Homozygus TT	9	5.2	18	10.7	
Freq All C	277	80.5	238	71.25	0.005
Freq All T	67	19.5	96	28.7	
OR= 0.59 (0.419-0.8568)					
(B) Genotype	**Control**		**Asthma**		
+2044G/A IL-13	N	%	N	%	
Homozygus GG	92	53.4	102	61	
Heterozygus GA	61	35.5	52	31.1	0.319
Homozygus AA	19	11	13	7.78	
Freq all G	245	71.2	256	76.6	0.108
Freq all A	99	28.7	78	23.3	
OR= 1.32 (0.9396-1.8718)					

Table 4. The Comparison of allele frequency of IL-13 -1112C / T and IL-13 + 2044 G / A polymorphisms with patients' gender

Single nucleotide polymorphisms	Allele	Female N (%)	Male	p-value
	CC	43 (27.7)	46 (27.5)	
-1112C/T IL-13	CT	33(19.8)	27 (16.2)	0.632
	TT	8 (4.8)	10(6)	
	GG	51 (30.5)	51 (30.5)	
+2044G/A IL-13	GA	28 (16.8)	24 (14.4)	0.608
	AA	5 (3)	8 (4.8)	

Discussion

IL-13 as a Th2- secreted cytokine is one of the main cytokines in asthma and is responsible for asthma's physiological and pathological events, including airway hyperresponsiveness, and airway remodeling. Besides, IL-13 can facilitate the differentiation and survival of eosinophils and mast cells, enhance IgM to IgG isotype switching and increase mucus secretion [9, 14]. Studies have shown that the IL-13-immunological pathway can be considered a therapeutic target in asthma, and also the administration of Lebrikizumab, IL-13 monoclonal antibody, in asthma-resistant individuals could improve and relieve asthma symptoms and increased the FEV1 [15].

IL-13 has been widely studied for its role in asthma, and some genetic studies point to the potential role of this cytokine in asthma and the effect of multiple polymorphisms of this gene regarding asthma in humans [16-18]. The most important IL-13 polymorphisms are IL-13-1112C/T, in the promoter gene region, and IL-13+2044 G/A, in the fourth exon of the IL-13 gene, which have been studied in several exercises and were associated with inconsistent results [19].

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IL-13-1112C/T polymorphism could increase the expression of IL-13 in Th2 cells and increase the secretion of this cytokine through stimulated mononuclear cells by mitogens [20]. On the other hand, IL-13 + 2044A / G polymorphism probably affected IL13 function, and studies have shown that the AA genotype of this polymorphism leads to a decrease of IL-13 affinity to binding IL-13Rα2 [21]. Therefore, the role of these polymorphisms is acceptable in increasing asthma sensitivity. The current study evaluated the comparison of frequency and distribution of genotyping of IL-13 -1112C / T and IL-13+2044G/A polymorphisms with asthma. Based on the results, IL-13 -1112C / T polymorphism can be considered a risk factor for asthma, but the present study's results showed no association between IL-13+2044G/A polymorphism and asthma.

Previous studies have shown the relationship between genetic variation in the IL-13 promoter region (IL-13 -1112C / T), RANTES genes, and leukotriene C4S with asthma and atopy on African Americans, indicating that this SNP can be used as a prognostic marker of asthma and atopy [22]. The current study also confirmed that IL-13 -1112C / T polymorphism is a risk factor for asthma. On the contrary, the other study evaluates the association of children's asthma, IgE level, and eosinophil with IL-13 polymorphisms in white Costa Rican children. It showed that the T allele of -1112 polymorphism only contributed to asthma's progress in corticosteroids consuming asthma children and did not affect asthma risk. Regarding IL-13+2044G/A polymorphisms, the A allele of this polymorphism was associated with an increase of IgE level and eosinophil, but no relation with asthma [23] showed that this polymorphism had no significant association with the disease in this study. Studies on IL-4 gene promoter (-590C > T) and IL-16 gene promoter (-295T > C) polymorphisms showed a significant correlation between IL-13 (R130Q) coding region polymorphisms and IL-4 gene promoter (-590C > T) with asthma disease in Tehran [24]. A cohort study on 2918 individuals to evaluate the relationship between genetic diversity of IL-13 and asthma, allergies and hay fever revealed a significant correlation between IL-13 -1112C / T polymorphism and IL-13+2044G/A, Q130R polymorphism with asthma and allergy. Results showed +2044G/A, Q130R polymorphism interfered with -1112C/T polymorphism function, and -1112C/T polymorphism did not have an independent role concerning asthma [25]. In our study, we did not find a significant association between +2044G/A and asthma. Besides, a comprehensive meta-analysis study revealed a meaningful relationship between +2044G/A and -1112C / T polymorphisms with the risk of Asthma [26]. The current project results showed the relationship between -1112C / T polymorphism and asthma, but we did not find any significant statistical difference between patient and control groups regarding genotype and allelic distribution of +2044G/A polymorphism with asthma, although the mentioned studies have reported this relationship. In other hand, mentioned markers can be just a sign and they are not the only direct cause of asthma in the population, therefore these outcomes also could be due to differences in the genetics of the

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Iranian population. Our suggestion to achieve better results is investigating other polymorphisms of IL13 cytokine, the measure of IL13 serum levels along with other polymorphisms and increase the sample size.

Conclusion

We showed that a significant IL-13 -1112C / T polymorphism in the ashma positively correlates with the induction of asthma while the IL13 + 2044G / A polymorphism results showed no significant difference between the ashma and control groups. Therefore, evaluation of these polymorphisms potentially can use to predict or control of disease sign.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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