The C-589T IL-4 Single Nucleotide Polymorphism as a Genetic Factor for Atopic Asthma, Eczema and Allergic Rhinitis in an Eastern Algerian Population.

Dahbia Ines Dahnani1,2,3, Karima Sifi4, Ikhllass Salem4, Jamila Chakli4, Sabah Hanachi4, Mounir Zhary Bachtarzi4, Laila Rouabah5, Noureddine Abadi6, Mohamed Bougrida6, Mahmoud Rouabha7

1 Research laboratory of metabolic diseases, Faculty of Medicine, University of Constantine-3, Algeria.
2 Research Laboratory of Biology and Molecular Genetics, Faculty of Medicine, University of Constantine-3, Algeria.
3 University Institute of Cardiology and Pneumology, Laval Hospital, Laval University, Quebec City, QC, Canada.
4 Private allergy clinic Constantine, Algérie.
5 Laboratory of cellular and molecular biology, Faculty of the sciences of the nature and the life, Constantine1 University, Algeria.
6 Research Group, Faculty of Dentistry, University Laval, Quebec City, Quebec, Canada.
7 *Corresponding author’s E-mail: mahmoud.rouabha@fmd.ulaval.ca

Accepted on: 14-01-2016; Finalized on: 29-02-2016.

ABSTRACT

The aim of this study was to investigate the potential link between the IL-4 gene, the levels of IgE and IL-4, atopic asthma and the incidence of eczema and allergic rhinitis in a young adult population. A total of 80 patients with atopic asthma and 80 non-atopic, non-allergic and non-asthmatic controls were included. Atopic asthma was confirmed by means of the skin prick test, The IgE levels were measured by Quantia IgE immunoturbidimetric assay, while, Interleukin (IL)-4 concentrations were determined by immunosorbent assay. IL-4 C-589T polymorphism was determined by the polymerase chain reaction-restriction fragment length polymorphism method (RFLP-PCR) using the Bsmf1 enzyme. Gene analyses revealed that, the IL-4 C-589T SNP shows a significant difference between atomatic and controls when comparing the TT vs CC (OR, 3.63; OR 95% CI, 1.16-11.63; p=0.01) and TT vs CT (OR, 2.48; OR 95% CI, 0.91-6.95; p=0.05) genotypes. Likewise, a significant association was found between the TT genotype and the positive family history of asthma (OR, 3.78; OR 95% CI 0.93-17.78; p=0.036) and positive parental smoking (OR, 3.16; OR 95% CI 0.85-12.80; p=0.05). On the other hand, the personal history of allergic rhinitis and eczema demonstrated a significant association of the CT and TT genotype with both allergic rhinitis (p = 0.04) and eczema (p=0. 005) in the astmatic group. We also demonstrated that patients with heterozygous CT (55%) and homozygous TT (23.75%) genotypes of the IL-4 C-589T polymorphism showed significantly higher of IgE levels (631.89±187.35 IU/ml, and >1000 IU/ml, respectively) (p=0.0000) and serum levels of IL-4 (>1000) for the TT genotype and (495. 96 ± 93.57) for heterozygote variant of IL-4 C-589T CT (p=0.0000). Our findings revealed that women are much more likely to develop asthma than men. We also showed a strong association between TT genotype and the incidence of eczema and allergic rhinitis in childhood.

Keywords: Asthma, allergic rhinitis, eczema, IL-4C-589T SNP, IL-4, IgE.

INTRODUCTION

Atopic asthma is a complex disease of the respiratory tract characterized by repeated episodes of bronchial obstruction due to chronic inflammation of the airways following exposure to various stimuli. It is thought to be caused by interactions between several genetic determinants, immune variations, and environmental factors.

Multiple cytokines such as IL-17, IL-22 play a crucial role in the development of allergic inflammation and immune response activations. IL-4 is a key component of the immune system involved in the regulation of allergic response through the control of immunoglobulin class switching in B-lymphocytes toward IgG and IgE expressions. Increased serum IgE levels are indicative of allergic response and correspond to a high level of IL-4 messenger RNA (mRNA) synthesis. It also acts as a growth factor for T-lymphocytes and mast cells and is a potent inducer that directs differentiation of naïve CD4+ T cells into CD4+ Th2 effector cells. These functional capabilities of IL-4 demonstrated the important roles of cytokines such as IL-4 in asthma.

Gene encoding IL-4 is located in the chromosome 5q23.3-31.2 region. It has been linked to atopy as well as to increased levels of total and allergy-specific immunoglobulin (IgE). In the last decade, the analysis of genetic variations, such as single nucleotide polymorphisms (SNPs) in different genes involved in asthma has become a new approach to detect and localize the genetic determinants of this disease. In this regard, several polymorphisms have been identified in IL-4 and it has been suggested that enhanced IL-4 transcription is derived from genetic variations in the promoter region. Notably, IL-4 gene promoter C-589T (also referred to as C-590T; rs number, 2243250) polymorphism has been reported to be associated with the susceptibility to atopic diseases including asthma. Compared to the C-589 allele, allelic variant T-589 is reportedly associated with higher IL-4 activity following modulation of IL-4 gene transcription.

In addition, the polymorphism is located in one of the five nuclear factors of activated T cell (NFAT) binding sites in the IL-4 promoter and plays a key role in the transcription of several cytokine genes. This SNP is placed inside the
inverted palindrome from \(-603\) to \(-588\), which may explain the increased accessibility of NFAT-1 dimers to the region and, subsequently, the increased IL-4 production by Th2 cells, which in turn activates B cells to produce high levels of IgE, resulting in atopy. Thus, the aim of this study was to shed light on the possible link between the IL-4 gene and atopic asthma in a young and adult population of the East of Algeria.

**PATIENTS AND METHODS**

**Study Participants**

The study enrolled 80 atopic asthma patients and 80 healthy control subjects of both sexes, whose average ages were \(25.94 \pm 16.32\) years and \(33.60 \pm 18.77\) years, respectively. The average duration of the disease was \(11.05 \pm 10.34\) years.

The controls included in our study were healthy volunteers, living in Constantine. The criteria for the healthy controls were the absence of atopy, which was defined by negative skin prick tests for common allergens (Food Allergens and pneumallergens), and blood specific IgE concentration lower than 100 IU/ml. The healthy subjects were also non-smokers with no personal history of asthma or any atopic diseases.

The atopic asthmatic subjects were subjected to clinical investigative in order to confirm the asthma. The inclusion criteria of patients were: asthma confirmed for at least 2 years as defined by the American Thoracic Society criteria\(^ {15}\), absence of respiratory tract infection during the four weeks leading up to this study. The absence of corticosteroid therapy in the previous 4 months must be untreated with antihistamines in the last 2 months, and a positive skin prick test with at least two pneum-allergens. It should be noted that some asthmatics have a personal history of eczema at a young age and allergic rhinitis. The eczema diagnosis and monitoring were made by their doctor using the Hanifin and Rajka diagnostic criteria\(^ {16}\), as well the SCORAD (SCORing Atopic Dermatitis)\(^ {17,18}\) was used to assess the extent and severity of atopic dermatitis. The allergic rhinitis diagnosis and management were made by the treating physician according to Allergic Rhinitis and its Impact on Asthma (ARIA recommendation)\(^ {19}\). Patients with other types of asthma, asthmatic patients with comorbidities including gastro esophageal reflux, chronic cardiorespiratory disease, pneumonia, nasal polyps, obesity, pregnant women and patients with a smoking history, and those on immunotherapy and with a following disease immune deficiency infection (bacterial, fungal, viral, mycobacteria) parasitic infestation, malignancy and systemic disease, which increase the IgE levels were excluded from the study. Written informed consent was obtained from all of the participants before being enrolled in this study.

**Methods**

During the visit, different information were collected from each participant, including weight, height, clinical examination that includes thorax and lung auscultation (morphology, expansion) and a search of allergic rhinitis and dermatitis atopic at a young age.

All participants were administrated a standardized questionnaire according to the criteria of the American Thoracic Society (ATS)\(^ {20}\). This questionnaire was about the past and present respiratory health of each participant. It includes the age, the severity of asthma and atopy, childhood respiratory infections with admission to the hospital, family history of asthma, atopy and other respiratory diseases, the history of allergic reactions or asthma symptoms while exposed to specific allergens, drugs, parental smoking, consanguineous marriages in the three previous generations, the major asthma symptoms such as wheezing, coughing, chest tightness, exacerbations frequency, sport activity in relation to asthma and asthma/allergic medications.

Patients were then subjected to clinical evaluation and physical examination to investigate asthma and assessed the level of asthma control in asthmatics during the previous four weeks, according to GINA (2008) guidelines criteria. The evaluation criteria include which were as follows: daytime and nocturnal symptoms, use rescue medications (short-acting bronchodilators), limitation of activities, frequency of exacerbations and lung function parameters\(^ {20}\). Furthermore, all the participants underwent pulmonary function tests (PFTs) (Zan 100, Megréta GmbH, Germany) according to American Thoracic Society/European Respiratory Society (ATS/ERS) (2005)\(^ {21}\). The detection of obstructive syndrome is confirmed by FEV1/FVC ratio or Tiffeneau index, FEV1/VC ≤ Low limit normal (LIN) or FEV1/FVC < 0.7(GINA 2008)\(^ {1,22}\). The predicted is invited to perform a reversibility test by administering four puffs of a beta-2-mimetic short-acting (Ventoline400µg). If lung function normalized showing 12% and 200 ml improvement of FEV1 or FCV as compared to the baseline, the bronchodilator reversibility may confirm asthma diagnosis. The level of disease control was defined according to the recommendations of the report of the working group (ATS/ERS 2005), and pulmonary function tests according to American Thoracic Society/European Respiratory Society (ATS/ERS) (2005)\(^ {21}\).

**Identification of atopic status using the prick test and measuring the levels of total IgE and IL-4**

The Atopic status was confirmed by at least two positive skin prick test to a panel of 15 specific pneumallergens (100 IR/ml, Laboratoires Stallerghénes® Paris, France). A positive result was defined as a wheal ≥ 4 mm diameter after 5-20 min of pneumallergen administration using a standardized procedure\(^ {23,24}\); see Figure 1.

The ranges of total IgE confirming atopic status differ in many papers (100 – 400) IU or higher\(^ {25}\). In our study, we decided to choose elevated total IgE, over 300 IU/ml\(^ {26}\), to
underlay the IgE overproduction. IgE levels were measured by Quantia IgE immunoturbidimetric assay (Biokit, S. A., Barcelona, Spain) using the analyzer Architect ci 8200 (Abbott Laboratories, Abbott Park, IL, USA). While IL-4 concentrations were determined by immunosorbent assay (ELISA) kit (DRG International, Inc., USA) as recommended by the manufacturer with a standard curve derived from known amounts of the relevant cytokine using absorbance readings at 450 nm using a spectrophotometer (Mindray MR 96 A, microplate reader). The minimum detection level for the cytokines was < 2 pg/ml.

Figure 1: Representative photo related to skin prick test in polysensitized patient.

a- Pneumallergens places on the skin of the flexor aspect of the forearm.

b- After 15-20 minutes of the application.

Genotyping of IL-4 C-589T SNP

All participants in the study provided EDTA-anticoagulated samples of whole blood. The blood samples were used to extract genomic DNA by a salting-out method. To detect IL-4 C-589T SNP, genotyping was carried out using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). PCR was performed with 200 ng of genomic DNA in a final volume of 20 μl. The PCR mixture contained 1X Gold Taq buffer, 25 mM of MgCl2, 0.2 mM of dNTPs, 10 μM of each primer, and 1.25 U of Got Taq® Hot start Polymerase. PCR was done using primers as previously described. Restriction enzyme digestion for the PCR products of IL-4 C-589T was carried out by adding 1X Buffer R, 0.1 mg/mL of bovine serum albumin, and 1 U of BsmF1 restriction enzyme to a PCR tube containing 10 μl of PCR product. The mixture was then spun down and incubated at 37°C for 16 h. Fragments were separated by electrophoresis in a 3% agarose gel and subsequently visualized with ethidium bromide under UV light and then photographed (Figure.2).

Figure 2: Results of PCR-RFLP analysis of C-589T promoter polymorphism of the IL-4 gene (rs 224350)

A 252 pb PCR fragment was digested with BsmF1. Lane 1, 3 and 5 are uncut PCR products; Lane 2: Allele TT homozygous (252pb); Lane4: Allele CT heterozygous (252pb, 192pb, 60pb); Lane 6: Allele CC homozygous (192pb and 60pb); Lane 7:100pb DNA ladder.

Statistical analyses

Data were analyzed with the software EPI INFO (version 6.0). The continuous variables were expressed as mean ± SD. Comparisons between the means were performed using the Student test, and for quantitative variables, the ANOVA test. However, the chi-square test was used to determine differences in genotype/allele frequencies and deviation from the Hardy-Weinberg equilibrium (HWE) and in the comparisons between groups. While the genotype between groups were analyzed with the Kruskal-Wallis. The significance level for all of the statistical tests was set at p ≤ 0.05.

RESULTS

The results shown in Table 1 represent the baseline and clinical characteristics of the study population. Our findings indicate that there was no association between age of subjects and the development of atopic asthma. Unlike the gender, we noticed a significant difference to develop atopc asthma in women than in men.

Similarly, the para clinical parameters of atopic asthma also shown a statistical difference for FEV1 (p=0. 0000), total IgE (p=0. 0000), IL-4 (p=0. 0000), when comparing the parameters of asthmatics with non-asthmatics.

In this paper, we have undertaken some genetic and environmental factors, which may influence the occurrence of atopic asthma. One of these factors is cigarette smoke exposure. Indeed, we demonstrated that childhood passively exposed to parental cigarette smoke (Table 1) showed a statistically significant difference (p=0.05) between early life exposures to cigarette smoke and subsequent atopic asthma development.
Another important factor involved in the occurrence of atopic asthma in the offspring is represented by consanguineous marriages and asthma family history. Our results have shown a significant difference between the consanguineous marriages in the three previous generations (p=0.0000), the asthma family history (p=0.0000) and the probability of developing atopic asthma. It should be emphasized that 71.25% of asthmatic subjects from consanguineous marriage have a positive family history of asthma (Table 1). Furthermore, children who had a RSV-respiratory infection at a young age that led them to hospitalization, are more susceptible to the subsequent development of atopic asthma, the difference obtained was statistically significant (p=0.0000) (Table 1).

Thereafter, we examined the association of the personal history of rhinitis and eczema in the development of atopic asthma during the life cycle of our patients. The results of this testing yielded a highly significant Wald chi-square statistic in patients and control subjects (Table 1).

Table 2 summarizes the statistical tests done to evaluate the association between the IL-4 SNP and atopic asthma. The IL-4 C-589T SNP was genotyped in healthy controls and subjects with atopic asthma, our findings show a significant difference between asthmatic and controls when comparing the TT vs CC (OR, 3.63; OR 95% CI, 1.16-11.63; p=0.01) and TT vs CT (OR, 2.48; OR 95% CI, 0.91-6.95; p=0.05) genotypes.

We have seen that T-589T homozygous genotype in the patient groups were significantly overrepresented in comparison with the control group (23.75% in patients vs. 10 % in controls), while the distribution of CC and CT genotype was the same between the cases and the control groups (21.25% in patients vs. 32.50% controls) and (55% in patients vs 57.50% in controls) respectively. Nonetheless, in the recessive genetic model (CT+TT) genotype vs. Genotype CC we notice the absence of statistically significant differences between the asthmatic and the control group in genotype distribution among C-589T polymorphism (OR, 0.56; OR 95% CI, 0.26-1.21; p=0.11). (Table 2)

However, the frequency of alleles differ significantly in the asthmatics and the control group. Our data show that the controls with C allele more frequently found when compared with the T allele, unlike to asthmatics which the T allele was more common than C (Table 2). In our population the IL-4 C-589T was found to be in Hardy–Weinberg equilibrium with P > 0.05 (X2=2.97).

Our analyses demonstrated also a significant association between the TT genotype and the positive family history of asthma (OR, 3.78; OR 95% CI 0.93-17.78; p=0.036) and positive parental smoking (OR, 3.16; OR 95% CI 0.85-12.80; p=0.05). By contrast, no association was found between the distribution of C-589T polymorphism and positive childhood respiratory infection. The outcomes achieved were presented in detail on the Table 3.

The personal history of allergic rhinitis and eczema analysis demonstrated a significant association of the CT and TT genotype with both allergic rhinitis (p = 0.04) and eczema (p=0.005) in the asthmatic group (Table 4).

We also showed that, patients with the heterozygous CT (55%) and homozygous TT (23.75%) genotypes of the IL-4 C-589T polymorphism showed significantly higher IgE levels (631.89±187.35 IU/ml, and >1000 IU/ml, respectively) than those with the wild type CC genotype (21.25%) (366.47±31.39) (p=0.0000). Whereas, non-significant association was found between IL-4 polymorphism at position C-589T and total IgE levels in the control group as is highlighted in Table 5.

Interestingly, the asthmatics revealed a noteworthy increase in serum levels of IL-4 among patients with the homozygous TT (>1000) and a heterozygote variant of IL-4 C-589T CT (495.96 ± 93.57) compared to patients with homozygous variant CC (340.08 ± 46.38) (p=0.0000). Unlike the control that showed no significant difference between the distribution of genotypes and increase of serum concentrations of IL-4 (P=0.63) (Table 5).

We also analyzed the distribution of heterozygous CT, wild type CC and homozygous TT genotypes of the IL-4 C-589T polymorphism with FEV1, which represents the major spirometric parameter in the determination of the level of asthma disease severity (according to the standard criteria). The analysis showed no significant difference between the distributions CT, CC and TT genotype of IL-4 C-589T polymorphisms and range of FEV1 values.

The genotype distribution of C-589T polymorphism was as follows: 21.25% of patients with mean FEV1 (77.58±11.42) have the CC genotype, 55% with mean FEV1 (77.42±10.35) have a CT genotype and finally 23.75% of asthmatics with TT genotype have mean FEV1 (75.16±7.60).

**DISCUSSION**

The atopic asthma is a heterogeneous and complex disease, on the etiologic as well as on the phenotypical side. It depends on the divers’ ages of the different environmental and genetic factors. The genetic study of this affection is difficult regarding its complexity and its close association with the allergy and the bronchial hyperactivity, for which a genetic component could also be involved.

In this present paper, we enrolled patients having an atopic asthma and healthy to demonstrate an increased incidence of the atopic asthma in women as previously reported. Indeed, the recent studies showed that the probability of developing atopic asthma in the women is approximately 10.5% higher as compared to men. Accordingly, the authors suggest a potential impact of female sex hormones on the immune function, thereby inducing an increased production of IL-4, IgE with a local influence on the resident lung cells.
### Table 1: Baseline Clinical and serological characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls n=80</th>
<th>Asthmatics n=80</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, ↑</td>
<td>35.93±19.07</td>
<td>33.48±16.32</td>
<td>0.38</td>
</tr>
<tr>
<td>Female, %</td>
<td>27(33.75%)</td>
<td>47(58.75%)</td>
<td>0.0012*</td>
</tr>
<tr>
<td>Males %</td>
<td>53 (66.25%)</td>
<td>33 (41.25%)</td>
<td></td>
</tr>
<tr>
<td>Sizes (cm) ↑</td>
<td>1.66±0.16</td>
<td>1.61±0.1616</td>
<td>0.04*</td>
</tr>
<tr>
<td>Weights (kg) ↑</td>
<td>69.75±19.73</td>
<td>68.86±23.72</td>
<td>0.80</td>
</tr>
<tr>
<td>Duration of asthma in years</td>
<td>0</td>
<td>10.75±10.07</td>
<td>0.00000*</td>
</tr>
<tr>
<td>Baseline FEV1 ↑</td>
<td>86.13±8.30</td>
<td>77±10</td>
<td>0.00000*</td>
</tr>
<tr>
<td>Mesured IgE, UI/ml ↑</td>
<td>26.48±8.90</td>
<td>578.64±216.30</td>
<td>0.00000*</td>
</tr>
<tr>
<td>Mesured IL4, pmol/mL ↑</td>
<td>31.86±15.74</td>
<td>655.76±414.91</td>
<td>0.00000*</td>
</tr>
</tbody>
</table>

#### Parental smoking
- Positive: 40(50%) vs 50(62.73%)  
  P value: 0.05
- Negative: 40(50%) vs 30(37.5%)  

#### Consanguinity
- Positive: 30(37.50%) vs 57(71.25%)  
  P value: 0.00000*
- Negative: 50(62.50%) vs 23(28.75%)  

#### Asthma family history
- Positive: 37(46.25%) vs 64(80%)  
  P value: 0.00000*
- Negative: 43(53.75%) vs 16(20%)  

#### Childhood respiratory infection
- Positive: 19(23.75%) vs 45(56.25%)  
  P value: 0.00000*
- Negative: 61(76.25%) vs 35(43.75%)  

#### Allergic rhinitis
- Positive: 0 vs 47(58.75%)  
  P value: 0.00000*
- Negative: 0 vs 33(41.25%)  

#### Eczema
- Positive: 0 vs 68(85%)  
  P value: 0.00000*
- Negative: 0 vs 12(15%)  

↑ Data are means ± standard deviations (SD); (n) refers to the number of subjects; (*) refers to: p < 0.05. **FEV1** = Forced expiratory volume during 1 sec. **P value** assessed via one way ANOVA and Chi-square with Fisher's Exact mid-P.

### Table 2: Determination of the genetic effects of C-589T polymorphisms on asthma

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Controls</th>
<th>Asthmatics</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C allele n (%)</td>
<td>98 (61.25%)</td>
<td>78 (48.75%)</td>
<td>0.60 (0.38-0.96)</td>
<td>0.02*</td>
</tr>
<tr>
<td>T allele n (%)</td>
<td>62 (38.75%)</td>
<td>82 (51.25%)</td>
<td>1.66 (1.04-2.66)</td>
<td>0.02*</td>
</tr>
<tr>
<td>TT vs CC n (%)</td>
<td>26(32.50%) vs 8(10%)</td>
<td>17(21.25%) vs 19(23.75%)</td>
<td>3.63 (1.16-11.63)</td>
<td>0.01*</td>
</tr>
<tr>
<td>CC vs CT+TT n (%) (recessive effect)</td>
<td>8(10%) vs 72(80%)</td>
<td>19(23.75%) vs 61(76.25%)</td>
<td>0.56 7 (0.26-1.21)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

(*) refers to significant value p < 0.05; (n) refers to the number of subjects
### Table 3: Genotype frequencies of C-589T polymorphism between asthma group and control group Genotype distributions with asthma in an eastern Algerian population.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>CT n (%)</td>
</tr>
<tr>
<td>Controls</td>
<td>19</td>
</tr>
<tr>
<td>Asthmatics</td>
<td>45</td>
</tr>
<tr>
<td>P</td>
<td>0.028</td>
</tr>
<tr>
<td>OR (95%CI)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

(n) refers to the number of subjects; (*) refers to significant value \( p < 0.05 \)

### Table 4: Association of C-589T promoter polymorphism of the IL-4 gene and common atopic disease.

<table>
<thead>
<tr>
<th>Atopic disease</th>
<th>n</th>
<th>Status</th>
<th>Genotype</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Status</td>
<td>Genotype</td>
<td></td>
</tr>
<tr>
<td>Eczema</td>
<td>80</td>
<td>Yes (n=64)</td>
<td>CC</td>
<td>0.030*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No (n=12)</td>
<td>CT</td>
<td></td>
</tr>
<tr>
<td>Rhinitis</td>
<td>80</td>
<td>Yes (n=47)</td>
<td>TT</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No (n=33)</td>
<td>CC</td>
<td></td>
</tr>
</tbody>
</table>

(n) refers to the number of patients; (*) refers to significant value (Statistical significance was defined as \( P \leq 0.05 \))

### Table 5: Analysis of associations between the -589 C/T IL4 promoter and serological measurement related to atopy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Genotype</th>
<th>Controls</th>
<th>Asthmatics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>( \bar{x} \pm \sigma )</td>
<td>n</td>
</tr>
<tr>
<td>Serum IgE (IU/ml)</td>
<td>CC</td>
<td>17(21.2%)</td>
<td>23.88±11.06</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>44(55%)</td>
<td>27.47±7.56</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>19(23.7%)</td>
<td>29.25±6.96</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
<td>80(100%)</td>
</tr>
<tr>
<td>Serum concentrations IL 4 (pmol/mL)</td>
<td>CC</td>
<td>17(21.2%)</td>
<td>34.31±19.49</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>44(55%)</td>
<td>30.74±13.73</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>19(23.7%)</td>
<td>30.37±13.92</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
<td>80(100%)</td>
</tr>
</tbody>
</table>

(n) refers to the number of subjects; \( \bar{x} \pm \sigma \) are means ± standard deviations (SD)

(*) refers to significant value; Significance using Kruskal Wallis test. Statistical significance was defined as \( P \leq 0.05 \)
Many environmental risk factors were involved in the etiology of atopic asthma, including some allergens, cigarette smoke, and air pollutants\(^{25}\).

In this study, we demonstrated that, there is an association between early cigarette smoke exposure and the occurrence of atopic asthma, which is in agreement with previous literature\(^{36,37}\) showing clear adverse effects of the parental smoking on their children’s respiratory health.

However, the role of cigarette smoke in the etiology of atopic asthma is controversial as reported by Hatakka\(^{38}\) and Vlaski\(^{39}\).

Thus, further studies are mandatory to demystify the link between cigarette smoke exposure and atopic asthma development.

Our study has shown that the consanguineous marriages and asthma family history are an important risk factor for the development of atopic asthma in the offspring (p<0.05). This important finding corroborates those previously reported studies\(^{40-42}\).

We then studied the frequency of the secondary respiratory infection, especially by the syncytial virus (VRS) and its role in the occurrence of the asthmatic disease demonstrating a clear relationship between asthma and the susceptibility contracting virus infections. These results support those previously reported by Gern\(^{43}\) and Busse\(^{44}\) who suggested that the viral respiratory tract infections could have a deep effect on the asthmatic exacerbation. It is important to note that 58.75% of our asthmatic population have presented rhinitis. These results joined those of Shaaban\(^{45}\) which suggested that the rhinitis is a predictive factor for atopic asthma. This group also demonstrated that people who have allergic rhinitis have 3.5 more chance developing asthma than non-affected subjects. Such observation was supported by Rochat\(^{46}\), suggesting that 41.5% of subjects who had allergic rhinitis in childhood or adolescence are more likely to develop subsequent asthma with adjusted relative risk of 3.8. From the study population we enrolled, 85% of the asthmatics have presented eczema in their young age. This factor may supports the March’s theory which suggested that asthmatic children having presented a dermatitis atopic before the age of 5 have a higher probability to present airway allergies\(^{47-49}\).

Another major area of study was the genetic association between C-589T promoter polymorphism of the IL-4 gene and the parameters previously cited.

The frequency of the IL-4 T-589 T genotype is higher in our patients (23.75%) compared to the healthy control whose IL-4 T-589 T genotypic frequency is of (10%). Our statistical analyses have shown that asthmatics who have T-589 T genotype are more susceptible of developing an atopic asthma compared to the wild type CC (OR, 3.63; OR 95% CI, 1.16-11.63; p=0.01) and heterozygous CT genotype (OR, 2.48; OR 95% CI, 0.91-6.95; p=0.05). This association has been established through several previous studies\(^{25,50-52}\) demonstrating the role of the polymorphisms of C-589 T of IL-4 gene promoter in the genesis of atopic asthma.

In addition, the results of the meta-analysis realized by Nie\(^{53}\) suggested that the individuals that have the allele T-589 can have an increased atopic asthma risk of 26% compared to the allele C-589 especially in the C-589 C homozygous genotype state.

On the other hand, no association has been published for the recessive genotype CT+TT vs CC of IL-4 - polymorphism and the atopic asthma, which joins the results of the meta-analysis realized by Nie\(^{53}\) which suggested that the recessive genotype CC vs CT+TT of the C-589T polymorphism in African Americans (OR, 1.20; CI 95% 0.72-2.00; p = 0.48) is not associated to the pathogenesis of atopic asthma.

These suggestions are incoherent with those of the meta-analysis of Tang\(^{54}\) which found significant associations between the recessive (genotype CT+TT vs CC) and the occurrence of the atopic asthma in the general population (OR,1.26; CI 95% 1.12 -1.42; P = 0.0001), in asthmatic Asian population (OR, 1.36; CI 95% 1.07-1.73; P = 0.01), Caucasian (OR, 1.30; CI 95% 1.09-1.54; P = 0.004) and European (OR, 1.29; CI 95%, 1.03-1.62; P = 0.03).

Likewise, the allele frequencies distribution was as follows 61.25% for C allele, 38.75% for T allele frequency controls and 48.75% for the C allele and 51.25% for T in atopic asthmatic patients (Table 2).

The present data suggest the possible association between the T allele at–589 of IL-4 and asthma, they also find that asthmatics who have T allele are more susceptible to developing the atopic asthma compared to the C allele (OR, 1.66; OR 95% CI, 1.04-2.66; p=0.02).

This result is similar to the one found in Pakistan\(^{55}\), Taiwan\(^{56}\), Iran\(^{57}\), China\(^{38}\) and Russia\(^{39}\) populations. However, some contradictory reports exist regarding the association of C-589T with atopic asthma susceptibility including those undertaken in Kuwait\(^{60}\) and India\(^{61}\).

Our result shown that the IL-4 (-589C/T) polymorphism in the eastern Algerian population was found to be in Hardy–Weinberg equilibrium with P > 0.05 (X \(^2\) = 0.08). The trend close to significance may be due to the mating system (mating, consanguinity) in Algeria where consanguinity is characterized by marriages that are performed (in 71.25% of asthmatics and 37.50% of controls) between first cousins.

This may be due to demographic, genetic, or structural features of this population.

With this study, we found evidence for positive associations between the early cigarette smoke exposure, family history of asthma and the TT genotype of C-589T.
promoter polymorphism in the IL-4 gene, which is concordant with the works realized by Smith.62

However these results are in disagreement with those reported with Egyptian population63.

Our results have shown that there is no association between the viral respiratory tract infections by SVR at a younger age and the CC, CT and TT genotypes of the variant C-589T of the IL-4 promoter. Such findings are in accordance with the results of Puthothu64 and in stark contrast with the results achieved by Zhang65, and Choi66, suggesting that the severe infection with the SVR could contribute enhancing Th2 immune response mediated by overexpression of IL-4. This may propose a preliminary evidence of a genetic link between a severe RSV infection and the development of subsequent wheezing65,66.

In the current study, a highly significant association was observed between C-589T and eczema. We noticed that the asthmatics with CT and TT genotype are more susceptible to develop eczema at a younger age than those with CC genotype, which lead us to suggest that CC and CT genotypes could be a contributing factor on the occurrence of eczema at a young age. The result of our study was similar to those conducted in Canada67 and Czech republic68 showing that the frequency of the IL-4 polymorphism C-589T tended to be higher in eczema. Nevertheless, other studies are not consistent with ours, reporting no association of this polymorphism with eczema69,70.

In addition, the results of the current study reveal that the asthmatic with CT genotype are more likely to develop allergic rhinitis at a young age compared to the other asthmatics with CC in the same initial group. These results were inconclusive owing to the TT genotype, which was less frequent in our population. Such findings are consistent with previous studies conducted in China71 and Iran72.

In our asthmatic patients the levels of interleukin 4 and total IgE are higher in the TT genotype (>1000 pmol/ml and >1000UI/ml, respectively) compared to the genotype CC that's between (340.08±46.39) pmol/ml for IL-4 and (366.47±31.39 U/l/ml) for the concentration log of total IgE level. These data revealed a significant association between the C-589T promoter polymorphism of the IL-4 gene and the increased levels of IL-4 and the total serum IgE (p value <0.05). Such association was previously reported69,72,73.

We showed that the variant C-589T of the promoter of the IL-4 gene was associates to higher levels of IgE than the wild genotype C-589C. This may be explained by the position of C-589T polymorphism, which is located in the upstream of the five NFAT-binding (P0 to P4) sites for the nuclear factor of activated T cell (NFAT) in the IL-4 promoter and lie precisely within the palindromic sequence going from −603 to −588. The substitution of a cytosine for a thymine in the position -589 in the IL-4 gene promoter creates a new fixation site close to the NFAT-1 binding site. That may explain the increased accessibility of NF-AT-1 dimers to the region leading to increased IL-4 production by Th2 cells, which in turn activate B cells producing high levels of IgE leading to atopy25.

In the present study, the measurements of pulmonary function, and more specifically the FEV1 values, have been used to determine asthma severity. Our results have not shown a significant difference between the FEV1 and the CC, CT and TT genotype of IL-4 C-589T polymorphism in our asthmatic group. It is possible that this polymorphism does not contribute to the variation in the FEV1. Our results joined those of Hosseini-Farahabadi who suggested that the non-association could be due to the limited size of the sample as well as the various grades of asthma severity74. Our results are discordant with those of Burchard74 and Berenguer75, which asserted the association between the FEV1 in asthmatic patients and some genetic determinants of asthma mapped in different loci including that of C-589T promoter polymorphism of the IL-4 gene, suggesting that this locus is associated with a slight decrease of pulmonary function.

CONCLUSION

Our findings revealed that women are much more likely to develop atopic asthma than men, and that consanguineous marriage and asthma family history are an important risk factor for developing atopic asthma in offspring. Patients being exposed to cigarette smoke in early childhood, allergic rhinitis, and eczema in early childhood are more susceptible to the subsequent development of atopic asthma. By contrast, no association was found between a respiratory infection with RSV in childhood and atopic asthma. For the IL-4 C-589T polymorphism, the TT genotype was significantly associated with atopic asthma and the incidence of eczema and allergic rhinitis in childhood.

We also demonstrated that patients with the heterozygous CT and homozygous TT genotypes of the IL-4 C-589T polymorphism showed significantly higher IgE and IL-4 levels than those with the wild type CC genotype.

A large-scale study with a greater number of patients and controls is necessary in order to better substantiate the phenotypic and genotypic characteristics of Algerian patients and understanding the etio-pathogenesis of atopic asthma.

Acknowledgment: The authors thank Dr. Mohcene Djamel and Hanan Benslimane for their excellent technical assistance.

Disclosure: All authors declare no conflict of interest. Funding for the Algerian government and the Fonds Émile-Beaulieu, Laval University Foundation to Dr Rouabhi.
REFERENCES


15. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This Official statement of the American Thoracic Society was adopted by the ATS Board of Directors, American Review of Respiratory Disease, 136, 1987, 225–244.


27. Miller SA, Dykes DD, Polesky HF, A simple salting out procedure for extracting DNA from human nucleated cells, Nucleic Acids Research, 16, 1988, 1215.


34. Kynk JA, Mastronarde JG, McCallister JW, Asthma, the sex difference, Current Opinion in Pulmonary Medicine, 17, 2011, 6–11.


41. Paaso EMS, Jaaokola MS, Lajunen TK, Hugg TT, Jaakokila JIK, The Importance of Family History in Asthma during the First 27 Years of Life, American Journal of Respiratory and Critical Care Medicine, 188, 2013, 624–626.


49. Watson W, Kapur S, Atopic dermatitis, Allergy Asthma Clinical Immunology, 7, 2011, 54.


57. Hosseini-Farahabadi S, Tavakkol-Afshari J, Rafatpanah H, Farid Hosseini R, Khaje Daluel M, Association between the polymorphisms of IL-4 gene promoter (-590C>T), IL-13 coding region (R130Q) and IL-16 gene promoter (-295T>C) and allergic asthma, Iranian Journal of Allergy, Asthma and Immunology, 6, 2007, 9–14.

58. Mak JCW, Ko FWU, Chu CM, Leung HCM, Chan HW, Cheung AHK, Polymorphisms in the IL-4, IL-4 receptor alpha chain, TNF-alpha, and lymphotoxin-alpha genes and risk of asthma in Hong Kong Chinese adults, International Archives of Allergy and Immunology, 144, 2007, 257–264.

59. Gervaziev YY, Kazmachee VA, Gervazieva VB, Allelic polymorphisms in the interleukin-4 promoter regions and their association with bronchial asthma among the Russian population, International Archives of Allergy and Immunology, 141, 2006, 190–194.

60. Hijazi Z, Haider MZ, Interleukin-4 gene promoter polymorphism ([C590T] and asthma in Kuwaiti Arabs, International Archives of Allergy and Immunology, 122, 2000, 190–194.


Source of Support: Nil, Conflict of Interest: None.