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


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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, interleukin-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-alpha.
Acute lower respiratory infections (ALRIs) are the leading infectious cause of childhood mortality worldwide. 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. A significant number of children with ALRIs develop sepsis, in which there is an ongoing balance between pro- and anti-inflammatory cytokines. 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.

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. 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.

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

In murine models, Khan et al 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 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. 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. 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.

A recent meta-analysis concluded that the IL4-590T allele may be a susceptibility allele in several respiratory infections. 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. 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 ALRI (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. Sepsis, SIRS and multi-organ dysfunction were defined according to the International pediatric sepsis consensus conference. 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 (bioMérieux, Marcy l‘Etoile, France) that uses MALDI-TOF mass spectrometry technology. 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.\(^\text{18}\) 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 \(\chi^2\) 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 122 (25%) had pneumonia and 182 (38%) were diagnosed with acute bronchiolitis.

According to disease severity, 110 (23%) of patients had severe ALRIs and 370 (77%) had mild to moderate ALRIs. During their hospital 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 (Table 1).

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%; \(P < 0.01\)).

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; \(P < 0.01\)).

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 -590TT 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.\(^\text{19}\) However, IL-4 promoter SNPs 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.\(^\text{13,20}\)

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.\(^\text{13}\) A pilot study performed by Choi et al\(^\text{20}\) reported that IL4 -590T allele carriers...
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 IL-4 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. 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.

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 suggesting that IL-4 -590C/T polymorphism was equally distributed in 131 German children with severe RSV infection and 270 control subjects.

Hobee et al. reported higher frequency of both the IL-4 590T allele among 207 Dutch children and the IL-4 -590TT genotypes. 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. *Student t-test.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n = 480)</th>
<th>Controls (n = 480)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>36 (6-60 months)</td>
<td>34 (6-58 months)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>245 (51)</td>
<td>254 (53)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>235 (49)</td>
<td>226 (47)</td>
<td></td>
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<tr>
<td>ALRIs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>298 (62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchiolitis</td>
<td>182 (38)</td>
<td></td>
<td></td>
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<tr>
<td>Etiologic pathogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral</td>
<td>77 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-infection</td>
<td>53 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified</td>
<td>115 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALRI severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild-moderate</td>
<td>370 (77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>110 (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe sepsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>379 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>101 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute respiratory failure</td>
<td>No</td>
<td>446 (93)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>34 (7)</td>
<td></td>
</tr>
<tr>
<td>ICU admission</td>
<td>No</td>
<td>355 (74)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>125 (26)</td>
<td></td>
</tr>
<tr>
<td>Hospital mortality</td>
<td>No</td>
<td>437 (91)</td>
<td></td>
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<tr>
<td></td>
<td>Yes</td>
<td>43 (9)</td>
<td></td>
</tr>
</tbody>
</table>

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.
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.

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

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

ALRI, acute lower respiratory infections; ARF, acute respiratory failure; ICU, intensive care unit.
P-value < 0.05 indicates a significant difference. Chi-square test.
*SSignificant difference between each three genotypes group.
\(^{3}\)ANOVA test.

## 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.

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### REFERENCES


