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ER22/23EK AND TTH111I POLYMORPHISMS IN THE GLUCOCORTICOID RECEPTOR GENE IN PATIENTS WITH BRONCHIAL ASTHMA WITH REGARD TO THE AGE OF ONSET

Vladyslava Kachkovska[1]*, Anna Kovchun[1], Viktor Kovchun[1], Ivan Klisch[2], Olha Marchuk[2], Iryna Dudchenko[1], Lyudmyla Prystupa[1].

[1]Sumy State University, 2, Rimskoho-Korsakova Str., Sumy, Ukraine, 40000.

[2]I. Horbachevsky Ternopil National Medical University, Voli Square, 1, Ternopil, Ukraine, 46001.

Abstract.

Objective: The objective of the study was to evaluate the frequency of the ER22/23EK and Tth111I polymorphisms in the glucocorticoid receptor gene (GR) in patients with early-onset and late-onset asthma (BA) and to assess the risk of its phenotype's development.

Materials and Methods: We examined 553 BA patients and 95 apparently healthy individuals. The patients were divided into 2 groups depending on the age of BA onset: Group I included 282 patients with late-onset asthma, and group II included 271 patients with early-onset asthma. The ER22/23EK (rs 6189/6190) and Tth111I (rs10052957) polymorphisms in the GR gene were determined using polymerase chain reaction-restriction fragment length polymorphism analysis. Statistical analysis of obtained results was performed using SPSS–17 program.

Results: The analysis of frequency of genotypes and alleles for the ER22/23EK polymorphism in the GR gene with regard to the age of BA onset demonstrated a significant difference between patients with early-onset and late-onset asthma ($p = 0.035$). A significant difference was revealed in the distribution of alleles and genotypes for the Tth111I polymorphism in the GR gene between patients with early-onset BA and late-onset BA ($p = 0.006$). No correlation was found between the ER22/23EK polymorphism in the GR gene and late-onset BA in all genetic models; also, there was a reduction in the risk of early-onset BA observed in the dominant and additive models. No association was demonstrated between the Tth111I polymorphism in the GR gene and late-onset asthma, while a statistically significant correlation was shown with the risk of early-onset asthma in the dominant and super-dominant models.

Conclusions: We established a significant difference in the distribution of alleles and genotypes for the ER22/23EK and Tth111I polymorphisms in the GR gene with regard to onset age; also, we found no association between these polymorphic variants and the development of late-onset asthma, but revealed a protective role of the ER22/23EK polymorphism in the GR gene in the dominant and additive inheritance models and of Tth111I polymorphism in the GR gene – in the dominant and super-dominant models.

Key words. Bronchial asthma, onset, ER22/23EK and Tth111I polymorphisms in the glucocorticoid receptor gene.

Introduction.

Bronchial asthma (BA) is a chronic inflammatory respiratory disease that affects 1 to 18% of population in various countries. The incidence of BA in developed countries is constantly growing. According to the GINA guidelines (2020), BA is a complex heterogeneous disease which is influenced by genetic factors. Due to polygenic nature of BA, the age of onset is of great importance in terms of diagnosis, prognosis, and treatment [1-3]. The differences known to date in the etiology, pathogenesis, and clinical manifestations of early-onset and late-onset asthma involve different genetic factors depending on the age of onset. Several studies on genetic factors in patients with early-onset and late-onset asthma identified specific variants of genetic risk factors for these asthma variants [4], which partly explains the differences in their pathogenesis [1-3].

Research on the ER22/23EK and Tth111I polymorphisms in the glucocorticoid receptor (GR) gene is attributable to the fact that the gene is located on the long arm of chromosome 5 (5q31-q32) which is associated with asthma risk and suggests its participation in the pathogenesis of this disease. More than 2,500 single-nucleotide substitutions were identified in the GR gene, of which only the following were observed in the Caucasian population with a frequency of > 1%: rs6190 (Arg23Lys), rs6195 (N363S), rs41423247 (BclI, C/G), and rs10052957 (-3807 C/T or Tth111I) [5]. Due to the high polymorphy of the GR gene, the frequency of alleles and genotypes differs statistically among different ethnic groups [6]. The involvement of some of its polymorphic variants in the development of asthma [7-9], obesity, and metabolic syndrome was demonstrated [10,11]. According to the results of a meta-analysis by Fu G., which included 4 studies on ER22/23EK polymorphism and 2 studies on Tth111I polymorphism in the GR gene, no association of these polymorphic variants with asthma was found [12]. However, some studies reported the association of these polymorphisms with the development of asthma, its course severity, and disease control [9]. Due to the lack of consistent data on the effect of the ER22/23EK and Tth111I polymorphisms in the GR gene on the risk of asthma, we aimed to study the association between these SNPs and the risk of different phenotypes of BA depending on the age of onset.

The objective of the study was to evaluate the frequency of the ER22/23EK and Tth111I polymorphic variants in the GR gene in patients with early-onset and late-onset BA and to assess the risk of BA phenotypes development.

Materials and Methods.

We examined 553 BA patients (experimental group) and 95 apparently healthy individuals (control group); all participants had previously signed an informed consent form. The patients were divided into 2 clinical groups depending on the age of BA onset. Group I included 282 patients with late-onset asthma

(late-onset asthma phenotype) and group II included 271 patients with early-onset asthma (early-onset asthma phenotype). Based on the absence of exact guidelines for asthma early and late-onset definitions, we use stratifications from the previously published data and consider asthma onset before the age of 12 as early and after the age of 12 as late [13].There was no significant difference in gender, age, severity, or control level between the groups ($p > 0.05$). BA was diagnosed according to the 2016 GINA recommendations and its later versions.

The study was approved by the Bioethics Committee of Medical Institute of Sumy State University and complied with the Declaration of Helsinki. The ER22/23EK (rs 6189/6190) and Tth111I (rs10052957) polymorphisms in the GR gene were determined using polymerase chain reaction-restriction fragment length polymorphism analysis. Statistical analysis of obtained results was performed using SPSS–17 program. We used Pearson's chi-squared test; the P-value of <0.05 was considered statistically significant. Binary logistic regression was used for risk assessment; analysis-of-variance method and F-test were also used in the study.

Results.

Given the fact that early-onset and late-onset asthma has its own phenotypic differences, we analyzed the frequency of alleles and genotypes for the ER22/23EK and Tth111I polymorphisms in the GR gene depending on the onset age in order to check for the association between the studied polymorphic variants and different phenotypes (Table 1).

The analysis of distribution of genotypes and alleles for the ER22/23EK and Tth111I polymorphisms in the GR gene with regard to the age of BA onset showed a significant difference between patients with early-onset and late-onset asthma ($p = 0.035$; $p = 0.006$). As can be seen from the table above, the frequency of GG-genotype for the ER22/23EK polymorphism in the GR gene was higher in the patients with early-onset asthma compared to late-onset asthma, while AG heterozygotes were more common with late-onset asthma. G-allele was more frequently observed in patients with early-onset asthma, while A-allele was more common among late-onset BA patients. The results of the study of the Tth111I polymorphism in the GR gene concerning the age of onset along with significant difference in the distribution of genotypes showed a higher frequency of TT homozygotes in patients with early-onset asthma compared to late-onset asthma.

Given the significant difference in the distribution of genotypes for the ER22/23EK and Tth111I polymorphisms in the GR gene depending on the age of onset, we performed a statistical analysis to identify a possible association between genetic markers and relative risk of early-onset and late-onset asthma (Table 2, 3).

No association was established between ER22/23EK polymorphism in the GR gene and the risk of late-onset asthma in any genetic model. However, there was a reduction in the risk of early-onset BA observed in the dominant, super-dominant, and additive models.

Analysis of the risk of late-onset and early-onset asthma depending on the Tth111I polymorphism in the GR gene showed no association between the development of late-onset asthma and Tth111I polymorphism in the GR gene but demonstrated a statistically significant association with the risk of early-onset asthma in the dominant ($p = 0.02$) and super-dominant ($p = 0.001$) models.

Table 1. *Distribution of genotypes and alleles for the ER22/23EK and TTH111I polymorphisms in the glucocorticoid receptor gene in patients with bronchial asthma with regard to the age of onset.*

Parameter	**Late onset, n = 282**		**Early onset, n = 271**	
Genotype	n	%	n	%
ER22/23EK polymorphism in the glucocorticoid receptor gene				
GG	244	86.5	252	93.0
AG	36	12.8	17	6.3
AA	2	0.7	2	0.7
$\chi^2 = 6.72$; $p = 0.035$				
Allele	%		%	
G	92.9		96.1	
A	7.1		3.9	
TTH111I polymorphism in the glucocorticoid receptor gene				
Genotype	n	%	n	%
CC	103	36.5	125	46.1
CT	141	50.0	99	36.5
TT	38	13.5	47	17.3
$\chi^2 = 10.2$; $p = 0.006$				
Allele	%		%	
C	61.5		64.4	
T	38.5		35.6	

Table 2. *The risk of early-onset and late-onset asthma with regard to the ER22/23EK polymorphism in the glucocorticoid receptor gene.*

Model	**P_{obs}**	**OR_{obs} (95% CI)**	**AIC**
Late onset			
Dominant	0.42	0.77 (0.41–1.49)	16.84
Recessive	0.27	0.33 (0.04–2.8)	16.34
Super-dominant	0.62	0.85 (0.44–1.7)	17.24
Additive	0.3	0.74 (0.43–1.34)	16.46
Early onset			
Dominant	0.01	0.37 (0.18–0.77)	15.82
Recessive	0.29	0.35 (0.04–2.91)	21.84
Super-dominant	0.01	0.39 (0.18–0.83)	17.05
Additive	0.01	0.44 (0.23–0.81)	16.18

Table 3. *The risk of early-onset and late-onset asthma with regard to the Tth111I polymorphism in the glucocorticoid receptor gene.*

Model	**P_{obs}**	**OR_{obs} (95% CI)**	**AIC**
Late onset			
Dominant	0.43	1.21 (0.75–1.94)	21.65
Recessive	0.07	2.31 (1.01–6.25)	18.22
Super-dominant	0.66	0.9 (0.56–1.43)	21.98
Additive	0.13	1.32 (0.92–1.92)	19.87
Early onset			
Dominant	0.02	0.67 (0.48–0.94)	25.65
Recessive	0.21	1.35 (0.85–2.15)	29.33
Super-dominant	0.001	0.58 (0.41–0.81)	20.67
Additive	0.34	0.89 (0.7–1.13)	30.01

Discussion.

The objective of our study was to provide supplementary modern knowledge about genetic aspects of BA with regard to

the age of onset taking into account the ER22/23EK and Tth111I polymorphisms in the glucocorticoid receptor gene. The study was performed by estimating the frequency of genotypes for the studied polymorphisms and assessing the risk of developing phenotypes of early-onset and late-onset BA. Taking into account the age of BA onset, we revealed a significant difference between early-onset and late-onset asthma in terms of the frequency of genotypes and alleles for the ER22/23EK polymorphism in the GR gene ($p = 0.035$). The frequency of GG-genotype was higher in the patients with early-onset asthma, while AG heterozygotes were more common with late-onset asthma. G-allele was more frequently observed in patients with early-onset asthma (96.1%) compared to late-onset asthma (93.1 %), while A-allele was observed in 3.9% and 6.9%, respectively. Preliminary we found no difference in the distribution of alleles and genotypes for the ER22/23EK polymorphism in the GR gene in patients with asthma with no regard to age of onset and in apparently healthy individuals ($\chi 2 = 4.14$; $p = 0.126$); apart from that, we revealed no statistically significant association with BA risk in all models of inheritance. Differentiated analysis of the association between the ER22/23EK polymorphism in the GR gene and different BA phenotypes demonstrated no correlation in patients with late-onset asthma, while patients with early-onset asthma revealed decreased BA risk in the dominant and recessive models ($p = 0.01$).

Taking into account the clinical and laboratory phenotypic peculiarities of early-onset asthma and late-onset asthma, we analyzed the distribution of alleles and genotypes for the Tth111I polymorphism in the GR gene with regard to onset age in order to check for the possible association between the studied polymorphic variant and these phenotypes. Preliminary analysis of BA risk with no regard to age of onset in recessive homozygotes showed a 2.69-fold increase vs. major allele homozygotes ($p = 0.02$). Taking into account the age of BA onset, we found a significant difference in the distribution of alleles and genotypes for the Tth111I polymorphism in the GR gene with regard to onset age ($p = 0.006$); also, we revealed no association between the development of late-onset asthma and Tth111I polymorphism in the GR gene, but demonstrated a statistically significant association with the risk of early-onset asthma in the dominant ($p = 0.02$) and super-dominant ($p = 0.001$) models.

The study of Szczepankiewicz A. (2008) did not find any association of the Tth111I polymorphism in the GR gene with asthma and an increased need for high doses of inhaled GC [14,15].

The inconsistency of findings that were obtained in different studies related to the role of the ER22/23EK and Tth111I polymorphisms in the GR gene in BA development and course and involving BA patients with and without regard to onset age can be attributable to the clinical heterogeneity of this disease, different age of onset, and pathogenetic differences among different phenotypes of the disease. Our findings show the differences in genetic factors in patients with early-onset and late-onset asthma. Therefore, an in-depth study of pathogenesis mechanisms and genetic factors causing the disease in adults and children will help to develop strategies for BA prevention and treatment.

Conclusion.

A significant difference was observed in the distribution of genotypes and alleles for the ER22/23EK and Tth111I polymorphisms in the GR gene between patients with early-onset and late-onset asthma.

No correlation was found between late-onset BA and the ER22/23EK polymorphism in the GR gene in all genetic models; also, there was a reduction in the risk of early-onset BA observed in the dominant and additive models.

No association was reported between late-onset asthma and the Tth111I polymorphism in the GR gene, while a statistically significant association was shown with the risk of early-onset asthma in dominant and super-dominant models.

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