Association of Interleukin 4 (IL4) Gene Polymorphisms with Asthma

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ABSTRACT
To verify the association of IL-4 (C-590T) gene polymorphisms with asthma severity in a sample of patients with atopic asthma. Aim of the study: This study aimed to verifying the association of CD14 (C-590T), gene polymorphisms with asthma. Methods: A clinical, laboratory, prospective study was performed in patients with atopic asthma, compared to a control group at allergy and asthma center also at Marjan teaching hospital between April and October 2014 in Babylon province/Iraq. IL4 C-590-T gene polymorphism was detected by PCR and restriction enzyme. Results: This study included 58 patients with persistent atopic asthma and 30 healthy blood donors. When distribution of C-590-T polymorphism genotype frequency (IL4) in asthma was compared with the control group, there was a result with the TT genotype. Conclusion: Our results indicate that C-590-T (IL4) polymorphism might be not involved in modulation of asthma.

KEY WORDS: Asthma genes, polymorphisms, SNP, ILA.

INTRODUCTION
Asthma is the most common chronic disease in childhood and adolescence. It is caused by genetic and environmental factors, and many genes have been identified in its pathogenesis (Sandford et al., 1996). Some studies, also including twins, have shown that a number of genes and their polymorphisms influence immune and pulmonary development and response to environmental factors, contributing to asthma occurrence and/or severity. The interleukin 4 (IL-4) gene, located on chromosome 5q31, has also been associated with atopy. IL-4 is the main cytokine responsible for change in B lymphocyte from immunoglobulin M (IgM) to IgE (Marsh et al., 1994). Nucleotide replacement (C-T) in position -590 of the IL-4 gene promoting region is present in approximately 27% of Caucasians. The IL4-590T allele was associated with increased expression of in vitro gene and with higher levels of in vivo IgE (Rosenwasser et al., 1995). The IL4-590T was associated with asthma, rhinitis and atopy in a study including children at risk for allergic diseases (Marsh et al., 1994). That allele has also been associated with low values of forced expiratory volume in 1 second (FEV1) in a Caucasian population with asthma (Burchard et al., 1999).These data suggest that the C-590T polymorphism could influence asthma severity.

Methods:
A clinical, laboratory and prospective study was carried out at DNA laboratory in the departments of biology, sciences college, university of Babylon.
Genomic DNA was extracted from 200 μl of whole blood using the genaid Blood Kit in accordance with manufacturer’s instructions.
Methods, primer sequence and restriction enzyme, as well as size of fragments generated by CD14, described in Table1.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Method</th>
<th>Primers</th>
<th>Amplified fragment(pb)</th>
<th>Restriction enzymes and fragments (pb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14 gene (C-590 T)</td>
<td>PCR +RE</td>
<td>5’-aaactttgagatcatgg-3’</td>
<td>195</td>
<td>Ava II, 177 and 19</td>
<td>(Hoebee et al., 2003)</td>
</tr>
</tbody>
</table>

Table 1: Description of methods, primer sequence, restriction enzymes and size of fragments generated by CD14 gene polymorphisms.

PCR = polymerase chain reaction; RE = restriction enzyme.
Statistical analysis:

Statistical analysis was carried out using SPSS version 18. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means with their 95% confidence interval (CI). Independent sample t-test was used to compare means between two groups. A p-value of ≤ 0.05 was considered as significant.

Results:

DNA extraction:

DNA extracted after collecting blood samples from the asthma patients and control individuals. Where we used gel electrophoresis device and 1% agarose substance also used 5 micro liter of DNA and 3 micro liter from loading dye for each well and we achieved the electrophoresis process with 75 V, 20 Am for 1 hour.

Cluster of differentiation (CD14) genotyping:

The polymorphism of IL4 gene (C-590T) gene was determined by polymerase chain reaction technique and agarose gel electrophoresis devise. Where used 1% agarose substance, 75 V, 20 Am for 120 min. (10 µl in each well) and appeared one band sized 495 bps. As shown in figure (2).

RFLP-PCR for CD 14 gene:

Genotyping of IL4 (C-590T) gene was determined by RFLP-PCR technique. Where used Ava I enzyme and appeared different bands sized 195 and 177 bps. As shown in figure (3).

The Genotype of IL4 gene polymorphism with allele frequency between the two group (patient vs control):

The frequencies of CC, CT and TT for IL4 gene polymorphism were 93.1%, 0% and 6.89% for the patient with asthma group, and 100%, 0% and 0%. As shown in table (5).
Fig. 4: Electrophoresis pattern of RFLP-PCR for PCR product (195 bps) with restriction enzyme Ava II, 1% agarose, 75 V, 20 Am for 120 min. (10 μl in each well).

**Lane 1**: DNA ladder 100 bp.

**Lane 2,4,5,6**: showing mutant homozygote (TT) genotype.

**Lane 3,7,8,9**: showing mutant heterozygote (CT) genotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient group</th>
<th>Control group</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>54 (93.1%)</td>
<td>30 (100%)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>C(93.1%)</td>
</tr>
<tr>
<td>TT</td>
<td>4 (6.89%)</td>
<td>0 (0%)</td>
<td>C(100%)</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100%)</td>
<td>30 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**:

From table (5), one can tell that homozygous CC genotype was more frequent in control groups, while homozygous TT genotype was only in asthmatic group.

In the IL-4 C-590T gene polymorphism, its variant is related to increased gene transcription. Incidence of such polymorphism in a study including an American population was 40%, and the T allele was associated with increased IgE production, positive allergic tests and asthma (Rosenwasser et al., 1995). Such association was not found in the present study, Australian and British study (Walley et al., 1996) and Italian individuals (Rigoli et al., 2004).

**REFERENCES**