Investigate the relation between Polymorphism of *IL-13* gene and Asthma at Thi-Qar province/ IRAQ

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Abstract:

The current study was conducted at the Al-Hussein Teaching Hospital in Thi- Qar province, during the period from October 2014 to May 2015. The study aimed to investigate polymorphism of IL-13 gene in patients with Asthma. The study included a total of 100 patients with asthma (32 males) and (68 females) and there aged between 17-62 years, and 50 individual healthycontrol. DNA was isolated and PCR was performed using primers specific for the *IL-13* gene, the results showed the presence of mutations in a sample of 48 out of 100 patients with asthma at site 1112C/T after using restriction enzyme BstUI. The results of the statistical analysis showed correlation between the occurrence of the disease and the emergence of mutation (C/T) in the promoter region of IL-13 gene when compared with the healthy control in population of Thi-qar province.

Key words: *IL-13*,polymorphism, Asthma,Thi-Qar.

الخلاصة:

اجريت الدراسة الحالية في مستشفى الحسين التعليمي في محافظة ذي قار وللاعوام ٢٠١٤ و٢٠١٥ . هدفت الدراسة للتحقق من تعدد الشكل الورثي للجين *IL-13* لدى المرضى المصابين بالربو. شملت الدراسة ١٠٠ مريض بواقع ٣٢ ذكور و ٦٨ اناث وباعمار تتراوح بين ١٧ و٦٢ سنة. و ٥٠ شخص كعينة اصحاء . تم اسخلاص الدنا وعمل تفاعل انزيم البلمرة المتعدد باستعمال بادئات متخصصه لجين الدراسة. اظهرت النتائج وجود طفرة ٤٨ عينه من اصل ١٠٠ عينة للمرضى بالربو في الموقع 1112C/T باستعمال انزيم القطع BstUI . نتائج التحليل الاحصائي اظهرت وجود علاقة بين وجود الطفرة C/T في الجين والمرض مقارنة بمجموعة السيطره بالنسبة لمجتمع محافظة ذي قار.

Introduction:

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. These episodes cause airflow obstruction, often reversible either spontaneously or with treatment [1] . it is a complex disease that is caused by a combination of genetic and environmental factors [2] .These factors influence its severity and its responsiveness to treatment. Asthma is a significant public health problem. Affecting approximately 300 million individuals worldwide [3] . Currently, the prevalence of allergic asthma is increasing globally due to air pollution and other environmental irritants. These environmental exposures are especially evident in developing countries. where industrialization is progressing rapidly [4] .

Chronic airway inflammatory processes result in intense recruitment of activated eosinophils and T-helper (Th2) lymphocytes at the site of injury and an inappropriate immune response to common allergens [5]. Recurrent inflammation and subsequent abnormalities in the tissue repair mechanisms lead to structural changes in the airway wall that manifest the clinically detectable features of epithelial injury, goblet cell hyperplasia, subepithelial thickening, airway hyperplasia and angiogenesis [6] . Thus, allergic asthma is characterized as a complex airway remodeling disease [7] . While there is currently no cure for asthma, the standard of care for asthma is limited to symptomatic control of disease mediators with potent inhaled corticosteroids (ICS), long-acting β-adrenergic agonists and leukotriene modifiers [8][9] .

***IL-13* gene**:

IL-13 is an immunoregulatory cytokine secreted predominantly by activated Th2 cells but also produced by a variety of cells, including, Th1 CD4+, CD8+ T cells, mast cells, Basophils and Eosinophils cells [10] . *IL-13* has been implicated in the pathogenesis of a variety of diseases characterized by inflammation and tissue remodeling, including asthma, idiopathic pulmonary fibrosis [11]. And chronic obstructive pulmonary disease (COPD) [12] . *IL-13* has many diverse functions on a wide variety of cell types and important in recruitment of inflammatory cells from the blood to the lung, immunoglobulin IgE production by B cells, airway hyper responsiveness, pulmonary fibrosis, and mucus hypersecretion[13].

The gene encoding *IL-13* is comprised of 4 exons and 3 introns and is located on chromosome 5q31, This chromosomal region also contains the genes encoding IL-3, IL-5, IL-9, and GM-CSF [14][15] . This region is rich in candidate genes involved in the IgE-mediated inflammatory response, in particular the genes encoding interleukin-4 and interleukin-13 [16][17] . Many studies report an association between single nucleotide polymorphisms (SNP) in *IL-13* and the effects on asthma in adults and in children, in the context of infections, atopy, IgE levels, or risk for asthma [18][19] . Studies utilizing multigene analysis have made similar associations of *IL-13* SNPs and asthma [20][21][22] . The transition of cytosine (allele C) to thymine (allele T) at the -1112 site in the promoter region, leads to a change in the binding rate of nuclear proteins to this region and to overproduction of IL-13 from Th2 lymphocytes, which may play a role in chronic inflammatory diseases and associated with genetic susceptibility to asthma in several populations [23][24] .

Material & Methods

DNA Extraction:

The method was used as Sambrook technique[25] in DNA extraction from blood samples.

Detecting Deoxyribonucleic acid (DNA):

Method is used, the electrical relay (Electrophoresis) according to the method of [25] using the gel (Agarose) for the detection of DNA was undiminished oxygen .

DNA template:

- Primers (forward & reverse) the kit provide by Idtdna company .

- Go Taq Green Master Mix, the kit provide by Bioneer company.

- Sterilized distilled water.

Table (1): Oligonucleotide primer sequences used for amplification of IL-13 gene.

Primers		Primer sequences
Primers *IL-13* Gene	F	5’GGAATCCAGCATGCCTTGTGAGG 3’
	R	5’GTCGCCTTTTCCTGCTCTTCCCGC 3’

Table (2): PCR condition for amplification of IL-13 gene.

No. of Steps	Steps	Temperature	Time	No. of Cycle
1	Denaturation 1	94 °C	5 min	1 Cycle
2	Denaturation 2	94 °C	50 s	35Cycles
3	Annealing	59°C	50 s	
4	Extension 1	72°C	30 s	
5	Final Extension 2	72°C	5 min	1 cycle

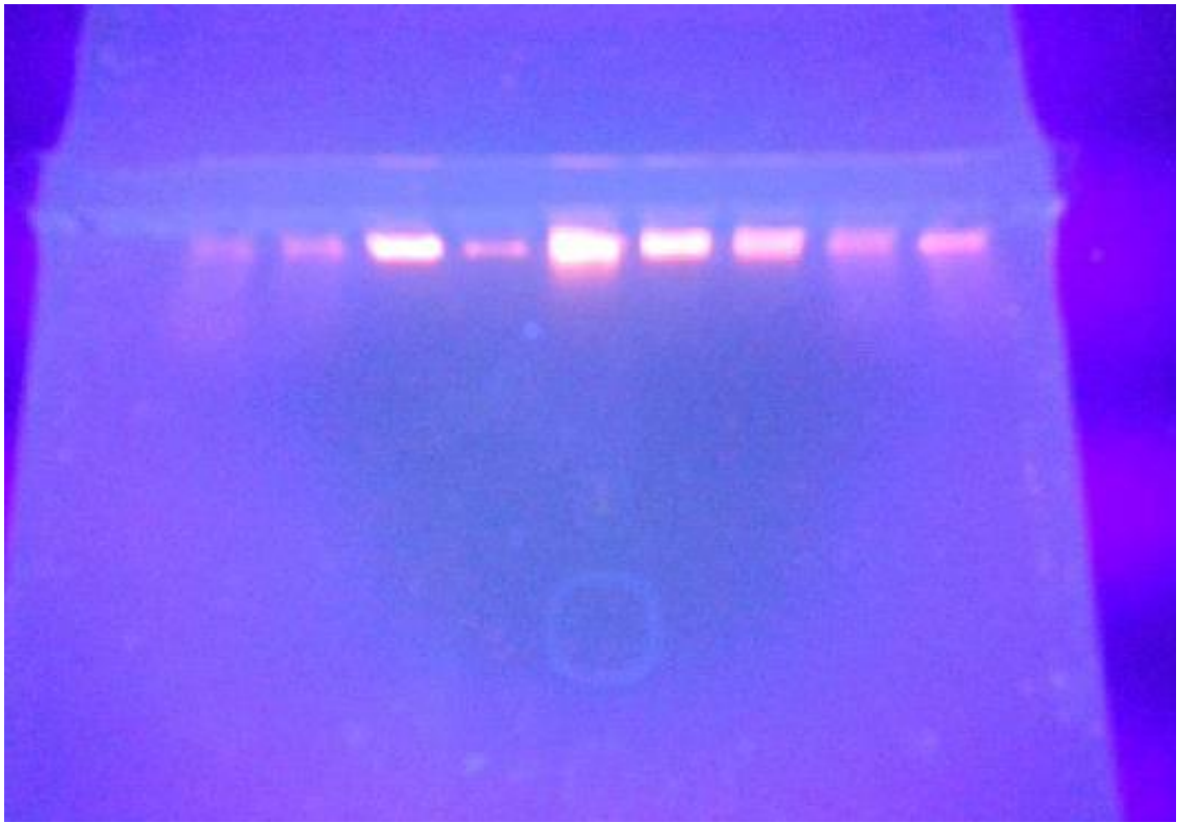
Restriction Fragment Length Polymorphism- Polymerase Chain Reaction

(RFLP - PCR):

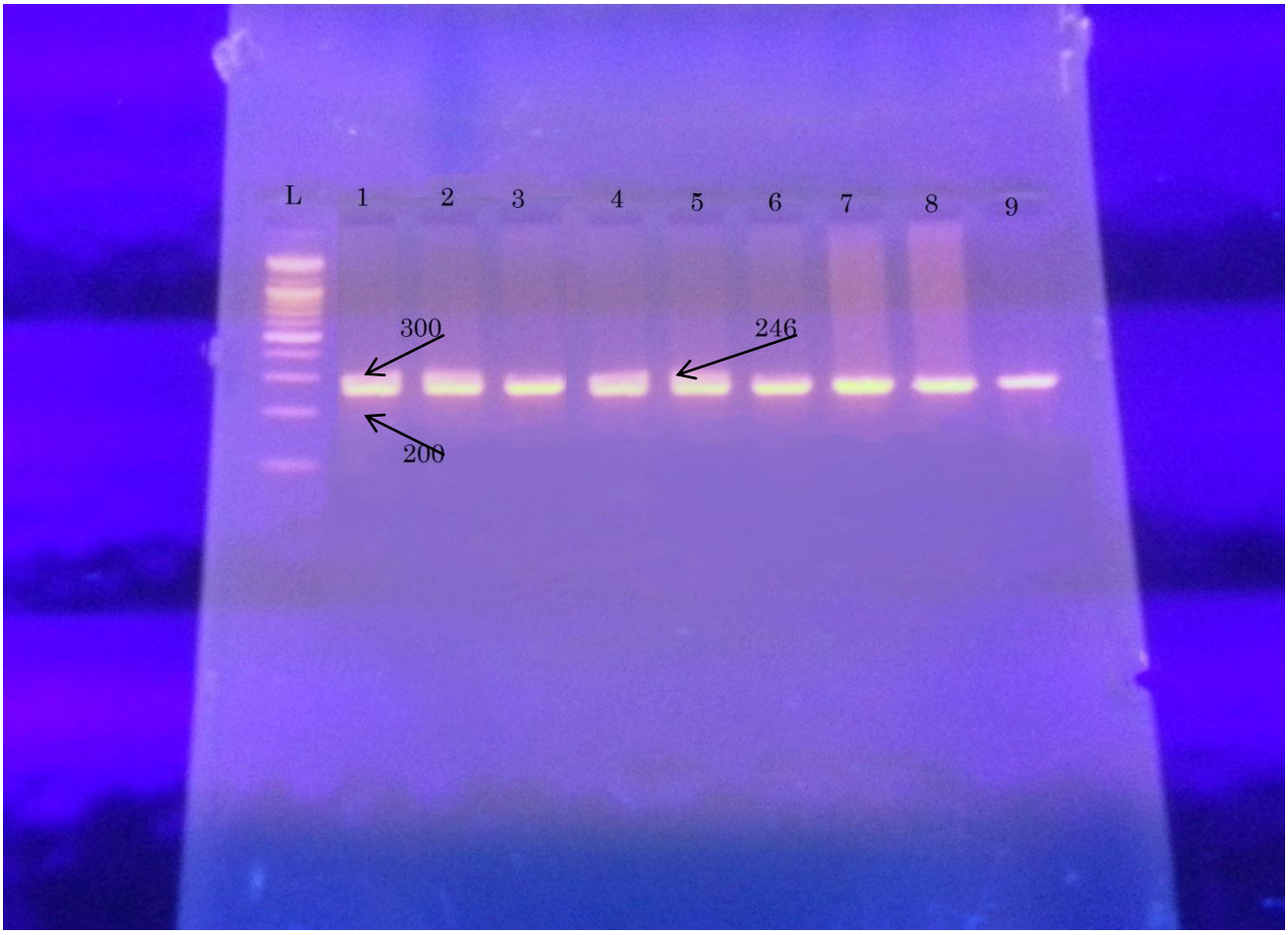
The primer pairs were included in the PCR for simultaneous amplification of fragments in the gene. The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after Ethidium bromide staining. The 246 bp product was digested for 6 h at 60°C with 5 U of the restriction enzyme BstUI. The fragment of the *IL-13* gene containing the C allel was digested into 223 and 23 bp fragments, and the fragment containing T allel remained intact. Digested products were electrophoresis on 3.0% agarose gel [26].

Results:

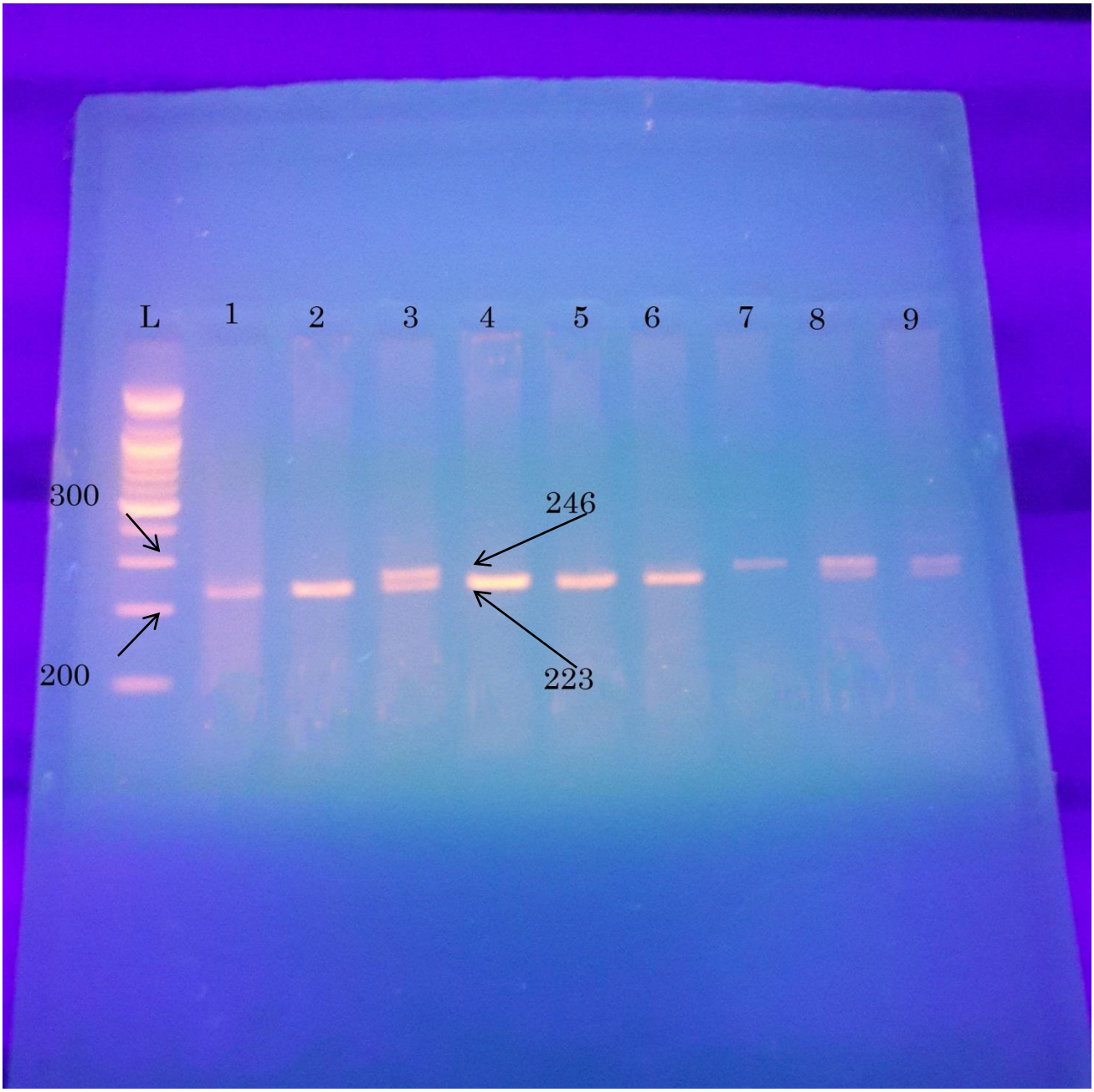
Lane	1	2	3	4	5	6	7	8	9



Figure(1):Agarose gel electrophoresis for Extract DNA products by electrophoresis on a 0.8 % agarose gel .



Figure(2):Agarose gel electrophoresis for amplified *IL-13* gene for asthmatic patients. Bands were fractionated by electrophoresis on a 2 % agarose gel (1 h., 80V/cm, 1X Tris-acetic buffer) and visualized under U.V. light after staining with ethidium bromide staining. (L:100 - 2000bp ladder); Lane : (1, 2,3,4,5,6,7,8,9) (product band).



Figure(3): Agarose gel electrophoresis for amplified *IL-13* gene of asthmatic patients. Bands were fractionated by electrophoresis on a 3% agarose gel (1 h., 80V/cm, 1X Tris-acetic buffer) and visualized under U.V. light after staining with ethidium bromide staining.(L :100 - 2000bp ladder); using restriction enzyme BstUI . Lane: 1, 2, 4, 5,6 (C Allele 223 bp fragment or wild homozygous C/C); Lane: 3, 8, 9 (mutant heterozygous C/T) ; Lane: 7 (Mutant sample T Allele

246 bp fragment or mutant homozygous T/T) .

1. Frequency of genotypes for the *IL-13* gene samples of patients and healthy controls.

The results of the current study show the presence of a correlation between the genotypes of the *IL-13* gene and the incidence of development asthma, as the results show a high significant difference between patients and healthy controls when genotype T / T (OR = 3.273)is more than three times, while the genotype C / T (OR = 1.781) show a significant difference between the two groups patients and control more than one and a half times (3).

Table (3): Distribution of genotypes for the *IL-13* gene samples of patients and healthy controls.

Geno type	Control	%	Pateints	%	OR	95% CI
C/C	36	72%	52	52%	1.0	
C/T	12	24%	36	36%	1.781	0.827-8.835
T/T	2	4%	12	12%	3.273	0.703-15.231

2. The distribution of genotypes of the *IL-13* gene in patient samples according to sex.

The results of the current study, show the absence of significant differences for sex and the risk of developing asthma, there were no significant differences in the genotype T/T genotype

C/T(OR= 0.901) also did not show significant difference in (OR=1.017) , as seem in table (4) .

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Table (4): Distribution of genotypes for the *IL-13* gene of patients according to the sex.

Genotype	Male	%	Female	%	OR	95% CI
C/C	17	17%	35	35%	1.0	
C/T	11	11%	25	25%	0.901	0.374 – 2.173
T/T	4	4%	8	8%	1.017	0.298– 3.859

3. Frequency of genotypes for the *IL-13* gene of patients according to residential areas.

Table (5) shows, there is a significant differences between the genotype C/T (OR=1.667) and the risk of developing asthma in the residential areas, while the genotype T/T show no significant difference (OR= 0.826).

Table (5): Distribution of genotypes for the *IL-13* gene of patient according to residential areas

Geno type	Rural	%	Urban	%	OR	95% CI

C/C	13	13%	39	39%	1.0	
C/T	6	6%	30	30%	1.667	0.587-4.731
T/T	3	3%	9	9%	0.826	0.203-3.356

4. The distribution of genotypes of the *IL-13* gene in patient samples according to smoking.

The results of the current study show no correlation between genotypes in people smoking and the risk of asthma in genotype C/T where there were not significant differences (OR= 0.232),alsogenotype T/T show no significant difference (OR=0.726) as seem in table (6).

Table (6) :Distribution of genotypes for the *IL-13* gene of patients according to the smoking.

Geno type	Smoking	%	Nonsmoking	%	OR	95% CI
C/C	16	16%	36	36%	1.0	
C/T	3	3%	33	33%	0.232	0.063- 0.854
T/T	2	2%	10	10%	0.726	0.147-3.601

5. The distribution of genotypes of the *IL-13* gene in patient samples according to family history.

The results of the current study, show a correlation between patients with a family history of asthma and genotype T/T and significant difference more than two and a half (OR= 2.632), While genotype C/T does not appear any significant difference (OR= 0.531) , as seem in table (7) .

Table (7): Distribution of genotypes for the *IL-13* gene of patients according to the family history of Asthma .

Genotype	Family history	%	Non – history	%	OR	95% CI
C/C	25	25%	27	27%	1.0	
C/T	13	13%	23	23%	0.531	0.230-1.228
T/T	8	8%	4	4%	2.632	0.737- 9.391

6 .The distribution of genotypes of the *IL-13* gene in patients samples according to education.

The results of the current study show no correlation between genotype of IL-13 gene for the educated and the uneducated and the risk of asthma, the genotype C/T shows no significant difference (OR=1.000) , and also the genotype T/T does not appear any significant differences (OR=0.439) .Table (8) .

Table (8): Distribution of genotypes for the *IL-13* gene of patients according to the education.

Genotype	Uneducated	%	Educated	%	OR	95% CI
C/C	12	12%	40	40%	1.0	
C/T	9	9%	27	27%	1.000	0.389– 2.568

T/T	5	5%	7	7%	0.439	0.126-1.528

Discussion

The results of statistical analyses show that there is significant difference between the mutant homozygous T / T (OR = 3.273, % 95 CI = 0.703-15.231), and the mutant heterozygous C / T (OR = 1.781, % 95 CI = 0.827-3.835) .These results indicate that there is correlation between polymorphism of IL-13 gene and risk of asthma in Thi-Qar population.

IL-13 was a critical cytokine in the development of asthma. IL-13 was one of the most studied of the candidate genes for asthma. IL-13 1112C/T polymorphism leads to increased IL-13 transcription in Th2 cells and enhanced IL-13 secretion [27].

The study show no significant difference in genotypes of IL-13 gene between males and females, where the genotype C / T (OR = 0.901, % 95 CI = 0.374-2.173), as well as genotype T/ T (OR = 1.017, % 95 CI = 0.298– 3.859).

The results of the current study showed the absence of significant difference for IL-13 gene polymorphism among urban people and rural people for individuals who have genotype T / T (OR = 0.826, % 95 CI = 0.203-3.356) , while genotype C / T (OR = 1.667,% 95 CI = 0.587-4.731). shows a significant difference of about more than one and a half,so there is a significant correlation between the incidence of asthma in urban areas and IL-13 gene polymorphism and this is due to the particles resulting from the exhaust of diesel during fuel combustion contribute significantly to air pollution in urban areas and the epidemiological studies have shown that inhalation of these particles was the reason for the high injury rates of respiratory system such as lung cancer and asthma [28] . The genetic and environmental factors play a role in the development of asthma as it found that the genetic changes which cause the risk of asthma have more profound impact on individuals when environmental exposures [29]. Studies also showed

that 60% cases of asthma resulting from genetic environment and gene interaction [30].

When the examination of the distribution of genotypes of patients according to smoking results shows statistically no significant differences between smokers and nonsmokers for individuals who have genotype C/T (OR= 0.232,% 95 CI= 0.063- 0.854) ,as well as genotype T/T (OR = 0.726,% 95 CI = 0.147-3.601) .

The study's results show that no significant difference for both genotype of IL-13gene between educated and uneducated patients .The genotype C / T(OR = 1.000,% 95 CI = 0.389–2.568), as well as the genotype T / T(OR = 0.439,% 95 CI = 0.126-1.528) .

The statistical analyses show the presence of a significant difference for genotype T/T of people who have a family history, where the genotype T / T have a correlation to asthma by more than two and a half times (OR = 2.632,% 95 CI = 0.737- 9.391) and these finding agree with [31][32] , while the genotype C / T (OR =0.531,% 95 CI = 0.230-1.228) has no significant difference .

The –1112C/T SNP is located in a region containing a binding site of the nuclear factor of activated T cells (NFAT) transcription factor that regulates IL13 and IL4 gene expression. The T allele at position–1112 may increase binding of the NFAT protein to this region. There is some evidence that the T allele at the –1112C/T polymorphism results in increased levels of IL-13. In a report to assess the functionality of this polymorphism, T cells were stimulated to produce higher amounts of IL-13. Recently, Cameron and colleagues showed that the IL13 –1112T allele enhanced promoter activity in primary human and murine CD41 Th2 lymphocytes [27].

Our results support the involvement of IL-13 gene in the pathogenesis of asthma in the Thi-Qar population. This result with the positive associations that have been reported for the IL-13 1112C/T polymorphism in populations including those Dutch[33][34], African Americans [35][36], British [37], German [38],Finland[39] On the other hand, no association has been reported in many populations including those, Chinese [45][46][47], Japanese [48], Korean[49] and Hispanic [51] These

contradictory results can be explained by the genetic heterogeneity among the studied populations, by the different environmental factors involved in the pathogenesis of asthma .

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