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Investigation of the Association of Gln27Glu Polymorphism of the B2-Adrenoreceptor Gene with Bronchial Asthma Phenotypes

Turdibekov Kh.I [1], Kuyliev K. U [2], Jurabaeva G. S [3], Ibragimov S. Kh [4], Urinov F. K [5]

[1, 2,3,4,5] Samarkand State Medical University, Uzbekistan, Tashkent State Pediatric Medical Institute, Uzbekistan

Abstract: In order to study the serum levels of cytokines IL-4, IFN-γ and IgE production in patients with bronchial asthma (BA) considering Gln27Glu polymorphism of β2-adrenoreceptor gene, we have genotyped 130 individuals of Uzbek population using PCR technology and restriction fragment analysis. In the group of patients with allergic bronchial asthma with Gln27Gln polymorphic variant of β2-adrenoreceptor gene, the IgE index (1664,2±293,29 pg/ml) was almost four times higher than the level registered in patients with this form of disease at Gln27Glu genotype (338,4±121,33 pg/ml; P<0,01). According to the results of our studies, no significant relationship between the Gln27Glu polymorphism of the β2-adrenoreceptor gene and the activity of inducible cytokines was found. The Gln27Gln genotype of the β2-adrenoreceptor gene is associated with hyperproduction of IgE in patients with BA, especially in the allergic form of the disease.

Keywords: bronchial asthma (BA), Gln27Glu polymorphism of the β2-adrenoreceptor gene, cytokines IL-4, IFN-γ, IgE, enzyme-linked immunosorbent assay (ELISA) method.

Introduction

Despite significant advances in the diagnosis and treatment of bronchial asthma (BA), the progression of the disease, changes in clinical forms towards the predominance of severe variants of the disease, the lack of radical treatment methods and insufficient developments in primary prevention are becoming a pressing public health problem [7]. According to modern concepts, BA like many diseases occurring in the population is considered to be a polygenic or a multifactorial disease with genetic predisposition. This is based on the combined effect of hereditary and environmental factors. With the development of the "Human Genome Program", it has become possible to conduct whole genome studies. As a result of these studies, candidate sites whose structural changes contribute to the formation of BA have been verified. Candidate genes for BA include IL-4, β2-adrenoreceptor (ADRB2), TNF-β, IFN-γ, T-cell receptor, mast cell proteins and others [3, 6, and 8].

It is known that activation of ADRB2 leads to rapid relaxation of bronchial smooth muscle cells and increases the lumen of the airways. Therefore, in BA the attention of researchers is focused on possible disorders of ADRB2 function, which may underlie the pathogenesis of the disease. The regulatory effect of β2-agonists on cytokine production by T cells has been studied [13,20,22] where β2-agonists have a stimulating effect on IFN-γ and IL-2 production, and also block IL-4, IL-13 production by isolated T cells. In this regard, the search for the association of the ADRB2 gene with predisposition to BA is an objective of many studies [9,10,11,18,26,25].

It is worth noting that the frequencies of ADRB2 gene genotypes differ markedly between populations [12, 15, 16, 17, 23, 24], and it should also be mentioned that there is no information on the features of the ADRB2 gene's Gln27Glu polymorphism in the Uzbek community. Meanwhile, the genotype Gln27Gln of the polymorphic locus Gln27Glu of the ADRB2 gene has been linked to the risk of BA in the population of Bashkir nationality [1]. According to genotyping data, a number of studies have established the association of the Gln27Glu polymorphism of the ADRB2 gene with bronchial hyperresponsiveness [4, 5, 19, 21, and 28]. Data on the association of immunological parameters with the Gln27Glu polymorphism of the ADRB2 gene are scanty and limited to the study of IgE levels. It should be noted that in the study of Kurenkeeva A.K. et al. (2006) showed that carriage of Gln27Gln genotype in BA patients of Kyrgyz nationality is associated with hyperproduction of IgE [2].

The aim of the research is to study serum cytokines levels of IL-4, IFN-γ and IgE production activity in BA patients considering polymorphism of ADRB2 gene.

2 Material and research methods

130 people of Uzbek nationality in the 3rd generation were examined (survey was conducted up to the 3rd degree of relationship), 83 of them had BA. The BA patients were divided into the groups according to WHO international classification and diagnostic criteria of GINA, 2006. 31 patients with allergic BA (ABA) (37%), 24 patients with non-allergic BA (NBA) (29%), and 28 patients with mixed BA (MBA) (34%) were selected for the comparative analysis of clinical and pathogenetic types of BA on the basis of differential-diagnostic criteria. The mean age of the patients was 42.5±1.41 years. The duration of the disease averaged 10.1±0.73 years. Among the patients, there were 36 (43.4%) men and 47 (56.6%) women. The control group consisted of 47 practically healthy persons.

Determination of total IgE in blood serum was performed by ELISA method using the principle of two-layer enzyme immunoassay using the Xema-Medica kit. The level of IFN-γ in blood serum was determined using ELISA-IFN-gamma (Vector-Best, Russia) test-systems for quantitative determination by solid-phase ELISA method. IL-4 level in blood serum was determined by ELISA method using ELISA-IL-4 test systems (Vector-Best, Russia).

DNA extraction from whole blood was performed using Diatom™ DNA Prep 200 reagent kit (produced by IsoGen Laboratory LLC). DNA extraction was performed according to the standard protocol of DNA extraction using Diatom™ DNA Prep 200 reagent kit. The supernatant with DNA was further subjected to direct genotyping by PCR amplification.

DNA samples were typed for the ADRB2 gene using two pairs of specific oligonucleotide primers with β2-AR gene sites - Forward 5'-CCGGACCACGACGACGTCACCCAG-3'; Reverse 5'-CCAGTGAAGTGATGAAGTAGTT-3'. PCR analysis was performed using GenePak™ PCR Core DNA amplification reagent kit (produced by Isogen Laboratory LLC). PCR amplification was performed according to the standard protocol. The data obtained during the research were statistically processed on a Pentium-IV personal computer using the Microsoft Office Excel-2003 software package, including the use of built-in statistical processing functions.

3 Results

When analyzing the Gln27Glu polymorphism of the ADRB2 gene among healthy individuals, it was ascertained that the frequency of the Gln27 allele in the population of Uzbek ethnicity is 70.2%, while the Glu27 allele is 29.8%. during genotyping BA patients, the Gln27 allele was detected in 76.7% of cases and the Glu27 allele in 23.3% of cases ($\chi 2$ =66.15; P<0.001) (Table 1).

Table 1. Distribution of allele and genotype frequencies of the Gln27Glu polymorphism of the ADRB2 gene in the BA patients and healthy individuals.

Study groups	Allele frequencies, abs. (rel.)		Allele frequencies, abs. (rel.)		
	27 Gln	27 Glu	Gln 27 Gln	Gln27Glu	Glu 27 Glu
BA patients	92 (0,767)	28 (0,233)	34 (0,567)	24 (0,4)	2 (0,033)
Healthy people	66 (0,702)	28 (0,298)	21 (0,446)	24 (0,51)	2 (0,044)
χ^2	1,9		1,5	1,3	0,06
Allergic BA (ABA)	38 (0,86)	6 (0,14)	16 (0,73)	6 (0,27)	-
Healthy people	66 (0,702)	28 (0,298)	21 (0,446)	24 (0,51)	2 (0,044)
χ^2	4,2		4,7	3,5	
Non-allergic BA (NBA)	25 (0,735)	9 (0,265)	8 (0,47)	9 (0,53)	-
Healthy people	66 (0,702)	28 (0,298)	21 (0,446)	24 (0,51)	2 (0,044)
χ^2	0,1		0,03	0,02	
Mixed BA (MBA)	29 (0,69)	13 (0,31)	10 (0,48)	9 (0,43)	2 (0,09)
Healthy people	66 (0,702)	28 (0,298)	21 (0,446)	24 (0,51)	2 (0,044)
χ^2	0,02		0,05	0,4	0,7

Note: χ^2 – difference criterion

While studying the frequency distribution of the ADRB2 gene polymorphic marker genotypes in healthy individuals, the Gln27Glu genotype was found in 44.6% of cases, Gln27Glu – in 51%, and Glu27Glu – in 4.4%.

When studying the frequency distribution of the ADRB2 gene polymorphic marker genotypes in healthy individuals, the Gln27Gln genotype was identified in 44.6%, Gln27Glu - in 51%, and Glu27Glu - in 4.4% of cases. In BA patients the Gln27Gln genotype was detected in 56.7%, the Gln27Glu in 40%, and the Glu27Glu in 3.3% ($\chi 2$ =40.2, P<0.001) of cases respectively.

Analysis of the frequency of alleles and genotypes of the Gln27Glu polymorphism of the ADRB2 gene among patients depending on clinical variants of the pathological process showed that the Gln27 allele was significantly more frequent in the group of ABA patients than in the group of almost healthy individuals (86% vs. 70.2%, respectively, $\chi 2$ =4.2; P0.05). Examination of genotype polymorphism showed that the frequency of the homozygous variant of the Gln27Gln gene in this patient subgroup was significantly higher than in the control group of healthy individuals (73% versus 44.6%, respectively, $\chi 2$ =4.7; P0.05). At the same time, the frequency of Gln27Glu heterozygotes in the ABA patient group was markedly lower than that in the healthy part of the examined population (27% versus 51%, respectively, $\chi 2$ =3.5). No Glu27Glu homozygous genotype variants were observed among ABA patients.

In NBA patients, the following polymorphic marker frequency values were revealed: Gln27 allele - 73.5%, Glu27 - 26.5%; Gln27Gln genotype - 47%, Gln27Glu - 53%; no patients with Glu27Glu genotype were observed. In MBA patients these figures were, respectively: 69%, 31%; 48%, 43%, and 9%. Thus, the analysis of genotype features in the groups of NBA and MBA patients showed no statistically significant differences in the frequencies of alleles and genotypes of the Gln27Glu polymorphism of the ADRB2 gene compared to the control group of healthy individuals.

In general, the obtained data indicate the existence of an association between the Gln27Gln genotype of the ADRB2 gene, on the one hand, and the carriage of the Gln27 allele with the allergic form of BA, on the other hand.

It was important to study the peculiarities of total IgE production and immunoregulatory cytokines in patients depending on the genotypes of the Gln27Glu polymorphism of the ADRB2 gene (Table 2).

Table 2. Immune status indexes depending on the genotypes of Gln27Glu polymorphism of the ADRB2 gene in the group of BA patients.

Groups	Indexes (pg/ml)	Genotypes		P_{1-2}
		Gln 27 Gln (1)	Gln27Glu (2)	
BA general group	IgE	996,9±186,49	453,7±125,59	<0,05
	IL-4	14,16±1,31	14,0±1,87	>0,05
	IFN-γ	0,13±0,02	0,13±0,018	>0,05
ABA	IgE	1664,2±293,29	338,4±121,33	<0,01
	IL-4	17,5±1,83	13,8±2,59	>0,05
	IFN-γ	0,06±0,01	0,09±0,02	>0,05
NBA	IgE	176,8±36,78	293,6±61,8	>0,05
	IL-4	8,98±1,64	12,9±2,85	>0,05
	IFN-γ	0,27±0,04	0,18±0,05	>0,05
MBA	IgE	728,1±221,1	666,9±300,11	>0,05
	IL-4	13,5±2,29	15,1±4,05	>0,05
	IFN-γ	0,12±0,02	0,12±0,02	>0,05

Note: ABA – Allergic BA, NBA – Non-allergic BA, MBA – Mixed BA.

It was determined that in the total group of patients with BA with Gln27Gln polymorphism the average level of total IgE was 996.9±186.49 pg/ml, significantly higher than in the group with the intermediate Gln27Glu genotype (453.7±125.59 pg/ml; P<0.05).

In the group of ABA patients with the Gln27Gln polymorphic variant of the ADRB2 gene, the values of IgE (1664.2±293.29 pg/ml) were almost fourfold higher than those registered in patients with this form of the disease with the Gln27Glu genotype (338.4±121.33 pg/ml; P<0.01). According to the results of our studies, no significant relationship between the Gln27Glu polymorphism of the ADRB2 gene and the activity of inducible cytokines was found. Thus, the Gln27Gln genotype is associated with hyperproduction of IgE in patients with BA and especially in the allergic form of the disease.

4 Discussion

Analyzing the above mentioned facts, we can conclude that the marked accumulation of the Gln27 allele, as well as the prevalence of the Gln27Gln genotype in BA patients, is comparable with the results presented in the works of scientists studying various populations of individuals living in Mexico, China, Kyrgyzstan, and Israel [2,23,24,27]. The prevalence of the Gln27 allele in the control group and the tendency towards the accumulation of the Gln27Glu genotype are close to those in the populations of New Zealand, Great Britain, and Iceland [14,15,16].

Considering the role of cytokines in the development of allergic inflammation, the association of alleles and genotypes of the polymorphic DNA locus of the ADRB2 gene with the levels of IL-4, IFN-γ and IgE in different pathogenic variants of BA in patients with a severe persistent course.

The analysis of immunological indices of BA patients depending on ADRB2 gene polymorphism revealed some differences in alleles and genotypes distribution. Thus, the association of this genotype with hyperproduction of IgE was found in patients in the general group of AD patients having predominantly the Gln27Gln genotype.

The results of our study of serum levels of IgE and cytokine antagonists IL-4 and IFN-γ produced by Th2 and Th1 in pathogenetic forms of BA, namely ABA, NBA and MBA, in relation to ADRB2 gene polymorphism indicate the multivariate nature of immunological deficiency in BA. This also reflects the relationship of immunological defects with clinical and genetic polymorphism of the pathology under study, i.e., evidence of genetic determinacy of pathogenetic forms of BA.

5 Conclusion

The study of Gln27Glu polymorphism of ADRB2 gene in the Uzbek population revealed the prevalence of Gln27Gln genotype frequency among BA patients compared to the healthy part of the population. The degree of association of the studied genetic markers varies depending on the clinical and pathogenetic variant of the disease. An increased risk of allergic BA is associated with the Gln27 allele and the Gln27Gln genotype of the Gln27Glu polymorphic locus of the ADRB2 gene.

Significant differences in the level of total IgE in the serum of BA patients depending on the polymorphism variants of the ADRB2 gene have been established. Among patients with BA of Uzbek ethnicity, hyperproduction of IgE is associated with the Gln27Gln genotype.

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