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Association of interleukin-17A genetic polymorphisms with risk of asthma: A case-control study in Iraqi patients

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ABSTRACT

Interleukin-17A (IL-17A) is pro-inflammatory cytokine that has been suggested to influence susceptibility to asthma. This study sought to explore serum level and three variants of *IL17A* gene (rs2275913 G/A, rs3819024 A/G and rs8193036 C/T) in Iraqi asthmatics to evaluate their association with susceptibility to disease. A case-control study was conducted on 104 asthmatics and 111 healthy controls. Serum level of IL-17A was determined with Enzyme linked-immunosorbent assay, while the ARMS-PCR method (tetra-primer-amplification-refractory-mutation system-polymerase chain reaction) was used for amplification and genotyping of *IL17A* variants. Median IL-17A level was significantly elevated in asthmatics compared to controls. Receiver operating characteristic curve analysis revealed that IL-17A was a significant predictor of asthma, and a very good area under curve was demonstrated. Logistic regression analysis revealed that allele and genotype frequencies of two variants (rs2275913 and rs8193036) showed significant differences between patients and controls. GA + AA and GA frequencies of rs2275913 variant were significantly increased in patients compared to controls. For rs8193036 variant, frequencies of *T* allele and CT genotype were significantly lower in patients than in controls. In conclusion, IL-17A was upregulated in sera of asthmatics. Moreover, the rs2275913 variant was associated with a higher risk of developing asthma, while the rs8193036 variant was possibly associated with protective effects.

1. Introduction

Asthma is an increasingly global respiratory disorder that affects about a third of the world's population and approximately 2.5 million asthmatics die annually from complications of disease (Rehman et al., 2018). It is defined as immune-mediated inflammation characterized by excessive airway response to environmental allergens and viral and bacterial respiratory infections (Patel and Teach, 2019). Asthma is a complex multifactorial disease largely driven by inappropriate immune responses to environmental challenges in genetically susceptible individuals (Hossain et al., 2019). Human and animal model studies of asthma indicate that multiple gene polymorphisms are involved in conferring susceptibility to or protection against the disease (Morales and Duffy, 2019). Genes encoding polymorphic proteins, particularly those involved in regulating immune responses, have been the focus of extensive research regarding genetic susceptibility to asthma (Demenais et al., 2018). More than 60 genetic loci have been linked to asthma, and genome-wide association studies (GWASs) in children and adults with asthma have identified that several of these genes are involved in immune responses (Schoettler and Strek, 2020). The GWASs have also identified that some of these genes encode the immune modulators cytokines and their variants have been proposed to influence the susceptibility to asthma (Laulajainen-Hongisto et al., 2020). Interleukin (IL)-17A is among these cytokines and has been shown to cause structural changes in airway smooth muscle cells, and thus may influence susceptibility to airway hyperresponsiveness (AHR) and development of asthma (Thompson et al., 2020).

IL-17A is a member of the IL-17 family of cytokines mainly produced by T helper 17 (Th17) cells (Gu et al., 2013). It is a cytokine with pro-inflammatory properties, and plays key roles in host's defenses against microbial pathogens and development of inflammation (Chen and Kolls, 2017). However, unrestrained signaling of IL-17A has been linked with immunopathology, autoimmune diseases, and cancer development (Amatya et al., 2017). Further, it has been reviewed that aberrant expression or overexpression of IL-17A is implicated in a number of pathological conditions, including asthma and other related pulmonary

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Table 1
Baseline characteristics of asthmatics and controls.

Characteristic	Asthmatics; *N* = 104	Control; *N* = 111	*p*
Age (mean ± SD); year	37.9 ± 12.1	35.9 ± 10.5	0.190
Gender; *N* (%)			
Male	50 (48.1)	54 (48.6)	1.000
Female	54 (51.9)	57 (51.4)	
Family history; *N* (%)			
Yes	54 (51.9)	NA	NA
No	50 (48.1)	NA	NA
Severity; *N* (%)			
Mild	49 (47.1)	NA	NA
Moderate/Severe	55 (52.9)	NA	NA
Atopy; *N* (%)			
Atopic	74 (71.2)	NA	NA
Nonatopic	30 (28.8)	NA	NA
Total IgE (mean ± SD); ng/mL	210.3 ± 48.4	146.8 ± 51.3	**<0.001**
Allergen type; *N* (%)			
Animal dander	2 (1.9)	NA	NA
Grasses	8 (7.7)	NA	NA
Mites	34 (32.7)	NA	NA
Molds	8 (7.7)	NA	NA
Mixed	22 (21.2)	NA	NA
Negative	30 (28.8)	NA	NA

SD: Standard deviation; NA: Not applicable; *p*: Student t-test or Fisher exact test probability (significant *p*-value is bold-marked).

disorders (Gurczynski and Moore, 2018). In addition, IL-17A is a critical cytokine in mediating neutrophilic inflammation; therefore, direct association between IL-17A and the processes of chronic airway inflammation and remodeling in severe asthma has been described (Ramakrishnan et al., 2019). It has also been shown that viral, bacterial and fungal infections of the respiratory system can affect the Th17/IL-17A axis, thus promoting the occurrence and development of asthma (Liu et al., 2020a, 2020b). It has also been revealed that IL-17A level and gene expression were elevated in the blood, sputum, and bronchoalveolar of asthmatic patients, and were positively correlated with disease severity (Hynes and Hinks, 2020).

IL-17A is encoded by a gene (*IL17A*) mapped to a region on the short arm of human chromosome 6 (6p12.2). Single nucleotide polymorphisms (SNPs) in the *IL17A* gene have been associated with susceptibility to various immune-mediated and inflammatory diseases (Pasha et al., 2019; Ruiz de Morales et al., 2020; Shao et al., 2020). Moreover, it has been proposed that these SNPs can modulate the expression of *IL17A* gene and may also affect Th17 cell functions (Chen and Kolls, 2017; Huang et al., 2017). A number of case-control and meta-analysis studies have been conducted in different ethnic and population groups to determine the role of *IL17A* gene SNPs in susceptibility to asthma but some results have not been replicated (Du et al., 2016; Liang et al., 2018; Nuolivirta et al., 2018; Zhai et al., 2018; Zhu et al., 2016).

This study sought to explore serum level and three SNPs of *IL17A* gene (rs2275913 G/A, rs3819024 A/G and rs8193036 C/T) in Iraqi patients with asthma to evaluate their association with susceptibility to disease.

2. Materials and methods

2.1. Populations studied

A case-control study was conducted on 104 adults with asthma at the Allergy Specialist Center (Rusafa, Baghdad, Iraq) during the period from January to June 2019. The Global Asthma Initiative (GINA) guidelines were followed in diagnosing asthma and grouping patients by disease severity (Becker and Abrams, 2017). Patients included in the study were those with a history of coughing accompanied by wheezing or shortness of breath (dyspnea) within the past 12 months. Patients with chronic respiratory infections or who had no apparent symptoms of asthma, as well as pregnant women, were excluded. The study also included a control group of 111 healthy individuals matched patients for age and gender. The controls were blood donors and had no signs or symptoms of allergy. Written consent was obtained from all participants after approval by the Ethics Committee at the Allergy Specialist Center.

Patients and controls were characterized for age and gender. The patients were also characterized for family history, asthma severity (mild and moderate/severe asthma), atopy, total IgE level, and allergen type (animal dander, herbs, mites, and molds). According to the type of allergen, the patient was considered non-atopic (seropositive) or atopic (seropositive) (Pillai et al., 2011). A family history of asthma was determined if one of the family members had a pulmonary allergy (siblings, parents, or grandparents) (Table 1).

2.2. Determination of serum total IgE, IL-17A and specific IgE

Sandwich enzyme-linked immunosorbent assay (ELISA) kits were used to determine levels of IgE and IL-17A in sera of participants (Sunlong Biotech Company, China). The detection ranges of kits were 6–400 ng/mL and 3.3–200 pg/mL, respectively. Serum evaluation for specific IgE was also performed in asthmatics (seronegative or seropositive) using multiplex immunoblot kit (Euroimmun, Germany). Twenty-five inhalation allergens were detected by the kit. For simplicity, the allergens were limited to four main types (animal dander, grasses, mites and molds). The tests were performed using the protocols recommended by the manufacturers.

Table 2
Primer sequences used in the tetra-primer amplification refractory mutation system-polymerase chain reaction for the detection of *IL17A* gene single nucleotide polymorphisms.

IL17A gene SNP	Position (Ch 6)[a]	Primer name	Sequence (5′–3′)	Product size (bp)
rs2275913 G/A	5′UTR: 52186235	Outer Forward	GGTACATGACACCAGAAGACCTACA	Outer: 375
		Outer Reverse	CCTGCTATGAGATGGACAAAATGT	
		Inner Forward	TTCCCATTTTCCTTCAGACGA	*G* allele: 246
		Inner Reverse	CCCAATGAGGTCATAGAAGAATCTATC	*A* allele: 177
rs3819024 A/G	5′UTR: 52185988	Outer Forward	ATCTCCATCACCTTTGTCCAGTC	Outer: 524
		Outer Reverse	GGAAGGGCAGAAATTCATGTTCCTA	
		Inner Forward	GGCCAAGGAATCTGTGATGA	*A* allele: 249
		Inner Reverse	TTGATTTTCCATTTGATCTTTCTGTC	*G* allele: 321
rs8193036 C/T	5′UTR: 52185695	Outer Forward	AGCATGTAGAATATGGGATACCAGC	Outer: 549
		Outer Reverse	AATTCCGGAAACTACTCAAGTTCC	
		Inner Forward	GCCCCCCTTTTCTCCATATT	*C* allele: 315
		Inner Reverse	TAGAGACTGGACAAAGGTGAGGG	*T* allele: 277

SNP: Single nucleotide polymorphism; Ch: Chromosome; bp: base-pair; T: Temperature.
[a] Location on chromosome based on dbSNP build 154 (Released April 21, 2020).

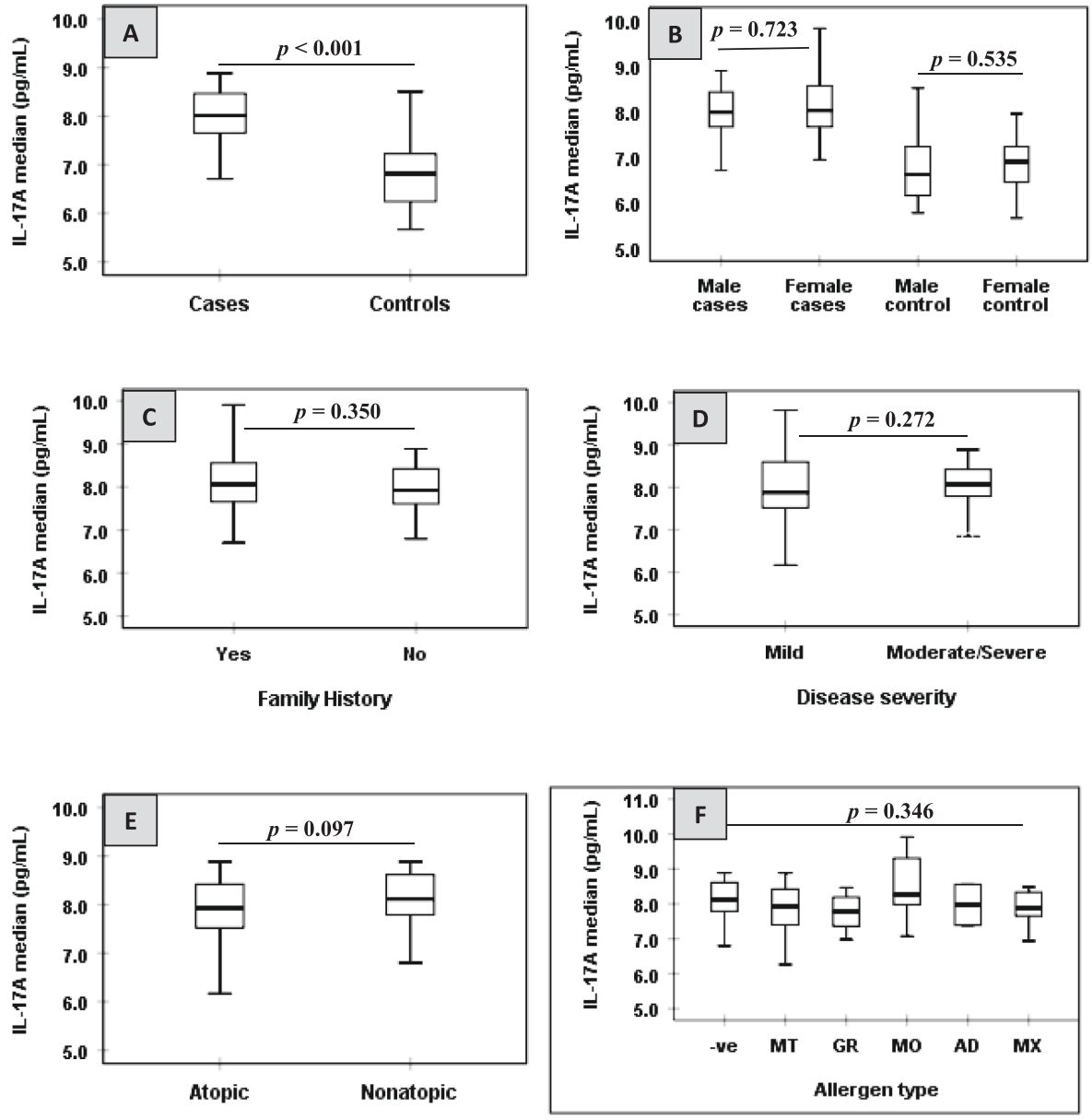

Fig. 1. Boxplot presentations of IL-17A median level in asthmatics and controls. The median was significantly increased in patients compared to controls (A), while no significant difference was found after stratification by gender (B), family history (C), disease severity (D), atopy (E) or allergen type (F). MT: Mites; GR: Grasses; MO: Molds; AD: Animal dander; MX: Mixed; *p*: Mann–Whitney *U* (to compare two groups) or Kruskal–Wallis (to compare more than two groups) test probability.

2.3. Genotyping of IL17A gene SNPs

Three *IL17A* gene SNPs associated with asthma were examined (rs2275913 G/A, rs3819024 A/G and rs8193036 C/T) as previously suggested (Du et al., 2016; Liang et al., 2018; Zhai et al., 2018; Zhu et al., 2016). The manual salting-out procedure was employed to isolate whole genomic DNA from EDTA blood (Kalousová et al., 2017). The ARMS-PCR method (tetra-primer amplification refractory mutation system-polymerase chain reaction) was used for amplification and genotyping of *IL17A* SNPs using two non-specific (outer forward and reverse) and two allele-specific (inner forward and reverse) primers as given in Table 2 (Medrano and De Oliveira, 2014). The primers were designed using primer design web-based service for tetra-primer ARMS-PCR (Collins and Ke, 2012), and validated using Primer-BLAST-NCBI database. The ARMS-PCR was conducted in 20 μL volume containing 2 μL of template DNA (20–50 ng/μL), 2 μL of AccuPower PCR PreMix (Bioneer, Korea), 0.5 μL of each primer (10 ρmol/μL) and 15 μL of DNAse-free water. The thermocycling was optimized for the following conditions: an initial denaturation (94 °C for 4 min), followed by 30 cycles of denaturation (94 °C for 30 s), annealing (58 °C for 30 s) and elongation (72 °C for 40 s), and a final single extension step (72 °C for 5 min). The ARMS-PCR products were electrophoresed on 2% agarose-gel, and migrating bands were visualized using a gel documentation system.

2.4. Statistical analysis

Pearson Chi-square test or two-tailed Fisher exact test were used to analyze categorical variables. Continuous variables were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed variables (parametric) were given as mean ± standard deviation (SD), and comparisons were made with Student *t*-test. Skewed variables (non-parametric) were given as median and interquartile range (IQR: 25–75%) and comparisons were made using either Mann–Whitney *U* (to compare to groups) or Kruskal–Wallis (to compare

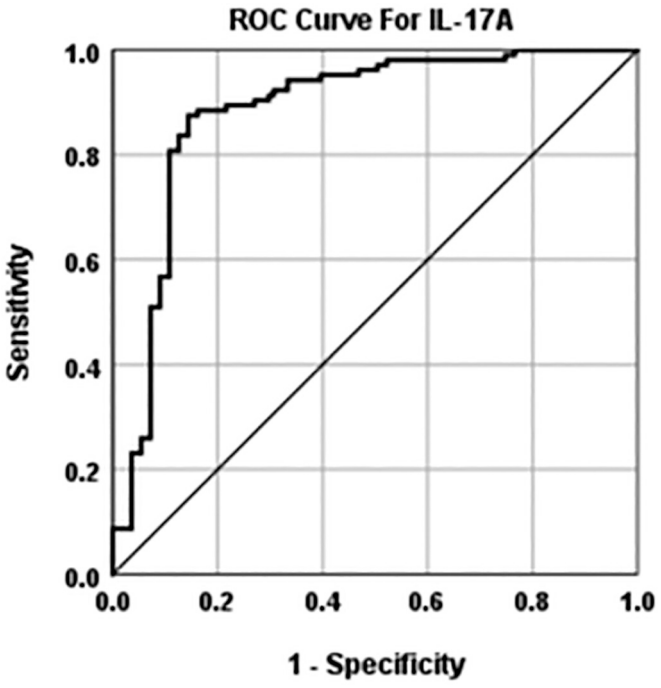

Fig. 2. Receiver-operating characteristic (ROC) curve analysis of IL-17A in asthmatics. The estimated area under curve was 0.884 (95% CI: 0.835–0.932; $p < 0.001$). At a cut-off value of 7.4 pg/mL, the sensitivity and specificity of IL-17A were 87.5 and 85.6%, respectively.

more than two groups) test. Hardy-Weinberg equilibrium (HWE) was assessed using Pearson Chi-square goodness of fit test. Receiver operating characteristic (ROC) analysis was employed to assess the diagnostic performance of IL-17A and to estimate area under curve (AUC), sensitivity, specificity and cut-off value. Genetic association between *IL17A* gene SNPs and asthma risk was evaluated using logistic regression analysis under five genetic models (allele, recessive, dominant, overdominant and codominant). The association was expressed as odds ratio (OR) and 95% confidence interval (CI). The statistical package IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) was used to perform these analyses. Pairwise linkage disequilibrium (LD) between *IL17A* gene SNPs were determined with SHEsis software (Shi and He, 2005). G*Power 3 software was used to estimate power of sample size (Faul et al., 2007). A probability value (p) ≤ 0.05 was considered statistically significant after adjustment for multiple comparisons (Bonferroni correction).

3. Results

3.1. Power of sample size

Under 0.05 α error of probability, a sample of 104 patients and 111 controls achieved an actual power of 0.73 (1 – β error probability).

3.2. IL-17A serum level

Median level of IL-17A was significantly elevated in asthmatics compared to controls (8.0 [IQR: 7.7–8.5] vs. 6.8 [IQR: 6.2–7.2] pg/ml; $p < 0.001$). Stratification of patients according to gender, family history, asthma severity, atopy or type of allergen revealed no statistically

Table 3
Genetic association and Hardy-Weinberg equilibrium analyses of *IL17A* gene SNPs in asthmatics and controls.

SNP/Genetic model	Allele/genotype	*N* (%)		OR	95% CI	*p* (adjusted *p*)
		Asthmatics; $N = 104$	Controls; $N = 111$			
rs2275913						
Allele	*G*	170 (81.7)	200 (90.1)	Reference		
	A	38 (18.3)	22 (9.9)	2.03	1.16–3.57	**0.017** (0.102)
Recessive	GG + GA	103 (99.0)	110 (99.1)	Reference		
	AA	1 (1.0)	1 (0.9)	1.07	0.07–17.30	0.963(1.000)
Dominant	GG	67 (64.4)	90 (81.1)	Reference		
	GA + AA	37 (35.6)	21 (18.9)	2.37	1.27–4.41	**0.007 (0.042)**
Overdominant	GG + AA	68 (65.4)	91 (82.0)	Reference		
	GA	36 (34.6)	20 (18.0)	2.41	1.28–4.53	**0.006 (0.036)**
Codominant	GG	67 (64.4)	90 (81.1)	Reference		
	GA	36 (34.6)	20 (18.0)	2.42	1.29–4.55	**0.006 (0.036)**
	AA	1 (1.0)	1 (0.9)	1.34	0.08–21.87	0.836 (1.000)
HWE-*p*		0.105	0.924			
rs3819024						
Allele	*A*	106 (51.0)	117 (52.7)	Reference		
	G	102 (49.0)	105 (47.3)	1.07	0.73–1.56	0.772(1.000)
Recessive	AA+AG	80 (76.9)	84 (75.7)	Reference		
	GG	24 (23.1)	27 (24.3)	0.93	0.50–1.75	0.830 (1.000)
Dominant	AA	26 (25.0)	33 (29.7)	Reference		
	AG + GG	78 (75.0)	78 (70.3)	1.27	0.70–2.32	0.438 (1.000)
Overdominant	AA+AG	50 (48.1)	60 (54.1)	Reference		
	AG	54 (51.9)	51 (45.9)	1.27	0.74–2.17	0.381 (1.000)
Codominant	AA	26 (25.0)	33 (29.7)	Reference		
	AG	54 (51.9)	51 (45.9)	1.34	0.71–2.55	0.366 (1.000)
	GG	24 (23.1)	27 (24.3)	1.123	0.53–2.40	0.753 (1.000)
HWE-*p*		0.692	0.409			
rs8193036						
Allele	*C*	197 (94.7)	190 (85.6)	Reference		
	T	11 (5.3)	32 (14.4)	0.33	0.16–0.68	**0.002 (0.004)**
Codominant	CC	93 (89.4)	79 (71.2)	Reference		
	CT	11 (10.6)	32 (28.8)	0.29	0.14–0.62	**0.001 (0.002)**
	TT	ND	ND			
HWE-*p*		0.569	0.076			

HWE: Hardy-Weinberg equilibrium; *p*: Two-tailed Fisher's exact or Chi-square test probability (significant *p*-value is bold-marked); Adjusted *p*: Bonferroni correction adjusted *p*; SNP: Single nucleotide polymorphism; OR: Odds ratio; CI: Confidence interval.

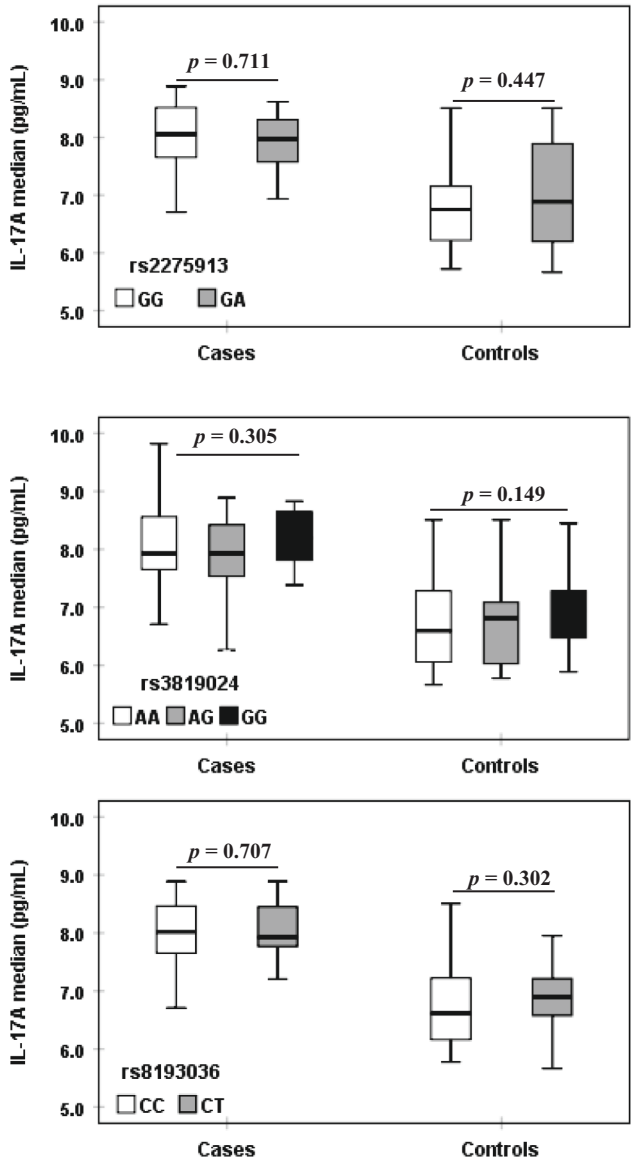

Fig. 3. Serum level of IL-17A stratified by *IL17A* gene SNP genotypes (rs2275913, rs3819024 and rs8193036) in asthmatics and controls. The genotype had no significant influence on IL-17A level. *p*: Mann–Whitney *U* (to compare two groups) or Kruskal–Wallis (to compare more than two groups) test probability.

significant differences with respect to the distributions of IL-17A median levels (Fig. 1). ROC curve analysis revealed that IL-17A was a significant predictor of asthma, and a very good AUC was demonstrated (AUC = 0.884; 95% CI: 0.835–0.932; $p < 0.001$). At a cut-off value of 7.4 pg/mL, IL-17A showed a sensitivity of 87.5% and a specificity of 85.6% (Fig. 2).

3.3. *IL17A* gene SNPs

Three SNPs of *IL17A* gene (rs2275913 G/A, rs3819024 A/G and rs8193036 C/T) were analyzed in asthmatics and controls under five genetic models (allele, recessive, dominant, overdominant and codominant). Genotype frequencies of the three SNPs were compatible with HWE and no significant differences were found between the observed and expected frequencies in patients or controls (Table 3).

Logistic regression analysis revealed that allele and genotype frequencies of two SNPs (rs2275913 and rs8193036) showed significant differences between patients and controls. Frequency of *A* allele of the SNP rs2275913 was higher in patients than in controls, but the adjusted *p* was not significant ($p = 0.017$; adjusted $p = 0.102$). However, GA + AA (dominant model) and GA (overdominant and codominant models) frequencies of the SNP rs2275913 were significantly increased in patients compared to controls. The corresponding ORs were 2.37 (95% CI: 1.27–4.41; $p = 0.007$; adjusted $p = 0.042$), 2.41 (95% CI: 1.28–4.53; $p = 0.006$; adjusted $p = 0.036$) and 2.42 (95% CI: 1.29–4.55; $p = 0.006$; adjusted $p = 0.036$), respectively. For SNP rs8193036, only two genotypes were detected (CC and CT). Frequencies of *T* allele and CT genotype were lower in patients than in controls, and the adjusted *p* of both differences was significant (0.004 and 0.002, respectively (Table 3).

When asthmatics stratified according to gender, family history, disease severity and atopy, allele frequencies of rs2275913, rs3819024 and rs8193036 SNPs showed no significant differences (data not shown). Further, genotypes of these SNPs had no influence on serum level of IL-17A in patients or controls (Fig. 3).

3.4. *Linkage disequilibrium*

Pairwise analysis of *IL17A* SNPs (rs2275913, rs3819024 and rs8193036) using SHEsis software revealed that the three SNPs did not show LD (Fig. 4).

4. Discussion

This study demonstrated that IL-17A was upregulated in sera of asthmatics. These findings are consistent with results of other studies, where elevated levels of IL-17A were found in peripheral blood and broncho-alveolar lavage fluids of asthmatics (Dimitrova et al., 2019; Hasegawa et al., 2017; Hynes and Hinks, 2020; Mansour et al., 2017; Shabana et al., 2019). Molecular studies confirmed that mRNA expression of *IL17A* gene was upregulated in patients with asthma (Alyasin et al., 2013; Eltaweel et al., 2018; Pandey and Prakash, 2020). Together, these findings indicate that IL-17A is a cytokine that plays a key role in immunopathogenesis of asthma. Functionally, IL-17A is a pro-inflammatory cytokine that can activate the airway epithelial cells and alveolar macrophages to produce pro-inflammatory cytokines and chemokines; for instance, IL-1, IL-6, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor-alpha (TNF-α), which enhance the recruitment of neutrophils into the lungs and thus inflammatory episodes are indirectly promoted by IL-17A. Therefore, neutrophilic airway inflammation has been shown to be an important asthma phenotype often associated with severe and acute exacerbation of asthma (Gao et al., 2017). Moreover, human lung endothelial cells also express IL-17A and IL-17F receptors (IL-17RA and IL-17RC), and when activated by IL-17, they release CXCL1, which is a chemoattractant for neutrophils (Hynes and Hinks, 2020). A further phenotype of asthma is mediated by eosinophils (eosinophilic asthma), and these cells also express IL-17RA and IL-17RC. Therefore, neutrophils and eosinophils are indirect targets for IL-17A in exaggerating the airway inflammation and AHR (Possa et al., 2013). Accordingly, the serum level of IL-17A has been proposed as an independent risk factor associated with asthma or the disease severity (Hynes and Hinks, 2020). This study confirms this suggestion and ROC curve analysis demonstrated that IL-17A was a very good predictor of asthma with a diagnostic sensitivity of 87.5% and a specificity of 85.6%. Another study showed that the IL-17A/IL-10 ratio could be used as a biomarker for improving asthma in patients receiving vitamin D supplements. The authors found that IL-17A levels were elevated in asthmatics with vitamin D deficiency, while IL-10 levels tended to be lower. When taking vitamin D supplements, IL-17A levels decreased while IL-10 levels increased (Shabana et al., 2019).

Case-control studies have also identified that *IL17A* gene variants (SNPs) are associated with susceptibility to asthma. These studies motivated us to make further understanding of IL-17A role in etiology and pathogenesis of asthma. Most of the studies investigated three SNPs,

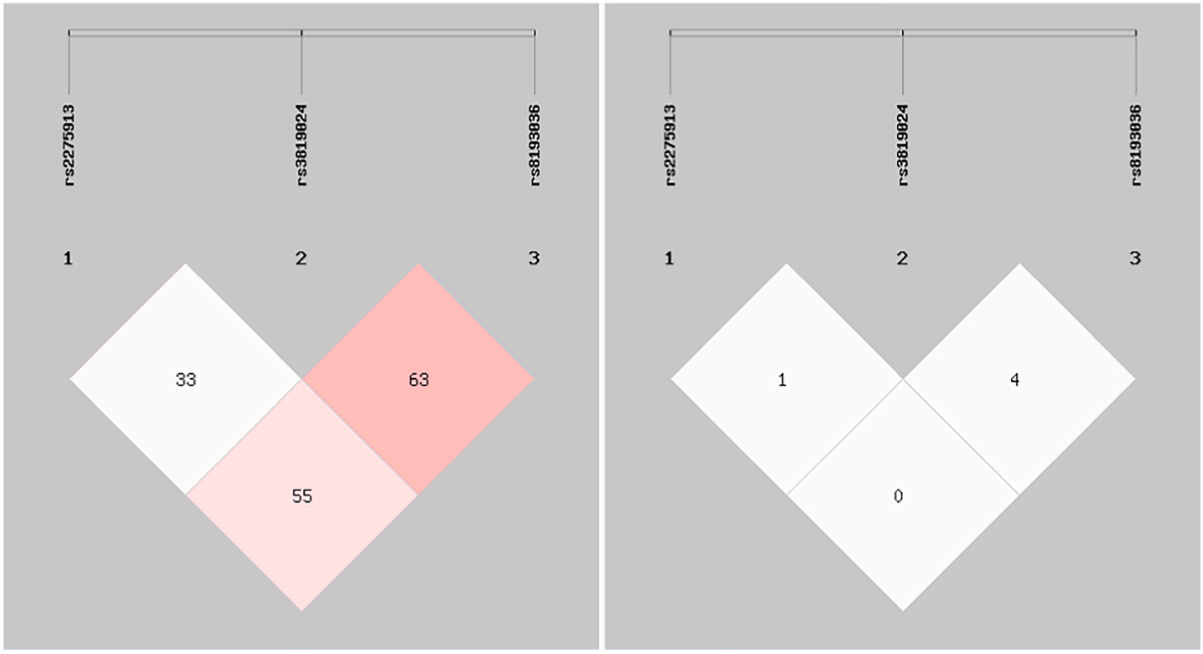

Fig. 4. Pairwise linkage disequilibrium (LD) map of three *IL17A* gene SNPs (rs2275913, rs3819024 and rs8193036) genotyped using SHEsis software. The LD between any pair of SNPs is expressed as D′ (Left) and r^2 (Right) values multiplied by 100. Values approaching zero indicate no LD, and those approaching 100 indicate complete LD. The square colored red represent varying degrees of LD and darker shades indicate stronger LD.

which were rs2275913, rs3819024 and rs8193036. Regarding the first SNP (rs2275913), genetic association analyses under dominant (GA + AA genotypes), overdominant (GA genotype) and codominant (GA genotype) genetic models suggested that GA genotype might have susceptibility role in asthma with an OR range of 2.37–2.42. In line with our observation, a significantly elevated frequency of GA genotype was reported in Chinese asthmatics (Du et al., 2016). Chinese asthmatic children with AA genotype were also at greater risk to have asthma (Chen et al., 2010). Additional three studies found conflicting results. In Tunisian asthma children, GG genotype of rs2275913 variant showed a significantly elevated frequency (Maalmi et al., 2014). In Portuguese adult asthmatics, allele and genotype frequencies of rs2275913 variant showed no significant differences between patients and controls, but the authors reported a significantly increased frequency of AA genotype in rhinitis patients compared to asthmatics (Resende et al., 2017). In study from Finland, the rs2275913 polymorphism was associated with a decreased risk of post-bronchiolitis asthma in children aged 11–13 years of age, but not in earlier ages (Holster et al., 2018). To clarify the association between *IL17A* rs2275913 polymorphism and susceptibility to asthma, a meta-analysis of 10 studies that included 5016 subjects was performed. Overall analysis indicated a significant association between rs2275913 SNP and risk of asthma. In subgroup analysis stratified according to age and ethnicity, the protective effects of *G* allele was suggested asthmatic children and Asians (Zhai et al., 2018). The present study support such conclusion, and the allele *G* had a lower frequency in asthmatics than in healthy controls (81.7 vs. 90.1%), but the difference was significant before correction of *p*-value ($p = 0.017$; adjusted $p = 0.102$).

In the case of rs3819024, neither alleles nor genotypes showed association with risk of asthma, and the frequencies showed no significant differences between patients and controls. However, in a Chinese study, the genotypes GA and GG were proposed to have protective effects against allergic rhinitis and comorbid asthma development (Wang et al., 2012). A meta-analysis of nine studies involving 3650 asthmatics and 3370 controls of Asian origin revealed that rs3819024 SNP was significantly associated with the risk of asthma in Asians (AA vs. GG: OR = 1.77, 95% CI = 1.39–2.25; $p = 0.02$) (Jin et al., 2015).

The third SNP was rs8193036, and the results revealed a significantly decreased frequency of *T* allele and CT genotype in asthmatics compared to controls. In line with our findings, the *T* allele frequency was significantly decreased in Japanese patients with aspirin-exacerbated respiratory disease (Kohyama et al., 2011). However, no association was found between rs8193036 variant and asthma of Chinese (Wang et al., 2011) and Finnish children (Nuolivirta et al., 2018). In Taiwanese patients, it was indicated that rs8193036 had an independent role in the association with pediatric asthma, but the association was weak (Wang et al., 2009). However, a further Asian study reported conflicting results and the TT genotype was presented with a significantly higher frequency in asthmatics compared to healthy controls (Du et al., 2016).

Although there have been some conflicting results, *IL17A* gene polymorphisms are suggested to play key roles in etiology of asthma, and their susceptibly and protective potentials have been indicated. The discrepancy might be related to ethnic variations, and most of the populations studied (including Iraqis) show ethnic-dependent variations in allele and genotype frequencies of cytokine genes (Ad'hiah et al., 2018). Besides, the calculated power of sample size was 0.73, which is less than the accepted power of 0.80, and this may not allow a representative picture of the association between *IL17A* gene SNPs and asthma to be drawn.

5. Conclusions

IL-17A was upregulated in sera of asthmatics, and this was accompanied by significant variations in the distributions of allele and genotype frequencies of two *IL17A* gene SNPs (rs2275913 and rs8193036) between patients and healthy controls. In this respect, the SNP rs2275913 was associated with an elevated risk to develop asthma, while rs8193036 was an SNP with protective potential. However, the study was limited by the number of SNPs investigated and further variants in *IL17A* gene meriting extensive investigations to determine their role in etiology of asthma.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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References

Ad'hiah, A.H., Ahmed, Z.A., Al-Naseri, M.A., Salman, E.D., Al-saffar, O.B., Ahmed, H.S., Hussain, T.A., Idan, E.M., Hassan, I.B., Shihab, B.A., Mohammed, L.O., Al-Nasiri, F. S., 2018. Cytokine gene polymorphisms in Iraqi Arabs. Hum. Immunol. 79, 91–92. https://doi.org/10.1016/j.humimm.2017.12.009.

Alyasin, S., Karimi, M., Amin, R., Babaei, M., Darougar, S., 2013. Interleukin-17 gene expression and serum levels in children with severe asthma. Iran J. Immunol. 10, 177–185.

Amatya, N., Garg, A.V., Gaffen, S.L., 2017. IL-17 signaling: the yin and the Yang. Trends Immunol. https://doi.org/10.1016/j.it.2017.01.006.

Becker, A.B., Abrams, E.M., 2017. Asthma guidelines: the global initiative for asthma in relation to national guidelines. Curr. Opin. Allergy Clin. Immunol. https://doi.org/10.1097/ACI.0000000000000346.

Chen, K., Kolls, J.K., 2017. Interluekin-17A (IL17A). Gene. https://doi.org/10.1016/j.gene.2017.01.016.

Chen, J., Deng, Y., Zhao, J., Luo, Z., Peng, W., Yang, J., Ren, L., Wang, L., Fu, Z., Yang, X., Liu, E., 2010. The polymorphism of IL-17 G-152A was associated with childhood asthma and bacterial colonization of the hypopharynx in bronchiolitis. J. Clin. Immunol. 30, 539–545. https://doi.org/10.1007/s10875-010-9391-8.

Collins, A., Ke, X., 2012. Primer1: primer design web service for tetra-primer ARMS-PCR. Open Bioinform. J. 6, 55–58. https://doi.org/10.2174/1875036201206010055.

Demenais, F., Margaritte-Jeannin, P., Barnes, K.C., Cookson, W.O.C., Altmüller, J., Ang, W., Barr, R.G., Beaty, T.H., Becker, A.B., Beilby, J., Bisgaard, H., Bjornsdottir, U.S., Bleecker, E., Bønnelykke, K., Boomsma, D.I., Bouzigon, E., Brightling, C.E., Brossard, M., Brusselle, G.G., Burchard, E., Burkart, K.M., Bush, A., Chan-Yeung, M., Chung, K.F., Couto Alves, A., Curtin, J.A., Custovic, A., Daley, D., De Jongste, J.C., Del-Rio-Navarro, B.E., Donohue, K.M., Duijts, L., Eng, C., Eriksson, J.G., Farrall, M., Fedorova, Y., Feenstra, B., Ferreira, M.A., Freidin, M.B., Gajdos, Z., Gauderman, J., Gehring, U., Geller, F., Genuneit, J., Gharib, S.A., Gilliland, F., Granell, R., Graves, P.E., Gudbjartsson, D.F., Haahtela, T., Heckbert, S. R., Heederik, D., Heinrich, J., Heliövaara, M., Henderson, J., Himes, B.E., Hirose, H., Hirschhorn, J.N., Hofman, A., Holt, P., Hottenga, J., Hudson, T.J., Hui, J., Imboden, M., Ivanov, V., Jaddoe, V.W.V., James, A., Janson, C., Jarvelin, M.R., Jarvis, D., Jones, G., Jonsdottir, I., Jousilahti, P., Kabesch, M., Kähönen, M., Kantor, D.B., Karunas, A.S., Khusnutdinova, E., Koppelman, G.H., Kozyrskyj, A.L., Kreiner, E., Kubo, M., Kumar, R., Kumar, A., Kuokkanen, M., Lahousse, L., Laitinen, T., Laprise, C., Lathrop, M., Lau, S., Lee, Y.A., Lehtimäki, T., Letort, S., Levin, A.M., Li, G., Liang, L., Loehr, L.R., London, S.J., Loth, D.W., Manichaikul, A., Marenholz, I., Martinez, F.J., Matheson, M.C., Mathias, R.A., Matsumoto, K., Mbarek, H., McArdle, W.L., Melbye, M., Melén, E., Meyers, D., Michel, S., Mohamdi, H., Musk, A.W., Myers, R.A., Nieuwenhuis, M.A.E., Noguchi, E., O'Connor, G.T., Ogorodova, L.M., Palmer, C.D., Palotie, A., Park, J.E., Pennell, C.E., Pershagen, G., Polonikov, A., Postma, D.S., Probst-Hensch, N., Puzyrev, V.P., Raby, B.A., Raitakari, O.T., Ramasamy, A., Rich, S.S., Robertson, C.F., Romieu, I., Salam, M.T., Salomaa, V., Schlünssen, V., Scott, R., Selivanova, P.A., Sigsgaard, T., Simpson, A., Siroux, V., Smith, L.J., Solodilova, M., Standl, M., Stefansson, K., Strachan, D.P., Stricker, B.H., Takahashi, A., Thompson, P.J., Thorleifsson, G., Thorsteinsdottir, U., Tiesler, C.M.T., Torgerson, D.G., Tsunoda, T., Uitterlinden, A. G., Van Der Valk, R.J.P., Vaysse, A., Vedantam, S., Von Berg, A., Von Mutius, E., Vonk, J.M., Waage, J., Wareham, N.J., Weiss, S.T., White, W.B., Wickman, M., Widén, E., Willemsen, G., Williams, L.K., Wouters, I.M., Yang, J.J., Zhao, J.H., Moffatt, M.F., Ober, C., Nicolae, D.L., 2018. Multiancestry association study identifies new asthma risk loci that colocalize with immune-cell enhancer marks. Nat. Genet. 50, 42–50. https://doi.org/10.1038/s41588-017-0014-7.

Dimitrova, D., Youroukova, V., Ivanova-Todorova, E., Tumangelova-Yuzeir, K., Velikova, T., 2019. Serum levels of IL-5, IL-6, IL-8, IL-13 and IL-17A in pre-defined groups of adult patients with moderate and severe bronchial asthma. Respir. Med. 154, 144–154. https://doi.org/10.1016/j.rmed.2019.06.024.

Du, J., Han, J.C., Zhang, Ya Jun, Qi, G. Bin, Li, H.B., Zhang, Yi Jie, Cai, S., 2016. Single-nucleotide polymorphisms of IL-17 gene are associated with asthma susceptibility in an Asian population. Med. Sci. Monit. 22, 780–787. https://doi.org/10.12659/MSM.895494.

Eltaweel, D., Hanna, K., Elnady, M., Awad, A., Elkholy, Y., 2018. Interleukin-17 gene expression and serum levels in asthma degenerative model. Egypt J. Immunol 25, 153–159.

Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. In: Behavior Research Methods. Psychonomic Society Inc., pp. 175–191. https://doi.org/10.3758/BF03193146

Gao, H., Ying, S., Dai, Y., 2017. Pathological roles of neutrophil-mediated inflammation in asthma and its potential for therapy as a target. J Immunol Res 2017, 3743048. https://doi.org/10.1155/2017/3743048.

Gu, C., Wu, L., Li, X., 2013. IL-17 family: cytokines, receptors and signaling. Cytokine. https://doi.org/10.1016/j.cyto.2013.07.022.

Gurczynski, S.J., Moore, B.B., 2018. IL-17 in the lung: the good, the bad, and the ugly. Am. J. Phys. Lung Cell. Mol. Phys. https://doi.org/10.1152/ajplung.00344.2017.

Hasegawa, T., Uga, H., Mori, A., Kurata, H., 2017. Increased serum IL-17A and Th2 cytokine levels in patients with severe uncontrolled asthma. Eur. Cytokine Netw. 28, 8–18. https://doi.org/10.1684/ecn.2017.0390.

Holster, A., Teräsjärvi, J., Lauhkonen, E., Törmänen, S., Helminen, M., Koponen, P., Korppi, M., Peltola, V., He, Q., Nuolivirta, K., 2018. IL-17A gene polymorphism rs2275913 is associated with the development of asthma after bronchiolitis in infancy. Allergol. Int. 67, 109–113. https://doi.org/10.1016/j.alit.2017.05.010.

Hossain, F.M.A., Choi, J.Y., Uyangaa, E., Park, S.O., Eo, S.K., 2019. The interplay between host immunity and respiratory viral infection in asthma exacerbation. Immune Netw. 19, 1–21. https://doi.org/10.4110/in.2019.19.e31.

Huang, H.T., Lu, Y.L., Wang, R., Qin, H.M., Wang, C.F., Wang, J.L., Xiang, Y., Guo, J., Lan, Y., Wei, Y.S., 2017. The association of IL-17A polymorphisms with IL-17A serum levels and risk of ischemic stroke. Oncotarget 8, 103499–103508. doi:10.18632/oncotarget.21498.

Hynes, G.M., Hinks, T.S.C., 2020. The role of interleukin-17 in asthma: a protective response? ERJ Open Res. 6, 00364–02019. https://doi.org/10.1183/23120541.00364-2019.

Jin, Y., Deng, Z., Cao, C., Li, L., 2015. IL-17 polymorphisms and asthma risk: a meta-analysis of 11 single nucleotide polymorphisms. J. Asthma 52, 981–988. https://doi.org/10.3109/02770903.2015.1044251.

Kalousová, M., Levová, K., Kuběna, A.A., Jáchymová, M., Franková, V., Zima, T., 2017. Comparison of DNA isolation using salting-out procedure and automated isolation (MagNA system). Prep. Biochem. Biotechnol. 47, 703–708. https://doi.org/10.1080/10826068.2017.1303613.

Kohyama, K., Abe, S., Kodaira, K., Yukawa, T., Hozawa, S., Sagara, H., Kurosawa, M., 2011. IL-13 and IL-17A gene polymorphisms in Japanese patients with aspirin-exacerbated respiratory disease. Ann. Allergy Asthma Immunol. 107, 510–516. https://doi.org/10.1016/j.anai.2011.09.003.

Laulajainen-Hongisto, A., Lyly, A., Hanif, T., Dhaygude, K., Kankainen, M., Renkonen, R., Donner, K., Mattila, P., Jartti, T., Bousquet, J., Kauppi, P., Toppila-Salmi, S., 2020. Genomics of asthma, allergy and chronic rhinosinusitis: novel concepts and relevance in airway mucosa. Clin. Transl. Allergy. https://doi.org/10.1186/s13601-020-00347-6.

Liang, T., Xu, Y.T., Zhang, Y., Cai, P.C., Hu, L.H., 2018. Interleukin-17A and -17F single nucleotide polymorphisms associate with susceptibility of asthma in Chinese Han population. Hum. Immunol. 79, 736–742. https://doi.org/10.1016/j.humimm.2018.07.227.

Liu, Z., Niu, C., Ying, L., Zhang, Q., Long, M., Fu, Z., 2020a. Exploration of the serum Interleukin-17 and Interleukin-27 expression levels in children with bronchial asthma and their correlation with indicators of lung function. Genet. Test. Mol. Biomarkers 24, 10–16. https://doi.org/10.1089/gtmb.2019.0155.

Liu, D., Tan, Y., Bajinka, O., Wang, L., Tang, Z., 2020b. Th17/IL-17 axis regulated by airway microbes get involved in the development of asthma. Curr Allergy Asthma Rep. https://doi.org/10.1007/s11882-020-00903-x.

Maalmi, H., Beraies, A., Charad, R., Ammar, J., Hamzaoui, K., Hamzaoui, A., 2014. IL-17A and IL-17F genes variants and susceptibility to childhood asthma in Tunisia. J. Asthma 51, 348–354. https://doi.org/10.3109/02770903.2013.876647.

Mansour, A.I., Abd Almonaem, E.R., Behairy, O.G., Gouda, T.M., 2017. Predictive value of IL-35 and IL-17 in diagnosis of childhood asthma. Scand. J. Clin. Lab. Invest. 77, 373–378. https://doi.org/10.1080/00365513.2017.1328739.

Medrano, R.F.V., De Oliveira, C.A., 2014. Guidelines for the tetra-primer ARMS-PCR technique development. Mol. Biotechnol. 56, 599–608. https://doi.org/10.1007/s12033-014-9734-4.

Morales, E., Duffy, D., 2019. Genetics and gene-environment interactions in childhood and adult onset asthma. Front. Pediatr. 7, 1–14. https://doi.org/10.3389/fped.2019.00499.

Nuolivirta, K., Holster, A., Teräsjärvi, J., Lauhkonen, E., Törmänen, S., Helminen, M., Koponen, P., Korppi, M., He, Q., 2018. IL17A gene polymorphisms rs4711998 and rs8193036 are not associated with postbronchiolitis asthma in Finnish children. Acta Paediatr. Int. J. Paediatr. 107, 1290–1291. https://doi.org/10.1111/apa.14340.

Pandey, R., Prakash, V., 2020. mRNA expression analysis of interleukins 17A and 17F in bronchial asthmatic patients from northern Indian population. J. Fam. Med. Prim. Care 9, 2258. https://doi.org/10.4103/jfmpc.jfmpc_35_20.

Pasha, H.F., Tantawy, E.A., Youssef, M.A., 2019. Osteopontin and interleukin-17A genes polymorphisms in Egyptian systemic lupus erythematosus patients: a relation to disease activity and severity. Gene 702, 107–113. https://doi.org/10.1016/j.gene.2019.02.100.

Patel, S.J., Teach, S.J., 2019. Asthma. Pediatr. Rev. 40, 549–565. https://doi.org/10.1542/pir.2018-0282.

Pillai, P., Corrigan, C.J., Ying, S., 2011. Airway epithelium in atopic and nonatopic asthma: similarities and differences. ISRN Allergy 2011, 1–7. https://doi.org/10.5402/2011/195846.

Possa, S.S., Leick, E.A., Prado, C.M., Martins, M.A., Tibério, I.F.L.C., 2013. Eosinophilic inflammation in allergic asthma. Front. Pharmacol. https://doi.org/10.3389/fphar.2013.00046.

Ramakrishnan, R.K., Al Heialy, S., Hamid, Q., 2019. Role of IL-17 in asthma pathogenesis and its implications for the clinic. Expert Rev. Respir. Med. https://doi.org/10.1080/17476348.2019.1666002.

Rehman, A., Amin, F., Sadeeqa, S., 2018. Prevalence of asthma and its management: a review. J. Pak. Med. Assoc. 68, 1823–1827.

Resende, E.P., Todo-Bom, A., Loureiro, C., Pinto, A.M., Oliveiros, B., Mesquita, L., Silva, H.C., 2017. Asthma and rhinitis have different genetic profiles for IL13, IL17A and GSTP1 polymorphisms. Rev. Port. Pneumol. (Engl. Ed.) 23, 10–16. https://doi.org/10.1016/j.rppnen.2016.06.009.

Ruiz de Morales, J.M.G., Puig, L., Daudén, E., Cañete, J.D., Pablos, J.L., Martín, A.O., Juanatey, C.G., Adán, A., Montalbán, X., Borruel, N., Ortí, G., Holgado-Martín, E., García-Vidal, C., Vizcaya-Morales, C., Martín-Vázquez, V., González-Gay, M.Á., 2020. Critical role of interleukin (IL)-17 in inflammatory and immune disorders: an updated review of the evidence focusing in controversies. Autoimmun. Rev. https://doi.org/10.1016/j.autrev.2019.102429.

Schoettler, N., Strek, M.E., 2020. Recent advances in severe asthma: from phenotypes to personalized medicine. Chest. https://doi.org/10.1016/j.chest.2019.10.009.

Shabana, M.A., Esawy, M.M., Ismail, N.A., Said, A.M., 2019. Predictive role of IL-17A/IL-10 ratio in persistent asthmatic patients on vitamin D supplement. Immunobiology 224, 721–727. https://doi.org/10.1016/j.imbio.2019.09.005.

Shao, M., Xu, Shanshan, Yang, H., Xu, W., Deng, J., Chen, Y., Gao, X., Guan, S., Xu, Shengqian, Shuai, Z., Pan, F., 2020. Association between IL-17A and IL-17F gene polymorphism and susceptibility in inflammatory arthritis: a meta-analysis. Clin. Immunol. 213 https://doi.org/10.1016/j.clim.2020.108374.

Shi, Y.Y., He, L., 2005. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res. 15, 97–98. https://doi.org/10.1038/sj.cr.7290272.

Thompson, E.E., Dang, Q., Mitchell-Handley, B., Rajendran, K., Ram-Mohan, S., Solway, J., Ober, C., Krishnan, R., 2020. Cytokine-induced molecular responses in airway smooth muscle cells inform genome-wide association studies of asthma. Genome Med. 12. https://doi.org/10.1186/s13073-020-00759-w.

Wang, J.Y., Shyur, S.D., Wang, W.H., Liou, Y.H., Lin, C.G.J., Wu, Y.J., Wu, L.S.H., 2009. The polymorphisms of interleukin 17A (IL17A) gene and its association with pediatric asthma in Taiwanese population. Allergy Eur. J. Allergy Clin. Immunol. 64, 1056–1060. https://doi.org/10.1111/j.1398-9995.2009.01950.x.

Wang, J., Zhou, J., Lin, L.H., Li, J., Peng, X., Li, L., 2011. Association of single nucleotide polymorphism of IL-17 gene promoter with childhood asthma. Acad. J. Second Mil. Med. Univ. 32, 481–484. https://doi.org/10.3724/SP.J.1008.2011.00481.

Wang, M., Zhang, Y., Han, D., Zhang, L., 2012. Association between polymorphisms in cytokine genes IL-17A and IL-17F and development of allergic rhinitis and comorbid asthma in Chinese subjects. Hum. Immunol. 73, 647–653. https://doi.org/10.1016/j.humimm.2012.03.010.

Zhai, C., Li, S., Feng, W., Shi, W., Wang, J., Wang, Q., Chai, L., Zhang, Q., Yan, X., Li, M., 2018. Association of interleukin-17a rs2275913 gene polymorphism and asthma risk: a meta-analysis. Arch. Med. Sci. https://doi.org/10.5114/aoms.2018.73345.

Zhu, M., Wang, T., Chen, R., Wang, C., Liu, S., Ji, Y., 2016. Association between interleukin-17a gene polymorphisms and asthma risk: a meta-analysis. Asian Pac. J. Allergy Immunol. 34, 115–123. doi:10.12932/AP0680.34.2.2016.