

An investigation of the association between *ADRB2* gene polymorphisms and asthma in Kazakh population

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Study conception and design RB, AA*. The acquisition and analysis of data MA, AA, BK, AP, AA*. Manuscript writing and editing RB, AA*. All authors have seen and approved the final version of the manuscript.

Conflict of interests

AA*, AA, MA, BK, AP and RB have no conflicts of interest with the contents of this article.

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Abstract

Introduction: Beta-2-adrenergic receptor (ADRB2) is present in the cells of the respiratory tract, including bronchial smooth muscle cells and bronchial epithelium, and is a target for endogenous catecholamines and drugs used to treat obstructive lung diseases.

Objectives: This study aimed to investigate the possible association of the Arg16Gly and Gln27Glu polymorphisms of the *ADRB2* gene with asthma and its endo-phenotypes in the Kazakh population.

Methods: A total of 70 asthmatic patients and 80 healthy controls were genotyped for Arg16Gly and Gln27Glu polymorphisms of the *ADRB2* gene by using quantitative real-time polymerase chain reaction. Statistical analysis was performed with the Graph Pad InStat 7 Software.

Results: No associations between asthma patients and healthy individuals were found when the allele and genotype distribution of Arg16Gly and Gln27Glu single nucleotide polymorphisms were compared. Analysis of the haplotype frequencies showed statistically significant differences between patients with asthma and controls for Arg16Gly/Gln27Gln and Arg16Gly/Gln27Glu haplotypes (odds ratio [OR] = 2.12, 95% confidence interval [CI] = 0.87-5.16 and OR = 2.25, 95% CI = 0.89-5.67, respectively). The Arg16 allele and Arg16Arg genotype frequencies were higher in patients with uncontrolled asthma than in controls ($\chi^2 = 5.17$, $df = 1$, $p = 0.02$ and $\chi^2 = 5.36$, $df = 1$, $p = 0.02$, respectively).

Conclusion: The results of this study support the possible involvement of Arg16Gly polymorphism in the development of uncontrolled asthma, and indicate that Arg16Gly/Gln27Gln and Arg16Gly/Gln27Glu haplotypes are more common in asthma patients in the Kazakh population.

Keywords: asthma, beta-2-adrenergic receptor, single nucleotide polymorphisms, Kazakhstan

1. Introduction

Asthma is a common heterogeneous disease characterised by chronic airway inflammation, bronchial hyperresponsiveness, and variable airflow obstruction. Approximately 300 million people currently suffer from asthma [1], and there is a trend towards increase in the prevalence of asthma in developing countries [1, 2].

Asthma therapy focuses on decreasing the symptoms and exacerbations and recovering normal pulmonary function. The aim is to achieve asthma control, which is possible with the prescription of drugs under long-term treatment as well as therapy to control acute symptoms [3]. Agonists of beta-2-adrenergic receptors (ADRB2) are recommended to relax the smooth muscle of the bronchi as the most effective and safe bronchodilators (short- and long-acting beta-agonists [SABAs and LABAs, respectively]) [4].

ADRB2 are cell membrane-spanning receptors, widely distributed in the lungs [5]. After the binding of ADRB2 to its ligand, the G-protein stimulates the adenylate cyclase signalling pathway, leading to airway smooth muscle relaxation. Additionally, it has been reported that activation of ADRB2 induces anti-inflammatory effects, such as mast cell stabilisation, cytokine production, and decrease of protein extravasation in the lungs [5, 6].

The *ADRB2* gene is located on chromosome 5q31-32, and has approximately 80 single nucleotide polymorphisms (SNPs), of which Arg16Gly and Gln27Glu are the most common non-synonymous polymorphisms [7, 8]. Polymorphisms of *ADRB2* gene, including Arg16Gly and Gln27Glu, disrupt the receptor function and contribute to increased risk of asthma and change the response to endogenous or inhaled adrenergic agonists [9, 10]. Studies have also shown the association between these SNPs and response to drug therapy [11], nocturnal asthma [12], and severe asthma [13]. However, large-scale population surveys and meta-analyses have demonstrated inconsistent results [14, 15, 16]. Despite the specific associations found in many populations with asthma, the nature of the relation between the Arg16Gly and Gln27Glu polymorphisms of the *ADRB2* gene and asthma in the Kazakh population is uncertain.

2. Materials and methods

Study participants

Patients with asthma were recruited from the City Hospital No. 2 of Nur-Sultan between January 2018 and July 2019. The survey comprised of 46 females and 24 males aged 20-68 years. The diagnosis of asthma was confirmed by the clinical profile and spirometry results in accordance with the Global Strategy for Asthma recommendations. The asthma control test was applied to determine controlled (score ≥ 20) and uncontrolled asthma (score < 20) [17]. The administration of drug therapy prior to the study was confirmed if patients were undergoing treatment with inhaled drugs (SABAs, LABAs, and inhaled corticosteroids [ICSs]) in the past 12 months [18]. We divided the asthma patients into two different groups depending on the Th2 inflammation levels: Th2-high and Th2-low. Th2-high was indicated by the presence of symptoms of an allergic component caused by allergen exposure, aggravated allergic history, and increased total immunoglobulin-E (IgE) and interleukin 4 (IL-4) in blood plasma [19]. The control group comprised of 80 healthy individuals. The inclusion criterion for the controls was absence of chronic respiratory, allergic, neurological, and systemic diseases, and other chronic diseases in the acute phase. All the participants, including asthma patients and healthy individuals, were of Kazakh descent. The control and study groups corresponded to each other with respect to age and sex, and their main characteristics are presented in Table 1. The study protocol was approved by the Ethics Committee of the Astana Medical University, and signed informed consent was provided by all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki, 2013 (<http://www.wma.net/en/30publications/10policies/b3/index.html>).

DNA isolation

Genomic DNA was isolated from frozen (-20°C) ethylenediaminetetraacetic acid-treated peripheral blood samples of patients with asthma and the control group using DNA-Extran-1 reagents (Synthol LLC, Moscow, Russia). Quantitative and qualitative assessment of the DNA preparations was carried out using spectrophotometric analysis. Aqueous solutions of DNA were used to study the genetic polymorphisms of the *ADRB2* gene.

Genotyping of ADRB2 by polymerase chain reaction-real time

The study of the polymorphic variants of the *ADRB2* gene (Arg16Gly - rs1042713; Gln27Glu - rs1042714) was carried out by the quantitative real-time polymerase chain reaction (qPCR) using LLC Syntol reagents kits (Moscow, Russia).

Reagents for DNA amplification were presented as components ($2.5 \times$ diluent, $2.5 \times$ reaction mixture, Taq DNA polymerase, and one negative and three positive control samples) that were mixed in the required volume immediately before the study. Principal components were calculated and introduced into the PCR tubes with $20 \mu\text{l}$ of the mixture components,

then mixed and centrifuged; 5 µl of control and test samples (asthma patients and control group) were added to each PCR tube in accordance with the label. Amplification was performed on a Bio-Rad instrument. HEX and FAM fluorophores were used. Analysis of the research results was performed using the Bio-Rad program.

Enzyme-linked immunosorbent assay (ELISA)

The content of IL-4 and IgE in blood plasma was determined by enzyme immunoassay using reagent kits of CJSC Vector-Best, Novosibirsk, Russia. The optical density of the solutions was measured at a main wavelength of 450 nm by a spectrophotometer. The calibration curve was drawn and the arithmetic mean of the IL-4 and IgE concentrations was calculated for assessing the results.

Statistical analysis

Statistical analysis was performed using GraphPad InStat 7 software (Graphpad Software Inc., San Diego, CA). The differences between the main characteristics of the study participants were determined using the student's t-test and χ^2 -criterion. The Hardy-Weinberg equilibrium was evaluated using the χ^2 -test. The odds ratio (OR), 95% confidence intervals (CI), and p-values were calculated to assess the differences between the frequency of genotypes between the cases and controls. Statistical significance was established at $p < 0.05$.

The Kolmogorov-Smirnov test was used to evaluate the immunological data. The student's t-test was applied in the case of normal distribution, while the Mann-Whitney test was used in the absence of a normal distribution.

3. Results

The current study recruited 150 asthma patients and healthy individuals. The characteristics of the study participants are presented in Table 1. Comparison between the cases and controls revealed significant differences in the family history of allergic diseases, allergic rhinitis, and IL-4 and IgE levels.

The distribution of the frequencies of the genotypes for the *ADRB2* Arg16Gly and Gln27Glu polymorphisms in the asthma and control group corresponded to the Hardy-Weinberg equilibrium (Table 2).

The frequencies of *ADRB2* alleles and genotypes in asthmatic patients and controls are presented in Tables 3 and 4. The frequencies of the allele A of the Arg16Gly SNP were 0.46 in patients with asthma and 0.50 in controls. The distribution of *ADRB2* genotypes were found to be 0.20 for A/A, 0.51 for A/G, and 0.29 for G/G in the asthma group, and 0.30 for A/A, 0.40 for A/G, and 0.30 for G/G in the control group. There were no statistically significant differences found in the distribution of alleles and genotypes between the asthma

patients and healthy participants ($\chi^2 = 0.53$, $df = 1$, $p = 0.47$ and $\chi^2 = 0.48$, $df = 1$, $p = 0.49$, respectively) (Table 3).

The allelic frequencies for the Gln27Glu polymorphism for C and G alleles, respectively, were 66.7 and 33.3 in the asthma group, and 70.3 and 29.7 in the control group. The genotype frequencies for the C/C, C/G, and G/G genotypes were 46.4, 40.6, and 13.0, in the asthma group and 46.8, 40.5, and 12.7 in the control group, respectively (Table 4).

The most remarkable difference between the groups in terms of haplotype frequencies of Arg16Gly/Gln27Gln was observed in 18 patients in the asthma group and 9 in the control group (OR = 2.12, 95% CI = 0.87-5.16), and Arg16Gly/Gln27Glu was detected in 17 asthma patients and 8 controls (OR = 2.25, 95% CI = 0.89-5.67) (Table 5).

Statistical analysis, based on the detected alleles and frequencies, showed that the allele A and genotype AA of *ADRB2* Arg16Gly polymorphism were significantly associated with uncontrolled asthma as compared to controlled asthma ($p = 0.02$). On the other hand, allele A of this SNP showed a lower frequency in patients with controlled asthma (Table 6).

We did not find any significant association between the alleles, genotypes, and groups of patients with various levels of Th2 inflammation showing a significant difference between Th2-high and Th2-low asthma (Table 7).

4. Discussion

Asthma is a multifactorial disease, which means that both genetic and environmental factors contribute to its development. In fact, genetic factors affect the responses to anti-asthma drugs in about 60-80% of individuals [20]. Currently, complete understanding of the asthma genetics and related pathologic processes has not yet been achieved; therefore, the existing system of diagnosis and treatment of asthma needs further development. Studies have shown that genetic polymorphisms mediate responses to drug therapy through different pharmacokinetic mechanisms [21]. The investigation of the drug target genes, one of which is the *ADRB2* gene, is of great interest. Genetic variants of *ADRB2* gene cause a change in the expression, function, and regulation of *ADRB2*, and affect the response to beta-2-agonist therapy to some extent [7].

Previous studies on the involvement of the *ADRB2* gene in the development and pharmacogenetics of asthma are conflicting [8, 14-16]. Perhaps this is because the genotype, allele, haplotype, and diplotype frequencies of the *ADRB2* gene polymorphisms Arg16Gly and Gln27Glu show different distributions among populations and ethnic groups [10, 12, 22]. We found that the frequencies of the Arg16Gly alleles identified in our study matched with the established allele frequencies of the American (A allele, 0.45 and G allele, 0.54) and

South Asian (A allele, 0.44 and G allele, 0.54) populations. The allele frequencies of Gln27Glu polymorphism were more close to the allele frequencies of the European population (C allele, 0.59 and G allele, 0.41) [www.ensembl.org]. We have not detected a statistically significant association between the allele and genotype frequencies of Arg16Gly and Gln27Glu polymorphisms of asthma patients and healthy individuals. The lack of association between these polymorphisms and the risk of asthma in many adult populations has been confirmed by several meta-analyses [14, 15, 23]. However, the most recent and more comprehensive meta-analysis showed that Gln27Glu polymorphism has a protective effect against asthma, and the influence of Arg16Gly SNP depends on ethnicity. Furthermore, it showed a disease predisposition in Arab and Hispanic-Latino populations, as well as reduced risk of asthma in the Indian population [24]. Besides, meta-analyses of the paediatric and young populations indicated that Arg16Gly and Gln27Glu SNPs were positively associated with asthma susceptibility and treatment response, demonstrating a significant contribution of the genetic component to the disease and therapeutic reactions at a young age [11, 16, 25, 26].

We identified a significant association of the Arg16 allele and Arg16Arg genotype with uncontrolled asthma, and this concurred with the results of previous studies. Rebordosa et al. [18] observed an increased risk of uncontrolled asthma with the Arg16 allele in patients of European descent. No relationship was revealed between the increased risk of uncontrolled asthma and the Arg16 allele in asthma patients using ICS and/or LABA. Additionally, Almomani et al. [27] reported that the Arg16 allele of Arg16Gly polymorphism is more common among patients with uncontrolled asthma in the Arab population. The influence of Arg16Gly polymorphism on asthma control could be related to impaired ADRB2 function due to the presence of arginine instead of glycine in its structure in the 16th amino acid position. It was reported that Arg16 causes a change in the conformation of the ADRB2, which leads to a decrease of the binding site availability for interactions with ligands [28]. It is assumed that the absence of the effect of the genotypes studied on asthma control among ICS users could be associated with reversed desensitisation of ADRB2 [18]. The materials for our study were collected in the city hospital providing specialized medical care, and the conditions were conducive for faster sampling and obtaining complete data of physical examination and laboratory and instrumental tests. More than half the asthma patients included in this study had not undergone basic treatment in the last 12 months, and they had been hospitalised for uncontrolled asthma and acute attacks. This probably led to some bias

in the structure of our sample, and possibly explained the similarity between our findings and the results obtained by Rebordosa et al. [18].

Beta-2-agonists are also widely used to manage the symptoms of chronic obstructive pulmonary disease (COPD). Patients with COPD and asthma have similar respiratory symptoms, which could lead to difficulties in the differential diagnosis between them in certain cases [29]. Previous studies do not allow drawing an unequivocal conclusion about the involvement of *ADRB2* polymorphisms in predisposition of COPD and response of COPD patients to treatment with beta-2-agonists. Meta-analyses [30] included 85,381 studies' participants and did not find a significant association of Arg16Gly and Gln27Glu polymorphisms with the risk of disease and therapeutic effects of inhaled beta-2-agonists. Hussein et al. [31] revealed an association of the Arg16 genotypes with highly pronounced respiratory symptoms and frequent exacerbations in Egyptian patients with COPD, and these findings were consistent with our data. However, a recent study by Karimi et al. [32] identified that Arg16/Gln27 haplotype reduces the risk of COPD exacerbation in patients using beta-2-agonists. The contradictory relationship between genetic variants of *ADRB2* and the predisposition to asthma and COPD could be because each of these diseases includes common and specific endotypes and phenotypes, where the alleles of various genes make different contributions [29, 33]. Furthermore, ethnicity traits of the allele frequencies, genetic interactions, different research designs, presence of comorbidities, and use of concomitant drugs could affect the results of studies.

Currently, the study of asthma endotypes and phenotypes defined using genetic and molecular markers is especially relevant. A detailed characterisation of asthma patients contributes to the investigation of the disease pathogenesis, selection of the most optimal therapeutic approaches, monitoring the treatment effectiveness, and assessing the risk of disease progression [33]. Arg16Gly and Gln27Glu *ADRB2* gene polymorphisms showed association with intermediate asthma phenotypes: nocturnal [12], severe [13], uncontrolled [18, 27], therapy-resistant [34], and atopic [35]. The most frequent asthma endotype is type 2 asthma [19]. No association was found between polymorphisms of *ADRB2* gene (Arg16Gly and Gln27Glu) and asthma patients with Th2-high and Th2-low inflammation types in our research.

Asthma is a polygenic disease of the respiratory system with treatment aimed at reducing the symptoms of bronchial obstruction [3]. The genetic predisposition of asthma requires clarification of many issues related to the ethnic and population features of allele combinations, intragenic, gene-gene and gene-environment interactions, as well as epigenetic

modulations [24, 36]. Genetic and epigenetic traits have been proposed as biological markers that can identify various subtypes of the disease. The *ADRB2* gene is considered as one of the most promising markers of an individual's susceptibility to asthma and therapeutic response. Despite the controversial results of the definition of *ADRB2* gene polymorphisms in different populations, the study of its variants provides new opportunities for the development of diagnostic and therapeutic strategies based on the genetic characteristics of patients. This study is the first investigation that showed that polymorphisms of the *ADRB2* gene could influence the asthma risk and levels of disease control in the adult Kazakh population. However, it should be noted that these results are preliminary and further researches conducted on a larger scale and for different types of asthma are needed to confirm them.

In conclusion, this study demonstrates that Arg16Gly polymorphism of the *ADRB2* gene contributes to uncontrolled asthma, and *ADRB2* haplotypes could be responsible for susceptibility in the Kazakh population.

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Table 1 – Demographic and clinical characteristics of study participants

Parameters	Control group	Asthma patients
Study participants, n	80	70
Age in years, SD	52.3±8.8	53.0±7.6
Male, n, %	20 (54.05%)	24 (40%)
Smokers, n, %	15 (18.8%)	16 (22.9%)
Family history of allergic diseases, n, %	6 (7.5%)	**22 (31.4%)
Allergic rhinitis, n, %	-	16 (22.9%)
FEV1%, SD	96.1 (±5.2)	82.6 ±13.7
FEV1/FVC, SD	0.89 (±0.5)	0.73 ±0.8
IL-4, pg/ml	3.6 (0.57-18.8)	**23.5 (0.7-151.5)
IgE, pg/ml	42.3 (1.2-145.6)	*102.6 (6-280)
Mild asthma, n, %	-	1 (1.4%)
Moderate asthma, n, %	-	66 (94.3%)
Severe asthma, n, %	-	3 (4.3%)
Inhaled SABAs, n, %	-	64 (91.4%)
Inhaled LABAs, n, %	-	42 (60%)
Inhaled steroids, n, %	-	37 (52.9%)
Controlled and partly controlled asthma, n, %	-	31 (44%)
Uncontrollable asthma, n, %	-	39 (55%)
Th2 "high" asthma, n, %	-	28 (40.6%)
Th2 "low" asthma, n, %	-	41 (59.4%)
FEV1: forced expiratory volume in first second; FVC: forced vital capacity; SABAs: short-acting beta-agonists; LABAs: long-acting beta-agonists. *p <0.05, ** p<0.01 when compared to the control group		

Table 2 - Compliance of the Arg16Gly and Gln27Glu ADRB2 polymorphisms genotypes distribution to the Hardy-Weinberg equilibrium in asthma patients and controls

Study participants	Genotypes of Arg16Gly polymorphism			χ^2	p
	<i>A/A</i>	<i>G/A</i>	<i>G/G</i>		
Controls	0.250	0.500	0.242	3.08	0.08
Asthma patients	0.209	0.496	0.295	0.09	0.76
	Genotypes of Gln27Glu polymorphism				
	<i>C/C</i>	<i>C/G</i>	<i>G/G</i>		
Controls	0.450	0.442	0.108	0.54	0.46
Asthma patients	0.444	0.444	0.111	0.52	0.47

Table 3 - Distribution of alleles and genotypes of the Arg16Gly ADRB2 polymorphism in asthma patients and in controls

Alleles and genotypes	Asthma patients	Controls	χ^2	p	OR	
	n = 70	n = 73			Value	95% CI
A allele	0.457	0.500	0.53	0.47	0.84	0.53 – 1.34
G allele	0.543	0.500			1.19	0.75 – 1.89
A/A genotype	0.200	0.301	0.48	0.49	0.58	0.27 – 1.25
A/G genotype	0.514	0.397			1.61	0.83 – 3.12
G/G genotype	0.286	0.301			0.93	0.45 – 1.91

Table 4 - Distribution of alleles and genotypes of the Gln27Glu ADRB2 polymorphism in patients with asthma and in the control individuals

Alleles and genotypes	Asthma patients	Controls	χ^2	p	OR	
	n = 69	n = 79			Value	95% CI
C allele	0.667	0.703	0.41	0.52	0.84	0.50 – 1.42
G allele	0.333	0.297			1.18	0.70 – 1.99
C/C genotype	0.464	0.468	0.01	0.94	0.98	0.51 – 1.88
C/G genotype	0.406	0.405			1.00	0.52 – 1.94
G/G genotype	0.130	0.127			1.04	0.39 – 2.72

Table 5 - The frequencies of ADRB2 gene polymorphisms combination (Arg16Gly and Gln27Glu) in patients with asthma and healthy subjects

Combination of polymorphisms	Asthma patients, (%)	Controls, (%)	OR	95% CI
Arg16Arg and Gln27Gln	9 (13.2)	14 (22.6)	0.52	0.21-1.31
Arg16Arg and Gln27Glu	3 (4.4)	5 (8.1)	0.53	0.12-2.30
Arg16Arg and Glu27Glu	1 (1.5)	2 (3.2)	0.45	0.04-5.06
Gly16Gly and Gln27Gln	4 (5.9)	5 (8.1)	0.71	0.18-2.78
Gly16Gly and Gln27Glu	8 (11.8)	14 (22.6)	0.46	0.18-1.18
Gly16Gly and Glu27Glu	7 (10.3)	4 (6.5)	1.66	0.46-5.98
Arg16Gly and Gln27Gln	18 (26.5)	9 (14.5)	2.12	0.87-5.16
Arg16Gly and Gln27Glu	17 (25.0)	8 (12.9)	2.25	0.89-5.67
Arg16Gly and Glu27Glu	1 (1.5)	1 (1.6)	0.91	0.06-14.87

Table 6 - The allele and genotype frequencies of ADRB2 Arg16Gly polymorphism in asthma patients with different phenotypes

Asthma phenotypes	Alleles		χ^2	p	Genotypes			χ^2	p
	A	G			AA	AG	GG		
Controlled asthma	0.372	0.628	5.17	0.02	0.077	0.590	0.333	5.36	0.02
Uncontrolled asthma	0.565	0.435			0.355	0.419	0.226		
Th2 "high" asthma	0.482	0.518	0.25	0.62	0.250	0.464	0.286	0.26	0.61
Th2 "low" asthma	0.439	0.561			0.171	0.537	0.293		

Table 7 - The allele and genotype frequencies of ADRB2 Gln27Glu polymorphism in asthma patients with different phenotypes

Asthma phenotypes	Alleles		χ^2	p	Genotypes			χ^2	p
	C	G			CC	CG	GG		
Controlled asthma	0.618	0.382	1.77	0.18	0.395	0.447	0.158	1.63	0.2
Uncontrolled asthma	0.726	0.274			0.548	0.355	0.097		
Th2 "high" asthma	0.679	0.321	0.29	0.59	0.464	0.429	0.107	0.26	0.61
Th2 "low" asthma	0.634	0.366			0.439	0.390	0.171		

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