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BcI1 POLYMORHPISM OF GLUCOCORTICOID RECEPTOR GENE IN PATIENTS WITH BRONCHIAL ASTHMA WITH OBESITY

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The objective of this investigation was to analyze possible association between BclI polymorphism of glucocorticoid receptor gene and obesity in patients with bronchial asthma (BA). The study involved 188 patients with bronchial asthma and 95 apparently healthy adult individuals. Generally accepted assessments and examinations for BA diagnosis, and anthropometric, molecular-genetic and statistic methods of investigation were used in the research. It was found out that the patients with BA demonstrated higher body mass index (BMI) and higher ratio of fat centralization much more often, than the control group. Genotypes distribution for BclI polymorphism in patients with BA showed a statistically significant difference between patients with different BMI unlike the control group. Comparison of genotype frequency for BclI polymorphism in glucocorticoid receptor gene in individuals with different ratio of fat centralization in the control group and in the patients with BA separately showed statistically significant differences in the distribution of gene allelic variations only among the patients with BA. It was demonstrated that G/G genotype in the patients with visceral obesity was associated with BA.

Key words: *bronchial asthma, obesity, BcI1 polymorphism, glucocorticoid receptor gene.*

Introduction. A plenty of evidence concerning the role of obesity in bronchial asthma occurrence (BA) [1, 2] and deterioration of BA control [3] has been reported in recent years. Thus, more than 40 crossover studies and case-control investigations showing the connection between BA and obesity have been carried out since 1990.

The mechanisms of this association have not been studied well yet, but their comprehension includes the following complex of factors: food with a low antioxidant value; low physical activity; gastroesophageal reflux disease; mechanical restrictions of respiratory movements volume caused by thoracicoabdominal fat depot; systemic inflammations induced by mediators of adipose tissue; genetic factors [1, 4, 5].

Obesity contributes not only to occurrence of BA, but also to more severe BA course due to causing a certain phenotype of disease and decreasing the effectiveness of treatment. Still, the associated pathogenic mechanisms in BA and obesity require further studies. This problem borders on three directions of scientific research: investigation of the metabolic disorders in obesity; further conceptual development of BA as a chronical disease, accompanied by a persistent inflammation; search for their common genetic determinants.

BA and obesity are multifactorial diseases, which are influenced by both genetic factors and environmental factors [6]. It is assumed that the common genetic factors of these two diseases can partly explain the data of epidemiological investigations, which confirm a close association between BA and obesity. More than 100 genes were found associated with BA occurrence and, in particular, with allergen-specific IgE production (atopy), development of bronchial hyperreactivity, synthesis of mediators of inflammation (cytokines, chemokins, growth factors), correlation between the types of Th1- and Th2-lymphocyte immune response [1, 2, 4].

Certain investigations showed that genetic factors of BA and obesity overlap each other [7–9]; this indicates that they have common genetic predisposition. Thus, BA and obesity are associated with the genes, which encode β-adrenergic receptor (locus 5q, ADRB2 gene), insulin-like growth factor (locus 12q, IGF1 gene), IL-1α (locus 12q, IL-1α gene), leukotriene A4 hydroxylase (locus 12q, LTA4H gene), GR (locus 5q, NR3C1 gene), signal transducers and activators of transcription (locus 12q), TNF (locus 6p, TNF gene), uncoupling protein (locus 11q13, gene UCP2 and 3), etc. [6].

Among candidate genes of obesity and BA there is a single copy of glucocorticoid receptor (GR) gene in humans and it is located on chro-

mosome 5 (locus 5q31.3). A few polymorphisms of this gene are described; they are related to anthropometric parameters [10, 11]. The most common and well-studied polymorphism is BclI (C647G, rs41423247). Depending on population, G-allele frequency of the polymorphism constitutes more than 30 %. Some investigations have proved the association of BclI polymorphism with body mass index (BMI) and abdominal obesity [10, 11]; a few studies have shown its association with BA [12]. Still, no investigations related to the connection of BclI polymorphism with body mass index in BA patients were carried out.

The objective of this investigation was to analyze possible association between BclI polymorphism of GR gene and obesity in patients with BA.

Materials and methods. Prior to the study, all patients provided written informed consent to participate. 188 patients with BA have been examined. The control group consisted of 95 apparently healthy adult individuals. Among 188 patients with BA were 124 women (66 %) and 64 men (34 %), in the control group – 50 (52.6 %) and 45 (47.4 %) respectively. The average age of patients with BA accounted 46,2 ± 0,83 years, and for the control group – 44,1 ± 1,53 years. There were no statistically significant differences in the ethnic component in the control group and in the patients with BA. We measured body mass, height, body mass index (BMI), and determined the ratio of fat centralization (RFC). BMI and RFC parameters were estimated according to WHO recommendations.

The determination of allelic polymorphism in exon 2 of BclI GR gene (C647G; rs41423247) was performed by means of polymerase chain reaction with subsequent analysis of restriction fragment length polymorphism (according to the instructions of Fleury I. et al.) with modifications. Statistical analysis of the results was performed using SPSS-17 program. The obtained results were subjected to descriptive statistical analysis with calculation of arithmetic means and standard deviations. The significance of differences between mean values was determined by means of Pearson χ^2, with $P < 0.05$ adopted as the significance level. Logistic regression, analysis of variance, Fisher criterion have been used as well.

Results. The compliance test for BclI polymorphism genotypes distribution and the Hardy-Weinberg equilibrium showed that deviations from the equilibrium were not statistically significant either in the control group ($\chi^2 = 0,01$; $p > 0,05$), or in the main one ($\chi^2 = 3,53$; $p > 0,05$), so the allele correlations in both groups didn't significantly differ from the predicted ($p > 0.05$). The control group had the following genotypes frequency for BclI 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 made: 0,228/0,426/0,346, respectively. Thus, the analysis of genotype frequencies for BclI 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 ($\chi^2 = 19,234$; $p = 0,001$).

Among patients with BA, normal body mass (NBM) was found in 50,5 % of individuals, overweight – 15,4 %, obesity – 34 %. In the control group, 76,8 % of the investigated had NBM, 20 % had overweight and 3,2 % had obesity. It was demonstrated that obesity occurs more often among BA patients, than in the control group ($p = 0,001$).

The patients with BA had higher BMI parameter, than the individuals in the control group

Table 1. **Body mass index in the groups depending on the variations of genotype for BclI polymorphism of glucocorticoid receptor gene**

Groups	C/C	C/G	G/G	F	p_1
Control	23.5 ± 0.46 (40)	23.6 ± 0.45 (43)	23.1 ± 0.61(12)	0.093	0.9126
BA	24.6 ± 0.68 (43)	25.2 ± 0.57 (80)	31.3 ± 0.74 65)	30.39	0.0001
p_2	0.1905	0.0608	0.0001		

Remarks: F – Fisher criterion; p_1 – significance of the differences among genotypes according to the data of the one-way analysis of variance; p_2 – significance of the differences between the control group and BA patients according to the t-test; figures in the braces – the number of patients.

(27,2 ± 0,44 kg/m² vs. 23,5 ± 0,29 kg/m²; p < < 0.001). Table 1 depicts indicators of BMI in control group and in patients with BA, which have different genotype by BclI polymorphism of the GR gene. The obtained data showed that BMI values didn't significantly differ in carriers with different genotypes for BclI polymorphism in the control group (p = 0,91). However, dependence between BclI polymorphism and BMI parameters was found in patients with BA: G/G genotype carriers had higher BMI (31,3 ± 0,74 kg/m²), than representatives with others genotypes. Comparing the groups, we found out that BMI values didn't differ significantly in C/C and C/G genotypes carriers. However, G/G homozygotes among BA patients had higher BMI, than those in the control group: 31,3 ± 0,74 kg/m² vs 23,1 ± 0,61 kg/m² (p = = 0,0001).

Table 2 represents the results obtained during the study of GR allelic variations distribution for BclI polymorphism in patients with BA and apparently healthy individuals having different BMI values. Differences in the distribution of different genotype variations for BclI polymorphism of GR gene between patients with BA and apparently healthy individuals with normal body mass were not statistically significant, because the p value determined by Pearson's chi-squared test (χ^2) was higher than 0,05 and equaled to 0,275, at same time this differences among BA patients and healthy individuals with increased body weight were statistically significant (p = 0,0001).

The analysis of genotypes distribution for this polymorphism in patients with BA showed significant differences among patients with different body mass. P was lower, than 0,0001 by Pearson's chi-squared test (χ^2). It was established that BA patients with obesity mostly had G/G genotype.

The research on the visceral obesity frequency demonstrated that 78,9 % of apparently healthy individuals had normal ratio of fat centralization, while the rest had a higher ratio. Among the patients with BA, 54,8 % had a high ratio of fat centralization, 45,2 % had normal value. It was established that the patients with BA demonstrated higher ratio of fat centralization much more often, than others (p = 0,0001).

As comparing the data of genotype frequency for BclI GR gene polymorphism in the control group and BA patients group with normal fat centralization parameters, it was observed that the differences in distribution of GR gene allelic variations were not statistically significant (p = 0,106). Where there was a higher ratio of fat centralization, a statistically significant difference in genotype distribution was reported (p = 0,001) (Table 3).

As comparing the data of genotype frequency for this polymorphism in exon 2 of GR gene in the control group and BA patients group with different fat centralization parameters, we found a statistically significant difference in the distribution of allelic variations only among the patients with BA. Thus, the analysis of genotypes distribution depending on the ratio of fat centralization in patients with asthma showed a statistically significant difference (χ^2 = 25,5; p = 0,001). C/G genotype was mostly found in patients without visceral obesity (56,3 %), while G/G genotype was peculiar for the patients with high ratio of fat centralization (52,9 %). Consequently, GG homozygotes have higher ratio of fat centralization, than the major allele homozygotes or heterozygotes.

Table 2. **Distribution of genotypes for BclI polymorphism of glucocorticoid receptor gene depending on body mass index**

Genotype	BMI < 25 kg/m²				BMI ≥ 25 kg/m²			
	Control		BA		Control		BA	
	n	%	*N*	%	*n*	%	*N*	%
C/C	29	39.7	27	28.4	11	50	16	17.2
C/G	34	46.6	55	57.9	9	40,9	25	26.9
G/G	10	13.7	13	13.7	2	9,1	52	55.9
	χ^2 = 2.58; p = 0.275				χ^2 = 17.641; p = 0.001			

We have studied BA risk depending on the genotype of Bcl1 GR gene polymorphism in patients. 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 (OR = 5.038, CI – 95 % 2,377–10.682, $p < 0.001$).

Odds ratio calculated for the association between Bcl1 gene polymorphism and predisposition to visceral obesity in BA patients, thus G/G genotype carriers showed a higher risk of visceral obesity comparing to the patients with C/C genotype (OR=3.13, CI – 95 % 1.4–6.97; $p < 0.01$).

Discussion. The objective of our research was to study the role of the most common and well studied polymorphisms – BclI polymorphism in intron 2 of GR gene – in occurrence of obesity in BA patients. It was reported in the literature that the BclI polymorphism of GR gene was associated with the accumulation of visceral fat, which is a risk factor for atherosclerosis, cardiovascular diseases, obesity and BA [10, 12].

A number of studies demonstrated an association between G/G genotype for the BclI polymorphism and obesity. Thus, Rosmond et al. [7] figured out that G allele was related to increase of BMI, ratio of fat centralization, saggital diameter. Other investigations also reported on the connection between G allele and visceral fat mass increase [8, 11]. Three large studies showed the connection between BclI polymorphism and visceral obesity among middle age individuals [7, 8, 11]; moreover, G allele was associated with higher mass of visceral fat independent of the total body fat mass [8].

BclI polymorphism was associated with abdominal obesity, though a few studies found no significant differences in BclI polymorphism genotype frequency in individuals with or without obesity [13]. Our investigation established that BMI values didn't differ much among the control group individuals with different genotypes for Bcl1 GR gene polymorphism. However, the analysis of genotypes distribution for this polymorphism in patients with asthma showed significant differences among patients with different body mass ($p < 0,0001$ by χ^2 Pearson's chi-squared test. Thus, G/G genotype for BclI polymorphism was related to higher BMI as compared with other genotypes and the control group ($p = 0,0001$). This indicated that body mass in patients with asthma depended on the genotype for Bcl1 GR gene polymorphism; besides, G/G genotype was related to the maximum BMI values.

The connection between BMI and the genotypes for BclI polymorphism of GR gene in patients with BA, as well as the absence of such connection in the control group, is explained by the fact that G/G genotype frequency in the patients was higher, than that in apparently healthy individuals. These observations are congruent with the data given by Polish scientists [12]. In its turn, this can testify that G allele homozygotes are more predisposed to developing BA and obesity, than C/C homozygotes and heterozygotes. Therefore, BclI polymorphism is associated with occurrence of BA and obesity in patients with BA.

The analysis of possible association between the genotypes for BclI GR gene polymorphism and visceral fat deposition stated no statistically significant differences in the distribution of GR gene allelic variations, if the ratio of fat centralization is normal ($p = 0,106$). In case of an increased ratio, differences in genotype distribution are present

Table 3. **Distribution of genotypes for Bcl1 polymorphism of glucocorticoid receptor gene depending on the ratio of fat centralization**

Genotype	Normal RFC				Increased RFC			
	Control, $n = 75$		BA, $n = 103$		Control, $n = 20$		BA, $n = 85$	
	n	%	*N*	%	*n*	%	*N*	%
C/C	29	38.7	25	24.3	11	55	18	21.2
C/G	36	48.0	58	56.3	7	35	22	25.9
G/G	10	13.3	20	19.4	2	10	45	52.9
	$\chi^2 = 4.5$; $p = 0.106$				$\chi^2 = 13.9$; $p = 0.001$			

(p = 0,001). Thus, C/G genotype was mostly found in the patients without visceral obesity, while G/G genotype was peculiar for the patients having visceral obesity. On the other hand, it was observed that in the group of BA patients: 58,1 % of C/C genotype carriers had normal ratio of fat centralization and 41,9 % had a high ratio; C/G genotype carriers had 72,5 and 27,5 %, respectively; G/G genotype carriers had 30,8 and 69,2 %, respectively. It was found out in the patients with BA that G/G homozygotes have higher ratio of fat centralization, than the major allele homozygotes or heterozygotes. The analysis of genotypes distribution depending on the ratio of fat centralization in patients with asthma showed a statistically significant difference (χ^2 = 25,5; p = 0,001).

Therefore, the obtained data concerning the association of G/G genotype for BclI GR gene polymorphism with the occurrence of BA, obesity (particularly, visceral type) verify possible common genetic origin of these two diseases and pleiotropic features of BclI polymorphism.

Molecular mechanisms of how BclI polymorphism influences the occurrence of obesity are not clearly determined yet; they can be related to the direct influence of the BclI polymorphism on GR gene expression, as well as to its possible influence on the transcriptional activity of target genes, involved in glucose and insulin homeostasis [10]. This polymorphism is probably connected with other polymorphisms in the promoter region of GR gene, as well as with other polymorphisms in other genes.

The mechanisms due to which the BclI polymorphism can influence BA occurrence remain unclear. This influence can be possibly explained by the results of the studies demonstrating the effect of this polymorphism on sensitivity to glucocorticosteroids and obesity occurrence, which can induce BA and complicate BA course [10, 12].

The obtained results as for the association between BclI GR gene polymorphism and occurrence of BA and obesity require further research on the complicated molecular mechanisms – this will enable better understanding of occurrence principles of obesity-associated BA, and will help to develop new individual therapy approaches, respectively.

Conclusions. It was found out that the patients with BA demonstrated higher BMI and ratio of fat centralization much more often, than the control group (p = 0,0001). Genotypes distribution for BclI polymorphism in patients with BA showed a statistically significant difference between patients with different BMI unlike the control group. G homozygotes had higher BMI, than those in the control group or with other genotypes. BMI didn't much differ between C/C and C/G genotypes carriers. Comparison of genotype frequency for BclI GR gene polymorphism in individuals with different ratio of fat centralization in the control group and in the patients with BA separately showed statistically significant differences in the distribution of gene allelic variations among the patients with BA only. Among BA patients with G/G genotype – 69.2 % had visceral obesity. In case of normal ratio of fat centralization, there were no statistically significant differences in the distribution of allelic variations between the control group and in the patients with BA. It was found out that G/G-homozygotes have a fivefold higher risk of BA, than those homozygous for C/C. We demonstrated that risk of visceral obesity in BA patients, homozygous for the minor allele, is 3.13 times higher, as compared with C/C-homozygotes (p < 0.001).

BclI ПОЛИМОРФИЗМ ГЕНА ГЛЮКОКОРТИКОИДНОГО РЕЦЕПТОРА У БОЛЬНЫХ БРОНХИАЛЬНОЙ АСТМОЙ С ОЖИРЕНИЕМ

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Исследована связь BclI полиморфизма гена глюкокортикоидного рецептора с ожирением у больных бронхиальной астмой (БА). Обследовано 188 больных на БА и 95 практически здоровых лиц. BclI полиморфизм гена глюкокортикоидного рецептора определяли методом полимеразной цепной реакции со следующим анализом длины рестрикционных фрагментов. Анализ распределения генотипов BclI полиморфизма у больных БА показал достоверное отличие между пациентами с разным индексом массы тела в отличие от контроля. Сравнение частоты генотипов BclI полиморфизма гена глюкокортикоидного рецептора у лиц с разным значением коэффициента централизации жира отдельно в контрольной группе и у больных БА показало наличие статистически значимого отличия в распределении аллельных вариантов гена лишь среди больных на БА. Доказано, что генотип G/G у больных с висцеральным ожирением ассоциирован с БА.

BclI ПОЛІМОРФІЗМ ГЕНА ГЛЮКОКОРТИКОЇДНОГО РЕЦЕПТОРА У ХВОРИХ НА БРОНХІАЛЬНУ АСТМУ З ОЖИРІННЯМ

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Досліджено зв'язок BclI поліморфізму гена глюкокортикоїдного рецептора із ожирінням у хворих на бронхіальну астму (БА). Обстежено 188 хворих на БА та 95 практично здорових осіб. BclI поліморфізм гена глюкокортикоїдного рецептора визначали методом полімеразної ланцюгової реакції з наступним аналізом довжини рестрикційних фрагментів. Аналіз розподілу генотипів за BclI поліморфізмом у хворих на БА показав вірогідну відмінність між пацієнтами з різним індексом маси тіла на відміну від контролю. Порівняння частоти генотипів за BclI поліморфізмом гена глюкокортикоїдного рецептора в осіб із різним значенням коефіцієнта централізації жиру окремо в контрольній групі та у хворих на БА показало наявність статистично значимої відмінності у розподілі алельних варіантів гена лише серед хворих на БА. Доведено, що генотип G/G у пацієнтів із вісцеральним ожирінням асоційований із БА.

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