BioGecko Vol 12 Issue 02 2023

ISSN NO: 2230-5807

Genetic association of IL-4 C590 T Gene polymorphism in patients with respiratory allergies In Basra Governorate / Iraq

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Abstract
The relationship between polymorphism C590 T for *IL-4* gene and respiratory diseases was studied in Basra Governorate, Iraq, where 150 blood samples were taken, including 50 blood samples as control elements and 100 blood samples for patients (50 blood samples for allergy patients and 50 blood samples for asthma patients) and genetic polymorphism was analyzed in the catalyst region (C_590T) using polymerase chain reaction (ARMS PCR), and the presence of the two nights, namely allele C and allele T and three genetic structures (CC, CT , TT)T allele frequency and TT genotype were higher in the patient group (allergies and asthma) compared to the control group at $P < 0.05$ It can also be suggested that the CT hybrid genotype may be associated with the disease .

Key words : polymorphism IL -4 C590T , allergy , asthma

1- Introduction

Allergies and asthma represent the most important chronic diseases affecting the respiratory system in the world and are the fourth major chronic disease according to the classification of the World Health Organization, as allergies cover a wide range of diseases, including allergic rhinitis, allergic asthma, and others, the prevalence of respiratory allergies among the entire population has increased alarmingly and at high rates worldwide (1) . Allergy in general is an abnormal immune reaction that occurs as a result of an antigen or foreign body, and allergy is not a disease but a series of immune reactions that lead to known symptoms of allergy (2 , 3). Allergic diseases are caused by allergens, whose chemical composition affects the human body through their effect on the immune system, which leads to an allergic reaction . It has recently been suggested that the molecular allergy approach may contribute to understanding the best mechanisms for asthma patients and accurate diagnosis (4) Heterogeneity in asthma between people can be attributed to complex interactions between the patient and the environment air allergens (5) . where allergens can improve the accuracy of diagnosis and thus educate the patient (6).

Genetic variation plays an important role in the development of sensitivity, so it is important to study polymorphism, and it is also important to look at proteins that are regulated by anti-inflammatory cytokines (7).
Asthma is defined as a chronic inflammation of the airways in which many cells and cellular elements play a role. Chronic inflammation is associated with airway overresponse, which leads to frequent episodes of wheezing, chest tightness, shortness of breath and coughing, especially in the early morning and at night, and these seizures are usually associated with airway obstruction and sputum secretion, in addition to chronic inflammation associated with airflow obstruction and bronchial over responsiveness. Inflammation is the determining factor (symptom) of allergies, and therefore the inflammatory agents are cytokines that play an important role in allergens. Previous studies have pointed to variation in a range of cytokine genes associated with allergies and asthma (8, 9 , 10 , 11)

Interleukin 4 (IL-4) gene is located on the long arm of chromosome 5 in the package 31.33 (5q31.33) (as in the figure) and consists of 4 encoded regions exons and 3 non-coding regions (intron) and this gene has the genetic Polymorphism ((590 C > T in the promoter region, i.e. this region has two alleles are (C, T), so it has three genetic structures are CC, CT , TT (12 , 13) This gene is encoded into a monomer glycoprotein with a molecular weight of 15 kDa consisting of 129 amino acids produced mainly from T cells (Th2) and also from basophils, eosinophils and mast cells (14). It has a vital role in the proliferation and differentiation of helper T cells (Th2) and the main

driver of the manufacture and secretion of immunoglobulin (IgE) from B cells, thus promoting the occurrence and development of inflammatory reactions, hyper adhesion of vascular cells and increased eosinophilic cells in the inflamed airways (15).

2- Materials and methods

2-1- Samples: 150 human blood samples were collected in a volume of (1-3 ml) in EDTA tubes distributed into two groups, the first group was represented by patient samples, and the number of blood samples was 100 blood samples divided into 50 blood samples from people with allergies and 50 blood samples from people with asthma patients, depending on the diagnosis of the specialist doctor and from the Allergy and Asthma Center, as well as from workers at the Nahran Omar site of the South Oil Company.As for the second group, it is the control group (healthy people), which included 50 blood samples from healthy people who do not have allergies and asthma, and the samples of the two groups included both sexes (males and females) and different age groups ranging between (20-70) years and for a period from March 2022 to September 2022.

2-2- Extracting DNA from the blood

DNA extraction from samples from the two groups (patients and control) using a DNA extraction kit prepared by Genaid Company .

2-3- ARMS – PCR (Amplification Refractory Mutation System Polymerase Chain Reaction)

The polymorphism of the interleukin-4 gene (IL-4) was studied using the following prefixes shown in the table (1).

Table (1) shows the prefixes used for the IL-4 gene for the genetic form C590T(13)

Gene	Allele	Primer sequences(5-3)	Length	TA
IL-4	T	ACACTAAACTTGGGAGAACATTGTT	25	65
IL-4	C	ACACTAAACTTGGGAGAACATTGTC	25	
IL-4	R	GAATTTGTTAGTAATGCAGTCCTCC	25	

The method of work was carried out with a reaction mixture with a volume of 20 microliters and as shown in Table (2) based on the leaflet attached with Bioneer Master Mix manufactured by Bioneer Reaction Mixture Company Here each sample is working two tubes, one of which carries the allele starter C and the other carries the allele starter T.

Table (2) represents the chemicals of the reaction mixture and their volumes

Chemicals	Volume
Master Mix	Bioneer
Premier Forward C or T	2 µl (10pc/ml)
Premier Reverse	2 µl (10pc/ml)
DNA	5 µl
D.W.	11µl
Total	20 µl

After completing all the additions, the samples were shaken slightly by the rapid shaking device for 5 seconds to ensure the homogeneity of all materials, then the samples were placed with a PCR Sprint Thermal Cycler device and the device was filled according to the following program for the interleukin 4 gene as in Table (3).

Table (3) shows the PCR program for the IL-4 gene

Steps	Temperature	Time/Cycle	Number of Cycles
Initial Denaturation	**95**	**1 min**	**1**
Denaturation	**95**	**1min**	**30**
Annealing	**65**	**40 sec**	
Extension	**72**	**1min**	
Final extension	**72**	**5min**	**1**
Hold	**4**	**10 min**	

After the end of the work of the device, the samples were electrophoresis migrated using agarose gel at a concentration of 2% and after detecting the beams with an ultraviolet light device and then recording the results**.**

2-4- Statistical Analysis

Fisher test was performed to test the homogeneity of the samples used and the Genepop program was used to estimate some genetic parameters of the studied samples.

3 - Results and discussion

Polymorphism of cellular motor genes

3-1- DNA extraction The genomic DNA of human blood samples was extracted for the three groups of allergy, asthma and control as in Figure (1).

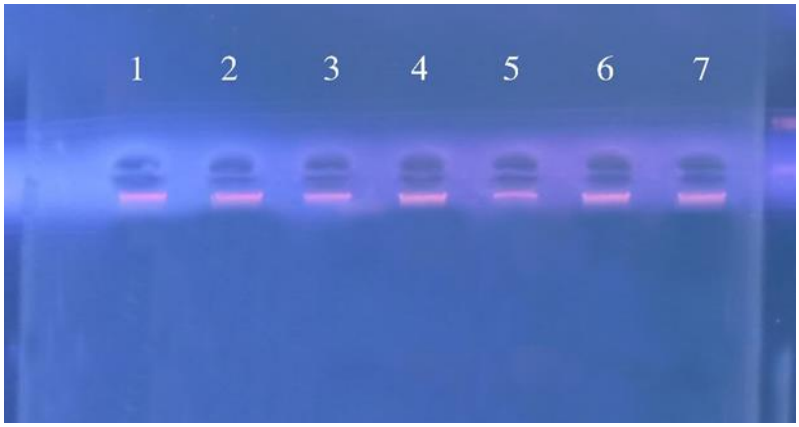

Figure (1) Electrophoresis of DNA on agarose gel at a concentration of (0.8%)

3 – 2- Interleukin-4 gene polymorphism for mutation site 590 C>T

The results of the electrical migration of the mutated gene IL-4 590 C>T enlarged by ARMS-PCR technique showed the presence of the two alleles, the T allele and the C allele, which gives three genetic structures (CC, CT, TT) with a size of 216 bp in each of the three groups as shown in Figure (2).

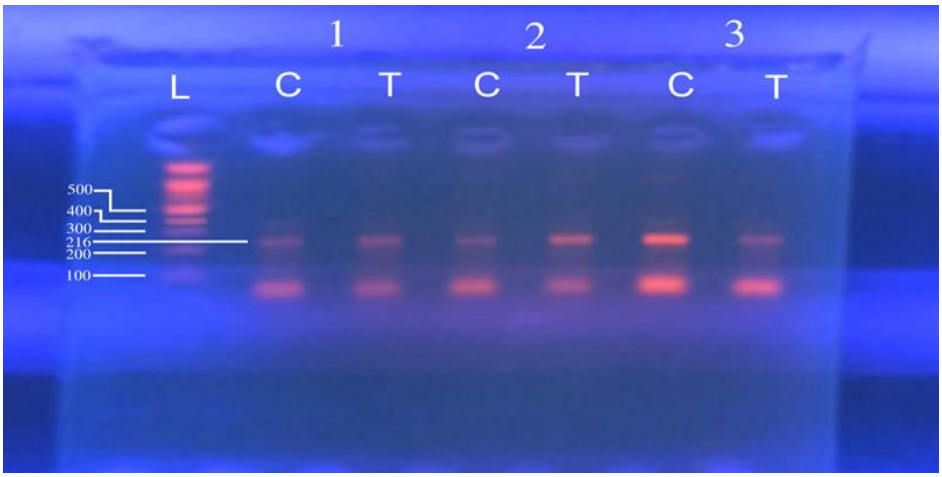

Figure (2) shows the electrophoresis of the products of the PCR technique of the genetic form 590 C>T) IL-4 (on the agarose gel at a concentration of (2%)

The results of the frequency distribution of the C and T alleles of the mutant gene IL-4 590 using the Hardy-Weinnberg equilibrium law showed heterogeneous results between samples of allergy and asthma patients and the control group, if the T allele was recorded (39% and 36%) in each of the allergy and asthma groups respectively compared to the C allele for the same two groups (61% and 64%) respectively.While the frequency of the T allele was recorded by 31% compared to the C allele which was recorded by 69% in the control group as shown in Table (4).Which shows no significant differences between the three groups and the frequency distribution of alleles, although there is little variation in the frequency of allele lines between the patient and control groups. The results show that the T allele showed an association with allergies and asthma using the Fisher s test, and the critical ratio (OR) Odds Ratio was 1.4 for the allergy group and approximately 1.25 for the asthma group with confidence intervals under 95% ranging between (0.7936-2.5519) for allergy patients and (0.6951-2.2552) for asthma patients.While the results showed that allele C was not associated with allergy and asthma, where the critical ratio (OR) was close between the two groups of the disease compared to the control group, where it was recorded 0.70 and 0.79 respectively under the confidence period of 95% CI (0.3919-1.2601) for allergy patients compared to control and (0.4434-1.4387) for asthma patients compared to the control group below the probability level $P < 0.05$.

Table (4) shows the repetition of alleles for the genetic form 590 C>T of the IL-4 gene for the three groups

P* Value	OR* (95%CI)*	Allele replication		Allele
		control%	allergy %	
0.2364	0.7027 (0.3919-1.2601)	69	61	C
	1.4231 (0.7936 -2.5519)	31	39	T
P*	OR*	Allele replication		Allele

Value	(95%CI)*	Allergy	Asthma	
0.4541	0.7987 (0.4434-1.4387)	69	64	C
	1.2520 (0.6951-2.2552)	31	36	T

*P<0.05 ***** OR Odd Ratios ***** 95 % CI Confidence Interval

These results are consistent with those obtained by (11, 16, 17 , 18 , 19) in terms of the appearance and association of the mutant allele T with allergy and asthma patients compared to the other allele C and control group, and the reason for the association of the mutant allele with the disease may be due to its location within the promoter region of the gene, which increases the binding of the nuclear transcription factor . Thus, it increases the expression of the IL-4 gene, which results in an increase in the production of the interleukin-4 protein, which proliferates T cells and stimulates B cells, and an increase in the synthesis and secretion of IgE and the activation of eosinophils in the airway, thus increasing inflammatory factors and allergic reactions (20, 21) There has been an association between the allele IL-4 T and increased production of interleukin-4 and elevated levels of IgE in individuals with asthma and allergic rhinitis, and this has been explained by the presence of the genetic form in the 5-flanking region of the IL-4 gene, where the genetic form C590 T of the IL-4 gene is located in one of the binding sites of the nuclear factor - active T cell (NF-AT), which plays an important role in the transcription of many cytokine genes (22) Interleukin-4 is a key factor for regulating the immune response through its control over the secretion of immunoglobulin E from B lymphocytes and is the main signal for the distinction of CD4+ cells to Th2 cells. These functional abilities make it one of the most important cytokines for the development of bronchial asthma, and the possession of the mutant allele (T 590) within the catalyst zone increases the production of the protein IL-4 compared to the normal allele (C 590) (23 ,24) noted that the level of interleukin-4 is regulated by the catalyst region of its coding gene at the transcription level after the second helper T cells are stimulated by pathogenic agents that activate the IL-4 gene for cytokine expression and secretion 4. IL-4 , IL-5 , IL-13 . (25) concluded during their analysis of the results of seven studies in different countries, including three studies in China and one each in Japan, Korea, Russia, and Poland, that an increased risk of asthma is associated with variation in a single base (Single Nucleotide Polymorphism (SNP) at the 590T C site for interleukin-4 and the association of the allele T and its genetic makeup TT increases the risk of possible asthma, especially in the Asian population. The results of the current study did not agree with the study conducted by (13) , which concluded that an increased risk of asthma is associated with allele C and that the T allele has a protective role against asthma.It also contradicts other studies such as those (24 , 26) whose study results showed that the risk of developing the disease is significantly associated with the recurrence of the C allele, where it has a positive association with the occurrence of allergic disease. It also contradicts the study of (27) which examined six genetic loci (IL-4 590). It was concluded that these genes do not contribute much to the development of asthma in Chinese children, as well as a study (28) that showed that genetic polymorphism was not associated with susceptibility to asthma. . The lack of correlation in our study with previous studies may be due to environmental impacts to our examined community in Basra Governorate.

The results of the genetic analysis of the results of the ARMS-PCR technology of the mutant gene IL-4-590 (C<T) using the Hardy-Weinberg law as in Table (5) showed three genotypes in the patient groups and the control group namely CC, CT AND TT. Although there was a clear difference in frequencies of genotypes between the two groups of patients compared to the control group. especially the frequency of the TT genotype in allergy patients was by (0.15) and in asthma patients (0.13) compared to the control group (0.09) Now this difference is insufficient for the latest variation in the frequency of genotypes from a statistical point of view between the two groups of patients and the control group at the probability level P = 0.1045 may be due to the small sample size . When using the Fisher test, it was shown that any of the genotypes were most associated with the onset of the

disease. The critical ratio of homologous mutant genotype OR=2.1622 with confidence interval (CI 95% 0.8522-5.4857) in allergy patients and the critical ratio in asthma patients OR=1.6911 with confidence interval (CI 95% 0.6951-2.2564) These results show that the TT genotype is twice as associated with the appearance of allergies and asthma compared to the control group. The results also recorded the highest genotype between the three genotypes is CT and it was(0. 48) and (0.46) in the two patient groups respectively compared to the control group, which appeared by (0.43), but there was no significant difference between the three groups at P = 0.2220 Statistical analysis when using the Fisher test showed that the repetition of the CT genotype may be related to the onset of allergic disease by about one and a half times and asthma by about 1.3 (OR=1.4481 and OR=1.2524) with confidence interval (CI 95% 0.7995 -2.6228 and CI 95% 0.6951-2.2564) respectively While the results showed that the CC genotype is more associated with the control group, as it was recorded at a higher frequency and by 48% among the three genotypes in the control group compared to the two patient groups, which was recorded at rates (0.37 and 0.41) respectively, but it did not reach the level of significance between the three groups at P = 0.2220, and also the critical ratio was low and almost close between the two groups of patients and the control group if it reached OR=0.6905 with confidence interval (CI 95% 0.331-1.2511) and OR = 0.7985 with confidence interval (CI 95% 0.4432-1.486)

Table (5) shows the relationship between the genotypes of the genetic form IL-4 C590 T and the three groups

P* Value	OR* (95%CI)*	Genotype replication		genotype
		Control %	allergy%	
0.2220	0.6905 (0.331 -1.2511)	48	37	CC
	1.4481 (0.7995 -2.6228)	43	48	CT
0.1045	2.1622 (0.8522-5.4857)	9	15	TT
P* Value	OR* (95%CI)*	Genotype replication		Genotype
		control	asthma	
0.4537	0.7985 (0.4432-1.486)	48	41	CC
	1.2524 (0.6951-2.2564)	43	46	CT
0.2767	1.6911 (0.6562-4.3578)	9	13	TT

***** OR Odd Ratios ***** 95 % CI Confidence Interval

From the above results, it is clear that homologous genetic type (TT) is associated with the appearance of the disease and that homologous genetic pattern CC may have a protective role against the risk of developing the disease, and this is consistent with the study of (16) which found that individuals with allergic diseases have a genetic pattern CC significantly associated with a lower level of IgE compared to the TT genotype. But it contradicts the results of our current study when it combined homologous and hybrid CC and CT genotypes with

a low level of IgE compared to TT genotype. This was explained by the fact that the design of his study was on a specific group in the hospital and did not represent the general population and to the small sample size. The results of our study also agreed with an analysis of a group of previous global studies by (24) who concluded that individuals with the TT genotype are more likely to develop allergies and asthmaIt also contradicts the results of our study that the CT genotype is not related to the disease and may be due to a wide statistical confidence field (CI 95%) that is randomly affected. While there is no association for variation in the genetic form IL-4 C590 T in Egyptian patients with bronchial asthma, this is what a study (29) showed.On the extent to which the genetic form IL-4 C590 T is associated with asthma severity in Egyptian patients, it has been stated that individual differences in cytokine production that may be related in variation in the genetic form affect the balance between pro-inflammatory and anti-inflammatory cytokines and thus affect the severity of asthma and its therapeutic outcomes. His study was in line with eight other studies, including a study in Kuwait (30), a study in Jordan by (31) and a study in India by (32).

Conclusions

Our current study indicates the relationship between IL-4 at the site of the C-590 T mutation and respiratory diseases and showed that the risk of asthma with the allele T and the TT genotype and the genetic makeup CT may have a link to the disease, while the allele C and the genetic makeup CC have a protective role for the disease and more studies on different races and cytokines of asthma patients are needed to confirm these results**.**

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