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Association of Genetic Polymorphism rs20541 at IL-13 gene with Susceptibility to Allergic Asthma

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Abstract: An imbalance exists between the forces preserving the patency of the airway and those attempting to constrict or close it in asthma, a lung deficiency illness. The purpose of this study is to determine if SNP (rs20541) in the IL-13 gene is linked to asthma in the sample of Iraqi population as well as to assess the relationship between total IgE serum level and eosinophils count with IL-13 SNP (rs20541). At the central allergy center in Baghdad, a cases-controls study was carried out from September 2019 to November of 2019. Asthma patients with ages of 10 to 65year, of both genders, were enrolled in this study and matched in age and gender with the control group of healthy individuals without a history of chronic disease. There were 60 subjects total, including 20 controls and 40 asthmatic sufferers. The average age of asthma patients was 34.9±15 years, compared to 37±12.4 years for the control group. According to the study, the difference in asthmatic mean age from the control group is not statistically significant (p=0.3). The rs20541 (AA+AG) genotype SNP was found to have a significant genetic connection in this study with IL-13 and the protective factor (GG) in the Iraqi population (AA+AG: OR=7.36, 95% CI= 1.50 - 36.04, p=0.006, GG: OR= 0.14, 95% CI= 0.03-0.64, p=0.008). It was concluded that A allele has a substantial relationship to the severity of asthma and is associated with higher levels of the biomarkers (Eosinophil count and Total IgE). In individuals with allergic asthma, this polymorphism (rs 20541) significantly linked with higher Eosinophil count and Total IgE levels.

Keywords: Asthma, IL-13, Total IgE, SNP, rs20541, Eosinophils count

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Introduction

Allergies are responses to an interaction of antigen-antibody. Allergens are antigens; defined as elements of the environment that provoke hyper-sensitivity reactions mediated by immunoglobulin-E following injection or ingestion or, inhalation. Some genetically predisposed people inherit from their parents the potential for allergies and become sensitized to single or multi an allergen (1). The most significant health issue in the world is asthma, one of the most common respiratory problem. An important part of the pathogenesis of asthma is played by the innate and adaptive immune systems (2). The most prevalent type of asthma is allergic asthma, which is characterized by elevated serum levels of IgE, eosinophil count, and inflammatory cytokines. Allergic asthma is one of several types of asthma in which symptoms are brought on by different allergens, such as indoor and outdoor allergens (3, 4). Asthma is a long-lasting inflammatory illness of the airways that is linked to Th-2, which produces cytokines like interleukin-4, IL-5, and IL-13 (5). An essential cytokine produced by type 2 helper T-lymphocytes is interleukin-13

(IL-13). In allergic asthma, exposure to allergens causes a complex combination of hereditary and environmental factors, and the immune system of the disease is mediated by a large number of cytokines. Allergic asthma is a chronic inflammatory disease of the airways (6,7). Previous research has demonstrated a connection between allergic asthma and IL-13 gene polymorphisms (8). Numerous SNPs were discovered for the IL13 gene, which is related to asthma, as SNP-IL-13 rs20541 has been demonstrated to be related to an increase in IL-13 expression and serum total IgE in asthma patients (9, 10). The current study's objectives are to assess the relationship between asthma in the sample of Iraqi population and the total IgE serum level, eosinophil count, IL-13, and SNP (rs20541) in the IL-13 gene.

Materials and methods

Sample collection

The study included two groups, 40 asthma patients (female and male), and 20 subjects who appeared to be healthy (control), as well as personal data like age, gender, family history, and other diseases. The patients were admitted to the Center for Asthma and Allergy in Baghdad between September and November of 2019. All individuals had their conditions determined by a doctor's clinical assessment and were chosen in accordance with the worldwide asthma initiating criteria.

Extraction of genomic DNA

Asthma patients and seemingly stable monitors had two ml of peripheral venous blood drawn using disposable syringes. After that, blood was retained in EDTA anticoagulant tubes and stored at -20 degrees Celsius to serve as a source for DNA extraction. Using the DNA Mini kit (blood) from Norgen®, (Canada), and DNA was extracted from blood samples of asthma patients and seemingly healthy participants. Then, using a Qubit 4 Fluorometer, DNA concentration and purity were evaluated.

Amplifications of SNP rs20541 at IL-13 Gene

In the current investigation, these were created as shown in (Table 1). The two primers were created utilizing online tools and databases from around the world, including the NCBI, as well as overseas databases.

Table (1): Primers Sequences to SNP (rs20541) in Gene.

Primer	Sequences
Forward primer	5′ TTT GTA AAG GAC CTG CTC TTA 3′
Reverse primer	5′ AGC TAA GGA ATT TTA CCC CTC 3′

The following PCR Thermocycler conditions were carried out utilizing the PCR Thermocycler apparatus, (Table 2). After electrophoresis on a 2% agarose gel stained with ethidium bromide, amplifications were examined at Gel Doc. (Biorad). The ABI Big Dye v.3.2 terminator sequencing kit (Applied Bio Systems) was used to sequence the PCR products. Using Geneious software, the sequence data were compared to the reference sequence (NCBI RefSeq; http://www.ncbi.nlm.nih.gov).

Table (2): Program for the IL-4 Gene PCR Profile.

Step	Temperature	Time	Cycle
Initial denaturation	94ºC	5 minutes	1
Denaturation	94ºC	30 second	20x
Annealing	56 ºC	1minutes	
Extension	72ºC	30 second	
Final extension	72ºC	7 minutes	1

Measurement of some of biomarkers levels

The total serum levels of IgE in the patients and control were examined. Each subject (patients and control) had two milliliters of blood removed, separated into sterile gel tubes, and allowed to clot for around two hours. After separating the serum, which was kept at -20°C until being tested, the sample was centrifuged at 3000 rpm for 15 minutes. To measure total IgE levels, ELISA kits were used (Euroimmun Company, Germany). Each individual had a vein punctured using an aseptic approach while two milliliters of venous blood were taken from them using a multi-sample syringe. According to the manual process of the manufacturing business (Beckman Coulter, United States), whole blood was deposited in EDTA tubes and examined by a hematology analyzer to estimate the eosinophil count.

Statistics analysis

Demographic and genotypic research was conducted using SPSS version 24 (Statistical Software for Social Sciences). The differences in participant answers were examined using the p-value. The chi-square test was used to assess deviations from the Hardy-Weinberg Equilibrium for all genetic variations. WinPepi software estimated the odds ratio and 95% confidence interval for the genotypes and allele frequencies. Data were examined for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests for the quantitative variables (level of total IgE and eosinophil count). The nonparametric (Mann-Whitney-U-test and Kruskal-Wallis test) was used to evaluate whether there were significant differences between the medians, with a p-value cutoff of 0.05.

Results and discussion

Demographic and clinical data

This study included 40 asthmatic patients and 20 persons who appeared to be in good health as a test sample. The demographic information for enrolled subjects is summarized in (Table 3); gender, age, and family history were comparable within these classes.

Table (3): Distribution of subjects accords to gender, age, age group, and family history.

Groups	Patients (n=40)	Control (n=20)	p-value
Gender (F/M)	20/20	9/11	0.14
Age (mean ± S. D.) year	34.9±15.8	37±12.4	0.3
Age Group (N,%): 10-25 y	13(23.5)	4 (20)	
26-41 y	18(45.0)	8(40)	0.001
<42 y	9(22.5)	8 (40)	
Family history (Yes/No)	25/15	0/20	0.001

M: Male; F: Female; N: Frequency: %: Percentage; S. D.; Standard deviation, p: Probability.

Hardy-weinberg equilibrium to subjects

As shown in (Table 4), the IL-13 gene's SNP was found in HWE in both patients and controls. As shown in Table (4), the Hardy Weinberg equilibrium of the IL-13 gene does not significantly differ between the genotypes expected and observed in patients and controls (P> 0.05).

Table (4): IL-13 Gene Genotypes and their Hardy-Weinberg Equilibrium in Subject.

SNP	Genotypes	Observed	Expected	Observed	Expected
		Patients (N=40)		Control (N=20)	
rs20541	GG	22	22.5	18	18.1
	AG	16	15.0	2	1.9
	AA	2	2.5	0	0.1
	X^2 value	0.17		0.055	
	HWE- P value	0.67		0.81	

N= Frequency, HWE- p value= Hardy-Weinberg Equilibrium- probability value, X^2= chi square

Characteristic of demographics by rs20541 genotype

As shown in (Table 5), the distribution of the IL-13 genotype of SNP rs20541 was examined in relation to demographic factors (Age, Gender, Age groups, and Family history) in forty patients with allergic asthma and twenty controls. At a p-value of 0.001, family history was significantly more important for patients with asthma than for those without asthma and for controls. As a result, the development of the disease is caused by the cumulative effects of multiple genes interfering with each other on one side and with environmental factors in the other side (11).

Table (5): Subject Demographics by Genotype rs20541.

	Groups		GG N (%)	AG N (%)	AA N (%)	P-value
Age	Patients (Mean± S.D)		31±10.0	34±16.4	40±4.1	0.7
	Control (Mean± S.D)		37.6±12.7	33± 2.5	-	
Gender	Patients	Male	11(55%)	8(40%)	1(5.0%)	0.1
		Female	11(55%)	8(40%)	1(5.0%)	
		Total	22 (57.9%)	16 (39.5%)	2 (2.6%)	
	Control	Male	10(90.9%)	1(9.1%)	-	0.71
		Female	8(88.9%)	1(11.1%)	-	
		Total	18 (90%)	2 (10%)	-	
Age Groups	Patients	10-25	5(38.5%)	7 (53.8%)	1(7.7%)	0.02
		26-41	15(83.3%)	3(16.7%)	0	
		>42	2(22.2%)	6(66.7%)	1(11.1%)	
	Control	10-25	3(75%)	-	1(25%)	0.3
		26-41	8(100%)	-	0	
		>42	7(87.5%)	-	1(12.5%)	
Family History	Patients	Yes	12(48%)	12(48%)	1(4%)	0.001
		No	10(66.7%)	4(26.7%)	1(6.7%)	
	Control		18(90%)	2(10%)	-	

%: Present, N: Frequency, S.D. = Standard division, p= Probability.

Genotyping and allele frequencies of SNP

The SNP (single nucleotide polymorphism) was employed to analyze the genotype distribution of SNP (rs20541) in asthmatic patients. The IL-13 gene polymorphisms on chromosome 5 are found via direct sequencing. Both groups' IL-13 genotypes and allelic frequencies were examined. The distribution of genotype and allele frequencies for the polymorphism rs20541 in participants is shown in (Table 6). The frequency of the GG (homozygous) genotype in allergic asthma is shown in (Table 6) in comparison to the control (57.9%, 90.0 %) with (OR = 0.14, 95% CI = 0.03-0.64), while Compared to controls, allergic asthma patients with

heterozygous AG genotype (39.5%, 10.0%) with (OR = 6.00, 95% CI = 1.27-28.41), and AA genotype (2.6%, 0%) with (OR =2.66 , 95% CI =0.13-54.30). There was a significant (P= 0.008) in (GG) genotype when compared allergic asthma to control, and significant (P= 0.01) in (AG) genotype, but in genotypes (AA) was non-significant (P= 0.4). A high significant allele frequency to G was also found in allergic asthma patients as compared to controls, with a p-value =0.0001. While the difference between allergic asthma patients and control in allele frequency to A was likewise statistically significant (p = 0.0001).

Table (6): Genotypes and Allele Frequencies Distribution of Polymorphism (rs20451) when compared between Subjects.

Genotype	Patients (N=40)		Control (N=20)		OR	95%CI	P-value
	N	%	N	%			
GG	22	57.9	18	90	0.14	0.03-0.64	0.008
AG	16	39.5	2	10	6.00	1.27-28.41	0.01
AA	2	2.6	0	0	2.66	0.13-54.30	0.4
Allele Frequencies							
G	60	75	38	95	0.16	0.06-0.43	0.0001
A	20	25	2	5	6.33	2.33-17.25	0.0001

N: Frequency, %: Present, OR: Odd Ratio, P: Probability, CI: Confidence interval.

Asthma sufferers who are exposed to certain triggers, such as animal dander, pollen, and other allergens, such as these, will experience symptoms of allergic asthma. Results of the current study showed that absolute eosinophil count, total immunoglobulin E, and interleukin 13 (IL-13) levels were significantly higher in the asthmatic patient group, especially when compared to controls. According to previous research, the AA genotype and A allele of rs20541 greatly enhanced the likelihood of developing asthma (12-16). The GG genotype may also be a protective factor against allergic asthma in the Iraqi population, and this agrees with a study that publishes by Resende *et al*. (17).

Risk of rs20541 polymorphisms in allergic asthma

The results of this test, which looked at the connections between the risk of polymorphisms (rs20541) and asthma, are displayed in the (Table 7). Four (4) ORs and their related 95% CIs were determined for each allele risk: OR allele versus allele, OR heterozygous, OR homozygous, and OR allele positivity. The investigation showed that an Iraqi population sample's "A" allele of rs20541 was associated with a higher incidence of allergic asthma (OR= 6.33 and P= 0.0007), and this association was significant under heterozygous (OR= 6.54 and P = 0.012), homozygous (OR= 4.11 and P = 0.2) and allele positivity (OR= 7.36 and P = 0.006) models of the genotype. Regarding the G allele of rs20541, people in the Iraqi group had a greater protective effect (OR= 0.158 and P= 0.007), and this association was statistically significant under heterozygous (OR= 1.320 and P = 0.6), homozygous (OR= 0.243 and P = 0.2) and allele positivity (OR= 0.376 and P = 0.3) models of the genotype. The findings of the arbitrage's trend test were significant for both the A and G alleles, with a p-value of 0.007.

Table (7): Analysis Association IL13 SNP (rs20541) with Allergic Asthma risk.

Tests for Risk and Association (OR and 95% C.I.)					
Risk	**Allele based**	**Genotype based**			**Test**
	Allele freq. Difference	**Heterozygous**	**Homozygous**	**Allele Positivity**	**Arbitrage's Trend Test**
Allele A	**(G)vs.(A)**	**(G/G)vs.(G/A)**	**(G/G)vs.(A/A)**	**(G/G)vs.(G/A+A/A)**	**Common OR**
OR	6.33	6.54	4.11	7.36	11.32
95% CI	1.40 - 28.6	1.32 - 32.30	0.18 - 91.08	1.50 - 36.04	-
X^2	7.12	6.31	1.57	7.35	7.14
p-value	0.007	0.012	0.2	0.006	0.007
Allele G	**(A)vs.(G)**	**(A/A)vs.(G/A)**	**(A/A)vs.(G/G)**	**(A/A)vs.(G/G+G/A)**	**Common OR**
OR	0.158	1.320	0.243	0.376	0.314
95% CI	0.03 - 0.71	0.048 - 36.32	0.011-5.389	0.017-8.200	-
X^2	7.12	0.25	1.57	1.03	7.14
p-value	0.007	0.6	0.2	0.3	0.007

P: Portability, OR: Odds ratio, C.I.: Confidence interval; X^2: Chi square, Freq.: Frequencies.

A strong correlation between minor A alleles (rs20541) and asthma risk was found in the current study's examination of the association polymorphisms (rs20541) with asthma risk (P-value = 0.007, OR, = 6.33) and genotype comparison (G/G vs G/A + A/A) was significantly correlated to allergic asthma risk with a P-value= 0.006 and an odds ratio= 7.36. Because only one copy of the minor allele was required to have an effect, people who inherited at least one copy of the minor risk allele (G/A+A/A) were more likely to be at risk for developing asthma. The minor A allele is substantially related with asthma susceptibility, according to latest studies from Halwani *et al.* The minor allele (G/A+A/A) carries a higher chance of developing asthma (18). According to Bottema *et al.*, this study provided the strongest support for a relationship between asthma and the rs 20541 (AG+AA) genotypes that were associated with an increased risk of developing asthma (16). Additionally, Sadeghnejad *et al*. believe that the (A) allele of IL13 SNPs rs20541 (R130Q) is risky based on earlier research, including a report on the Isle of Wight cohort (19). Also, Andrews *et al.*, at IL13 rs20541, the A allele was similarly closely related to late wheeze but not early wheezing (20). According to recent findings, homozygosis for the (G) allele reduces asthma susceptibility as well as may have a protective effect on the Iraqi population, and several studies have confirmed that (Andrews *et al*,). This study shows that the "G" allele had lower inflammatory activity than the glutamine (A) allele, supporting the idea that the G allele has a weaker connection with asthma susceptibility (20). Also, Heinzmann *et al.*, and Wang *et al.*, refer that the arginine (Arg) variation is connected to the homozygous G-allele, which may lessen asthma susceptibility (12,21). In addition, Resende *et al*, suggest that according to this study's findings, the IL13 rs20541 GG genotype is associated with a decreased risk of acquiring asthma (17).

Association the rs20541 of IL-13 gene with T-IgE with eosinophils

According to table (8), this study not only looked into the relationship between rs20451 (R130Q) variations and genetic susceptibility to disease, but also into how much total IgE and eosinophils were found to be circulating in the blood. The effect of the R130Q (rs20541) polymorphism on levels of serum total IgE and eosinophil count in patient-groups with asthma has been

examined. This polymorphism results in a change in amino acid function. Total IgE levels in patient-subjects (AA, GG, and GA) were increasing in a trend direction and were significant (P=0.042). Additionally, patients with genotypes GG, AG, and AA showed greater levels of circulating eosinophils when the eosinophil count was significant at p-value = 0.034 (Table 8).

Table (8): Genotype of IL-13 Polymorphism (rs20451) Comparison with Levels of Total IgE and Eosinophils in Allergic Asthma Patients.

Genotypes	N, (%)	Eosinophils Mean ± S.D.	Total IgE Median, (min- mix)
GG	22, (55%)	5.5 ± 2.5	374.5, (100-500)
AG	16, (40%)	7.0 ± 3.4	283.9, (100-500)
AA	2, (5%)	12.5 ± 0.7	497.5, (497.5 - 500)
P-value	-	0.034	0.042

N: Frequency, %: present, S.D. = Standard division, p= Probability, min: Minimum, mix: Maximum

The current study is in line with studies that demonstrate a substantial correlation between SNPs (rs20541) and elevated serum IgE levels in asthma patients (22-26), whereas others have not-confirmed such findings (27,28). Furthermore, Hunninghake *et al*.'s research in Costa Rica and a prior work by DeMeo DL *et al.* in CAMP are agreement with this study's findings that rs20541 has a role in the rise in eosinophil levels (27,28). This rise in total IgE and eosinophil levels in patients is related to the fact that the (R130Q) SNP has functional importance for changes in protein levels in different types of cells, enhanced protein activity, or transcriptional activity. Additionally, SNPs can have a variety of biological effects depending on the cell that the cytokine works on or the state of the immune cell that is affected (16).

Conclusion

Up to our knowledge, this study is the first to look at the relationship between allergic asthma patients and rs20541 in the IL-13 gene in samples from the Iraqi population. Additionally, it is discovered that in the Iraqi subjects under study, the G allele was a protective factor against this illness and the allele A was a risk factor for allergic asthma. Additionally, there was a highly significant correlation between this polymorphism (rs 20541) and higher T-IgE and Eosinophil counts in patients.

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