

ORIGINAL ARTICLE

ARG16GLY POLYMORPHISM IN THE β_2 -ADRENOCEPTOR GENE IN PATIENTS WITH BRONCHIAL ASTHMA

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SUMY STATE UNIVERSITY, SUMY, UKRAINE**ABSTRACT**

The aim: The objective of the study was to analyze the frequency of Arg16Gly polymorphism in the β_2 -adrenoceptor (β_2 -AR) gene in patients with bronchial asthma (BA) and to assess the association of the polymorphism with BA risk.

Materials and methods: We examined 553 BA patients and 95 apparently healthy individuals. Arg16Gly polymorphism in the β_2 -AR gene (rs1042713) was determined using polymerase chain reaction-restriction fragment length polymorphism analysis. Statistical analysis of obtained results was performed using SPSS-17 program.

Results: It was established that distribution of Arg/Arg, Arg/Gly, and Gly/Gly genotypes for Arg16Gly polymorphism in the β_2 -AR gene was 44.2%, 40.0%, 15.8% in the control group vs. 31.3%; 45.7% and 23.0 among BA patients, respectively ($\chi^2 = 6.59$; $p = 0.037$). No significant difference was observed with regards to the distribution of genotypes for Arg16Gly polymorphism in the β_2 -AR gene in men and women controls ($\chi^2 = 4.05$; $p = 0.13$) and BA patients ($\chi^2 = 4.34$; $p = 0.11$). BA risk was 1.74 times higher in the minor allele carriers (Arg/Gly + Gly/Gly genotypes) for Arg16Gly polymorphism in the β_2 -AR gene.

Conclusions: Analysis of Arg16Gly polymorphic variants in the β_2 -AR gene showed a statistically significant difference in the distribution of Arg/Arg, Arg/Gly, and Gly/Gly genotypes in patients with BA and apparently healthy individuals due to the higher frequency of Arg/Arg genotype in controls and higher frequency of Gly/Gly genotype in patients with asthma. No difference with regard to gender was found in the distribution of genotypes.

KEY WORDS: bronchial asthma, Arg16Gly polymorphism in the β_2 -adrenoceptor gene

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INTRODUCTION

β_2 -adrenoceptors (β_2 -AR) are an integral part of the sympathetic nervous system and are important for the respiratory and cardiovascular systems functioning, as well as for some immune and metabolic processes [1]. The gene encoding ADRB2 was cloned by Kobilka et al. The gene encoding β_2 -AR is one of the most well-studied candidate genes for bronchial asthma (BA) and has more than 500 single nucleotide substitutions in the coding part. Among them, the most extensively studied non-synonymous nucleotide substitutions are arginine for glycine at position 16 (Arg16Gly (rs1042713)) and glutamine for glutamic acid at position 27 (Gln27Glu (rs1042714)) [1,2], which play an important role for receptor functioning. The results of numerous studies among different ethnic groups on the Arg16Gly polymorphism in the β_2 -AR gene are contradictory, since many studies have shown the role of this single nucleotide polymorphism in the pathogenesis of asthma, bronchial hyperreactivity, pulmonary dysfunction and impaired response to β_2 -agonists, while other studies dismissed this association [3,4]. The main reasons why the role of this polymorphism has been shown discrepant in various studies are population heterogeneity and insufficient sample size. Therefore, the clinical application of genetic testing for polymorphic variants in the β_2 -AR gene is of special interest.

Although several studies revealed no association between Arg16Gly polymorphism and asthma, the former

has been shown to correlate with some disease phenotypes such as severe and nocturnal asthma [5,6]. Given the discrepant results regarding the role of Arg16Gly polymorphism in the β_2 -AR gene in asthma development and since they were not found in our population, the objective of our study was to compare the frequency of this polymorphism in patients with asthma and in apparently healthy individuals, as well as to assess the influence of the polymorphism on BA risk.

THE AIM

The objective of the study was to analyze the frequency of Arg16Gly polymorphism in the β_2 -adrenoceptor (β_2 -AR) gene in patients with bronchial asthma (BA) and to assess the association of the polymorphism with BA risk.

MATERIALS AND METHODS

553 patients with bronchial asthma were examined. All of them had previously signed an informed consent form. The control group consisted of 95 apparently healthy individuals with no history of asthma symptoms, symptoms of allergies and atopy, hypersensitivity to aspirin and nonsteroidal anti-inflammatory drugs; the subjects were non-smokers and had no history of smoking, no acute or chronic somatic diseases in the acute stage, or chronic

Table 1. Genotype and allele distribution for Arg16Gly polymorphism in the β_2 -adrenergic receptor gene with regard to gender

rs 1042713	Control group, n = 95				BA patients, n = 553			
	Men, n = 45		Women, n = 50		Men, n = 193		Women, n = 360	
Genotype	n	%	n	%	n	%	n	%
Arg/Arg	21	46.7	21	42.0	71	36.8	102	28.3
Arg/Gly	14	31.1	24	48.0	83	43.0	170	47.2
Gly/Gly	10	22.2	5	10.0	39	20.2	88	24.5
	$\chi^2 = 4.05$; $p = 0.13$				$\chi^2 = 4.34$; $p = 0.11$			
Arg allele	62.2		66.0		58.3		51.9	
Gly allele	37.8		34.0		41.7		48.1	

Table 2. Analysis of the association between Arg16Gly polymorphism in the β_2 -adrenergic receptor gene and BA risk

Model	P_{obs}	OR _{obs} (95% CI)	AIC
Dominant	0.01	1.74 (1.11 – 2.71)	19.7
Recessive	0.12	1.59 (0.91 – 2.96)	23.0
Super-dominant	0.29	1.27 (0.82 – 1.99)	24.4
Additive	0.02	1.47 (1.08 – 2.01)	19.4

Notes: P_{obs} – observed p-value (not adjusted for covariates); OR_{obs} – observed odds ratio; CI – confidence interval; AIC – Akaike's information criterion.

infectious diseases of the upper airways, or autoimmune and oncological diseases. Among the studied patients, there were 360 women (65.1%) and 193 men (34.9%), and the control group consisted of 45 men (47.4%) and 50 women (52.6%). The mean age of BA patients was (42.3 ± 0.71) years, and the mean age of controls was (44.1 ± 1.53) years. The study has been approved by the Bioethics Committee of Medical Institute of Sumy State University. Arg16Gly polymorphism in the β_2 -AR gene (rs1042713) was determined using polymerase chain reaction-restriction fragment length polymorphism analysis. Statistical analysis of obtained results was performed using SPSS-17 program. Comparison of the distribution of genotypes in the experimental and control groups and verification of the correspondence of this distribution to the Hardy-Weinberg equilibrium was performed using Pearson's chi-squared test. In order to determine BA risk, the odds ratio (OR) and 95% confidence interval (CI) for dominant, recessive, superdominant and additive inheritance models were calculated. The relevance of the obtained results was assessed with Akaike's information criterion. All tests were two-sided, the p-value < 0.05 was considered statistically significant.

RESULTS

The analysis of correspondence of the distribution of genotypes for Arg16Gly polymorphism in the β_2 -AR gene to Hardy-Weinberg equilibrium in the control group and in BA patients showed no statistically significant deviations from the expected genetic population values ($p > 0.05$). It was established that the distribution of Arg/Arg, Arg/Gly,

and Gly/Gly genotypes for Arg16Gly polymorphism in the β_2 -AR gene was 44.2%, 40.0%, 15.8% in the control group vs. 31.3%; 45.7% and 23.0% among BA patients, respectively. The frequency of the major (Arg) and minor (Gly) alleles in patients with asthma was 48.4% and 51.6% vs. 52.6% and 47.4% in the control group. By means of the chi-square test, a statistically significant difference was found with regards to the distribution of genotypes for this polymorphism in the β_2 -AR gene in patients with asthma and in the group of apparently healthy individuals ($\chi^2 = 6.59$; $p = 0.037$).

The results of the analysis of genotype and allele distribution for Arg16Gly polymorphism in the β_2 -AR gene in BA patients and controls are presented in Table 1.

Thus, no significant difference was observed for the distribution of alleles and genotypes for Arg16Gly polymorphism in the β_2 -AR gene in men and women controls ($\chi^2 = 4.34$; $p = 0.11$) and BA patients ($\chi^2 = 4.05$; $p = 0.13$). In addition, a significant difference was found with regard to the distribution of genotypes for the studied polymorphism in women with asthma and apparently healthy women ($p = 0.03$; $\chi^2 = 6.77$). In particular, women with BA had Gly/Gly genotype more often (24.5%) than those from the control group (10%). Thus, a gender-dependent statistically significant difference was found only for the distribution of genotypes among women both in the case and control groups.

Asthma is known to be a genetically determined disease, and β_2 -AR gene plays a special role among the candidate genes that take part in BA development [1,4]. Given the discrepant results obtained in a number of studies in different populations regarding the correlation of Arg16Gly polymorphism in the β_2 -AR gene with asthma development, we assessed the influence of the polymorphism on BA risk in the study subjects.

Table 2 represents the results of the analysis as for the association between genotypes for Arg16Gly polymorphism in the β_2 -AR gene and BA risk using binary logistic regression in four models of inheritance.

A statistically significant correlation was found for dominant ($P_{obs} = 0.01$) and additive ($P_{obs} = 0.02$) models. The risk of developing asthma in minor allele carriers (Arg/Gly and Gly/Gly genotypes) was 1.74 times higher (95% CI = (1.11 – 2.71) than in the major allele homozygotes (Arg/Arg) (according to the dominant model).

DISCUSSION

Sufficient data has been obtained indicating that a large number of genes are involved in the pathogenesis of asthma, and each of them individually makes a certain contribution to the realization of this mechanism. This confirms that asthma development involves a genetic mechanism. Along with genetic factors which determine the level of immunoglobulin E and directly define predisposition to atopy, the most significant role in the development of asthma is played by genes that control the degree of bronchial reactivity. The key place with regard for bronchial contractility belongs to β_2 -AR, which determines the contractile capacity of the bronchi and is a target for the most commonly used bronchodilators – β_2 -agonists. Numerous studies demonstrated that the risk of asthma, the clinical course of the disease, variability of the response to β_2 -agonist depend on polymorphisms in the β_2 -AR gene [5,6]. Therefore, our study was aimed to study the distribution of alleles and genotypes for Arg16Gly polymorphism in the β_2 -AR gene in BA patients and apparently healthy individuals, as well as to analyze possible relationships of this polymorphic variant with the risk of asthma. Arg16Gly polymorphism in the β_2 -AR gene is one of the most studied SNPs and is due to arginine-to-glycine amino acid substitution at position 16. The choice of this polymorphism was conditioned by its high frequency in different populations and insufficient study data in our population [7]. Moreover, it is a non-synonymous polymorphism, which is responsible for the differences in protein composition. This, in turn, can influence BA development as well as response to the drugs [8].

The study of the frequency of alleles and genotypes for Arg16Gly polymorphism in the β_2 -AR gene in patients with asthma and apparently healthy individuals showed a significant difference according to Pearson's chi-squared test ($\chi^2 = 6.59$; $p = 0.037$). According to the data obtained, the Gly/Gly genotype was more frequent in BA patients (23.0%) vs. controls (15.8%). The frequency of Arg16Gly mutation variant equaled 45.7% in patients with asthma vs. 40.0% in healthy individuals, which is comparable to the data obtained for the European population (37–40%), but differs from the Asian population data (73%) [1,9].

In addition, a correlation was found between this polymorphism and the risk of asthma. In particular, BA risk for the minor allele carriers (Arg/Gly + Gly/Gly genotypes) is 1.74 times higher ($p = 0.01$) than for homozygous Arg/Arg allele carriers. Similar data were previously obtained in the study of Zh. A. Mironova et al. The study demonstrated the association of allelic variants of Arg/Gly polymorphism in the β_2 -AR gene with clinical phenotypes of asthma. Thus, Gly/Gly genotype carriers probably have increased risk of blood eosinophilia as an indirect marker of eosinophilic inflammation, and the risk of respiratory failure development and progression [10]. This is confirmed by the study of N. N. Chakova et al. (2017), which revealed the association of Gly16 allele of Arg16Gly polymorphic variant with an increased risk of atopic diseases (OR 1.28; 95% CI 1.01–1.63) [11]. This is indicative of its role in the development of asthma. The higher frequency of Arg/Arg genotype

observed in our study in controls vs. BA patients was also demonstrated in other studies. Thus, the protective role of this genotype in relation to the risk of allergic diseases has been confirmed [1,11]. A meta-analysis of data obtained in 28 studies showed that Gly16 allele did not contribute to the predisposition to asthma in general or to BHR, but it had a strong association with nocturnal (OR 2.20; 95% CI 1.56–3.11) and severe to moderate asthma (OR 1.42; 95% CI 1.04–1.94). Gly16 homozygotes had a 5.15-fold higher risk of nocturnal and 2.84-fold higher risk of severe asthma as compared to Arg16 homozygotes [5]. The association of 16Gly allele with nocturnal asthma has also been found in Mexicans, Taiwanese, and Caucasians living in the United States, and with severe BA in Caucasians from New Zealand [11,13]. This confirms that the polymorphism in the β_2 -AR gene does not significantly contribute to the occurrence of asthma in some populations, but may influence the development of certain phenotypes of the disease. Therefore, during the further studies conducted in order to identify the possible association of Arg16Gly polymorphism in the β_2 -AR gene with BA risk, it is important to consider phenotypes of the disease, time of onset, body weight, and concomitant pathology. This may represent the basis for further study of this polymorphism in order to detail the features of asthma phenotypes taking into account genetic determinants. This will help to create a more personalized, recommendation-based treatment of selected groups of patients with reduced response to treatment.

CONCLUSIONS

Analysis of Arg16Gly polymorphic variants in the β_2 -AR gene showed a statistically significant difference in the distribution of Arg/Arg, Arg/Gly, and Gly/Gly genotypes in patients with BA and apparently healthy individuals due to the higher frequency of Arg/Arg genotype in controls and higher frequency of Gly/Gly genotype in patients with asthma. No difference with regard to gender was found in the distribution of genotypes. Women with BA had Gly/Gly genotype more often (24.5%) than those from the control group. BA risk was 1.74 times higher in the minor allele carriers (Arg/Gly + Gly/Gly genotypes) than that in the carriers of the Arg/Arg genotype for Arg16Gly polymorphism in the β_2 -AR gene. This finding can be used for BA risk prediction.

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Conflict of interest

The Authors declare no conflict of interest.

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