Effect of Polymorphism in the Interleukin-13 Gene on Serum IL-13 Levels, and Its Association with Asthma Disease

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Abstract
Asthma is a chronic respiratory disorder characterized by a heterogeneous and multifactorial background. Asthma is a complex genetic disorder with a strong environmental impact, and symptoms clinically associated with asthma include wheezing, coughing, chest tightness and shortness of breath. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for the detection of Interleukin-13 (*IL-13*) gene polymorphism -1112 C>T (rs1800925). The genotype distribution results of the *IL-13* gene showed a significant increase (p<0.05) in the CT genotype and T allele of the -1112 C>T single nucleotide polymorphism (SPN) in asthma patients than in controls, and they were significantly more likely to have the mutant allele than controls (OR=34.211 95%CI= 4. 576 -255.771, p=0.01). The results showed that the levels of the IL-13 recorded a significant increase (P<0.05) in patients. The study showed that the *IL-13* heterozygote genotype (CT) and the homozygote TT significantly had higher IL-13 levels compared to the IL-13 CC genotype in patients and controls.These results imply an association between the polymorphism of the *IL-13* -1112 C>T and IL-13 cytokine levels in the Iraqi asthma patient.
Keywords: Asthma, Interleukin-13, IL-13gene polymorphism, ARMS –PCR.

1. Introduction

Asthma is a very common chronic airway disease leading to shortness of breath, chest tightness, and cough. Symptoms fluctuate over time and can worsen and lead to respiratory failure during periods of exacerbation, which are often precipitated by viral upper respiratory tract infections or less commonly by exposure to aeroallergens or air pollution. Asthma has long been considered a prototypical T helper 2 (Th2)-cell-mediated disease (1).

The interleukin-13 (IL-13) gene, located on human chromosome 5q31-33, is produced by innate lymphoid cells and T-helper type 2 (Th2) cells during allergic inflammation, containing four exons and three introns and encoding an unglycosylated protein composed of 132 amino acids. The most prominent effects of IL-13 include the promotion of differentiation and survival of eosinophils and mast cells, activation of fibroblasts, the elevation of bronchial hyperresponsiveness, and switching of B-cell antibody production from IgM to IgE (2). The IL-13 gene can be considered a candidate for the development, progression, or clinical signs of asthma (3). One SNP is the promoter of the gene in the (-1112C >T) (rs1800925) region and seems to play a role in regulating the gene expression (4).

The current study aimed to determine the frequencies of (-1112C >T) (rs1800925) SNP in the IL-13gene in asthmatic patients by ARMS-PCR technique and to investigate whether there is an influence of each SNP on variations in serum protein production level of IL-13 in asthmatic patients according to genotypic and allelic variation.

2. Materials and Methods

This study had carried out in the laboratory of molecular biology in the Department of Biology / Faculty of Science –University of Kufa, and in Al-Hakim Hospital/ Chest and Respiratory Diseases Center and Al-Sadr Teaching Hospital/ Allergy and Asthma Center and in Al-Najaf governorate -Iraq during the period from April 2021 through May 2022.

Sampling of Cases

The study population included 70 Iraqi Asthma patients and 20 apparently healthy individuals. Tests had performed on 3 ml of venous blood, which had been collected from asthma patients and the control group. 2 ml had collected in gel tubes centrifuged at 5000 rpm for 5 minutes; the obtained serum had used to detect the concentrations of IL-13. 1 ml had collected in tubes with anticoagulant EDTA and used for PCR test.

Measurement of IL-13 Serum Level

The levels of IL-13 in the serum were measured by enzyme-linked immunosorbent assay (ELISA) kit (SunLong Biotech, China).

Determination of the *IL-13* -1112 C>T gene polymorphism

Genomic DNA was isolated from blood using protocol from Genomic DNA Kit (Geneaid Biotech Ltd., Taiwan) designed specifically for purifying DNA

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from blood. The concentration and purity of the DNA were measured by the NanoDrop spectrophotometer (THERMO.USA). Amplification of *IL-13* gene (for fragment which contained the SNP location) was done by using a conventional PCR thermocycler (BIO-RAD/USA). The Amplification refractory mutation system polymerase chain reaction (ARMS-PCR) technique was performed for the detection of the *IL-13* gene polymorphism (-1112C>T). The amplification steps (Table 1) consisted of a first denaturation step where DNA was initially denatured at 95 ◦C for 3min., and then 15 cycles had performed as follows: The first Denaturation 94°C for 30 sec, first annealing 63°C for 60 sec, first extension 72◦C for 60 sec. Then, 20 cycles had performed as follows: The second denaturation 94°C for 30 sec, the second annealing 60°C for 60 sec, 72°C for 60 sec, and The PCR amplification had completed by a final extension 72◦C for 7 min. Amplification of the *IL-13* genes resulted in 396bp products

Table 1: Reaction conditions for IL-13 gene.

Gene	Initial Denaturation	Numbers of Cycles	The compositions of each cycle			Extension Step
			Denaturation	Annealing	Extension	
IL-13 gene -1112 C>T rs1800925 Polymorphism	94°C for 2 min.	15	94°C for 30 sec.	63°C for 60 sec.	72°C for 60 sec.	72°C for 5 min
		20	94°C for 30 sec.	60°C for 60 sec.	72°C for 60 sec.	

The primer pairs listed in table 2 were synthesized by Macrogen/China. Electrophoresis was used to separate the PCR products with a ladder marker (Promega, USA). The product was loaded onto a 2% agarose stained with a DSRed Nucleic Acid Stain and run at 70 volts for an hour and a half. The DNA bands were photographed using a photo documentation system after being visualized by a UV transilluminator.

Table 2: Primers sequences for *IL-13* polymorphism.

Gene	Primers sequences		PCR product bp	Ref.
IL-13 -1112C>T (rs1800925)	Forward 1 (C allele)	5′ -TTCTGGAGGACTTCTAGGAAAAC-3′	396	Gleń et al. (5) Choto et al. (6)
	Forward 2 (T allele)	5′ -TTCTGGAGGACTTCTAGGAAAAT-3′		
	Reverse	5′-GGAGATGGGGTCTCACTATG-3		

Statistical analysis

Statistical analyses of all results were done by the Statistical Package for the Social Sciences (SPSS-version 23) software statistical package by using Chi-square test (with a P-value at a level of significance less than 0.05). Result values were expressed as mean± SE and number of patients or percentages.

3. Results and Discussion

The *IL-13* gene polymorphism -1112C >T (rs1800925)

Among the 20 healthy subjects; 20 (100%) had found as homozygous CC alleles, no healthy subject was found as heterozygous genotype (with the C and T alleles (CT), and no healthy subject had found as homozygous genotype TT alleles; (CC: n=20, 100%; CT: n=0; TT: n=0). Asthma patients: Among the 70 patients; 6(8.57%) had found as homozygous CC alleles, 63(90%) were found as a heterozygous genotype with the C and T alleles (CT), and 1(1.42%) had found as homozygous genotype TT alleles; (CC: n=6, 8.57%; CT: n=63, 90%; TT: n=1, 1.42%) (Table 3 & figure1)
gene in patients and controls.

Table 3: The results of genotypic frequencies polymorphism -1112 C>T the *IL-13*

Genotypes	Healthy controls (n=20)	Asthma patients (n=70)
CC	20 (100%)	6 (8.57%)
CT	0	63(90%)
TT	0	1 (1.42%)
p-value	0.001*	
Alleles frequency	n (%)	n (%)
C allele	40 (100%)	75 (53.57%)
T allele	0 (0%)	65 (46.42 %)
X2 p-value OR (95%CI)	26.197 0.001* 34.211 (4. 576 -255.771)	
Data were expressed as number and a percentage (n%). *P <0.05 significant. Abbreviations: X2= chi-square, OR= odds ratio, CI= confidence interval.		

That means the frequencies of -1112 C>T (rs1800925) SNP in the IL-13 gene in the 70 Iraqi Asthma patients in Al-Najaf province were significant differences from that of the 20 healthy controls group (p<0.05). The results showed an increase in genotype CT of the -1112 C>T in asthma patients than controls, and they were significantly more likely than controls to have the mutant allele (T allele) (OR=34.211 95%CI= 4. 576 -255.771, p=0.01). This indicates a possible role for the CT genotype in asthma disease.

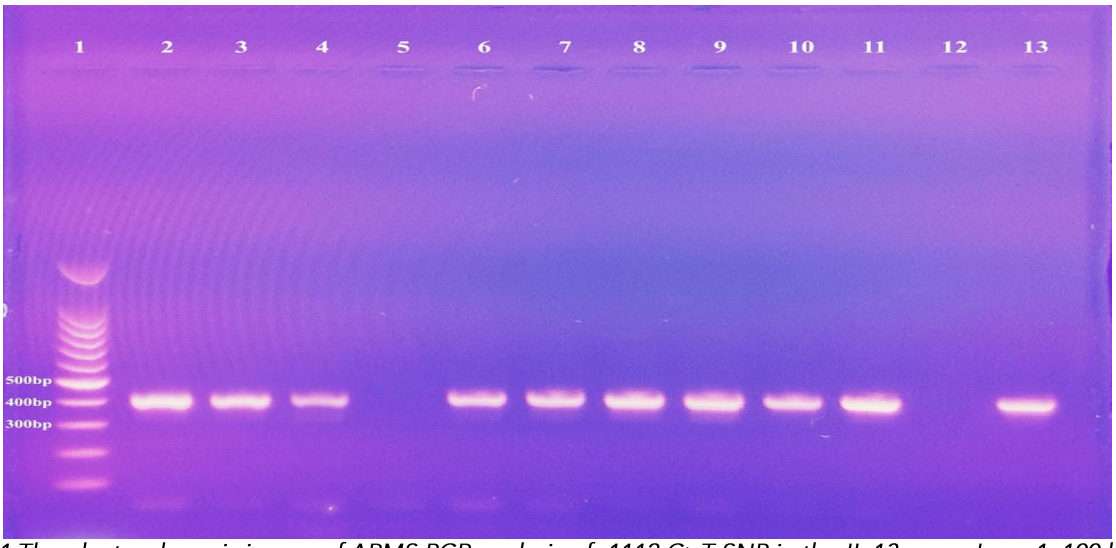

Figure 1:The electrophoresis image of ARMS-PCR analysis of -1112 C>T SNP in the IL-13 gene. Lane 1: 100 bp DNA Ladder. Lane 4&5 (sample 2): homozygous genotype, CC (396 bp in the first lane of the sample); Lane 2&3(sample 1); Lane 6&7(sample 3), Lane 8&9(sample 4); Lane 10&11(sample 5): heterozygous genotype, CT (396 bp in both lanes of each sample). Lane 12&13 (sample 6): homozygous genotype, TT (396 bp in the second lane of the sample).

Moreover, these results highlight the potential role of -1112C>T SNP in the IL-13 gene promoter regions, and then the IL-13 protein as the promoter region of the IL-13 gene contains an -1112 C>T SNP that is understood to interact with nuclear transcription factors and regulate IL-13 expression (7).

The IL-13 is a Th2-derived pro-inflammatory+ cytokine known to induce apoptosis in many cell types (8). The IL-13 gene has many SNPs, including -1112C>T which is mainly associated with allergy and atopic diseases, as well as effector functions that induce airway hyper-responsiveness, allergic inflammation, and IgE production (9).

Based on the key roles of IL-13 in the IgE pathway, Bottema et al., have focused on the contribution of IL-13 polymorphisms -1112C>T to the risk of allergic rhinitis and asthma (10).

Cui et al. investigated IL-13 polymorphisms in rhinitis and asthma populations; their results showed that IL-13 rs1800925 was significantly associated with rhinitis and asthma (11).

In agreement with the present study, the results of a study done by Gleń et al. reviled that in the group of patients with atopic dermatitis; the CT genotype was most prevalent at 81.1%, while TT and CC genotypes were found in 7.8% and 11.1% of patients, respectively. In the control group of healthy subjects, the CT (56.9%) and CC (40.1%) genotypes were most frequent; and the TT genotype was found only in 3% of subjects (P=0.00001) (5).

These results agreed with Alsaid et al. study which found that the polymorphism at -1112 of IL-13 showed a highly significant increase in the frequency of CT genotype in diabetic patients compared to controls (76.3% vs 51.5%, p<0.001). The frequency of the IL-13 promoter -1112 C>T polymorphism (genotype CC) was significantly lower in diabetic patients compared with control (20.7% vs. 31.7%, p=0.04) (12).

These results were consistent with Choto et al. study about the effect of the IL-13 -1112C>T polymorphism in 50 schistosomiasis-infected participants. They recorded that the frequencies of the genotypes CC, CT, and TT, were 20%, 58%, and 22%, respectively (6).

This study is in accordance with Deng et al. study, which recorded that IL-13 -1112 C>T polymorphism had a possible role in hepatocellular carcinoma disease. The frequencies of the genotypes CC, CT, and TT in their study were 67.9%, 29.7%, and 2.4%, respectively in infected participants, while CC, CT, and TT were 66.6 %, 31.8 %, and 1.6 %, respectively in controls (13).

The Serum Levels of Interleukin-13 cytokine.

The result showed that the levels of the IL-13 recorded a significant increase (p<0.01) in the patients (13.05±0.44 pg/ml) compared with the healthy group (8.50 ± 0.48 pg/ml) (Table 4).

Table 4: The serum level of IL-13cytokine in Patients and Controls.

Groups Parameters	Patient (n=70)	Controls (n=20)	p-value
Interleukin-13 cytokine(pg/ml)	13.05±0.44	8.50 ± 0.48	0.0001*

Results values had expressed as mean± SE, *: p< 0.05 or significant differences between values of parameters.

The result of this study agreed with Jebur and Saud the results revealed that Interleukin-13 (IL-13) level was significantly increased (p<0.001) in Allergic Asthma patients in comparison to healthy individuals (14).

The result of this study also agreed with Fuschiotti et al. this study showed high levels of interleukin-13 (IL-13) in Systemic Sclerosis patients as compared with normal controls or with patients with rheumatoid arthritis (15). IL-13 is an immunoregulatory cytokine that is predominantly secreted by activated Th2 cells. It is known to play a prominent role in mediating

tissue fibrosis and participating in the pathogenesis of fibrosis in many diseases (16).

IL-13 is a pleiotropic type 2 cytokine that has been considered to be essential in the pathogenesis of allergic asthma and another eosinophilic disease (17).

The result of this study in agreement with Nabavi et al study results, which revealed that the serum level of IL-13 in the patients with chronic rhinosinusitis with nasal polyps (CRSwNP) was significantly higher than the controls (0.98 ± 1.56 vs. 0.34 ± 0.16 pg/ml, respectively, p = 0.002) (18).

Interleukin-13 is believed to play a central role in orchestrating airway inflammation more clearly in asthma (19). Interleukin-13 is implicated in the production of IgE that binds to mast cells, basophils, and inflammatory cells in exposed sites to aeroallergens like upper airways (20).

The result of this study agreed with Martínez-Reyes et al. the results revealed that Insulin-resistant patients showed 2.5-fold higher serum levels of IL-13 than controls (P < 0 0001) (21).

Association between Genotypes of *IL-13* and the serum level of IL-13 cytokine.

In the current study, IL-13 serum level was the highest level in asthma patients with CC variants (11.68±1.34pg/ml) compared to the homozygous CC variant of controls (8.50±0.48 pg/ml). Additionally, a combination of the heterozygote CT and variants of homozygote TT significantly had higher IL-13 cytokine levels in patients (13.09±0.71 pg/ml&18.41±0.0 pg/ml, respectively) compared to the IL-13 CC variant (11.68±1.34pg/ml) in patients. These results imply an association between the polymorphism of the *IL-13* 1112 C>T and IL-13 cytokine levels (Table 5).

Table 5: The results of IL-13 levels according to the genotype of IL-13 1112 C>T in asthma patients and controls

Genotype	IL13 level (pg/ml)		*p*-values
	Healthy controls (n=20)	Asthma patients (n=70)	
CC	8.50±0.48	11.68±1.34	0.010**
CT	0	13.09 ±0.71	
TT	0	18.41 ±0.0	
Results values were expressed as mean± SE, *: p< 0.05 or significant differences between mean values.			

Single nucleotide polymorphisms substitution of the nucleotide cytosine (allele C) to thymine (allele T) at the IL-13 rs1800925 (-1112C>T) site in the promoter region, results in the binding of nuclear proteins. This causes overproduction of IL-13 cytokine in Th2 lymphocytes that may play a role in allergic and chronic inflammatory diseases (22).

Howard et al., found that the IL-13 -1112 T allele was associated with asthma they observed a borderline association between four IL13 SNPs, including the IL-13-1112 C>T variant, and asthma severity (23).

Cytokine gene polymorphisms such as single nucleotide polymorphisms located in the promoter region of encoding genes may modify gene transcription and cytokine production in autoimmune diseases (24).

The IL-13 -1112 T allele resulted in increased IL-13 transcription and was associated with allergic phenotypes in multiple studies (25)

The associations between the IL-13 -1112 T allele and increased plasma concentrations of IL-13 can influence many allergic phenotypes, such as high IgE serum levels, bronchial hyperresponsiveness, and positive skin tests (23). And susceptibility to the development of many allergic diseases like asthma, Atopic Dermatitis, and allergic rhinitis (26).

Through several mechanisms, this increase in IL-13 plays a critical role in many immunoregulatory pathways. The IL-13 is well known as a Th2 anti-inflammatory cytokine that is involved in mediating B cell and mast cell proliferation and correlates with IgE synthesis, which is a major regulator in Th2-mediated disease (27).

In agreement with the present study, the results of a study done by Gleń et al. in Atopic Dermatitis reviled that patients with TT and CT genotypes had an increased serum IL-13 level, contrary to the CC genotype, both in the control and Atopic Dermatitis groups; however, these differences were not statistically significant (5).

Choto et al. evaluated the frequency of the IL-13 -1112 C>T SNP among schistosomiasis-infected individuals and assessed the association of the variants on IL-13 cytokine levels. They found significantly (p<0.05) higher IL-13 cytokine levels among infected participants with the CC and CT genotypes (6).

4. Conclusion

Based on the results and findings, the study has drawn that the frequencies of -1112 C>T SNP in the IL-13 gene in asthma patients were with significant differences with controls (p<0.05). The results showed an increased in CT genotype and T allele which may represent a risk factor in asthma patients. The IL-13 gene polymorphism (-1112 C>T) had direct effect on increased expression and secretion of IL-13 in serum of asthmatic patients. Patients with TT and CT genotypes had an increased serum IL-13 level, contrary to the CC genotype

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