Received: 16 October 2018 | Accepted: 12 December 2018
DOI: 10.1002/ppul.24235

ORIGINAL ARTICLE: INFECTION AND IMMUNITY

WILEY PEDIATRIC PULMONOLOGY

Interleukin-4 -590C/T gene polymorphism in Egyptian children with acute lower respiratory infection: A multicenter study

Ahmed A. Emam[1] | Mohamed M. M. Shehab[1] | Mayy A. N. Allah[1] | Mohamed A. Elkoumi[1] | NourEldin M. Abdelaal[2] | Amira A. A. Mosabah[3] | Mervat T. Zakaria[3] | Ashraf M. Sherif[3] | Mohammed M. Soliman[3] | Rania M. H. El-Kaffas[3] | Heba Abouzeid[1] | Mohammed A. Abdou[1] | Nermin Abdalmonem[1] | Heba R. Abdelbaset[4] | Soma A. Mohamed[5] | Attia A. Soliman[1] | Shaimaa S. A. Elashkar[1] | Mohamed S. Hegab[1] | Atef M. Khalil[1] | Alsayed Abdel-Aziz[1] | Heba G. Anany[1] | Hossam E. Salah[6] | Adel M. Abdou[7] | Naglaa A. Elshehawy[8] | Hany A. A. Elbasyouni[9] | Sahbaa F. M. Hafez[1] | Doaa A. Abo-Alella[10] | Maggie M. Fawzi[6] | Samar S. Morsi[10]

[1] Faculty of Medicine, Department of Pediatrics, Zagazig University, Zagazig, Egypt
[2] Department of Pediatrics, Ain-Shams University, Cairo, Egypt
[3] Faculty of Medicine, Cairo University, Cairo, Egypt
[4] Faculty of Medicine, Benha University, Benha, Egypt
[5] Faculty of Medicine, Al Azhar University, Cairo, Egypt
[6] Faculty of Medicine, Zagazig University, Zagazig, Egypt
[7] Faculty of Medicine, Al Azhar University, Cairo, Egypt
[8] Faculty of Medicine, Al Azhar University, Cairo, Egypt
[9] Faculty of Medicine, Menoufia University, Shibin El Kom, Egypt
[10] Faculty of Medicine, Zagazig University, Zagazig, Egypt

Correspondence
Ahmed A. Emam, MD, Faculty of Medicine, Department of Pediatrics, Zagazig University, Zagazig, Egypt.
Email: ahmedemam1111@yahoo.com

Funding information
none reported

Abstract

Background: Acute lower respiratory infection (ALRI) is the leading cause of child mortality, especially in the developing world. Polymorphisms in the interleukin 4 (IL-4) gene have been linked to a variety of human diseases.

Objectives: To investigate whether the IL-4 -590C/T (rs2243250) polymorphism could be a genetic marker for susceptibility to ALRIs in young Egyptian children.

Methods: This was a multicenter study conducted on 480 children diagnosed with pneumonia or bronchiolitis, and 480 well-matched healthy control children. Using *PCR-RFLP* analysis, we genotyped a -590C/T (rs2243250) single nucleotide polymorphism of the *IL-4* gene promoter, meanwhile the serum *IL-4*concentration was measured by *ELISA*.

Results: The frequency of the IL-4 -590 T/T genotype and T allele were overrepresented in patients with ALRIs in comparison to the control group (OR = 2.0; [95% confidence interval [CI]: 1.38-2.96]; for the T/T genotype) and (OR: 1.3; [95%CI: 1.07-1.56]; for the T allele; $P < 0.01$). The IL-4 -590 T/T genotype was associated with significantly higher mean serum *IL-4* concentration (58.7 ± 13.4 pg/mL) compared to the C/T genotype (47.6 ± 11 pg/mL) and the C/C genotype (34.8 ± 9.6 pg/mL); $P < 0.01$.

Conclusion: The IL-4 −590C/T (rs2243250) polymorphism may contribute to susceptibility to ALRIs in young Egyptian children.

KEYWORDS
acute lower respiratory infections, children, gene polymorphism, interleukin 4

Abbreviations: ALRI, acute lower respiratory infection; ARF, acute respiratory failure; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IL-4, interluekin-4; MODS, multiple organ dysfunction syndrome; NK, natural killer; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RSV, respiratory syncytial virus; SNPs, single nucleotide polymorphisms; SIRS, systemic inflammatory response syndrome; Stat6, signal transducer and activation of transcription factor-6; Th2, T-helper Type 2 lymphocytes; TNF-α, tumor necrosis factor-α.

1 | INTRODUCTION

Acute lower respiratory infections (ALRIs) are the leading infectious cause of childhood mortality worldwide.[1] Annually, about 1.4 million children die from ALRIs. One in five of these deaths occur in the under-five age group. Pneumonia and bronchiolitis account for the majority of the ALRIs burden. Half the world's deaths due to ALRIs in young children occur in Africa.[2] A significant number of children with ALRIs develop sepsis, in which there is an ongoing balance between pro- and anti-inflammatory cytokines.[3] Since the inflammatory mediators are believed to be a key pathway in sepsis pathophysiology, recent studies have focused on cytokine milieu and cytokine gene polymorphisms.[4]

Interleukin 4 (IL-4) is a pleiotropic anti-inflammatory cytokine secreted mainly by activated T-helper type 2 (Th2) lymphocytes. It suppresses the secretion of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-12, and tumor necrosis factor-α (TNF-α). Moreover, IL-4 acts as a growth factor for B-lymphocytes and stimulates the release of class-specific immunoglobulin.[5] The biological effects of IL-4 are mediated by a cell surface receptors (IL4R) expressed in most cell types including endothelial, hematopoietic, muscular, and neuronal cells.[6]

IL-4 and its signaling pathway have been involved in the pathogenesis of a variety of human diseases including allergy, autoimmunity, cancer and infections.[7]

In murine models, Khan et al[8] demonstrated that animals overexpressed IL-4 and were infected with *S pneumoniae* experienced a delay in bacterial clearance and an increase in pulmonary immunopathology. Haridzhuk[9] reported that plasma IL-4 levels were significantly higher in 80 children during the acute phase of complicated pneumonia compared with 20 age-matched, healthy control subjects.

Despite these reports, the association of elevated IL-4 levels with ALRI does not constitute proof of cause. A genetic approach could be the only way to clarify this issue. The human IL-4 gene has been mapped to chromosome 5q31.1 that codes for a cluster of Th2-related cytokines such as IL-5, IL-9, IL-13, and IL-15.[10] Three major polymorphisms in the IL4 gene at −590(C/T; rs2243250), +33(C/T, 5′-untranslated region and a variable number of tandem repeats (VNTR) of 70-bp located in the intron 3 have been identified.[11] Among them, a functional −590C/T polymorphism, located in the IL-4 gene promoter region has been shown to increase *IL-4* transcriptional activity and expression.[12]

A recent meta-analysis concluded that the IL4-590T allele may be a susceptibility allele in several respiratory infections.[13] Given the sparse data on IL-4 and ALRI, we aimed to investigate whether the IL-4-590C/T (rs2243250) polymorphism could be a genetic marker for susceptibility to ALRI in young Egyptian children, and we also estimated the serum IL-4 levels for its potential relation to such polymorphism.

2 | METHODS

This was a multicenter case-control study carried out in Zagazig University, Cairo and Ain-Shams University hospitals from January 2017 through April 2018. The study was approved by Ethical Committees in Faculty of Medicine at Zagazig, Cairo and Ain-Shams Universities. The parent of each participant provided written informed consent in accordance with the Declaration of Helsinki.

Four hundred and eighty unrelated children aged 6-60 months admitted to the study hospitals with a diagnosis of either pneumonia or bronchiolitis, were enrolled in this study. Pneumonia was diagnosed by the presence of clinical (febrile respiratory illness of less than 2 weeks plus lower respiratory signs) and radiological evidence (consolidation or new pulmonary infiltrates on a chest radiograph) confirmed independently by two pediatric radiologists.[14] Children aged less than 24 months with a clinical diagnosis of bronchiolitis were also recruited. Bronchiolitis was defined as audible wheeze and/or crackles on auscultation, and increased respiratory effort in the absence of consolidation on chest radiographs in children less than 2 years of age.

2.1 | Exclusion criteria

Children were excluded if they had a clear alternative respiratory diagnosis or any chronic underlying diseases that could complicate ALRIs (eg, immunodeficiency, congenital heart disease).

Patients were further classified into two categories “mild to moderate or severe” ALRI according to severity criteria sourced from previously published guidelines of the British Thoracic Society.[15]

Sepsis, SIRS and multi-organ dysfunction were defined according to the International pediatric sepsis consensus conference.[16] Acute respiratory failure was defined as PaO2 ≤50 mmHg in room air or [PaO2/FiO2] ratio ≤250 under oxygen administration in the absence of cyanotic CHD.

The control group included 480 unrelated healthy children aged 6 months to 5 years who attended the outpatient clinics in the study hospitals for routine care (all without a previous history or diagnosis of ALRI at the time of recruitment). Patients and controls belong to the same ethnic group: African Caucasian.

Upon enrollment, detailed information was recorded together with blood sampling for patients and control children for laboratory evaluation. Screening of respiratory fluids (eg, nasopharyngeal swabs) for viral and bacterial etiologies of ALRIs was performed for patients only. Routine bacterial identification was carried out by culture and the VITEK® MS system (bioMerieux, Marcy l'Etoile, France) that uses MALDI-TOF mass spectrometry technology.[17] For respiratory viruses' identification FilmArray™ respiratory panel-multiplex PCR system (Biomérieux) was used.

2.2 | Estimation of serum IL-4 levels

The concentrations of IL-4 in serum were determined using an enzyme-linked immune-sorbent assay (ELISA) technique (kit provided by Bender Medsystem, S: Germany) using standard curve with a sensitivity < 2 pg/mL.

2.3 | Genomic DNA extraction

Genomic DNA was extracted from the peripheral blood leukocytes using the Flexigene Kit (Qiagen, Germany). The extracted DNA was stored at −20°C before genotyping.

2.4 | Genotyping

All subjects were genotyped for the IL-4 -590C/T (rs2243250) polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously.[18] DNA was amplified using the sense primer 5′-ACT AGG CCT CAC CTG ATA CG-3′ and the antisense primer, 5′-AGG TGT CGA TTT GCA GTG AC-3′ as a product with 646 base-pair length. The PCR products were then digested with BsmFI restriction enzyme (New England Biolabs, Hitchin, UK) and separated on 3% agarose gel. Genotypes were described as CC (601 + 45 bp), CT (646 + 601 + 45 bp), and TT (646 bp).

2.5 | Statistical analysis

All statistical analyses were performed using the SPSS software for windows, version 18.0 (SPSS Inc., Chicago, IL). Patient and control populations were tested for conformity to Hardy-Weinberg equilibrium (HWE). Genotype distribution and allele frequencies were compared using the χ^2 test or *Fisher exact* test, as appropriate, and odds ratios with 95% of confidence intervals [OR, 95%CI] were calculated. Continuous clinical data were compared by Student's *t*-test or *Analysis of variance* (*ANOVA*) test. Logistic regression analyses were used for evaluation of the independent effect of IL-4 genotypes on the clinical outcome of ALRIs. A $P < 0.05$ was considered statistically significant.

3 | RESULTS

Four hundred and eighty patients with ALRIs (245 (51%) were males; age range 6-60 months, median age 36 months) were enrolled. The control group included 480 healthy children with matched age, and gender (254 (53%) were males; age range 6-58 months, median age 34 months). The main demographic and clinical data of studied patients and controls are summarized in Table 1. Of these patients, 298 (62%) had pneumonia and 182 (38%) were diagnosed with acute bronchiolitis. Bacterial pathogens were detected in 235 (49%) of the cases while viral pathogens were detected in 77 (16%). Co-infection with more than one respiratory pathogen was found in 53 patients (11%). No organisms were identified in 115 (24%) patients.

The most common bacterial pathogens of pneumonia were *S pneumoniae* (18.6%), followed by *H influenzae* (7.9%), *S aureus* (6.5%), and *Klebsiella pneumoniae* (3.6%). The commonest atypical bacteria were *Mycoplasma pneumoniae* (9%).

According to disease severity, 110 (23%) of patients had severe ALRIs and 370 (77%) had mild to moderate ALRIs. During their inpatient stay, 101 patients (21%) suffered from severe sepsis and 34 patients (7%) had ARF. One hundred and twenty-five patients (26%) required admission to the ICU and 43 patients (9%) died (Table1).

The genotype frequencies of IL4-590C/T in patients with ALRIs and control group were compatible with Hardy-Weinberg expectations. The IL-4 -590C/T genotype distribution differed significantly between studied patients and the control group as shown in Table 2. The IL-4 TT genotype was overrepresented among patients, compared to the control group (25% vs 12%, respectively). The TT homozygous subjects had a two- fold increased susceptibility to ALRIs (OR = 2.0; [95%CI: 1.38-2.96]; $P < 0.001$), meanwhile the IL-4 CT genotype was significantly underrepresented in patients with ALRIs (OR = 0.66 [95% CI: 0.49-0.89]; $P < 0.01$). There was a significant increase in the frequency of the IL-4 T allele among patients (39.5% vs 29%; OR: 1.3; [95%CI: 1.07-1.56]; $P < 0.01$). On the other hand, a concomitant significant decrease in the frequency of IL-4C allele was found compared to the control group (60.5% vs 71%; $P < 0.01$) (Table 2).

Serum IL-4 levels in our patients were significantly higher in comparison with control children (49.7 ± 8.5 pg/mL vs 16 ± 3.8 pg/mL respectively; $P < 0.01$) (Table 2).

Sixty-nine (58%) of the TT homozygous subjects had severe ALRIs; meanwhile the heterozygous CT gene variant was more frequent among mild to moderate cases 130 (94%), $P < 0.001$; Table 3. Moreover, patients carrying the TT genotype were more likely to have severe sepsis than those with the CT or CC genotypes (OR: 6.9; [95%CI: 4.16-11.47]; $P < 0.001$). No significant association was evident between the IL-4 -590C/T genotypes and the risk of ARF among our patients ($P = 0.690$). ICU mortality was more frequent in patients with the TT genotype (23%) compared to those with the CT genotype (4.3%), or CC genotype (4%); $P < 0.01$ (Table 3).

Of note, mean serum IL-4 level was significantly higher in patients with the IL-4 -590 TT genotype (58.7 ± 13.4 pg/mL) compared to those with the CT genotype (47.6 ± 11 pg/mL) and CC genotype (34.8 ± 9.6 pg/mL); $P < 0.01$, respectively (Table 3).

However, we did not find any association between the IL4-590C/T alleles, genotypes and IL-4 serum levels and either a bacterial or viral etiology of ALRIs among studied patients (All $P > 0.05$) (data not shown).

4 | DISCUSSION

Polymorphisms in the IL4 gene have been linked to a variety of the immunoglobulin-mediated allergic and inflammatory diseases, including childhood asthma, atopic dermatitis, allergic rhinitis, and autoimmune disorders.[19] However, IL-4 promoter *SNPs* has not yet been investigated, to our knowledge, in Caucasian children with ALRIs. In the current study, we found an over-expression of the IL-4 TT genotype and T allele at position -590 in patients with ALRIs compared to the control group. Moreover, children carrying the TT genotype had twofold higher risk for ALRI, indicating that patients were more susceptible to ALRIs. Of interest, there was a significant negative association between the IL4-C allele at the same position and susceptibility to ALRI suggesting that IL4-590C allele confers in some fashion protection against ALRIs. A few studies in the literature concerned the association of IL-4 gene polymorphisms and susceptibility to ALRIs in young children.[13,20]

In accordance with our results, a recent meta-analysis by Patarčić et al reported an allelic association between the IL4 -590T allele and susceptibility to several respiratory infections.[13] A pilot study performed by Choi et al[20] reported that IL4 -590T allele carriers

TABLE 1 Demographic and clinical characteristics of patients with acute lower respiratory infections (ALRI) and healthy controls

Characteristics		Patients (n = 480)	Controls (n = 480)	P
Age, median (range)		36 (6-60 months)	34 (6-58 months)	>0.05
Gender		n (%)	n (%)	
	Male	245 (51)	254 (53)	>0.05
	Female	235 (49)	226 (47)	
ALRIs	Pneumonia	298 (62)	–	
	Bronchiolitis	182 (38)		
Etiologic pathogen	Bacterial	235 (49)	–	
	Viral	77 (16)	–	
	Co-infection	53 (11)	–	
	Unidentified	115 (24)	–	
ALRI severity	Mild-moderate	370 (77)	–	
	Severe	110 (23)	–	
Severe sepsis	No	379 (79)	–	
	Yes	101 (21)	–	
Acute respiratory failure	No	446 (93)	–	
	Yes	34 (7)	–	
ICU admission	No	355 (74)	–	
	Yes	125 (26)	–	
Hospital mortality	No	437 (91)	–	
	Yes	43 (9)	–	

ALRI, acute lower respiratory infections; ICU, intensive care unit.
Values in parentheses are percentages or data are presented as median (range).
P-value < 0.05 indicates a significant difference.

TABLE 2 Distribution of the IL-4-590C/T genotypes, allele frequency, and serum IL-4 in patients with ALRIs and controls

Genotype	Patient group, n (480) %	Control group, n (480) %	OR (95%CI)	P
IL-4 (-590 C/T)				
C/C	221 (46)	216 (45)	Referent	
C/T	139 (29)	206 (43)	0.66 (0.49-0.89)	<0.01
T/T	120 (25)	58 (12)	2.0 (1.38-2.96)	<0.001
Allele				
C	581 (60.5)	638 (71)	Referent	<0.01
T	379 (39.5)	322 (29)	1.3 (1.07-1.56)	
Serum IL-4 (pg/mL)	49.7 ± 8.5	16 ± 3.8		<0.01[a]

ALRI, acute lower respiratory infections; OR, odds ratio; CI, 95% confidence interval.
Values in parentheses are percentages or data are presented as mean ± SD.
P-value < 0.05 indicates a significant difference. Chi-square test.
[a]Student t-test.

have been found to have more risk and severity of RSV disease in a cohort of Korean infants and young children.

In an attempt to explain our results, we assessed the serum IL4 concentrations in patients with ALRIs which were significantly elevated compared to the healthy control children. This finding was in agreement with a recent study investigated the cytokine profile during the acute phase of complicated pneumonia among Ukrainian children. The authors reported that serum levels of IL-4 increased by 2.2 times exceeding the indices of healthy children.[9] They concluded that the synthesis of IL-4 in the dynamics of ALRIs significantly increased, indicating the structural rebuilding of the human immune response towards the anti-inflammatory cascade with stimulation of humoral and cellular defense.[21]

In our study, we speculate that the effect of this IL-4 *SNP* is due to altered IL-4 transcription and expression as we found that the C to T switch at position -590 was associated with increased serum IL-4 level and the TT, TC, and CC gene variants were associated with high, intermediate, and low IL-4 production in our patients. These findings confirm and extend previous studies[22,23] suggesting that IL-4 promoters harboring -590 T allele plays a unique role in the activation of IL-4 transcription, and that the homozygous individuals of IL-4-590 TT had increased IL-4 production. Gonzales et al[23] stated that *SNPs* in the IL4 gene promoter could be functional, as demonstrated by increased IL4 concentration and STAT6 mRNA, as well as IL-4 protein levels, in CD4+ cells of individuals with −34TT and −590TT genotypes.

In vitro studies have demonstrated that pretreatment with extrinsic IL-4 inhibited phagocytosis and decreased the killing capacity of alveolar macrophages which are the first line of host defense response against bacteria in the lungs.[24]

Antibodies to IL-4 increased cytotoxic lymphocyte activity and attenuated RSV bronchiolitis in animal models challenged with respiratory syncytial virus.[25]

Our study demonstrates that the presence of the IL-4 T allele or the TT genotype; being associated with higher serum IL-4 concentrations; constitute risk factors for more severe clinical course as well as developing severe sepsis, and ICU mortality among studied ALRI patients.

Hobee et al[26] reported higher frequency of both the IL-4 590T allele and the IL-4Rα R551 allele among 207 Dutch children hospitalized with severe RSV disease compared to 447 adult control subjects. Earlier studies demonstrated that a more prominent Th2 cytokine response, in particular IL-4, may play a key role in the immune-pathogenesis of severe RSV disease as well as early wheezing in infants and young children.[27,28] By contrast, Puthothu et al[29] found that IL-4 -590C/T polymorphism was equally distributed in 131 German children with severe RSV infection and 270 control subjects.

TABLE 3 Association of the IL-4-590C/T genotypes with disease severity, clinical outcome, and serum IL-4 in patients with ALRIs

IL-4-590 C/T genotype	C/C (*n* = 221) *n* (%)	C/T (*n* = 139) *n* (%)	T/T (*n* = 120) *n* (%)	*P*
ALRIs severity				
Mild-mod	189 (85)	130 (94)[a]	51 (42)	<0.001
Severe	32 (15)	9 (6)	69 (58)[a]	
Severe sepsis	29 (13)	14 (10)	58 (48)[a]	0.001
ARF	18 (8)	9 (6)	7 (5.8)	0.690
Clinical outcome				
ICU mortality	9 (4)	6 (4.3)	28 (23)[a]	<0.01
Serum *IL-4* (pg/mL)	34.8 ± 9.6	47.6 ± 11	58.7 ± 13.4[a]	<0.01[b]

ALRI, acute lower respiratory infections; ARF, acute respiratory failure; ICU, intensive care unit.
P-value < 0.05 indicates a significant difference. Chi-square test.
[a]Significant difference between each three genotypes group.
[b]ANOVA test.

However, the authors added that this *SNP* seems to be particularly important in the context of *IL13 SNPs* which support the necessity of performing haplotype analysis. Song et al[30] demonstrated that IL-4 induces the activation of the signal transducer and activation of transcription factor-6 (Stat6) pathway, which contributes to the suppression of cell-mediated immune response and death in animal models of sepsis. Moreover, neutralization of IL-4 markedly decreased the mortality rate in septic animals.[31] Gu et al[32] reported that IL-4 -590T/C *SNP* may modulate T-helper1/T-helper2 balance and increase susceptibility to sepsis in Chinese patients. Bozza et al[33] added that higher serum IL-4 concentrations had a good accuracy for predicting early mortality in severe sepsis.

Among the host molecules that control the clinical course during sepsis is the major anti-inflammatory cytokine IL-4. It promotes TH2 cell differentiation while suppressing TH1 cell response primarily through the activation of Stat6 pathway. IL-4 also down-regulates the expression of the genes for TNF-α and IL-1. Moreover, it inhibits antigen presentation by monocytes.[34] This unique property of IL-4 may be critical in modulating the host immune response to sepsis. However, the cytokine milieu and cytokine polymorphisms are only a part of complex molecular mechanisms that may be involved in the pathogenesis of sepsis.

Combining clinical data, bio-informatics and different population genomics may help to discover some genetic *SNPs* that could influence the susceptibility to sepsis in young children with ALRIs.

The first limitation of this study was evaluating only IL-4 -590 T/C (rs2243250) polymorphism in the IL-4 gene promoter which could be in linkage-disequilibrium (LD) not only with another, as of yet unidentified, IL-4 gene variant but also with other genomic markers of the 5q31 cytokine gene cluster. Another limitation in our study was the lack of sufficient data on environmental risk factors that may predispose to ALRIs in a genetically susceptible child such as parental smoking, low socioeconomic status, overcrowding, incomplete immunization, prematurity, poor nutrition and breastfeeding history. Therefore, our findings require independent replication in future genome-wide association studies on different ethnicities all around the world.

5 | CONCLUSION

The IL-4-590C/T (rs2243250) polymorphism may contribute to susceptibility to ALRIs in young Egyptian children.

Finally, identification of IL-4 promoter genotype may be useful marker which could provide insight into a novel therapeutic strategy for ALRIs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

AAE submitted the manuscript. MAE designed the study. MANA, HAZ, AMK, HGA, and MSH collected clinical data and coordinated the sample collection (Zagazig University). NMA collected clinical data and coordinated the sample collection (Ain-Shams University). MMS and RMHE collected clinical data and coordinated the sample collection (Cairo University). NAE coordinated the samples collection. MAA and HAAE performed the statistical analysis. NAA, AMS, and AAS helped to draft the manuscript. MMMS, AAM, SAM, and HRA wrote the manuscript. AAA, MTZ, SSAE, and SFMH critically revised the final version. DAA, MMF, and SSM performed microbiology screening. AMA and HES performed laboratory analysis and genotyping. All authors read and approved all the manuscript.

ORCID

Ahmed A. Emam http://orcid.org/0000-0001-9890-0346

REFERENCES

1. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015;385:117–171.

2. UNICEF/WHO. UNICEF; 2006. Pneumonia: the forgotten killer of children. Available at: http://whqlibdoc.who.int/publications/2006/9280640489_eng.pdf
3. Gallagher PM, Lowe G, Fitzgerald T, et al. Association of IL-10 polymorphism with severity of illness in community acquired pneumonia. *Thorax*. 2003;58:154–156.
4. Solé-Violán J, de Castro FV, García-Laorden MI, et al. Genetic variability in the severity and outcome of community-acquired pneumonia. *Respir Med*. 2010;104:440–447.
5. Lupaltsova OS, Senatorova GM. Inflammatory markers (IL-4, IL-6, IL-8, IL-10) in inductive sputum of children with bronchitis. *Zdorovie Rebenka*. 2013;3:46–49.
6. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol*. 1999;17:701–738.
7. Haukim N, Bidwell JL, Smith AJ, et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 2. *Genes Immun*. 2002;3:313–330.
8. Khan AQ, Shen Y, Wu ZQ, Wynn TA, Snapper CM. Endogenous pro- and anti-inflammatory cytokines differentially regulate an in vivo humoral response to *Streptococcus pneumoniae*. *Infect Immun*. 2002;70:749–761.
9. Haridzhuk LI. Dynamics of interleukin-4 and interleukin-8 in young children with complicated pneumonia secondary to iron-deficiency anemia. *Galician Med J*. 2016;23:133–138.
10. Kelly BL, Locksley RM. Coordinate regulation of the IL-4, IL-13, and IL-5 cytokine cluster in Th2 clones revealed by allelic expression patterns. *J Immunol*. 2000;165:2982–2986.
11. Kawashima T, Noguchi E, Arinami T, et al. Linkage and association of an interleukin 4 gene polymorphism with atopic dermatitis in Japanese families. *J Med Genet*. 1998;35:502–504.
12. Paffen E, Medina P, de Visser MC, et al. The -589C>T polymorphism in the interleukin-4 gene (IL-4) is associated with a reduced risk of myocardial infarction in young individuals. *J Thromb Haemost*. 2008;6:1633–1638.
13. Patarčić I, Gelemanović A, Kirin M, et al. The role of host genetic factors in respiratory tract infectious diseases: systematic review, meta-analyses and field synopsis. *Sci Rep*. 2015;3:16119.
14. Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. *Bull World Health Organ*. 2005;83: 353–359.
15. British Thoracic Society of Standards of Care Committee. BTS Guidelines for the management of community acquired pneumonia in childhood. *Thorax*. 2011;57:1i––1i–.
16. Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med*. 2005;6:2–8.
17. Seng P, Drancourt M, Gouriet F, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis*. 2009;49:543–551.
18. Hegab AE, Sakamoto T, Saitoh W, et al. Polymorphisms of IL4, IL13, and ADRB2 genes in COPD. *Chest*. 2004;126:1832–1839.
19. Haukim N, Bidwell JL, Smith AJ, et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 2. *Genes Immun*. 2002;3:313–330.
20. Choi EH, Lee HJ, Yoo T, Chanock SJ. A common haplotype of interleukin-4 gene IL4 is associated with severe respiratory syncytial virus disease in Korean children. *J Infect Dis*. 2002;186:1207–1211.
21. Gähler A, Stallmach T, Schwaller J, Fey MF, Tobler A. Interleukin-8 expression by fetal and neonatal pulmonary cells in hyaline membrane disease and amniotic infection. *Pediatr Res*. 2000;48:299–303.
22. Rosenwasser LJ, Klemm DJ, Dresback JK, et al. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy*. 1995;25:74–78.
23. Gonzales JR, Gröger S, Haley G, Bödeker RH, Meyle J. The interleukin-4 −34TT and −590TT genotype is correlated with increased expression and protein production in aggressive periodontitis. *Mol Immunol*. 2010;47:701–705.
24. Song Z, Zhang J, Zhang X, et al. Interleukin 4 deficiency reverses development of secondary *Pseudomonas aeruginosa* pneumonia during sepsis-associated immunosuppression. *J Infect Dis*. 2015;211:1616–1627.
25. Tang YW, Graham BS. Anti-IL-4 treatment at immunization modulates cytokine expression, reduces illness, and increases cytotoxic T lymphocyte activity in mice challenged with respiratory syncytial virus. *J Clin Invest*. 1994;94:1953–1958.
26. Hoebee B, Rietveld E, Bont L, et al. Association of severe respiratory syncytial virus bronchiolitis with interleukin-4 and interleukin-4 receptor alpha polymorphisms. *J Infect Dis*. 2003;187:2–11.
27. Renzi PM, Turgeon JP, Yang JP, et al. Cellular immunity is activated and a TH-2 response is associated with early wheezing in infants after bronchiolitis. *J Pediatr*. 1997;130:584–593.
28. Román M, Calhoun WJ, Hinton KL, et al. Respiratory syncytial virus infection in infants is associated with predominant Th-2-like response. *Am J Respir Crit Care Med*. 1997;156:190–195.
29. Puthothu B, Krueger M, Forster J, Heinzmann A. Association between severe respiratory syncytial virus infection and IL13/IL4 haplotypes. *J Infect Dis*. 2006;193:438–441.
30. Song GY, Chung CS, Chaudry IH, Ayala A. IL-4-induced activation of the Stat6 pathway contributes to the suppression of cell-mediated immunity and death in sepsis. *Surgery*. 2000;128:133–138.
31. Song GY, Chung CS, Chaudry IH, Ayala A. MAPK p38 antagonism as a novel method of inhibiting lymphoid immune suppression in polymicrobial sepsis. *Am J Physiol Cell Physiol*. 2001;281:C662–C669.
32. Gu W, Zeng L, Zhang LY, et al. Association of interleukin 4-589T/C polymorphism with Th1 and Th2 bias and sepsis in Chinese major trauma patients. *J Trauma*. 2011;71:1583–1587.
33. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. *Crit Care*. 2007;11:R49.
34. van der Poll T, van Deventer SJ. Cytokines and anticytokines in the pathogenesis of sepsis. *Infect Dis Clin North Am*. 1999;13:413–426.

How to cite this article: Emam AA, Shehab MM, Allah MA, et al. Interleukin-4 -590C/T gene polymorphism in Egyptian children with acute lower respiratory infection: A multicenter study. *Pediatric Pulmonology*. 2019;1–6. https://doi.org/10.1002/ppul.24235