THE ARG16GLY POLYMORPHISM IN THE B2-ADRENERGIC RECEPTOR GENE IS ASSOCIATED WITH BRONCHIAL HYPERRESPONSIVENESS AND ALLERGIC RHINITIS IN THE BULGARIAN POPULATION

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ABSTRACT
Bronchial Hyperresponsiveness (BHR) is a risk factor for asthma but it can be observed in allergic rhinitis (AR) and healthy subjects, too. The mechanisms of genetic susceptibility to BHR are unknown. In general, it is thought to result from both genetic and environmental factors. Some studies have demonstrated that nonspecific airway hyperresponsiveness is associated with a specific β2-adrenergic receptor (β2-AR) genotype in asymptomatic healthy subjects. The present study was performed to determine the impact of single nucleotide polymorphism (SNP) on allergic rhinitis patients with evidence for bronchial hyperresponsiveness. One hundred allergic rhinitis patients analyzed for BHR and forty healthy controls were genotyped for polymorphism of the β2-AR gene. Nonspecific airway hyperresponsiveness was measured using the methacholine bronchoprovocation (BPT) test. Polymerase chain reaction (PCR) was used to identify (Arg16/Gly) polymorphism at codon 16 in the β2-AR gene. It was observed that allergic rhinitis patients who are homozygous for the Gly16 allele are more responsive to methacholine than patients who carry the Arg16 allele.


Keywords: bronchial hyperresponsiveness, single nucleotide polymorphism, arginine, glycine, methacholine, β2-adrenergic receptor gene

Introduction
Single nucleotide polymorphisms (SNPs) represent a difference in a single nucleotide. DNA sequence changes observed in more than 1 % of the population are defined as polymorphisms. SNPs are the most common type of genetic variation among people. They occur normally throughout a person’s DNA and may help in predicting an individual’s response to particular drugs, susceptibility to environmental factors and predisposition to some diseases. The role of SNPs for complex diseases, such as allergic conditions simultaneously affecting the upper and lower airways, is unclear.

A significant amount of genetic research related to respiratory allergy, especially asthma, is devoted to SNPs in the coding region of the β2 adrenergic receptor (β2-AR) gene. ADRβ2 agonists alone or in combination with inhaled corticosteroids have extensive use (1) and variations in responses can, in part, be attributed to genetic variation with different polymorphisms. Clinical studies of ADRβ2 polymorphisms are predominantly pharmacogenetic, concerning effects on acute bronchodilator response to short-acting β-agonists or regular use of them and clinical response to regular long-acting β-agonists.

β2-AR is a product of a gene consisting of 1242 bases located on 5q31.32 chromosome long arm. The adrenergic receptor is a member of the seven-transmembrane, G-protein-coupled receptor family (1). The adrenergic receptor is composed of 413 amino acid residues, 7 transmembrane-spanning helices, 3 extracellular and 3 intracellular loops (4). SNPs of β2-AR were first identified in 1993 and 49 different polymorphisms have been identified till now. Their importance remains controversial (1). Functional genetic polymorphisms may be clinically relevant in terms of susceptibility to disease, bronchial hyperresponsiveness or therapeutic response. Most observed polymorphisms are arginine 16 to glycine (Arg16/Gly) and glutamine 27 to glutamic acid (Gln27/Glu) (7).

Clinical observations mainly focus on the receptor regulatory ability on bronchial function and the possibility to downregulate the airways through bronchodilator responses (4). Since in the diploid genome there are two copies of the β2-AR gene, an individual can be homozygous or heterozygous for a given polymorphism. Recent clinical studies tracked genotype-specific response to monotherapy with long-acting B2 agonists or combined therapy with inhaled corticosteroids (8).

Bronchial hyperresponsiveness (BHR) is a hallmark of asthma and can be observed in normal subjects, too, probably because of genetic predisposition (2). A study on 120 healthy subjects analyzed for BHR and genotyped indicated that a specific β2-AR polymorphism at codon 16 might be a genetic determinant of airway hyperresponsiveness (3). To our knowledge, data about the effect of β2-AR SNPs on BHR in allergic rhinitis is very limited.
patients with allergic rhinitis (AR) have not been published so far. The focus of the present study was to determine whether the β2-AR gene Arg16Gly polymorphism influences pulmonary function parameters and BHR in Bulgarian patients with AR.

Materials and Methods

Subjects
The research project was approved by the University Review Board and written informed consent was obtained from all individuals involved in the study. We studied 100 patients with allergic rhinitis (mean age of 37.6, range of 18–59 years) and 40 healthy controls (mean age of 40.1 ± 9.2, range of 18–40 years). The studied population was subdivided into two groups according to their results for bronchial hyperresponsiveness. Positive results from the bronchoprovocation test (BPT) with methacholine were shown by 50 subjects (mean age of 37.52 ± 12.2) and another 50 (mean age of 37.68 ± 12.82) were negative for BPT. None of them had overt asthma or other allergic co-morbidities.

Study design
Inclusion criteria for all patients were clinical data for AR in accordance with the ARIA document and positive skin prick tests to perennial allergens, in order to prove the presence of atopy. Before genotyping, the patients with AR underwent pulmonary function testing. Forced expiratory volume for 1 second (FEV1), forced vital capacity (FVC), forced expiratory flow at 25 % and 75 % (FEF25-75) and peak expiratory flow rate (PEF) adjusted for age, height and gender presented as a percentage of predicted values, were taken into account.

Bronchial methacholine challenge

Equipment and reagents. Spirometer “Spirovit sp-10” (Schiller, Switzerland), which meets the criteria of the European Respiratory Society, was used. Methacholine chloride solutions were prepared from a commercial powder (Methacholine chloride; 98 %; abcr GmbH & Co. KG, Karlsruhe, Germany). A standard dosimeter protocol (SDP) was used, following a detailed laboratory procedure with incremental concentrations of methacholine. BHR was expressed as the provocation concentration of methacholine causing a 20 % decrease in the FEV1 (PC20) (5). PC20 concentrations lower than 8 mg/mL were considered as positive for BPT.

Molecular methods
DNA isolation. Salt precipitation method for DNA extraction from whole blood was used.

Genotyping. Genotyping was performed by allele-specific polymerase chain amplification and agarose gel electrophoresis by a standard protocol. Polymerase chain reaction (PCR) was carried out to amplify a 167 bp region of the β2-AR gene using a forward primer (F): 5' GCCCTCTTCTGCGTGGCACCAGGT 3', and a reverse primer (R): 5' AGACGCTGAACTTGGCCATG 3'. The protocol included six successive steps. The first stage was preparing the PCR mixture for amplification of the DNA region possibly carrying β2-AR Arg/Gly, followed by amplification. The amplified products were analyzed in 2.5 % agarose gels. Restriction reaction with 12 μL of PCR product, 2.4 μL of buffer and 0.24 μL of Nco I 10 IU/μL (Fermentas UAB, Vilnius, Lithuania) was carried out through incubation for 6 h. Electrophoretic separation and visualization of the restriction products preceded the final interpretation of the results.

Statistical analysis
Allelic and genotype frequencies among cases and controls were compared by χ2 Pearson’s test and p-values lower than 0.05 were considered significant. To determine statistical significance, when comparing quantitative variables in independent samples including a small number of cases, Fisher’s exact test was used. The statistical analyses were done with EpiInfo 2008, Statgraphics v 3.5.1 and SPSS for Windows v.16.1 and EXCEL.

Results and Discussion
The objective of our study was to examine the influence of a variation in the β2-AR gene at codon 16 on the airway hyperresponsiveness and pulmonary function in patients with allergic rhinitis.

All patients and controls were genotyped for SNP affecting the codon for amino acid 16 (Gly or Arg). The gender distribution in the group of patients was in favour of women: 63.6 % (n = 63); 36.4 % (n = 36) were men (p = 0.009) in the studied population. The healthy controls showed a similar proportion: 85 % women (n = 34) and 15 % men (n = 6). The frequency of the Gly/Gly genotype was found to be higher in the studied patients (82.6 %) in comparison with that in the group of healthy subjects (17.4 %), p = 0.01 (Fig. 1).

![Fig. 1. Genotypes of the β2-AR gene in patients and controls (in numbers).](image)
The results were different in the negative bronchoprovocation test group. The wild Arg16 genotype had significantly lower frequency (20 %) and only 2 % of the studied individuals were homozygous for Gly/Gly. Twenty-seven (54 %) rhinitic patients were heterozygous (FET; p = 0.001).

The pulmonary function of all subjects (patients and controls) measured with spirometry showed normal values. However, the flow rates of the patients were lower than those of the healthy control subjects.

The pulmonary lung function did not differ from the normal values in both groups of AR and healthy subjects, but the lung volumes (FEV1, PEF and FVC) of the patients were significantly lower (Fig. 3).

We were prompted to test the hypothesis about a possible link between rhinitis pathology, BHR and β2-AR gene polymorphism by a study on 120 healthy subjects which indicated that a specific β2-AR polymorphism at codon 16 might be a genetic determinant of BHR (3). This provoked us to examine the established link between upper (AR) and lower airway pathology (BA) from a genetics point of view. Some studies demonstrated that a gene governing BHR is located on chromosome 5q31 (6) – the same chromosome that the β2-AR gene is located on. It is well known that SNPs within a gene or in a regulatory region near a gene may affect the gene’s function and may, thus, play a more direct role in disease. The β2-adrenergic receptor expression might also affect the function of cholinergic receptors controlling airway contractility, which might influence the genotypic hyperresponsiveness to methacholine (3). Data on the functionality of ADRB2 polymorphisms are limited to genotypes rather than haplotypes. The effect of a single SNP may be negated by other polymorphisms and it is the haplotype that determines the overall characteristics of the receptor (1). Nevertheless, we accept the possible role of a single arg16gly polymorphism in the β2-adrenergic receptor in the regulation of airway responsiveness in rhinitis patients.

In our study patients with AR were found to have positive response to methacholine and alleged association with receptor gene polymorphism was explored. It was identified that Allergic Rhinitis patients who are homozygous for the Gly16 allele are more responsive to methacholine than patients who carry the Arg16 allele. A continuous clinical observation on AR patients with Mch+ who are homozygous for the Gly16 allele in a subsequent period of time would be of significant interest.

Some limitations of this study are that the role of β2-AR single gene polymorphism on pulmonary function was not precisely estimated because of lack of information about SNP impact on each spirometric value.

Conclusions
The Gly16Gly genotype variant of β2-AR gene was shown to be more frequent than the wild-type Arg16Arg in a Bulgarian population of Allergic Rhinitis patients. Specific genotypes of the receptor gene may be associated with bronchial hyperresponsiveness in patients with Allergic Rhinitis without overt phenotype asthma. The findings of our study suggest such a relationship, but they should be interpreted with caution.

REFERENCES