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Association of IL-4 gene Polymorphism (rs2243250) and its Level as Genetic Risk Factor to Asthma in Iraqi Patients

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Abstract

Asthma is a chronic, complex respiratory disorder in which allergen-triggered inflammatory reactions in the airways contribute to the development of symptoms, including breathlessness, cough, wheezing, and dyspnea. It has been estimated that asthma affects about 300 million people in the world.Amplification refractory mutation system techniques (ARMS –PCR) was used for detection Interleukin-4(*IL-4*) gene polymorphism -590 C>T (rs2243250). The genotype distribution results of the *IL-4* gene showed significant differences $p<0.05$ between controls (CC: n=19, 95%; CT: n=1, 5%; TT:n=0) and asthma patients (CC: n=35, 50%; CT: n=33, 47.14 %; TT: n=2, 2.86%). The result showed an increase in CT, TT genotype, and T allele of -590 C>T in asthma patients than in controls, and they were significantly more likely than in controls to have the mutant allele (OR=14.010, 95%CI= 1.858 -105.627, p=0.01).Results revealed that serum IL-4 levels were significantly higher (P<0.05) in patients (9.65±0.27pg/ml) compared with the healthy group (5.47±0.32 pg/ml).The result showed the levels of the IL-4 recorded a significant increase (P<0.05) in the patients (9.65±0.27pg/ml) compared with the healthy group (5.47±0.32pg/ml). The study showed that the*IL-4* genotype variants are indicative of IL-4 cytokineconcentrations, with the highest levels in asthma patients with CC variants (9.37±0.35pg/ml) compared to the homozygous CC genotype of controls (5.39±0.32 pg/ml). The heterozygote CT and variants of homozygote TT significantly had higher IL-4 cytokine levels in patients (9.88±0.02pg/ml&10.87±1.11pg/ml, respectively) compared to the *IL*-4 CCvariant (9.37±0.35pg/ml) in patients, and heterozygote CT in controls had higher IL-4 cytokine levels (7.16±0.0 pg/ml) compared to *IL*-4 CCvariant (5.39±0.32pg/ml) in controls. These results imply an association between the polymorphism of the *IL-4* -590 C>T and IL-4 cytokine levels in the Iraqi asthma patient.

Keywords: Asthma,Interleukin 4, *IL-4* gene polymorphism,ARMS –PCR.

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Introduction

Asthma is a chronic, heterogeneous inflammatory disease with complex pathological mechanisms and diverse clinical phenotypes. Severe asthma is one of the phenotypes, which is defined as uncontrolled asthma despite adherence to maximally optimized therapy and asthma worsens when high-dose treatment is decreased (1).Asthma is one of the most common major non-communicable diseases and for many, has a substantial impact on quality of life. Globally, asthma is ranked 16th among the leading causes of years lived with disability and 28th among the leading causes of burden of disease, as measured by disability-adjusted life years. Around 300 million people have asthma worldwide, and it is likely that by 2025 a further 100 million may be affected (2). Asthma was a problem worldwide with estimated 495,000 deaths every year, thus possessing a significant public health burden (3).

The evidence suggests that asthma is a complex multifactorial disorder, and its etiology has increasingly been attributed to interactions between genetic susceptibility, host factors, and environmental exposures. These include environmental factors (air pollution, pollens, mold and other aeroallergens, and weather), host factors (obesity, nutritional factors, infections, allergic sensitization), and genetic factors (asthma susceptibility loci on genes) (4). Although underlying mechanisms of asthma have not yet been fully understood, they may include airway inflammation, control of airway tone, and reactivity (5). It has also now been recognized that asthma may not be a single disease but a group of heterogeneous phenotypes with different etiologies and prognoses (6).

Interleukin (IL)-4 is the greatest T-helper (Th)2 secreted cytokine and has determinative roles in asthma disease pathogenesis (7). Many asthma-related genes have been linked to susceptibility of this disease. The role of the IL-4 is represented by its effect that seems of particular importance for asthma including stimulation of mucus-producing cells and fibroblast, thus also implicating IL-4 in the pathogenesis of airway remodeling (8). Another potentially important activity of IL-4 in allergic inflammation is its ability to induce the expression of vascular cell adhesion molecule-1 on endothelial cells this will produ enhanced adhesiveness of the endothelium for T-cells, eosinophils, basophils, and monocytes, which are characteristics of allergic reactions (9).Because of these properties, IL-4 has long been considered a potential target in allergies and asthma, and numerous in vivostudies highlighted its role in IgE production (10). *IL-4* has originally been discovered as a low molecular weight T cell-derived polypeptide of 129 amino acids these characteristics of IL-4 accentuate the crucial roles of cytokines in the pathogenesis of asthma (11, 12). Additionally, IL4 gene polymorphisms, like promoter region (C + 33 T) SNP(13), and 3017 G/T SNP in intron 2 (14),The *IL-4* gene is located on chromosome 5q31(15). The -590C>T (rs2243250) polymorphism has been recognized upstream of the transcription initiation site (16).

The current study aimed to determine the frequencies of -590 C>T (rs2243250) SNP in the *IL-4* genein 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-4in asthmatic patients according to genotypic and allelic variation.

Materials and methods

Sampling and data collection

This study had carried out in Al-Sadr Teaching Hospital/ Allergy and Asthma Center and Al-Hakim Hospital/ Chest and Respiratory Diseases Center in Al-Najaf province -Iraq and the laboratory of molecular biology in the Department of Biology / Faculty of Science – University of Kufa, during the period from April 2021 through May 2022. The study population included 70 Asthma and 20 healthy subjects.

The levels of IL-4 in the blood were measured by enzyme-linked immunosorbent assay (ELISA)kit SunLong Biotech, china.

Genomic DNA was isolated by using the protocol of DNA Mini Kit, which was designed for purifying DNA from Geneaid Biotech. Ltd., Taiwan The concentration (ng/ml) and purity (260/280 nm) of the DNA extracts were measured at 260 nm and 280 nm with a NanoDrop spectrophotometer (BioDrop\England). Amplification the of SNPS fragments in the IL-4 590 C>Tgene was done by using a conventional PCR thermocycler (Labnet/USA), The ARMS PCRTechnique was performed for the detection of the *IL-4* gene polymorphism (-590

C>T).The amplification stepsconsisted of a first denaturation step where DNA was initially denatured at95 ◦C for 3min, and then 10 cycles had performed as follows: First Denaturation 95 ◦C for 15 sec, First Annealing 65◦for50 sec, First Extension72◦C for 40 sec. Then, 20 cycles had performed as follows: The second Denaturation 96◦C for 50 sec, Second Annealing59◦C for50 sec, Extension 72◦C for 50 sec, The PCR amplification was completed by a final Extensionof 72◦C for 7 min. Amplification of the *IL-4* genes resulted in 248 bp (C allele) and 216bp (T allele) products. The primer pairs listed in (table, 1), 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% agarosestained 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.

Table1: Primers sequences for *IL-4* polymorphism.

Gene	Primers sequences		PCR product	Ref.
IL-4 -590 C>T rs2243250	Forward 1 (C allele)	5'-ACA CTA AAC TTG GGA GAA CAT TGT C -3'	248bp	Alsaid*et al.* (2013)(17)
	Forward 2 (T allele)	5'-ACA CTA AAC TTG GGA GAA CAT TGT T -3'	216bp	Hussein & Jaber (2017) (18)
	Reverse	5'- GAA TTT GTT AGT AAT GCA GTC CTC C- 3'		Badshah *et al.* (2018)(19)

Statistical analysis

Statistical analyses of all results were carried out with the help of Statistical Package for the Social Sciences (SPSS) version 23 software statisticalpackage using t- test and Chi-square test (with a P-value at a level of significance less than 0.05) to compare values of results between groups. Result values were expressed as mean + SE, anumber of patients, or percentages.

Results and Discussion

The *IL-4* gene polymorphism -590 C>T (rs2243250)

The 20 healthy subjects; 19 (95%) had found as homozygous CC alleles, 1 (5%) 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=19, 95%; CT: n=1, 5%; TT: n=0) **(**Figure1).Asthma patients**:** Among the 70 patients; 35(50%) had found as homozygous CC alleles, 33(47.14%) were found as heterozygous genotype (with the C and T alleles (CT), and 2(2.86%) had found

as homozygous genotype TT alleles; (CC: n=35, 50%; CT: n=33, 47.14 %; TT: n=2, 2.86%) (Table 3 & figure1).

Table 3: The results of genotypic frequenciespolymorphism -590 C>Tthe*IL-4*genein patients and controls.

Genotypes	Healthy controls (n=20)	Asthma patients (n=70)
CC	19(95%)	35(50%)
CT	1(5%)	33(47.14%)
TT	0	2(2.86 %)
***p*-value**	0.001*	
Alleles frequency	**n (%)**	**n (%)**
C allele	39(97.5%)	103(73.57%)
T allele	1(2.5 %)	37(26.43 %)
X^2	10.696	
***p*-value**	0.001*	
OR (95%CI)	14.010 (1.858 -105.627)	

Data were expressed as number and a percentage (N %). *P<0.05 significant. Abbreviations:X^2= chi-square, OR= odds ratio, CI=confidence interval.

That means the frequencies of -590 C>T (rs2243250) this SNP in the *IL4* gene in the 70 Iraqi Asthma patients in Al-Najaf province were with significant differences from that of the 20 healthy controls group (p<0.05). The result showed an increase in genotype CT, TT, and T allele of the -590 C>T in Asthma patients than controls, and they were significantly more likely than controls to have the mutant allele (OR=14.010, 95%CI= 1.858 -105.627, p=0.01). This result indicates a possible role for the SNP in asthma disease.

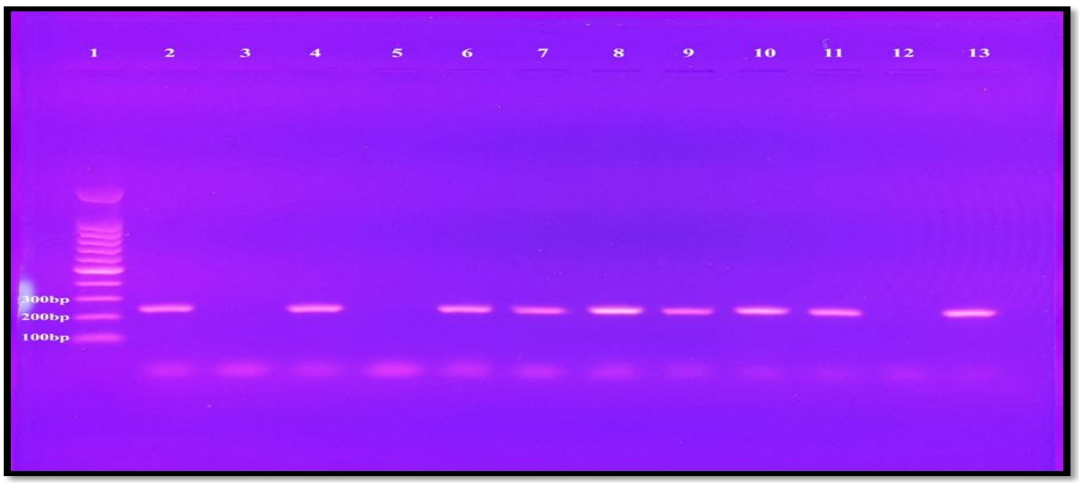

Figure 1 The electrophoresis image of ARMS-PCR analysis of -590 C>T SNP in the *IL-4* gene. Lane 1: 100 bp DNA Ladde. Lane 2&3 (sample 1); Lane 4&5(sample 2):homozygous genotype, CC (248bp in the first lane of each sample). Lane 6&7 (sample 3); Lane 8&9(sample 4); Lane 10&11(sample 5):heterozygous genotype, CT (248 bp in the first lane, and 216 bp in the second lane of each sample). Lane 12&13 (sample 6): homozygous genotype, TT (216 bp in the second lane of the sample).

That means the frequencies of -590 C>T (rs2243250) this SNP in the *IL4* gene in the 70 Iraqi Asthma patients in Al-Najaf province were with significant differences from that of

the 20 healthy controls group (p<0.05). The result showed an increase in genotype CT, TT, and T allele of the -590 C>T in Asthma patients than controls, and they were significantly more likely than controls to have the mutant allele (OR=14.010, 95%CI= 1.858 -105.627, p=0.01). This result indicates a possible role for the SNP in asthma disease.

The promoter region of the *IL-4* gene that contains a -590C>T SNP interacts with nuclear transcription factors and regulates IL-4 expression. Specifically, the T allele has been shown to enhance the binding of nuclear transcription factors to the promoter region, ultimately upregulating the IL-4 expression (20, 21, 22). The substitution of C with T in the -590 position of the *IL-4* promoter has been observed to increase IgE levels in patients with asthma (23,24, 25).

The result of this study agrees withHussein and Jaber study which found that the *C* allele frequency in asthma patients was highest in comparison with the Tallele, in spite of that, the T allele may play role in the development of the disease(18).

These results are consistent withGuia and Ramos study, which showed that the CC genotype was observed to have a significantly increased frequency in patients in spite that CT and TT genotype frequencies were found in asthma(26).

These results are consistent with Dahmani*et al.* study, which reviled that in allergic rhinitis patients from an Iranian population in Tehran the CC genotype in the -590 C>T SNP within the *IL-4* gene was associated with an increased risk of allergic rhinitis (27).

These results were agreed with Zhang*et al*. who showed thatthe *IL-4* -590 C>T SNP shows a significant difference between asthmatic and controls when comparing the TT vs. CC (OR, 3.63; OR 95% CI, 1.16-11.63; p=0.01) and TT vs. CT (OR, 2.48; OR 95% CI, 0.91-6.95; p=0.05) genotypes. As well as Allergic rhinitis and eczema, the same, study demonstrated a significant association of the CT and TT genotype with both allergic rhinitis (p = 0.04) and eczema (p=0. 005) in the asthmatic group(28).

These results were consistent withNeelofar *et al*.who recorded that the genotype and each allele frequency of *IL-4* gene -590 C>T locus had statistically significant differences between the two groups (P=0.007, P=0.002, <0.05). The probability of asthma in children with TT homozygous genotype was higher than that in children with CC genotype (OR=8.91, 95% CI=1.89, 41.98), and the probability of asthma in children with T allele was significantly higher and was 3.07 times more than that in children with C allele (OR=3.07, 95% CI=1.50, 6.27). They suggested that the T allele might increase the IgE level and the probability of asthma in children(29).

Fila-Danilow*et al*. found through the study on IL-4 gene polymorphisms -590 C>T in 250 asthma children and 200 normal children that the T allele is related to asthma, and it can increase the serum IgE concentration. The above indicates that the allele of T is closely related to asthma, which is consistent with the results of this study(30).

This result agrees withPawankar*et al.*study, which reviled that, the distribution of the observed *IL-4* -590 C>T genotype and alleles frequencies in the asthma case was heterozygous genotype CT 42.7% for homozygous CC 36.0% and TT 21.3%. Their results revealed significant differences in the frequency distributions of the heterozygous CT among case and control (0.019) at p<0.05. Although there were no significant differences in wild homozygous (CC) and mutant homozygous (TT) polymorphisms between case and controls(31).

These results were consistent with the results ofAlsaid *et al.*study, which recorded the distribution of alleles and genotypes of *IL4* -590 C>T polymorphism (rs2243250) between the controls and patients with paranoid schizophrenia has been compared. The CC genotype and the C allele were the most represented in both groups. As the homozygous TT genotype was extremely rare in both tested groups, the distribution of genotypes of *IL4* -590 C>T polymorphism was compatible with schizophrenic patients (p = 0.5379), but not for the control group (p<0.01)(17).

These results were agreed with Al Rushood*et al.*study which reported that the frequency of the homozygous CC genotype in type 2 Diabetes Mellitus was significantly lower in diabetic patients compared with the controls (10.4% vs. 26.7%, p=0.002). On the other hand the frequency of the heterozygous (CT) at -590 of the *IL-4* was significantly higher in patients than in controls (85.2% *vs.* 66.3%, p=0.001). The frequency of the TT genotype was 4.4% in patients compared to 6.9% in the controls with anon-significant statistical difference(32).

These results were agreed withMahmood& Abdullastudy which reviled that the polymorphism of the *IL-4* -590 C>T gene showed high CT and TT genotype inankylosing spondylitis in comparison with control while CC genotype showed high in controls(33).

These results were conducted withBadshah*et al*. study which showed that the genotypic and allelic distributions of *IL-4* -590 C>T promoter regions among Hepatitis C Virus Infection patients as followings: CT and TT, were prevalent in HCV-infected patients (42.1% and 15.1%, respectively) compared with the control population (22.3% and 10.8%, respectively), whereas CC genotype predominated the overall population(19).

These results were agreed with Kazemi study which showed thatin a total of 153 Idiopathic Nephrotic Syndrome subjects and 64 controls, the CC genotype of *IL-4* gene polymorphism was detected in 64% of the patients compared to 69.5% in the controls (P = 0.57). The heterozygous CT genotype was detected in 30% of patients compared to 25.5% in the controls (p= 0.61). The TT-genotype was detected in 6% of patients and in 5% of the controls (p= 1.00)(34).

These results have disagreed withHussein*et al.*study which showed that despite the frequency of the CC genotype was 69 (69.0%) in the patients and 108 (72.0%) in the controls, the frequency of the CT genotype was 29 (29.0%) in the patients and 38 (25.3%) in controlsand the frequency of the TT genotype was 2 (2.0%) in the patients and 4 (2.7%) in the controls(35).

These results partially disagreed with Ramroodi*et al.*study which reviled that the polymorphism of the *IL-4* -590 C>T gene was showed high CC and CT genotype inrheumatoid arthritis in comparison with control while TT genotype showed high in controls(36).

These results disagreed withRockman*et al.*study which reviled that, The CC, CT, and TT genotypes of 590 C>T were found in 45%, 44%, and 11% of healthy control, in comparison with 71.5%, 18%, and 10.5% in migraines patients, respectively. The allele frequency of IL-4 rs2243250 (CT) were 67% (C), 33% (T) in healthy controls and 80.5% (C), 19.5% (T) in migraines, respectively(37).

Table 4.5: The results of IL-4 levels according to the genotype of *IL4*-590 C>T in asthma patients and controls.

	IL-4 level (pg/ml)	***p*-values**

Genotype	Healthy controls (n=20)	Asthma patients (n=70)	
CC	5.39±0.32	9.37±0.35	0.0001*
CT	7.16±0.0	9.88±0.02	0.315 *
TT	0	10.87±1.11	

Results values were expressed as mean± SE, *: $p< 0.05$ or significant differences between mean values.

The rs2243250 (−590C>T) SNP in the promoter region of the *IL-4* gene considers a regulatory polymorphism, that affects the binding of the nuclear factor for activated T cells, one of the main transcriptional activators of IL-4 in T cells. In vitro and in vivo studies show that the presence of the minor allele T contributes to the appearance of a supplementary NFAT binding site, which will affect the transcription rate (a threefold greater expression than the −590C allele) (36). This is one of the explanations for the high level of *IL-4* in asthma patients that carry the T allele than in those with the CC genotypes.

Another study, which included patients of north European heritage, reported the influence of −590 C>T promoter polymorphisms on *IL-4* gene expression and IL-4 secretion. The presence of −590 C>T TT genotypes were associated with increased expression and secretion compared with −590 C>T CC (38).

Study Tsai *et al.*indicated that the T allele of the SNP -590C > T has been associated with increased transcriptional activity in vitro(39).

While studyRenauldmentioned that, the exact mechanism for this increment is not known. However, since the SNP is located within 5'UTR of the gene, it may be possible that alterations in this gene could be influencing its transcription and/or mRNA stabilization(40).

Increased plasma concentrations of IL-4 can influence the immune status of asthma patients through several mechanisms and result in many phenotypes beyond the scope of the immune system, IL-4 was originally identified as a B cell growth factor, which drives the optimal stimulation of B cells by antigen. IL-4 induces the immunoglobulin isotype switch from IgM to IgE. IL-4 is also deeply involvedin IgE synthesisfrom B-lymphocytes and plasma cells, and it could be hypothesized that total serum IgE is a biomarker for asthma phenotypes. In addition to its direct activity on IgE production, IL-4 promotes the development of TH2 responses by modulating the differentiation of T cells. Another important activity of IL-4 is its ability to induce the expression of vascular cell adhesion molecule 1, which is expressed by IL-4-activated endothelial cells. This will produce enhanced adhesiveness of the endothelium for T cells, eosinophils, basophils, and monocytes, which is characteristic of allergic reactions. Because of these properties, IL-4 has long been considered a potential target in asthma (41).

In addition to −590C>T promoter polymorphisms on the *IL-4* gene direct effect on increased expression and secretion of IL-4 in asthma patients, suggested that the T allele may increase the IgE level and the probability of asthma(29).

Study Pawankar*et al.*investigated the association between *IL-4*-590 genotype in asthmatic patients and control. Their study showed that the presence of IL-4 serum levels was significantly associated with the genotype of *IL-4*-590 (CC, CT, and TT) among patients and control at ($P \leq 0.05$). They recorded the highest levels of IL-4 in bronchial asthma

patients with CT and TT variants compared to the controls, which is in agreement with the current study results(3).

Mahmood and Abdulla results were inconsistent with the findings of this study, which recorded that the IL-4 concentrations were significantly lower in ankylosing spondylitis patients carrying the rs2243250 TT genotype than in those with the CC and CT genotypes (both *P*< 0.05) (33).

Table 2: the serum level ofIL-4 cytokinein Patients and Controls

Groups Parameters	Patient (n=70)	Controls (n=20)	*p*-value
Interleukin-4pg/ml	9.65 ± 0.27pg/ml	5.47 ± 0.32 pg/ml	0.0001*

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

The result of this study showed that the mean serum level of IL-4 in asthma patients was significantly higher (*P*<0.05) than in controls (9.65±0.27pg/ml vs. 5.47±0.32 pg/ml) respectively (table, 2).

These results are in consonance with Tavakol *et al*. study that found that the mean serum IL-4 levels in asthmatics patients and controls were 15.73 pg/ml and 13.07pg/ml, respectively. Immune and inflammatory responses mediated by cytokines, play important roles in the pathophysiology of asthma. These responses are associated with the overexpression of Th2 cytokines such as IL-4 and IL-13 (42).

The result of this study agreed withRen*et al*.study, which showed that the levels of serum IL-4 in the asthmatics group were obviously higher than in the control group(43).

The result of this study agreed withMahmood& Abdullastudy, which found that the levels of serum IL-4 in the ankylosing spondylitis group were remarkably higher than in the control group(33).The results of this study just as Pawankar*et al.*study showed that the mean serum level of IL-4 was significantly higher in Asthma (*P* ≤ 0.01) than in controls (445.1978± 356.39316pg/ml vs. 54.4255± 19.80227pg/ml) respectively(31). Additionally, a highly significant (*P*≤ 0.01)IL-4 has a critical role in the pathogenesis of allergic rhinitis, particularly in the late phase of the disease (44).

Conclusion

The genotype distribution results of the -590 C>T SNP of the *IL-4* gene showed a significant increased (p<0.05) in genotype CT, TT, and T allele of the -590 C>T in asthma patients than controls. This indicates a possible role of this genotype in the risk of asthma occurrence in these Iraqi patients.

The -590C>T promoter polymorphisms on the *IL-4* gene had a direct effect on increased expression and secretion of IL-4 in asthma patients.There was a high level of IL-4 in asthma patients that carry the T allele than in those with the CC genotypes.

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References

1. Boulet, L. P., Reddel, H. K., Bateman, E., Pedersen, S., FitzGerald, J. M., & O'Byrne, P. M. (2019). The global initiative for asthma (GINA): 25 years later.*European respiratory Journal*, *54*(2).
2. Network, G. A. (2018). The Global Asthma Report, Auckland, New Zealand.
3. (GINA) Global Strategy for Asthma Management and Prevention. 2020. Available from: https://www.ginasthma.org. Accessed 20 Dec 2020.
4. Eder, W., Ege, M. J., & von Mutius, E. (2006). The asthma epidemic. New England Journal of Medicine, 355(21), 2226-2235.
5. Dharmage, S. C., Perret, J. L., & Custovic, A. (2019). Epidemiology of Asthma in Children and Adults. Frontiers in pediatrics, 7, 246-246. DOI: 10.3389/fped.2019.00246.
6. Pavord, I. D., Beasley, R., Agusti, A., Anderson, G. P., Bel, E., Brusselle, G., ... & Bush, A. (2018). After asthma: redefining airways diseases. *The Lancet*, *391*(10118), 350-400.
7. 7.Doran, E., Cai, F., Holweg, C. T., Wong, K., Brumm, J., & Arron, J. R. (2017). Interleukin-13 in asthma and other eosinophilic disorders. *Frontiers in medicine*, *4*, 139.
8. Tavakol, A. J., Hosseini, F. S., Khonavazi, R., Ghayour, K. E., Ghassemi, G. R., Farid, H. R., ... & Razavi, A. (2007). Association of the expression of IL-4 and IL-13 genes, IL-4 and IgE serum levels with allergic asthma

9. Arzu, D. ; Yalcin, B. ; Atil, B. and Reginald, M. (2012) . IL-8 , IL – 10 , TGFβ and GCSF levels were increased in severe persistent allergic asthma patients with anti IgE treatment .
10.Akbari, O. ; Dekruyff, R.H. and Umetsu, D.T. (2001) . Pulmonary dendritic cells producing IL_10 mediate tolerance induced by respiratory exposure to antigen. Nat. Immunol. 2:725_731.{pub Med}.
11.Faith, A. *etal.,* (2003) . Impaired secretion of interlekin-4 and interleukin -13 by allergen-specific T cells correlates with defective nuclear expression of NF-AT2 and jun B: relevance to immunotherapy. Clin. Exp. Allergy.33:1209-1215. {pub Med}12.Rengarajan, J., Szabo, S. J., & Glimcher, L. H. (2000). Transcriptional regulation of Th1/Th2 polarization. *Immunology tToday 21*(10), 479-483.
13.Weiss, D. L., & Brown, M. A. (2001). Regulation of IL-4 production in mast cells: a paradigm for cell-type-specific gene expression. *Immunological reviews*, *179*(1), 35-
14.Suzuki, I., Hizawa, N., Yamaguchi, E., & Kawakami, Y. (2000). Association between a C+ 33T polymorphism in the IL-4 promoter region and total serum IgE levels. *Clinical & Experimental Allergy*, *30*(12), 1746-1749.
15.Basehore, M. J., Howard, T. D., Lange, L. A., Moore, W. C., Hawkins, G. A., Marshik, P. L., ... & Bleecker, E. R. (2004). A comprehensive evaluation of IL4 variants in ethnically diverse

populations: association of total serum IgE levels and asthma in white subjects. *Journal of Allergy and clinical Immunology*, *114*(1), 80-87

16. Hegab, A. E., Sakamoto, T., Saitoh, W., Massoud, H. H., Massoud, H. M., Hassanein, K. M., & Sekizawa, K. (2004). Polymorphisms of IL4, IL13, and ADRB2 genes in COPD. *Chest*, *126*(6), 1832-1839.

17. Nie, W., Zhu, Z., Pan, X., & Xiu, Q. (2013). The interleukin-4− 589C/T polymorphism and the risk of asthma: a meta-analysis including 7345 cases and 7819 controls. *Gene*, *520*(1), 22-29.

1.Alsaid, A., El-Missiry, M., Hatata, E. S., Tarabay, M., & Settin, A. (2013). Association of IL-4-590 C> T and IL-13-1112 C> T gene polymorphisms with the susceptibility to Type 2 diabetes mellitus. *Disease markers*, *35*(4), 243-247

19. Hussein, I. A., & Jaber, S. H. (2017). Genotyping of IL-4− 590 (C> T) gene in Iraqi asthma patients. *Disease markers*, *2017*. Bagnasco D, et al. A critical evaluation of anti-IL-13 and anti-IL-4 strategies in severe asthma. Int Arch Allergy Immunol. 2016;170(2):122–31.

19. Badshah, Y., Arshad, N., Khan, M., Jannat, A., & Hayat, H. (2018). Association of interleukin-4 (-590 C/T) and interleukin-6 (-174 G/C) polymorphism in pathogenesis and prognosis of hepatitis C virus infection. Journal of Interferon & Cytokine Research, 38(12), 559-565

20. Rosenwasser, L J, Klemm, D. J., Dresback, J. K., Inamura, H., Mascali, J. J., Klinnert, M., & Borish, L. (1995). Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clinical & Experimental Allergy, 25, 74–78.

21. Lu, M.-P., Chen, R.-X., Wang, M.-L., Zhu, X.-J., Zhu, L.-P., Yin, M., Zhang, Z.-D., & Cheng, L. (2011). Association study on IL4, IL13 and IL4RA polymorphisms in mite-sensitized persistent allergic rhinitis in a Chinese population. PloS One, 6(11), e27363

22.Chanez, P., Wenzel, S. E., Anderson, G. P., Anto, J. M., Bel, E. H., Boulet, L.-P., Brightling, C. E., Busse, W. W., Castro, M., & Dahlen, B. (2007). Severe asthma in adults: what are the important questions? Journal of Allergy and Clinical Immunology, 119(6), 1337–1348

23. Kim, J. J., Min, J. Y., & Lee, J. H. (2007). Polymorphisms in the IL-13 and IL-4 receptor alpha genes and allergic rhinitis. European Archives of Oto-Rhino-Laryngology, 264(4), 395– 399.

24. Movahedi, M., Amirzargar, A. A., Nasiri, R., Hirbod-Mobarakeh, A., Farhadi, E., Tavakol, M., Behniafard, N., Ansaripour, B., Moradi, B., & Zare, A. (2013). Gene polymorphisms of Interleukin-4 in allergic rhinitis and its association with clinical phenotypes. American Journal of Otolaryngology, 34(6), 676–681.

25. Howard, T. D., Koppelman, G. H., Xu, J., Zheng, S. L., Postma, D. S., Meyers, D. A., & Bleecker, E. R. (2002). Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. *The American Journal of Human Genetics*, *70*(1), 230-236.

26. Guia, R. M., & Ramos, J. D. A. (2010). The-590C/TIL4 single-nucleotide polymorphism as a genetic factor of atopic allergy. International Journal of Molecular Epidemiology and Genetics, 1(1), 67.

27. Dahmani, D. I., Sifi, K., Salem, I., Chakir, J., Hanachi, S., Bachtarzi, M. Z., ... & Rouabhia, M. (2016). The C-589T IL-4 single nucleotide polymorphism as a genetic factor for atopic asthma, eczema and allergic rhinitis in an eastern Algerian population. *Int J Pharm Sci Rev Res*, *37*(1), 213-23.

28. Zhang, J. H., Zhang, M., Wang, Y. N., & Zhang, X. Y. (2019). Correlation between IL-4 and IL-13 gene polymorphisms and asthma in Uygur children in Xinjiang. Experimental and therapeutic medicine, 17(2), 1374–1382. https://doi.org/10.3892/etm.2018.7096
29. Neelofar, K., Ahmad, J., Ahmad, A., & Alam, K. (2017). Study of IL4-590C/T and IL6-174G/C Gene Polymorphisms in Type 2 Diabetic Patients With Chronic Kidney Disease in North Indian Population. *Journal of cellular biochemistry*, *118*(7), 1803–1809.
30.Fila-Danilow, A., Kucia, K., Kowalczyk, M., Owczarek, A., Paul-Samojedny, M., Borkowska, P., ... & Kowalski, J. (2012). Association study of interleukin-4 polymorphisms with paranoid schizophrenia in the Polish population: a critical approach. *Molecular biology reports*, *39*(8), 7941-7947.
31.Pawankar, R., Mori, S., Ozu, C., & Kimura, S. (2011). Overview on the pathomechanisms of allergic rhinitis. *Asia Pacific Allergy*, *1*(3), 157-167.
32. Al Rushood, M., Al-Eisa, A. A., & Haider, M. Z. (2020). Interleukin-4 and interleukin-13 gene polymorphisms in children with idiopathic nephrotic syndrome. Frontiers in Pediatrics, 787.
33.Mahmood, N. S., & Abdulla, A. A. (2021). Association of IL-4− 590 (C> T) Gene polymorphism with the Levels of Serum IL-4 and IgE on the Risk of Bronchial Asthma in Babylon Province/Iraq. *Annals of the Romanian Society for Cell Biology*, 391-400
34. Kazemi, A. M. (2010). Interleukin-4 gene polymorphisms in type 2 diabetic patients with nephropathy.
35. Hussein, Y. M., Amal, S., Rezk, N. A., Galil, S. M. A., & Alzahrani, S. S. (2013). Influence of interleukin-4 gene polymorphisms and interleukin-4 serum level on susceptibility and severity of rheumatoid arthritis in Egyptian population. *Cytokine*, *61*(3), 849-855.
36. Ramroodi, N., Javan, M. R., Sanadgol, N., Jahantigh, M., Khodakheir, T. N., & Ranjbar, N. (2017). Association between interleukin-4 (IL-4), gene polymorphisms (C-589T, T+ 2979G, and C-33T) and migraine susceptibility in Iranian population: A case–control study. *Egyptian Journal of Medical Human Genetics*, *18*(1), 29-34.
37. Rockman, M. V., Hahn, M. W., Soranzo, N., Goldstein, D. B., & Wray, G. A. (2003). Positive selection on a human-specific transcription factor binding site regulating IL4 expression. *Current biology : CB*, *13*(23), 2118–2123.
38. Gonzales, J. R., Gröger, S., Haley, G., Bödeker, R. H., & Meyle, J. (2010). The interleukin-4 -34TT and -590TT genotype is correlated with increased expression and protein production in aggressive periodontitis. Molecular immunology, 47(4), 701–705.
39. Tsai, M. H., Chen, W. C., Tsai, C. H., Hang, L. W., & Tsai, F. J. (2005). Interleukin-4 gene, but not the interleukin-1 beta gene polymorphism, is associated with oral cancer. *Journal of clinical laboratory analysis*, *19*(3), 93–98.
40. Renauld J. C. (2001). New insights into the role of cytokines in asthma. *Journal of clinical pathology*, *54*(8), 577–589.
41. Bagnasco, D., Ferrando, M., Varricchi, G., Passalacqua, G., & Canonica, G. W. (2016). A Critical Evaluation of Anti-IL-13 and Anti-IL-4 Strategies in Severe Asthma. *International archives of allergy and immunology*, *170*(2), 122–131.
42.Ren, Y. F., Li, H., Xing, X. H., Guan, H. S., Zhang, B. A., Chen, C. L., & Zhang, J. H. (2015). Preliminary study on pathogenesis of bronchial asthma in children. *Pediatric research*, *77*(4), 506-510.
43.Liu, X. L., Ren, J. K., & Su, Y. L. (2016). Association between IL-4 gene polymorphisms, IL-4 serum levels, and ankylosing spondylitis. *Genet Mol Res*, *17*(15), 4.

.

44.Shirkani, A., Mansouri, A., Hosseini, R. F., Azad, F. J., Mahmoudian, R. A., Montazer, M., Samimi, A., Momtazi-Borojeni, A. A., Abbaszadegan, M. R., & Gholamin, M. (2019). The role of interleukin-4 and 13 gene polymorphisms in allergic rhinitis: a case control study. Reports of Biochemistry & Molecular Biology, 8(2), 111.