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BCL1 POLYMORPHISM OF GLUCOCORTICOIDS RECEPTOR GENE AND BRONCHIAL ASTHMA

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Bcl1 polymorphism of glucocorticoids receptor gene (GR, h-GR/NR3C1) was first described by Murray et al. in 1987 [5]. The investigation of GR polymorphism frequency in different general populations has found that Asians have a high C allele frequency - 32,8%, Caucasians have 28,9%, and South Americans have 15,2% [7]. Bcl1 polymorphism of GR gene is associated with increased body mass index and body fat centralization ratio, dyslipidemia, insulin resistance, cardiovascular and autoimmune diseases, sensitivity to glucocorticosteroids (GCS), endothelial dysfunction and inflammation activity [1, 3, 6, 10, 12, 13, 14]. As for respiratory diseases, there are some publications on the association between BclI polymorphism and bronchial asthma (BA) [8, 9], chronic obstructive pulmonary disease (COPD) [11], and cystic fibrosis [2].

In the opinion of Pietras T. et al. (2010), GR gene polymorphism can play an important role in BA development and severity, and can influence response to corticosteroids [8]. The study carried out in Polish population among individuals that have no history of BA and atopy [9], and in patients with BA [8] demonstrated heterogeneous distribution of genotypes among patients with BA and in the general population. Patients with BA were more likely to have G allele of the studied Bcl1 polymorphism. G allele frequency, as compared to C allele, correlated with a high prevalence of BA in the study population. BA developed more frequently in the carriers of allele G, both homozygotes and heterozygotes, as compared to the individuals with C/C genotype. C allele was associated with a lesser risk of BA, since C allele carriers (C/C+C/G) had BA less often than G allele carriers [8]. Thus, this study despite the small size of the study groups confirmed that the substitution of G allele for C allele contributed to the development of BA among the Polish population.

Some scientists conducted research of BclI polymorphism in the GR gene and found out that distribution of alleles and genotypes did not statistically differ between a control group and groups of boys and girls with BA of varying severity [15]. So far in Ukraine, there has been no research on the study of GR gene polymorphisms (h-GR/NR3C1), including Bcl1, in patients with BA. Therefore, the aim of our study was to investigate frequencies of alleles and genotypes of Bcl1 GR gene polymorphism and their correlation with prevalence of BA.

Materials and methods. The study has been approved by the Bioethics Committee of Medical Institute of Sumy State University. Prior to the study, all patients provided written informed consent to participate. 188 patients with BA have been examined. BA was diagnosed in accordance with the GINA guidelines. The control group consisted of 95 healthy adult individuals. Bcl1 (rs41423247) polymorphism in exon 2 was determined by means of polymerase chain reaction with subsequent RFLP analysis (restriction fragment length polymorphism) by Fleury I. et al. with modifications [4].

Statistical analysis of the results was performed using SPSS-17 program. To evaluate the influence of polymorphism genotype frequencies, the odds ratio (OR) and 95% confidence interval (CI) were calculated. Values of $p<0,05$ were considered statistically significant.

Results. The frequency of the three possible genotypes for Bcl1 polymorphism of GR gene and compliance of main and minor alleles distribution with Hardy-Weinberg equilibrium were identified (table 1).

Compliance test for Bcl1 polymorphism genotypes distribution and the Hardy-Weinberg equilibrium showed that deviations from the equilibrium were not statistically significant neither in the control group, nor in the main one. It was found out that alleles spreading in both groups didn't significantly differ from the predicted ($p>0,05$).

The control group had the following genotypes frequency for Bcl1 polymorphism of GR gene: C/C, C/G, G/G – 0,421/0,453/0,126, respectively. In patients with BA the frequency of studied genotypes were: 0,228/0,426/0,346, respectively. Thus, the analysis of genotype frequencies for Bcl1 polymorphism of GR gene asserted that there is a statistically significant difference in the distribution of allelic variants of the gene between patients with BA and healthy individuals: homozygous for the minor allele had a higher risk of the disease than the major allele carriers (C/C i C/G) (χ^2=19,234; p=0,001).

The distribution of allele frequencies for Bcl1 polymorphism of GR gene in the general population and in patients with BA are shown in table 2.

Using Pearson's chi-squared test revealed association between the G allele of Bcl1 polymorphism of GR gene and development of BA. Distribution of alleles between patients with BA and the control group demonstrated a statistically significant difference (p=0,001).

Distribution of allele frequencies for Bcl1 polymorphism of GR gene in male and female comparison groups is presented in table 3.

Gender-stratified analysis showed a statistically significant difference in the distribution of genotypes for Bcl1 GR gene polymorphism among women in the control group and women with BA (χ^2=6,1; p=0,047). Males demonstrated comparatively higher statistically significant difference in the distribution of genotype frequencies for C647G polymorphism of GR gene (χ^2 = 14,1; p = 0,001) in the control group and in patients with BA.

By studying the prevalence of individuals in groups, formed according to different variants of polymorphism, it was ascertained that homozygotes for C/C allele comprised 47,5% of women and 52,5% of men in the control group, and 69,8% and 30,2%, respectively, – in the group of patients with BA. Heterozygotes were constituted by 51,2% of women and 48,8% of men in the control group and 62,5% and 37,5%, respectively, – among patients with BA. 75% of women and 25% of men were homozygous for the minor allele in the control group, and 67,7% and 32,3%, respectively, – among patients with BA.

We have studied BA risk depending on the genotype of Bcl1 GR gene polymorphism in patients with BA as a whole and stratified by gender. Taking C/C genotype as a reference one, we demonstrated that G/G homozygous type of GR gene is likely to cause a fivefold increase of BA risk in patients regardless of gender (OR=5,039, CI – 95% 2,377-10,682, p<0,001). The results are given in table 4.

Female carriers of G/G genotype showed a much lesser risk of BA (OR=3,096, CI 95% 1,235-7,761, p=0,016). As is evident from the data, OR is more than 1 that indicates the significance of G-allele of Bcl1 GR gene polymorphism with regard to the BA risk in women. Odds ratio calculated for men with BA showed the association between GR gene polymorphism (C647G) and predisposition to BA in G/G genotype carriers (OR=11,308, CI 95% 2,807-45,558, p<0,001).

Discussion. Identification of modifier genes that may influence the development and progression of the disease is an important issue for patients with respiratory diseases. It helps not only for understanding the pathophysiology of progression, but also for identifying patients who may benefit from new therapeutic strategies and adaptation of treatment to their genetic profile. Among the candidate genes of interest there are genes that may influence the inflammatory cascade and response to anti-inflammatory drugs, in particular, genes that influence the effect of exogenous and endogenous GCS. The key factor of GCS action are steroid hormone receptors. According to

the U.S. National Institute of Health, 2571 SNPs of GR gene are known up to now, of which only 161 has the minor allele frequency more than 10%, and 127 - more than 1%. The most common and well-studied polymorphism is BclI. Depending on population, G-allele frequency of the polymorphism constitutes more than 30%.

The mechanisms due to which the BclI polymorphism affects disease occurrence and progression, and lung function remain unclear. The results of several studies demonstrating the effect of this polymorphism on sensitivity to GCS may serve as an explanation [7, 14]. BclI GR gene polymorphism may cause changes in the receptor expression level and, respectively, affect sensitivity to GCS (either increase or decrease it, by tissue-specific manner in particular). This is ascertained by the studies of Panarelli et al. (1998) in healthy humans [7].

The data from the studies, carried out in patients with chronic respiratory diseases, mainly focused on BA. The study carried out in Polish individuals with no history of BA and atopy showed that the genotype frequency of BclI GR gene polymorphism was as follows: CC/CG/GG – 0,400/0,471/0,129 [8]. The following study by Pietras T. (2011) investigated the relationship between this polymorphism and the prevalence of BA; the following genotypes frequency for BclI GR gene polymorphism was found: CC/CG/GG–0,128/0,462/0,410, respectively. BA developed significantly more often in allele G carriers (GG + GC) as compared to CC carriers (OR = 5,44, CI: 95%, CI = 2,05–14,41, χ^2 = 13,16, p=0,00029) [9]. Our results are congruent with the data of Polish scientists. However Pietras T. et al. (2011) found no gender-dependent statistical difference in genotypes distribution of BclI polymorphism, which was found in our study.

At the same time, the researches of BclI GR gene polymorphism, conducted children with BA, found out that distribution of alleles and genotypes did not statistically differ between a control group and groups of children with BA of varying severity; gender-dependent statistical difference was not also noticed. However, when comparing the results of clinical and instrumental examination and molecular genetic testing, it was asserted that children with G/G genotype had BA earlier than C/C and C/G genotype carriers; they were also more likely to demonstrate bronchial hyperreactivity due to physical examination and histamine challenge tests. It was also reported that children with C/C genotype had milder disease, less severe exacerbations and adequate control of the disease, as compared with children who had C/G and G/G genotypes [15]. Thus, the distribution of alleles and genotypes of BclI GR gene polymorphism in children with BA did not differ according to sex or severity, and was identical to that in the control group. Despite this fact, however, clinical and functional data, laboratory examination and assessment of the control level in children with different genotypes of BclI polymorphism make it clear that increasing number of G-

alleles in genotype combinations in children with BA is associated with more severe course, acute exacerbations and decreased control level.

Thus, genotyping of BclI GR gene polymorphism together with clinical, laboratory and instrumental methods of examination can be used to assess the risk of BA, peculiarities of its clinical course and, in the future, for the selection of individual therapy and predicting the effectiveness of treatment in these patients.

Conclusions.

We have identified statistically significant differences in genotypes distribution of BclI polymorphism of GR gene in the control group and in patients with BA, homozygous for the minor allele had a higher risk of the disease than the major allele carriers.

It was found out that G/G-homozygotes have a fivefold higher risk of BA, than those homozygous for C/C regardless of gender.

We demonstrated that BA risk in females, homozygous for the minor allele, is three times higher and in males – 11,3 times higher, as compared with C/C-homozygotes. That is, men with G/G genotype of Bcl1 GR gene polymorphism have the highest risk of BA.

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BCL1 ПОЛИМОРФИЗМ ГЕНА РЕЦЕПТОРА ГЛЮКОКОРТИКОИДОВ И БРОНХИАЛЬНАЯ АСТМА

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Целью нашего исследования было изучение частоты аллелей и генотипов Bcl1 полиморфизма гена глюкокортикоидного рецептора и связи с распространенностью бронхиальной астмы. Обследовано 188 больных бронхиальной астмой и 95 практически здоровых лиц. Bcl1 (rs41423247) полиморфизм 2-го экзона определяли методом полимеразной цепной реакции с последующим анализом длины рестрикционных фрагментов по Fleury I. Статистическую обработку результатов проводили с использованием программы SPSS-17. Результаты исследования показали, что существует разница в распределении аллельных вариантов гена между больными с бронхиальной астмой и практически здоровыми лицами. Частота генотипов по Bcl1 полиморфизмом гена ГР в контроле составляла: C/C, C/G, G/G – 0,421/0,453/0,126, а у больных бронхиальной астмой – 0,228/0,426/0,346 соответственно (p=0,001). Установлено, что у гомозигот по минорному аллелю G/G риск возникновения бронхиальной астмы в 5 раз выше по сравнению с гомозиготами по основному аллелю. Доведено, что у женщин существует тенденция к увеличению риска возникновения бронхиальной астмы при наличии G/G генотипа (p=0,016), а самый высокий риск развития существует у мужчин с G/G генотипом. Таким образом, G/G генотип Bcl1 полиморфизма гена глюкокортикоидного рецептора ассоциирован с развитием бронхиальной астмы.

КЛЮЧЕВЫЕ СЛОВА: BclI полиморфизм, ген глюкокортикоидного рецептора, бронхиальная астма.

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The aim of our study was to investigate frequencies of alleles and genotypes of Bcl1 GR gene polymorphism and their correlation with prevalence of BA. Study involved 188 patients with BA and 95 healthy individuals. Bcl1 (rs41423247) polymorphism in exon 2 was determined by means of polymerase chain reaction with subsequent RFLP analysis (restriction fragment length polymorphism) by Fleury I. et al. with modifications. Statistical analysis of the results was performed using SPSS-17 program.

The results showed statistically significant differences in genotypes distribution of BclI polymorphism of GR gene in the control group and in patients with BA. The frequency of genotypes distribution of Bcl1 polymorphism of GR gene were in controls: C/C, C/G, G/G - 0,421/

0,453/0,126, and in patients with bronchial asthma - 0.228/0.426/0.346, respectively (p = 0.001). It was found out that G/G-homozygotes have a fivefold higher risk of BA than those homozygous for C/C regardless of gender.

We demonstrated that BA risk in females, homozygous for the minor allele, is higher (p = 0.016) and men with G/G genotype of Bcl1 GR gene polymorphism have the highest risk of BA. Thus, G/G genotype of Bcl1 polymorphism of the glucocorticoid receptor gene is associated with the development of asthma.

KEY WORDS: Bcl1 polymorphism, glucocorticoid receptor gene, bronchial asthma.

Table 1. **The frequency of allelic variants and alleles for Bcl1 polymorphism of glucocorticoid receptor gene**

Genotype/ allele	Control group n (%)	Patients with bronchial BA, n (%)
C/C	40 (42,1)	43 (22,8)
C/G	43 (45,3)	80 (42,6)
G/G	12 (12,6)	65 (34,6)
C allele	0,65	0,44
G allele	0,35	0,56
	χ^2=0,01; p>0,05	χ^2=3,53; p>0,05

Remarks: n – number of patients; χ^2 and p denote deviations from Hardy-Weinberg equilibrium in each group.

Table 2. **Allele frequencies for Bcl1 polymorphism of GR gene in the general population and in patients with bronchial asthma**

Allele		Control group, n = 95		Patients with bronchial asthma, n = 188	
		n	%	n	%
C	present	83	87,4	123	65,4
	none	12	12,6	65	34,6
χ^2 =15,343; p=0,001					
G	present	55	57,9	145	77,1
	none	40	42,1	43	22,9
χ^2 =11,263; p=0,001					

Remarks: n – number of patients; p – statistical significance of difference (according to Pearson's chi-squared test (χ^2)).

Table 3 **Distribution of various genotypes of Bcl1 GR gene polymorphism according to sex in the control group and in patients with bronchial asthma**

Genotype	Women (n, %)		Men (n, %)	
	Control group	Bronchial asthma	Control group	Bronchial asthma
C/C	19 (38%)	30 (24,2%)	21 (46,7 %)	13 (20,3 %)
C/G	22 (44%)	50 (40,3%)	21 (46,7 %)	30 (46,9 %)
G/G	9 (18%)	44 (35,5%)	3 (6,7 %)	21 (32,8 %)
Total	50 (100%)	124 (100 %)	45 (100 %)	64 (100 %)
Women	χ^2 =6,1; p_1 =0,047		χ^2 =14,1; p_2 = 0,001	

Remarks: n – number of patients; p_1 – statistical significance of difference in genotype distribution between female control group and female patients with BA; p_2 – statistical significance of difference in genotype distribution between male control group and male patients with BA.

Table 4 **Analysis of risk of BA depending on the genotype of Bcl1 GR gene polymorphism**

Genotype	CR	SE	WS	P	OR	95% CI for OR lower	95% CI for OR upper
Patients with bronchial asthma							
C/G	0,549	0,290	3,581	0,058	1,731	0,981	3,054
G/G	1,617	0,383	17,794	0,001	5,039	2,377	10,682
Females							
C/G	0,364	0,389	0,876	0,349	1,439	0,671	3,086
G/G	1,130	0,469	5,811	0,016	3,096	1,235	7,761
Males							
C/G	0,836	0,453	3,403	0,065	2,308	0,949	5,611
G/G	2,425	0,711	11,638	0,001	11,308	2,807	45,558

Remarks: the comparison was performed for the major allele (C/C) homozygous subjects; CR – regression coefficient; SE – standard error; WS – Wald statistic; P – statistical significance; OR – odds ratio; CI – confidence interval