ASSOCIATION ANALYSIS OF ANTI-INFLAMMATORY CYTOKINE GENES WITH THE DEVELOPMENT OF ATOPIC ALLERGIC RHINITIS

Vasila Alieva

Republican Scientific-Specialized Allergy Center, Healthcare Ministry, Uzbekistan

Key words: Allergic rhinitis, gene, mutation, polymorphism, RANTES, TNF-α.

UDC: 616.248-053.2-056.7

Allergic rhinitis (AR) is related to atopic conditions, described by IgE-mediated inflammation and developed as a result of hitting of different allergens into mucous membrane of nose (Freidin et al., 2006; Khuzina, 2007; Hopkin, 1996). Currently AR is accepted to be multifactorial disease. Inherited susceptibility plays a major role along with the other environmental factors in the development of this disease. Therefore, it is important to search for genetic markers, associated with the development of AR that enables us to understand the pathogenesis, treatment and prevention of the disease. Several gene-candidates, which proteins participate in pathogenesis of AR, are known to us. Anti-inflammatory cytokines RANTES and TNF-α, considered as natural immunity regulators, are given special consideration among others (Fryer et al., 2000a; Fryer et al., 2000b; Nickel et al., 2000).

Taking into consideration the priority of immune system dysfunction in AR genesis as the main diagnostic sign of disease and the data from previous investigations of atopic genetics, and their major role in the development of many allergic diseases, we assume the existence of possible correlation between pathological variants of these genes with the development of AR.

It is important to emphasize that molecular-genetic research of AR was performed for the first time in Uzbekistan.

The work pursues molecular analysis of polymorphous variants (types) of cytokine genes - RANTES and 308 G>A TNF-α and evaluation of the role of these genetic markers in the development of allergic rhinitis.

Materials of research

DNA molecules separated from peripheral blood of 44 patients with AR disease served as a material for molecular genetic research. Among the observed patients with AR, 19 cases with seasonal form and 25 cases of year-round form were discovered. 70 (TNF-α) and 91 (RANTES) conditionally healthy donors constituted the control group.

DNA molecule separation was conducted as per standard methodology (Sambrook et al., 1989) with some modifications. Polymorphous locus amplification with the usage of polymerase chain reaction was performed in a programmable thermal cycler of “Applied Bio-systems” (USA), there were further breakdown of DNA fragments with restriction endonuclease under the conditions recommended by the firm-producer.
Amplification products of DNA fragments and restriction completeness were partitioned in 2-3% agarose gel and evaluated in progressing through ultraviolet rays (UR) after staining with ethidiumbromide.

Allele and genotype variant frequencies were calculated as per formula ($f$):

$$f = n/2N \text{ and } f = n/N$$

Where, $n$ - occurrence of variant (allele or genotype), $N$ - sample size

Comparative risk (CR) of disease development was calculated using standard formula:

$$OR = a/b \times c/d$$

Where, $a$ and $b$ - number of patients, who have and do not have mutant genotype respectively; $c$ and $d$ - number of people in control group, who have and do not have mutant genotype, respectively.

**Research results and discussion**

RANTES is chemokine related to a class of anti-inflammatory cytokines produced with an activation of T-cells. RANTES gene is located in chromosomes locus 17q11.2-q12 (Donlon et al., 1990). Our observed polymorphism (mutation) is located in proximal part of functional gene, and as per authors, may have relation to increased expression to CC-chemokine, detected in many patients.

The distribution analysis results of allele and genotypes of polymorphism RANTES, which are considered to be a potential risk factor in AR patients, as well as in control group, are shown in Table 1. Occurrence frequency of “N” (normal) and “M” (mutant) alleles in AR patients and in control group were equal to 71.6% and 28.4% against 79.3% and 20.7% respectively.

The frequency of genotypes N/N, N/M and M/M of this genetic marker was equal to 52.3%, 38.6% and 9.1% respectively within the observed patients group. At the same time, the frequency of these genotypes in a control group was equal to 66.0%, 27.5 and 6.5% respectively.

Total frequency of hetero or homozygous variant of mutant genotype RANTES gene carriage was equal to 34% in a conditionally healthy group and 47.7% in patients with AR (OR=1.4, p<0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Allele frequency</th>
<th>Genotype distribution frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Main group</td>
<td>44</td>
<td>71.6</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seasonal form</td>
<td>19</td>
<td>76.3</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>year-round form</td>
<td>25</td>
<td>68.0</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>91</td>
<td>79.7</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Note: n - number of observed people; N - normal allele; M - mutant allele.

Significant statistical distinctions were determined also in a comparative prevalence analysis of allele variants and genotypes of RANTES gene, subject to AR forms (Table 1). It is established that such mutation is reliably discovered more frequently in patients with year-round form of AR (52% cases) in comparison with the control group (34%) and in patients with seasonal form (42.1%). Moreover, the probability of risk for development of year-round form of AR in carriers of this marker is 1.5 times higher than in control group (OR=1.5 p<0.05). The chance of disease development among patients with seasonal form
of AR was statistically inauthentic (OR=1.2; p>0.05) in comparison with the control group.

Comparative analysis of distribution of alleles and genotypes of polymorphism 308G>A TNF-α gene was performed at the second stage of work within main and control groups. TNF-alpha (tumor necrosis factor) is a substance, playing a major role in regulation of normal differentiation, growth and metabolism of various cells and at the same time, is the powerful anti-inflammatory cytokine (Sandford et al., 2004). TNF-α necrosis gene factor is localized in short sixth (6) chromosome arm (6p21.3). According to authors (Freidin et al., 2006; Sandford et al., 2004) allele variant of 308G>A gene is considered as functionally significant in atopic diseases.

The obtained results of frequency distribution of allele and genotype polymorphism 308G>A gene are presented in Table 2. As seen in Table 2, during a comparative analysis of frequencies in alleles and genotypes of polymorphism 308G>A TNF-α gene, no statistically significant differences were determined between the general group of patients with AR and the control group. Total occurrence frequency of polymorphism 308G>A TNF-α gene among patients was equal to 11.4%, and in control group, it was 7.1% (OR=1.15; p>0.05). The frequency of occurrence of alleles A and G was: 5.7% and 94.3% respectively in a group of patients with AR; and 6.4% and 93.6% - in a control group. The frequency of genotypes of A/A, G/A and G/G was: 0.0 %, 11.4 % and 88.4% - in a group of patients; and 2.9%, 7.1% and 90% - in a control group. It is important to note that homozygosis on rare allele genotype A/A was not detected in any group of observed patients.

Thus, no statistically significant differences were determined between the general group of patients and control group (11.4% and 7.1% respectively) in comparative analysis of genetic variants of polymorphism 308G>A TNF-α gene.

### Table 2. Frequency of alleles and distribution of genotypes of -308G>A TNF-α gene among different forms of AR

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Allele frequency</th>
<th>Genotype distribution frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Main group:</td>
<td>44</td>
<td>5.7</td>
<td>94.3</td>
</tr>
<tr>
<td>seasonal form</td>
<td>19</td>
<td>10.5</td>
<td>89.5</td>
</tr>
<tr>
<td>year-round form</td>
<td>25</td>
<td>2.0</td>
<td>98.0</td>
</tr>
<tr>
<td>Control group</td>
<td>70</td>
<td>6.4</td>
<td>93.6</td>
</tr>
</tbody>
</table>

Note: n - Number of observed people; N - normal allele; M - mutant allele.

**Association analysis of RANTES and 308 G>A TNF-α genes in patients with AR**

Today, gene-to-gene interactions remain the least investigated components that would enable us linking the inherited factors with the presence, character and intensity of organ affection in atopic diseases. Simultaneous carrier of alleles “RANTES + TNF-α” was discovered in 5 cases out of 44 observed patients, whereas such combination was not revealed in any observed cases within control group. Moreover, it is important to emphasize that pathological allele RANTES genes, characterized by the increased risk of AR origin in the observed group, were simultaneously discovered in all patients with hetero or homozygous carriers of mutant allele 308G>A TNF-α gene. Statistical analysis of such gene-to-gene interactions showed that the AR origin risk in carriers of adverse “RANTES + TNF-α” genotype genes is 11.5 times more frequent than in patients without such combination (OR=11.7, p<0.05).

Thus, obtained results prove the simultaneous role of “RANTES + TNF-α” alleles’ carrier as a risk factor of AR.
Conclusion

1. Association of polymorphous variant of RANTES - chemokine gene with the
development of year-round (as well as general group) form of AR in Uzbek
population has been discovered.

2. No statistically significant differences have been determined between the general group
of patients and control group in comparative analysis of genetic variants of
polymorphism 308G>A TNF-α gene.

3. For the first time has been determined intergenic interaction of polymorphous
variant of cytokine genes “RANTES + TNF-α” in determination of development
risk of AR (OR=11.7, p<0.05). The presence of such mutant alleles combinations in
patients genotypes significantly rises the risk of disease development for more than
11.5 times (OR=11.7, p<0.05).

References


Harbor Laboratory Press.