INVESTIGATION OF ASSOCIATION BETWEEN BRONCHIAL ASTHMA AND GENE FcεRIβ 109 C/T POLYMORPHISM IN UZBEK POPULATION

The molecular bases of predictive medicine are being actively developed at present. Conceptual basis for predictive medicine is presented by notion of genetic polymorphism. Among the set of candidate genes, the FcεRIβ gene being an important component of the hereditary component of susceptibility to asthma and atopy occupies big interest.

We studied the distribution of allele and genotype frequencies of polymorphic gene variant of high affinity IgE receptor (FcεRIβ) in patients in with different phenotypic variants of bronchial asthma and healthy individuals of Uzbek nationality. Material for the study included DNA samples from patients with asthma and healthy adolescents of Uzbek nationality. Amplification of loci was performed by PCR, genotyping was performed by polymorphism of length of restrictive fragments.

The results of investigations of the polymorphic gene variant of high affinity IgE receptor have shown that markers of increased risk of allergic form of bronchial asthma in adolescents of Uzbek ethnicity are genotype FcεRIβ-109T/T and allele FcεRIβ-109T, markers of low-risk are FcεRIβ-109C/C and allele FcεRIβ-109C.

Keywords: Asthma, gene, polymorphism.
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Introduction

Currently, bronchial asthma (BA) is regarded as multifactorial polygenic disease and its transmission to posterity is realized by a group of genes (Ivaschenko et al., 2001; Kolchanov et al., 2000; Puzyrev, 2003). A detailed description of the pathogenesis of the studied diseases contributes largely to genetic studies of multifactorial diseases. Knowing the molecular mechanisms of the disease, we can identify genes whose protein products are in the greatest value. Such genes would be the “candidates” for the role of genes susceptible to the disease. The study of their variability in connection with pathologic condition will identify molecular hereditary features predisposing to the development of the disease.

Molecular and cellular mechanisms of implementation of atopic states have been well studied today. An important role is played by cytokines (Puzyrev et al., 2002; Freidin, 2001; Fedorova, 2005; Freidin et al., 2000), responsible for humoral immunity factors of antigenic recognition (Hill and Cookson, 1996), lymphocyte receptors, metabolic enzymes (Lyakhovich et al., 2000; Lyakhovich et al., 2002; Safronova et al., 2003), inflammatory mediators, etc. As suggested above, the interaction of antigen-specific IgE to the receptor on mast cells (FceRI) plays a central role in the pathogenesis of BA and its accompanied signs (Hill and Cookson, 1996; Hopkin, 1996). Association of polymorphisms of FcεRIβ gene with BA have not been studied to the end, including the Uzbek population, however, we can ascertain in advance that this gene is an important component of hereditary constituent of susceptibility to asthma.

In this regard, the objective of this study was to examine the association of asthma in adolescence due to the influence of FcεRIβ gene polymorphism in the Uzbek population, to improve diagnosis and optimize therapy.
**Materials and methods**

According to the goal, we have studied the distribution of allele and genotype frequencies of polymorphic gene variant of high affinity IgE (FcεRIβ) receptor in the total group of patients and with different phenotypic variants of bronchial asthma and healthy individuals of Uzbek nationality. In total, more than 150 teenagers and young adults suffering from bronchial asthma at the age of 12 to 19 years have been studied. The control group consisted of 48 healthy individuals of Uzbek nationality. Patients with asthma were allocated into groups according to the international classification of WHO (X revision, ICD-10) and in accordance with the diagnostic criteria of the Global Strategy for the treatment and prevention of bronchial asthma (GINA, 2007) and the criteria of national policy papers on the diagnosis, treatment and prevention of BA.

DNA extraction was carried out by the standard protocol for DNA extraction using a set of reagents Diatom™ DNA Prep 200. Genomic DNA was visualized in 0.9% agarose gel and its concentration was estimated visually by comparison with a standard concentration of the marker - DNA of phage lambda and diluted to a working concentration of 10 ng/ml.

The supernatant was then subjected to DNA directly genotyping by PCR amplification using two pairs of specific oligonucleotide primers with the sites of the gene FcεRIβ - Forward - 5'-GTG GGG ACA ATT CCA GAA GA-3 ', Reverse - 5'-CCG AGC TGT CCA GGA ATA AA-3'. Oligonucleotides were synthesized by IDT (Integrated DNA Technologies, Iowa, USA). Products of PCR amplification were subjected to restriction analysis using specific restriction endonucleases Tru9I. Endonuclease production “SibEnzyme” (Russia). Incubation temperature was 65°C. Amplification program: 5 min preliminary denaturation at 94°C, 35 cycles at 94°C - 30s, 55°C - 45s, 72°C - 1min, elongation at 72°C for 7 min.

Detection of RFLP products and genotyping was performed using a horizontal or vertical electrophoresis system in a 2% agarose gel or 6% acrylamide gel. For photographic documentation gels were stained for a short time (3-5 min.) Solution of ethidium bromide with a concentration of 0.1 mg/ml, washed in tap water (15-20 minutes) and photo documented using Alpha Imager 3400 (Alpha Innotec, USA). For further processing the data of electrophoresis were transferred to a digital format and put tables Microsoft Excel. These studies were conducted at the Center for Genome Technology Institute of Biochemistry, Academy of Sciences of Uzbekistan (Director Mukhamedov R.S.).

**Results**

The analysis of the distribution of allele and genotype frequencies of polymorphic variants - 109C/T FcεRIβ gene showed a tendency to accumulate homozygous genotype 109T/T and 109T allele with statistically significant difference between the total group of patients with BA and control sample in the Uzbek population.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Groups</th>
<th>N</th>
<th>Allele</th>
<th>Genotypes</th>
<th>(X^2)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>109C/T</td>
<td>Control</td>
<td>46</td>
<td>109C</td>
<td>109T</td>
<td>109C/T</td>
<td>109C/C</td>
</tr>
<tr>
<td></td>
<td>109C</td>
<td>49-106.5%</td>
<td>43-93.5%</td>
<td>13-28.2%</td>
<td>18-39.1%</td>
<td>15-35.6%</td>
</tr>
<tr>
<td></td>
<td>109T</td>
<td>43-93.5%</td>
<td>13-28.2%</td>
<td>18-39.1%</td>
<td>15-35.6%</td>
<td>2.33</td>
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</tbody>
</table>

Differences in the distribution of allele and genotype frequencies of polymorphic loci-109C/T FcεRIβ gene were reliable between groups of patients with allergic form of BA and healthy individuals of Uzbek ethnicity. Association with allergic form of asthma has been defined for FcεRIβ-109T/T genotype and FcεRIβ-109T allele. Homozygous FcεRIβ-109C/C genotype and FcεRIβ-109C allele are markers of reduced risk of allergic asthma in
Uzbek ethnicity. It should be noted that a high degree of cohesion of the FcεRIβ-109T/T genotype and FcεRIβ-109T allele was observed in a family history of hereditary disease. Analysis of the distribution of allele and genotype frequencies of polymorphic variants - 109C/T gene FcεRIβ revealed no significant differences between the control and sample groups with different clinical-pathogenic form of the disease.

<table>
<thead>
<tr>
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<th>Groups</th>
<th>N</th>
<th>Allele</th>
<th>Genotypes</th>
<th>X²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>109C/T</td>
<td>Control</td>
<td>46</td>
<td>49-106.5%</td>
<td>109C/T</td>
<td>109C/C</td>
<td>13-28.2%  18-39.1%</td>
</tr>
<tr>
<td></td>
<td>Allergic asthma</td>
<td>103</td>
<td>43-20.8%  153-79.1%</td>
<td>31-30.1%</td>
<td>6-5.8%</td>
<td>66-64.1%</td>
</tr>
</tbody>
</table>

The obtained data suggest that markers of increased risk of an allergic form of bronchial asthma in adolescents Uzbek ethnicity are genotype FcεRIβ-109T/T and allele FcεRIβ-109T, markers of low-risk - FcεRIβ-109C/C and allele FcεRIβ-109C.

**Discussion**

The results of genomic studies of atopy are an example of the success that can be achieved by using modern concepts and technologies. Perhaps no other multifactorial condition in humans is characterized in terms of genetics as well as atopy. Success in other sciences a in particular, immunology, describing in detail the mechanisms of atopic reactions contributes to it. Of course it is early to argue that now there is a full understanding of the hereditary basis of atopy and associated diseases, but progress in this area is obvious: many genes are characterized by susceptibility to atopy, revealing their “reasons” for polymorphisms, wide-genomic screening for atopy and positional cloning of 4 genes of this state have been successfully performed, model of atopic diseases has been developed and is actively exploited, publications on the analysis of gene expression of atopy are collected. Moreover, approaches to therapy of atopic diseases based on genomic technologies are already “groped”.

The gene encoding the β-subunit was recognized as a candidate for atopy for two main reasons: 1) the function of its protein product is a significant (up to 7 times) amplification of the signal by γ-chains, 2) it is localized on chromosome llql3 near the marker D11S97, which had displayed a close genetic linkage with the hypothetical locus of asthma/atopy (Hill and Cookson, 1996). It has been suggested that molecular variants of FcεRI-β may favor the development of atopic status by increasing the release of inflammatory mediators by mast cells or stimulating the expression of IL-4 and SE40-ligand (Hopkin, 1996). In the sixth exon of FcεRI-β gene missense mutations of Leul81Ile and Leul83Val have been revealed. In a random sample of unrelated individuals from England, the authors of the study found a significant association of Leu181 allele with high total IgE and positive allergic tests to pollen. In the study of the Japanese population Glu237Gly variant showed an association with high levels of total IgE and atopic, but not endogenous asthma (Shirakawa et al., 1996). Association of polymorphisms of FcεRI-β gene with severe atopic dermatitis (Fedorova, 2005) has been established. Studies on 109 C/T polymorphism of FcεRI-β gene, according to available literature, have been carried out (Li et al., 2009) in a Chinese population while the risk of asthma in individuals with this substitution was 2.3 times higher compared with subjects without it (p=0.005).

**Conclusion**

To sum up, a molecular genetics study of asthma has been performed and it shows the contribution of the polymorphic variant of FcεRIβ gene, the formation of the genetic structure of the predisposition to asthma in the Uzbek population.
References


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