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Original Article

Association between IL-10 (at position -592) and IL-4 (at position -589) genotype polymorphism with atopic and non-atopic asthma in children

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Abstract: Objectives: Asthma is the most prevalent respiratory disease, caused by chronic bronchial inflammation. Cytokines are known to play an important role in the pathophysiology of asthma. This study aimed to compare interleukin-4 (IL-4) and interleukin-10 (IL-10) gene polymorphisms between Iranian pediatric asthmatic patients and healthy controls and to investigate *IL4* and *IL10* gene variations in children with atopic and non-atopic asthma phenotypes. Methods: In this prospective case-control study, a total of 95 unrelated pediatric asthmatic patients were recruited according to the Global Initiative for Asthma (GINA) criteria. The control group comprised two subgroups of 538 and 491 healthy individuals, undergoing *IL4* and *IL10* polymorphism assessments, respectively. The *IL4* -589C/T (rs2243250) and *IL10* -592A/C (rs1800872) gene polymorphisms were evaluated using the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) assay. Results: The findings indicated a significant difference in *IL4* gene polymorphisms at position -589 between the asthmatic and healthy control groups. However, no significant difference was found in terms of *IL10* gene polymorphisms, and they were not associated with atopy in the patients. Conclusion: The *IL4* -589C/T polymorphism (rs2243250) can be a risk factor for asthma susceptibility, whereas the *IL10* -592A/C polymorphism (rs1800872) is not a risk factor in the Iranian pediatric population. The results also showed that these polymorphisms are not risk factors for atopy in asthmatic children.

Keywords: Asthma, interleukin-4 (IL-4), interleukin-10 (IL-10), genetic polymorphism, single-nucleotide polymorphism, atopy

Introduction

Asthma is a chronic inflammatory disease of the airways, characterized by reversible airway obstruction, hyperresponsiveness, and inflammation. To date, two asthma phenotypes have been described, including atopic asthma associated with immunoglobulin E (IgE) production and non-atopic asthma [1, 2]. A positive skin prick test (SPT) can distinguish between these two phenotypes, with a positive response representing atopic asthma [3]. Increased *IL4* and *IL5* gene expression, activated T cells, eosinophils, and high-affinity IgE receptor-bearing cells have been described in bronchial biopsy samples, collected from atopic and non-atopic asthmatic patients. Although increased IL-4 levels have been only detected in the bronchoalveolar lavage specimens of patients with atopic asthma [4], this finding might suggest a shared pathophysiological mechanism underpinning the two variants [5].

Viruses and aeroallergens are two major environmental triggers of airway inflammation in asthma, causing bronchial hyperresponsiveness by assembling type 1 helper (TH1) and TH2-associated cytokines, respectively [6]. These cytokines promote eosinophilic airway inflammation, mucus overproduction, bronchial hyperactivity, and IgE synthesis [7]. T lymphocytes, mast cells, and basophils secrete IL-4,

which plays a significant role in the production of IgE by B cells, as well as differentiation of naive T cells into TH2 [6, 8, 9]. Generally, IL-4 is recognized as a typical pleiotropic Th2 cytokine, involved in IgE-dependent inflammatory reactions. The *IL4* gene resides on chromosome 5q (5q31-33), where many single-nucleotide polymorphisms (SNPs) have been described.

The *IL4* SNPs have been reported in patients with allergic diseases in numerous countries. Several SNPs have been reported in the promoter region, located upstream of the transcription start site of *IL4* gene, including C-590T, C-285T, A-81G, and C-589T. Additionally, some studies have confirmed that differentiation of C to T cells at 589 loci of *IL4* gene can lead to an increase in the concentration of total serum IgE. Consequently, it has been inferred that the single nucleotide variant (C-590T) in the *IL4* promoter region is associated with asthma in both children and adults [9-14]. Nevertheless, it is important to note that studies on different populations have yielded variable results [15] and that determination of SNPs associated with atopy is a challenging task [16, 17].

Regulatory T cells (Treg cells) play a pivotal role in coordinating immune responses to maintain and acquire immune tolerance to allergens through several mediators, especially IL-10. Generally, IL-10 is one of the key immune system regulators, modulating both innate and humoral immunity. It is an anti-inflammatory cytokine [18], secreted by TH1, TH2, monocytes, and tissue macrophages. The most essential function of IL-10 is the inhibition of inflammatory cytokines, such as IL-1, IL-6, tumor necrosis factor-α (TNF-α), and interferon-γ (IFNγ), as well as cytokines related to allergic inflammation, including IL-4 and IL-5 [14, 19, 20].

In allergic patients, the production of IL-10 is reduced. Meanwhile, the *IL10* gene is one of the candidate genes for asthma, located on chromosome 1 (1q31-1q32). Three SNPs in the promoter regions of *IL10* gene, namely, rs1800896 (-1082A>G), rs1800871 (-819C>T), and rs1800872 (-592C>A), have been linked to effective IL-10 production [15, 21]. Moreover, the -592C/A variation of *IL10* gene is associated with higher serum levels of IgE in asthmatic patients, who are homozygous or heterozygous for the SNPs [18, 21]. However, the effects of *IL10* gene polymorphisms on asthmatic patients from different populations remain unknown [14, 15].

The current study aimed to identify and compare *IL4* (589C/T) and *IL10* (592A/C) gene SNPs between asthmatic children and healthy controls and to investigate whether these variations are associated with atopy.

Materials and methods

Study population

This prospective case-control study was performed on 95 unrelated asthmatic patients, who were referred to the Allergy Clinic of Children's Medical Center, Tehran, Iran. Diagnosis of asthma was established according to the Global Initiative for Asthma (GINA) 2023 criteria [22]. The diagnosis was based on the presence of variable respiratory symptoms, such as wheezing, chest tightness, shortness of breath, and cough, along with confirmation of variable airflow limitations, based on one or more of the following tests in children >5 years: (1) positive bronchodilator reversibility test, indicated by an increase in the forced expiratory volume in 1 second (FEV1) >10-12%; (2) positive exercise challenge test, characterized by a decrease in FEV1 >10-12% or peak expiratory flow (PEF) >15%; (3) average daily diurnal PEF variability >13% in children; (4) increase in FEV1 >12% or 200 mL of the predicted value after four weeks of anti-inflammatory treatment; and (5) a clinical diagnosis of asthma by an allergist.

The exclusion criteria were chronic pulmonary diseases other than asthma, congenital heart disease, cystic fibrosis, inborn errors of immunity, prematurity, and history of neonatal intensive care unit (NICU) admission with intubation. We evaluated 75 patients for atopy using the SPT test to identify common aeroallergens and food allergens. Patients showing a wheel diameter of ≥ 3 mm to at least one allergen extract were classified as atopic [23], whereas patients with no skin reaction were considered non-atopic.

In this study, *IL4* (589C/T) and *IL10* (592A/C) gene polymorphisms were assessed in 538

Table 1. Primers used in the tetra-primer ARMS-PCR technique

Loci	SNP	Oligo	Primer sequencing (5'-3')	Tm (°C)	Amplicon (bp)
IL-4	rs2243250 (C/T)	IC-F	AGCCTAGGCAACATAGTGAGACTCTTATC	60.7	466 bp
		IC-R	CAGGTGGCATCTTGGAAACTGTC	61.7	
		Wild Allele-R	AAACACCTAAACTTGGGAGAACATTTTC	61.7	133 bp
		Mutant Allele-F	TCTCCTACCCCAGCACTGGTGA	62.8	382 bp
IL-10	rs1800872 (A/C)	IC-F	AAGCTTTCAGCAAGTGCAGACTACTC	60.5	402 bp
		IC-R	GGTTCTCATTCGCGTGTTCCTAG	60.9	
		Wild Allele-R	TTCCAGAGACTGGCTTCCTACGGT	63	172 bp
		Mutant Allele-F	ACATCCTGTGACCCCGCCTATC	63	275 bp

and 491 unrelated healthy controls, respectively. These individuals, as well as their first-degree relatives, did not report a history of atopy or autoimmunity. The control group was selected among blood transfusion clients, as they represent healthy individuals in the community. We also made sure that genetic polymorphisms in the control group were not influenced by age.

Written informed consent was obtained from the parents or legal guardians of all patients, and the Ethics Committee of Tehran University of Medical Sciences approved the study (Ethics Committee Reference No.: IR.TUMS.CHMC.REC.1398.033).

Molecular methods

After obtaining written consent from the participants, a 5-cc whole blood sample was collected in ethylenediaminetetraacetic acid (EDTA) tube to evaluate the gene polymorphisms. Additionally, 2 cc of the serum was collected separately for the serum total IgE tests. Total genomic DNA was extracted from the peripheral blood of all participants using the salting-out method, as previously described [24]. To ensure the quality of extracted DNA, optical densities (ODs) were determined at 260/280 nm, and DNA concentrations were measured using a NanoDrop 2000 spectrophotometer (Eppendorf, Germany). Moreover, amplification of *IL4* -589C/T (rs2243250) and *IL10* -592A/C (rs1800872) SNP regions was performed using polymerase chain reaction with sequence specific primers (PCR-SSP), as previously discussed [25].

The isolated DNA was amplified using a Techne Flexigene thermal cycler (Rosche, Cambridge, UK) under the following conditions: initial denaturation at 94°C for two minutes, denaturation at 94°C for 10 seconds, annealing and extension at 65°C for one minute (10 cycles), denaturation at 94°C for 10 seconds, annealing at 61°C for 50 seconds; and extension at 72°C for 30 seconds (20 cycles). The presence or absence of PCR products was visualized via 2% agarose gel electrophoresis using an ultraviolet transilluminator. Amplification was carried out using the Techne Flexigene thermal cycler (Roche, Cambridge, UK). The characteristics of the primers are presented in **Table 1**. The PCR products were analyzed via 2% agarose gel electrophoresis; the results were excluded if the quality of the agarose gel was deemed unacceptable.

Statistical analysis

The association between genotypes and variables was evaluated using the Chi-square test. The genotype frequencies were determined in the patient and control groups, using student's *t*-test; a *P*-value <0.05 was considered statistically significant. The odds ratios (ORs) and 95% confidence intervals (CIs) were also measured in a multivariate logistic regression analysis.

Results

A total of 95 children with asthma (64.4% males and 35.6% females) were recruited in this study. The age range of the patients was 2.5-16 years. The results indicated a significant association between sex and the presence of asthma. Atopic dermatitis, food allergies, and allergic rhinitis were observed in 35%, 22%, and 67% of the patients, respectively.

IL4 genotype frequency

The allele frequencies of *IL4* -589C/T (rs2243250) were compared between asthmatic

Table 2. Genotype and Alleles frequencies of IL-4 polymorphisms in asthma children in comparison with normal controls

Loci	Genotypes or Alleles	Children with asthma (n=95), N (%)	Controls (n=538), N (%)	*p*-value
IL-4	C	128 (85.3%)	684 (63.6%)	0.000
	T	T	392 (36.4%)	
rs2243250 (C/T)	CC (Wild type)	67 (70.5%)	148 (27.5%)	0.000
	CT (Heterozygote type)	26 (27.4%)	388 (72.1%)	
	TT (Homozygote type)	2 (2.1%)	2 (0.4%)	

Table 3. Genotype and Alleles frequencies of IL-10 polymorphisms in asthma children in comparison with normal controls

Loci	Genotypes or Alleles	Children with asthma (n=95), N (%)	Controls (n=491), N (%)	*p*-value
IL-10	A	66 (34.7%)	299 (30.4%)	0.243
	C	124 (65.3%)	683 (69.6%)	
AC (Heterozygote type)	AA (Wild type)	11 (11.6%)	41 (8.4%)	0.471
	AC (Heterozygote type)	44 (46.3%)	217 (44.2%)	
	CC (Homozygote type)	40 (42.1%)	233 (47.5%)	

children and healthy controls (**Table 2**). The genotype and allele frequencies of *IL4* gene at -590C/T locus were significantly different between the two groups (P=0.001 and P= 0.001, respectively). The most significant positive allelic association with asthma was attributed to *IL4* -589C allele. The present results indicated a significant correlation between IL4 -589 polymorphism and asthma.

The most frequent genotype in children with asthma was the *IL4* CC genotype at position -589, with a significant difference between the groups (70.5% of the patients vs. 27.5% of the controls; P=0.001). The homozygous TT genotype for this variant was detected in 2.1% of the patients versus 0.4% of the controls. Additionally, a significant difference was observed in the heterozygous CT genotype between the patient and control groups (27.4% vs. 72.1%) (**Table 2**). The probability of asthma in children with C allele was 3.33 times higher than that of children with T allele, and the difference was statistically significant (OR=3.33, 95% CI: 2.86-5.33).

IL10 genotype frequency

The *IL10* -592A/C (rs1800872) allele frequency and genotypes were compared between asthmatic children and healthy controls (**Table 3**). No significant differences were observed in terms of *IL10* -592A/C allele frequency between asthmatic children and healthy controls (P=0.903) (**Table 3**). Moreover, the genotype frequency of *IL10* gene at -592A/C locus was not significantly different between asthmatic children and the controls (P=0.544). Among children with asthma, 46.3% were heterozygous for the AC genotype, 11.6% were homozygous for the A genotype, and 42.1% were homozygous for the C genotype. In the control group, the frequencies of AC, AA, and CC genotypes were estimated at 44.2%, 8.4%, and 47.5%, respectively.

Genotype frequencies in children with atopic and non-atopic asthma

The atopic status of patients with asthma was evaluated using the SPT test. The SPTs were performed on the forearm for 21 inhalants and food allergens in the patients, as well as positive and negative controls. The following allergens were used in this study: tree mix, grass mix, weed mix, mites (Dermatophagoides pteronyssinus and Dermatophagoides farina), cockroaches, Alternaria Alternaria, Penicillium, Aspergillus mix, Cladosporium, feather, cat fur, wheat flour, soya, cow's milk, egg (whole), walnut, hazelnut, peanut, codfish, and tomatoes.

Table 4. Genotypes and Alleles frequencies of IL-4 polymorphisms in atopic asthma patient in comparison with non-atopic asthma children

Loci	Genotypes or Alleles	Atopic asthma children (n=37), N (%)	Non-atopic asthma children (n=38), N (%)	*p*-value
IL-4	C	61 (82.4%)	67 (88.2%)	0.322
	T	13 (17.6%)	9 (11.8%)	
rs2243250 (C/T)	CC	25 (67.6%)	29 (76.3%)	0.476
	CT	11 (29.7%)	9 (23.7%)	
	TT	1 (2.7%)	0 (0%)	

Table 5. Genotype and Alleles frequencies of IL-10 polymorphisms in atopic asthma patient in comparison with non-atopic asthma children

Loci	Genotypes or Alleles	Atopic asthma children (n=37), N (%)	Non-atopic asthma children (n=38), N (%)	*p*-value
IL-10	A	27 (36.5%)	27 (35.5%)	0.903
	C	47 (63.5%)	49 (64.5%)	
rs1800872 (A/C)	AA	3 (8.1%)	5 (13.2%)	0.374
	AC	21 (56.8%)	17 (44.7%)	
	CC	13 (35.1%)	16 (42.1%)	

Atopy was confirmed by a positive reaction (wheal diameter of 3 mm or higher than the negative control) to one or more allergens. The patients were considered to have atopic asthma if they had at least one positive SPT result; otherwise, they were considered non-atopic. Overall, 75 out of 95 patients in the age range of 4-16 years underwent SPT, which indicated 37 cases of atopic asthma and 38 cases of non-atopic asthma; these subgroups were matched in terms of sex, age, and family history of allergic diseases.

Moreover, the frequencies of *IL4* (-589) and *IL10* (-592) genotypes and alleles were investigated in patients with atopic asthma and non-atopic asthma (**Tables 4**, **5**). The homozygous CC genotype of *IL4* gene was observed at position -589 in 66.7% of patients with atopic asthma and 76.3% of patients with non-atopic asthma (OR=1.58, 95% CI: 0.634-3.973, P=0.476). No significant difference was found regarding heterozygous CT and homozygous TT genotypes between the two groups (35.5% and 2.3% in atopic asthmatic patients vs. 23.7% and 0% in non-atopic asthmatic patients, respectively).

On the other hand, the analysis of *IL10* gene polymorphism at position -592 revealed that 58.3% of patients with atopic asthma were heterozygous for the AC genotype, 36.11% were homozygous for the C genotype, and 5.5% were homozygous for the A genotype. In non-atopic asthmatic patients, the frequencies of AC, CC, and AA genotypes were measured to be 44.7%, 42.2%, and 13.6%, respectively (OR= 1.043, 95% CI: 0.555-2.031, P=0.544). The results indicated that *IL4* (-589) and *IL10* (-592) gene polymorphisms were not associated with atopy in asthmatic patients (P>0.05).

Comparison of the serum IgE level between different *IL4* genotype groups at -589C/T locus in asthmatic patients and also atopic and non-atopic asthmatic patients showed no significant difference in terms of the IgE level between the CC, CT, and TT genotypes (mean total IgE of asthmatic patients were, 164.77 IU/mL in CC genotype and 179.38 IU/mL in CT genotype, *P* value: 0.867). Similarly, a comparison of serum IgE levels, between different *IL10* genotype groups at -592A/C locus showed no significant difference in asthmatic patients or the two subgroups of asthmatic patients (mean total IgE of asthmatic patients were, 106.60 IU/mL in AA, 214.25 IU/mL in AC, and 131.63 IU/mL in CC, *P* value: 0.811).

Discussion

In this study, we first analyzed two SNPs of *IL4* gene polymorphism in 95 asthmatic children

and 538 healthy individuals in the control group. Additionally, we examined 491 individuals in the control group for *IL10* gene polymorphism. Next, we compared the genotype and allele frequencies between atopic asthmatic and non-atopic asthmatic patients. Our findings revealed a significant difference between asthmatic children and healthy controls regarding the *IL4* gene SNPs. However, there was no significant difference regarding the *IL10* gene SNP. Also, there was no significant difference between the two SNPs in patients with atopic and non-atopic asthma.

The *IL4* gene polymorphism (-589C/T), located in the promotor region of *IL4* gene, has been found to be associated with asthma [11, 12, 26]. Compared to the C-589 variant, the T-589 allelic variant is associated with an increased production of *IL4* gene. Several case-control studies and meta-analyses have reported the higher frequency of T allele in the C-589T promoter region of the *IL4* gene, as well as the higher frequency of homozygous TT genotype in patients with asthma compared to normal subjects [9, 13, 14]. Additionally, a significant relationship was detected between the T allele in the asthma group and the increased risk of asthma in pediatric and adult patients of different ethnicities [27, 28]. However, Zhang et al. showed no significant association between *IL4* gene promoter polymorphisms and asthma in Chinese children [29]. Meanwhile, a meta-analysis by Zheng X-Y suggested that homozygous individuals for the CC allele had an increased risk of asthma compared to T-allele carriers [15]. In our study, we found that the C allele of *IL4* -589C/T polymorphism (rs2243250) was associated with asthma.

In a meta-analysis by Kousha et al., evaluating *IL4* gene polymorphism (-589C/T) and the risk of asthma revealed that the TT genotype of *IL4* -589C/T polymorphism increased the risk of asthma compared to the CC genotype. In the subgroup analysis by ethnicity, they found a significant association in Asian, American, and European ethnicities, whereas no significant association was found among Arabs [9]; these conflicting results highlight the importance of gene-gene and gene-environment interactions in the pathogenesis of asthma. Environmental and epigenetic factors may also contribute to the discrepancy between the results regarding asthma risk factors. Therefore, further studies on a larger sample size, representing different ethnicities and age groups, are needed to investigate this association in different populations.

Regulatory T (Treg) cells play a pivotal role in coordinating immune responses to maintain and acquire tolerance toward allergens through several mediators, especially IL-10. IL10 is one of the key regulators of the immune system which regulates both innate and humoral immunity. In allergic patients, the amount of IL-10 production is reduced. Meanwhile, the *IL10* gene is one of the candidate genes for asthma. Certain SNPs in the *IL10* gene, namely, *IL10* G-1082A, C-819T, and C-597A, seem to affect the amount of IL-10 production and may be potential risk factors for asthma [14]. However, our findings indicated that *IL10* -592A/C (rs1800872) polymorphism was not associated with asthma, and no significant difference was observed between the two asthma groups. The results reported by Kim also support our findings, as they found no significant differences in the allele or genotype frequencies of these *IL10* polymorphisms between the atopic asthma, non-atopic asthma, and normal control groups [30].

According to some research, there is no evidence of a significant association between *IL10* -592A/C polymorphism and pediatric asthma [15, 29]. Conversely, some studies have demonstrated that *IL10* -592A/C polymorphism is related to asthma phenotypes [15, 16, 31]. In a more recent meta-analysis of rs1800896, rs1800871, and rs1800872 SNPs of *IL10* gene and susceptibility to asthma, rs1800871 was the only *IL10* gene SNP, decreasing the risk of asthma in the European population. The overall analysis of other SNPs and rs1800871 in other populations indicated no significant association between these SNPs and the risk of asthma [19]. Therefore, it is probable that the *IL10* -592A/C locus does not cause any changes in the amount of IL-10 production and does not exert any significant effects on asthma prevention.

The present results indicated no significant relationship between *IL4* -589 and *IL10* -592 polymorphisms and the severity of asthma in the patients. The findings of some previous research are consistent with our results [14], while some others suggested that the *IL4*

-589T allele is associated with the severity of asthma [32, 33]. In this regard, a previous study reported that *IL10* gene polymorphism at position -592 is associated with the regulation of *IL10* expression. Moreover, Katsuyuki Tomita et al. indicated that IL-10 synthesis is attenuated in severe persistent asthma compared to mild asthma [20, 34]. Therefore, *IL10* gene polymorphism at position -592 may be associated with the severity of asthma; nevertheless, further studies on larger populations are recommended to obtain more accurate results.

In the present study, we investigated the allele frequencies and genetic polymorphisms of *IL4* (position 589) and *IL10* (position 592) genes in two groups of asthmatic patients (atopic and non-atopic) and found no significant differences between the groups. Conversely, Yafei Li et al. reported that the CC genotype of *IL4* gene (position 589) was almost 21% less likely to be associated with atopic asthma compared to the CT and TT genotypes [28]. Additionally, Dahmani et al. found that the TT genotype of *IL4* C589T polymorphism was significantly related to atopic asthma [26]. Also, in a meta-analysis by Song Liu et al., it was suggested that *IL4* C-589T polymorphism probably contributes to atopic asthma susceptibility, especially in Caucasians [35]. Besides, Y.M. Hussein et al. found a significant association between *IL10* 1082G/A polymorphism and atopic asthma [36]. Due to differences in the results, it is important to consider gene-environment interactions and racial differences. Further investigations are recommended to reach a better understanding of the relationship between these genetic polymorphisms and atopic asthma susceptibility.

Multiple studies have reported that asthmatic patients have high total IgE levels, regardless of their atopy status [4, 5, 37-39]. In the present study, no significant difference was found in the serum IgE levels of patients with atopic and non-atopic asthma. Moreover, the serum IgE levels were not significantly different between the groups of asthmatic patients with different *IL4* (position 589) and *IL10* (position 592) polymorphisms. These findings are consistent with the results of some other studies, which also reported no significant differences in the serum IgE level and *IL4* gene polymorphisms of asthmatic patients [27, 40]. Additionally, in a study by Kim on asthmatic children, the *IL10* genotype or haplotype had no significant effects on the serum IgE level [30].

Conclusion

In this case-control study, the results revealed that the *IL4* -589C/T (rs2243250) polymorphism was associated with an increased risk of asthma in Iranian children, unlike the *IL10* -592A/C polymorphism (rs1800872). On the other hand, these polymorphisms were not risk factors for atopy in asthmatic patients. Considering the discrepancy between the results of studies on the role of polymorphisms of these two interleukins in the pathogenesis of asthma and atopy in different populations from different countries, further research is needed on asthma subgroups, based on atopy or asthma severity. Also, future studies need to elucidate the functional significance of these SNPs in the pathogenesis of asthma.

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Disclosure of conflict of interest

None.

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References

[1] Sobeih AA, Behairy OG, Abd Almonaem ER, Mohammad OI and Mn Abdelrahman A. Clusterin in atopic and non-atopic childhood asthma. Scand J Clin Lab Invest 2019; 79: 368-371.

[2] Potaczek DP, Miethe S, Schindler V, Alhamdan F and Garn H. Role of airway epithelial cells in

the development of different asthma phenotypes. Cell Signal 2020; 69: 109523.
[3] Castro-Rodriguez JA, Ramirez AM, Toche P, Pavon D, Perez MA, Girardi G and Garcia-Marcos L. Clinical, functional, and epidemiological differences between atopic and nonatopic asthmatic children from a tertiary care hospital in a developing country. Ann Allergy Asthma Immunol 2007; 98: 239-244.
[4] Böttcher MF, Bjurström J, Mai XM, Nilsson L and Jenmalm MC. Allergen-induced cytokine secretion in atopic and non-atopic asthmatic children. Pediatr Allergy Immunol 2003; 14: 345-350.
[5] Morahan G, Huang D, Wu M, Holt BJ, White GP, Kendall GE, Sly PD and Holt PG. Association of IL12B promoter polymorphism with severity of atopic and non-atopic asthma in children. Lancet 2002; 360: 455-459.
[6] Jung JS, Park BL, Cheong HS, Bae JS, Kim JH, Chang HS, Rhim T, Park JS, Jang AS, Lee YM, Kim KU, Uh ST, Na JO, Kim YH, Park CS and Shin HD. Association of IL-17RB gene polymorphism with asthma. Chest 2009; 135: 1173-1180.
[7] Beghé B, Barton S, Rorke S, Peng Q, Sayers I, Gaunt T, Keith TP, Clough JB, Holgate ST and Holloway JW. Polymorphisms in the interleukin-4 and interleukin-4 receptor α chain genes confer susceptibility to asthma and atopy in a Caucasian population. Clin Exp Allergy 2003; 33: 1111-1117.
[8] Lambrecht BN, Hammad H and Fahy JV. The cytokines of asthma. Immunity 2019; 50: 975-991.
[9] Kousha A, Mahdavi Gorabi A, Forouzesh M, Hosseini M, Alexander M, Imani D, Razi B, Mousavi MJ, Aslani S and Mikaeili H. Interleukin 4 gene polymorphism (-589C/T) and the risk of asthma: a meta-analysis and met-regression based on 55 studies. BMC Immunol 2020; 21: 55.
[10] Bijanzadeh M, Mahesh PA and Ramachandra NB. An understanding of the genetic basis of asthma. Indian J Med Res 2011; 134: 149-61.
[11] Berenguer AG, Fernandes AT, Oliveira S, Rodrigues M, Ornelas P, Romeira D, Serrão T, Rosa A and Câmara R. Genetic polymorphisms and asthma: findings from a case-control study in the Madeira island population. Biol Res 2014; 47: 40.
[12] Ruan Z, Shi Z, Zhang G, Kou J and Ding H. Asthma susceptible genes in children: a meta-analysis. Medicine (Baltimore) 2020; 99: e23051.
[13] Zhang JH, Zhang M, Wang YN and Zhang XY. Correlation between IL-4 and IL-13 gene polymorphisms and asthma in Uygur children in Xinjiang. Exp Ther Med 2019; 17: 1374-1382.
[14] Smolnikova MV, Smirnova SV, Freidin MB and Tyutina OS. Immunological parameters and gene polymorphisms (C-590T IL4, C-597A IL10) in severe bronchial asthma in children from the Krasnoyarsk region, West Siberia. Int J Circumpolar Health 2013; 72.
[15] Zheng XY, Guan WJ, Mao C, Chen HF, Ding H, Zheng JP, Hu TT, Luo MH, Huang YH and Chen Q. Interleukin-10 promoter 1082/-819/-592 polymorphisms are associated with asthma susceptibility in Asians and atopic asthma: a meta-analysis. Lung 2014; 192: 65-73.
[16] Lyon H, Lange C, Lake S, Silverman EK, Randolph AG, Kwiatkowski D, Raby BA, Lazarus R, Weiland KM, Laird N and Weiss ST. IL10 gene polymorphisms are associated with asthma phenotypes in children. Genet Epidemiol 2004; 26: 155-165.
[17] Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M and Borish L. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995; 25 Suppl 2: 74-78.
[18] Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J and Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. J Allergy Clin Immunol 1996; 97: 1288-1296.
[19] Imani D, Dashti N, Parvari A, Shafiekhani S, Alebrahim F, Razi B, Makoui MH, Motallebnezhad M, Aslani S and Aliyu M. Interleukin-10 gene promoter polymorphisms and susceptibility to asthma: systematic review and meta-analysis. Biochem Genet 2021; 59: 1089-1115.
[20] Karimabad MN, Arababadi MK, Hakimizadeh E, Daredori HY, Nazari M, Hassanshahi G and Kennedy D. Is the IL-10 promoter polymorphism at position -592 associated with immune system-related diseases? Inflammation 2013; 36: 35-41.
[21] Vázquez-Villamar M, Palafox-Sánchez CA, Hernández-Bello J, Muñoz-Valle JF, Valle Y, Cruz A, Alatorre-Meza AI and Oregon-Romero E. Frequency distribution of interleukin-10 haplotypes (-1082 A>G, -819 C>T, and -592 C>A) in a Mexican population. Genet Mol Res 2016; 15.
[22] Levy ML, Bacharier LB, Bateman E, Boulet LP, Brightling C, Buhl R, Brusselle G, Cruz AA, Drazen JM, Duijts L, Fleming L, Inoue H, Ko FWS, Krishnan JA, Mortimer K, Pitrez PM, Sheikh A, Yorgancıoğlu A and Reddel HK. Key recommendations for primary care from the 2022 Global Initiative for Asthma (GINA) update. NPJ Prim Care Respir Med 2023; 33: 7.
[23] Almeida ALM, Perger ELP, Gomes RHM, Sousa GDS, Vasques LH, Rodokas JEP, Olbrich Neto J and Simões RP. Objective evaluation of immediate reading skin prick test applying image

planimetric and reaction thermometry analyses. J Immunol Methods 2020; 487: 112870.
[24] Miller SA, Dykes DD and Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.
[25] Amirzargar AA, Naroueynejad M, Khosravi F, Dianat SS, Rezaei N, Mytilineos J and Nikbin B. Cytokine single nucleotide polymorphisms in Iranian populations. Eur Cytokine Netw 2008; 19: 104-112.
[26] Dahmani DI, Sifi K, Salem I, Chakir J, Hanachi S, Bachtarzi MZ, Rouabah L, Abadi N, Bougrida M and Rouabhia M. 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 2016; 37: 213-223.
[27] Chiang CH, Tang YC, Lin MW and Chung MY. Association between the IL-4 promoter polymorphisms and asthma or severity of hyperresponsiveness in Taiwanese. Respirology 2007; 12: 42-48.
[28] Li Y, Guo B, Zhang L, Han J, Wu B and Xiong H. Association between C-589T polymorphisms of interleukin-4 gene promoter and asthma: a meta-analysis. Respir Med 2008; 102: 984-992.
[29] Zhang J, Chen H, Hu L, Fu J, Zhang H and Chen Y. Correlation between polymorphism of IL-4 and IL-10 gene promoter and childhood asthma and their impact upon cytokine expression. Zhonghua Yi Xue Za Zhi 2002; 82: 114-118.
[30] Kim KW, Lee KE, Hong JY, Kim MN, Heo WI, Sohn MH and Kim KE. Involvement of IL-10 gene promoter polymorphisms in the susceptibility for childhood asthma. Lung 2011; 189: 417-23.
[31] Movahedi M, Mahdaviani SA, Rezaei N, Moradi B, Dorkhosh S and Amirzargar AA. IL-10, TGF-ß, IL-2, IL-12, and IFN-γ cytokine gene polymorphisms in asthma. J Asthma 2008; 45: 790-794.
[32] Sandford AJ, Chagani T, Zhu S, Weir TD, Bai TR, Spinelli JJ, FitzGerald JM, Behbehani NA, Tan WC and Paré PD. Polymorphisms in the IL4, IL4RA, and FCERIB genes and asthma severity. J Allergy Clin Immunol 2000; 106: 135-140.
[33] Steinke JW and Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. Respir Res 2001; 2: 66-70.
[34] Tomita K, Lim S, Hanazawa T, Usmani O, Stirling R, Chung KF, Barnes PJ and Adcock IM. Attenuated production of intracellular IL-10 and IL-12 in monocytes from patients with severe asthma. Clin Immunol 2002; 102: 258-266.
[35] Liu S, Li T and Liu J. Interleukin-4 rs2243250 polymorphism is associated with asthma among caucasians and related to atopic asthma. Cytokine 2012; 59: 364-369.
[36] Hussein YM, Alzahrani SS, Alharthi AA, Ghonaim MM, Alhazmi AS, Eed EM and Shalaby SM. Association of serum cytokines levels, interleukin 10 -1082G/A and interferon-γ +874T/A polymorphisms with atopic asthma children from Saudi Arabia. Cell Immunol 2014; 289: 21-26.
[37] Lavoie ME, Meloche J, Boucher-Lafleur AM, Bégin P, Morin C, Boulet LP, Madore AM and Laprise C. Longitudinal follow-up of the asthma status in a French-Canadian cohort. Sci Rep 2022; 12: 13789.
[38] Contoli M, Santus P, Menzella F, Rocchi C, Radovanovic D, Baraldi F, Martelli C, Casanova S, Barbetta C, Micheletto C, Scichilone N, Beghè B, Carpagnano E and Papi A. Effects of anti-IL5 biological treatments on blood IgE levels in severe asthmatic patients: a real-life multicentre study (BIONIGE). Clin Transl Allergy 2022; 12: e12143.
[39] Melscoet L, Khayath N, Migueres N, Goltzene MA, Meyer N and de Blay F. Severe non-atopic asthma: omalizumab can reduce severe asthma exacerbations. J Asthma 2023; 60: 881-889.
[40] Elsaid A, Shoaib RMS, Badr SS, Wahba Y and Ayyad SN. Polymorphisms of interleukin 4 and interleukin 4 receptor genes and bronchial asthma risk among Egyptian children. Clin Biochem 2021; 93: 66-72.