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Gene polymorphism of glutathion S-transferase T1, M1 and non-specific bronchial hyperresponsiveness in case of eosinophilic bronchial asthma in children

Key words: *children, bronchial asthma, genes of the system of xenobiotics biotransformation.*

Abstract

114 patients of school age were observed at the serum and pulmonary department of Chernivtsi regional child clinical hospital with the aim of detecting the correlation between *GSTT1* and *GSTM1* gene deletion and non-specific bronchial hyperresponsiveness in children, suffering from eosinophilic bronchial asthma. Eosinophilic asthma (I clinical group) was diagnosed in 68 patients. 46 patients formed the II clinical group (non-eosinophilic asthma). It has been found that genotype *T1delM1+* was more often registered with eosinophilic BA, and genotype *T1+ M1del –* in patients with non-eosinophilic phenotype of the disease. Coincidently, genotype *T1delM1del* equifrequently occured in patients with different types of respiratory inflammation, and in patients with eosinophilic asthma was connected with more severe course of the disease. In patients, suffering from eosinophilic bronchial asthma, who were the carriers of defective alleles of *GSTT1* and *M1*genes in homozygous state, there appeared a tendency to increased bronchial lability due to more distinctive bronchial spasm, and the index of bronchial hyperresponsiveness proved to be higher than in children with functionally adequate alleles of these genes. Thus, the genetically determined absence of activity of certain xenobiotic-metabolizing enzymes, namely, *GSTT1* and *M1*, may cause the more definite bronchial lability.

Thus, genotype determination of glutathione S-transferases in patients with eosinophilic bronchial asthma with due regard to bronchial hyperresponsiveness allows to predict the subsequent course of the disease and to devise the individualized therapeutic approach.

Introduction

Bronchial asthma (BA) is one of the most common conditions of a human in all age groups, the severe forms of which lead to disability and falling off in patients' health-related quality of life, thus creating a serious sociomedical problem. It's a typical multifactor disease, rested on a complex character of interrelation between genetic and environmental factors [1].

BA prevention is built with due regard to attainments of modern allergology but is still characterized by a quite low efficiency. In fact, the weaknesses of the current therapy and prevention complexes, presented as treatment regimen and prescriptions, depend significantly on the fact of existing of different variations of BA and different phenotypes of the disease. All these facts demand the development of the individual approach to BA therapy. In this dimension, a complex account of respiratory ways hyperresponsiveness indices and the character of inflammation, the factors which mainly determine BA polymorphism, may seem promising as to the development of individual therapy and prevention measures.

Given the ecological and toxicological basis of many multifactor diseases [2], including asthma, it seems appropriate to study the involvement of genes abnormal effects of which on phenotypic level occur under the influence of factors of chemical nature. These genes include genes of enzymes of biotransformation of xenobiotics (XBE) and, in particular, glutathione-S-transferase (GST). It is established that GST in humans is encoded in a large multygene family including more than 20 genes [3, 4], the function of many of which still needs further study. Relatively well studied is the cytoplasmic

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isoform *GSTT1* and *GSTM1*, taking part in the detoxification of many toxins, products of oxidative stress, carcinogens and drugs.

In human populations widespread are polymorphisms in the genes *GSTM1* and *GSTM1*, which are associated with deletions of both large fragments of genes, making them functionally deficient (null allele *T1del* and *M1del*). It has been proved that in homozygous individuals for the deletion of *GSTT1* genes *GSTM1* enzymatic activity of the respective isoform is lost [5, 6].

Special literature describes studies on the interrelation between polymorphisms of genes *GSTT1*, *GSTM1* and asthma in children and adults [7–9]. However, they are ambiguous and do not take into account the peculiarities of different phenotypes of asthma that arise from the interaction of environmental factors and genetic susceptibility to the disease. Particularly now the question remains as to phenotypic features of the disease and their interrelation of polymorphisms of genes XBE.

The purpose of the study

To find out the relationship between the presence of deletions in the genes *GSTM1* and *GSTT1* and non-specific bronchial hyperresponsiveness in children with eosinophilic asthma.

Materials and methods

114 patients of school age were observed at the serum and pulmonary department of Chernivtsi regional child clinical hospital. The average age of patients was 11,1 ± 0,3 year; surveyed was 79,4 % of boys. All children underwent a comprehensive survey, which included evaluation of non-specific bronchial hiperresponsiveness. The diagnosis of asthma was set on international standards GINA-2009 [10] and the Order of the Ministry of Health of Ukraine [11]. Taking into account the published data on the probability of the existence of different phenotypes depending on the type of airway inflammation, which determine the features of asthma in children, 2 phenotypes of the disease in a cohort of patients indicated were established [10, 12, 13, 14]. Eosinophilic asthma phenotype was defined in patients whose sputum obtained after inhalation of hypertonic solution of NaCl, contained 3 % or more of eosinophilic leukocytes (I clinical group). Non-eosinophilic inflammation of the bronchial tree (II clinical group) was diagnosed with a relative content of cell sediment in cytogram less than 3 % of eosinophils, or in their absence [15].

Among the patients studied eosinophilic asthma was diagnosed in 68 patients. The average age of children in I clinical group was 11,4 ± 0,8 years, the proportion of city residents was 45,5% and the boys – 67,6 %. II clinical group (non-eosinophilic asthma) formed 46 patients, whose average age was 10,9 ± 0,5 ($p > 0,05$) years, including boys – 67,4 % ($p > 0,05$), and city residents – 39,1 % ($p > 0,05$).

Genotyping by the genes *GSTM1* and *GSTT1* was performed for 88 patients with asthma. The total genomic DNA was isolated from blood by standard protocol using proteinase K and sodium dodecyl sulfate as detergent [16].

Detection of deletions in the genes *GSTM1* and *GSTT1* was performed using multiplexed polymerase chain reaction (PCR). As a positive control of PCR success amplification of gene fragments BRCA1 was used. Analysis of PCR was performed by electrophoresis in 2 % agarose gels [17]. To visualize DNA fragments they were stained with ethidium bromide gel and photographed under ultraviolet light using the facility GelDoc 2000 (BioRad, USA). To determine the length of the fragments obtained their electrophoretic mobility was compared with the mobility of DNA Marker Gene Ruler DNA Leader Mix (Fermentas, Lithuania).

Expected length of DNA fragments (431 np for *GSTT1* and 120 np for *GSTM1*) were calculated using the software package DNASTAR computer data using sequences of genes *GSTT1* and *GSTM1*, which are available in the database Genbank. Homozygous deletion forms of both copies of the genes *GSTM1* and *GSTT1* were identified in the absence of appropriate fragment on electrophoregram. These genotypes designated as *T1del* and *M1del*. Accordingly, the presence of these fragments on electrophoregram showed homo- or heterozygosity in normal copies of the gene. The genotypes of patients were designated as T1 + and M1 + (Fig. 1).

Figure 1.

Nonspecific bronchial hyperresponsiveness was assessed by spirographic tests with histamine considering as hypersensitive airways (RK20H) and their hyperactivity, according to a dose-dependent curve (DDC) [18]. Bronchial lability was studied as bronchial lability index (BLI), which was an estimate of spasm after dosed physical activity (IBS – index of bronchospasm) and bronchodilation following inhalation of salbutamol (IBD – index of bronchodilation) [18].

Statistical analysis of the results was performed on a personal computer using the application package Statistika 5.0.

Results and Discussion

Taking into account the fact that BA is a typical multifactor disease in the development of which the significant role is played by both environmental factors and genetic predisposition to it, it was thought advisable to study gene polymorphism of glutathione-S-transferase M1 and T1 in patients with different types of respiratory tract inflammation (Table 1).

The results of genotyping give reason to believe that the genotype *T1delM1+* is more (15,5 %) often registered with eosinophilic asthma than the type of neutrophilic airway inflammation (11,6 %). Genotype *T1+ M1del* was more often recorded in children with the non-eosinophilic disease phenotype (32,6 %) than in their peers suffering from eosinophilic asthma (28,9 %). Genotype *T1delM1del*, which leads to the simultaneous loss of activity of the two isoforms of GST, was equally recorded in patients with different types of airway inflammation. Therewith the severe form of the disease was detected in 4 of 5 *T1delM1del* genotype carriers (80 %) in patients with eosinophilic asthma and in 2 of 5 carriers (40 %) with neutrophilic type of airway inflammation. At the same time, in *T1+M1+* genotype carriers severe form of the disease was diagnosed in 12 of 21 (57,1 %) patients with eosinophilic asthma and in 8 of 19 (42,1 %) with non-eosinophilic type of airway inflammation. Thus, in patients with eosinophilic asthma phenotype, having *T1delM1del* genotype, the disease often ran into severe.

Table 1

The frequencies of different genotypes of GSTM1 and GSTT1 in children surveyed

BA phenotypes	Patients number, N хворих, N	Distribution of genotypes of GSTT1 and GSTTM1							
		T1+ M1+		T1del M1+		T1+ M1del		T1del M1del	
		А	Б	А	Б	А	Б	А	Б
Eosinophilic	45	21	46,6	7	15,5	13	28,9	5	11,1
Non-eosinophilic	43	19	44,2	5	11,6	14	32,6	5	11,6

Note. A – the absolute number of patients with a particular genotype GST; B – % relative to the total number of patients with a particular phenotype of asthma.

In general, it should be noted that in almost half of the patients of both groups there was a deletion of genes of glutathione-S-transferases.

Taking into consideration the presence of deletions in the genes of xenobiotics biotransformation enzymes in children surveyed, it could be suggested the slowdown in detoxification processes, leading to changes in non-specific bronchial hyperresponsiveness.

It is proved that with eosinophilic asthma phenotype the proportion of children who are sick at the age of 6 years comprised 51,2 ± 5,4 %, and with non-eosinophilic asthma it was 52,6 ± 5,7 % ($p > 0,05$). Meanwhile, in the children with the "early-onset" asthma (up to 3 years) eosinophilic phenotype of the disease was more frequently recorded – in 32,1 ± 5,2 % of cases. In contrast, in patients with no n-eosinophilic asthma type "early onset" disease was recorded in 26,3 ± 5,0 % of observations ($p > 0,05$). Despite the fact that light and severe courses of the disease were recorded more often with eosinophilic asthma phenotype, the proportion of children with moderate course of disease was more likely with non-eosinophilic type of airway inflammation (55,3 ± 5,7 %) compared with eosinophilic phenotype (39,3 ± 5,3, $p < 0,05$).

The study of sputum revealed that the percentage of eosinophils in patients of I clinical group was 12,7 ± 1,7 %, and in the comparison group – 1,57 ± 0,1 %.

Table 2 shows the results of the study of bronchial hyperresponsiveness indices under the circumstances of eosinophilic and non-eosinophilic asthma in children.

Despite the fact that the rate of bronchial lability was not significantly different in the comparison group, though there was a trend towards a higher index of bronchodilation in patients with non-eosinophilic asthma and index of bronchospasm in children with eosinophilic asthma. Thus, the index of bronchodilation less than 10,0 % was recorded in 56,0 % of patients in I clinical group and 40,0 % of children in the II group. Index of bronchial hypersensitivity PK20H less than 1,0 mg/ml met more frequently in patients with eosinophilic asthma (60,9 %) when compared with patients whose type of bronchial inflammation was defined as non-eosinophilic (47,0 %). Index of bronchial hyperreactivity according to a dose-dependent curve over 2,5 RVU in patients of I clinical group was recorded in 32,5 % of cases versus 18,1 % in the II group.

The dependence of indicators of nonspecific bronchial hyperresponsiveness in patients with eosinophilic asthma in the presence of deletions in the genes of glutathione-S-transferase *M1* and *T1* has also been studied (Table 3).

Based on these data it can be argued that in patients with eosinophilic asthma who are carriers of the defective alleles of genes *GSTT1* and *M1* in the homozygous state, a recorded tendency to increase bronchial lability due to more expressive bronchospasm and bronchial hyperreactivity rate was significantly higher than in children with full functional alleles of these genes. Thus, due to the lack of genetic activity of some xenobiotics biotransformation enzymes, including *GSTT1* and *M1*, can cause a higher lability of the bronchi.

The obtained results suggest that the definition of GST genotypes in patients with eosinophilic asthma based on their bronchial hyperresponsiveness will predict the future course of the disease and to develop individualized treatment approaches.

Table 2

Bronchial hyperresponsiveness indices in children of comparison groups

BA phenotypes	Bronchial lability indices, %			Bronchial hyperresponsiveness indices	
	Broncho-dilation index	Broncho-spasm index	Bronchial lability index	Hyperresponsiveness index, RK20H (mg/ml)	Hyperreactivity index, DDC (RVU)
Eosinophilic n = 68	12,9 ± 1,4	10,8 ± 1,8	24,6 ± 2,7	1,55 ± 0,4	2,1 ± 0,1
Non-eosinophilic n = 46	17,5 ± 2,9	8,8 ± 1,2	24,6 ± 3,4	2,2 ± 0,4	1,9 ± 0,1
Pt	НВ	НВ	НВ	НВ	НВ

Table 3

Bronchial hyperresponsiveness indices in children with eosinophilic asthma regarding the presence of deletions in the genes GSTM1 and GSTT1

Genotype GSTT1 and GSTM1	Bronchial lability indices, %			Bronchial hyperresponsiveness indices	
	Bronchodilation index	Bronchospasm index	Bronchial lability index	Hyperresponsiveness index, RK20H, (mg/ml)	Hyperreactivity index DDC, (RVU)
T1+M1+ n = 21	12,5 ± 2,4	10,9 ± 2,5	21,6 ± 3,5	0,25 ± 0,1	2,0 ± 0,2
T1+M1del; T1delM1+; T1delM1del n = 25	11,4 ± 2,0	14,7 ± 2,9	25,4 ± 4,1	1,3 ± 0,4	1,9 ± 0,2
Pt	HB	HB	HB	< 0,05	HB

Conclusions

1. Genotype *T1delM1+* is more often registered with eosinophilic asthma and genotype *T1+M1del* – in patients with non-eosinophilic phenotype of the disease.

2. Genotype *T1delM1del* is equifrequently recorded in patients with different types of airway inflammation.

3. The presence of genotype T1delM1del in eosinophilic asthma patients associates with severe disease course.

4. Among patients with eosinophilic asthma there was a tendency of increasing the index of bronchospasm, and among patients with non-eosinophilic phenotype – the growth of bronchodilation index.

5. Patients with eosinophilic asthma – carriers of the defective alleles of genes *GSTT1* and *M1* in the homozygous state, showed a tendency to increased bronchial lability due to more expressive bronchospasm and bronchial hyperreactivity rate was significantly higher than in children with functional full alleles of these genes.

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ПОЛИМОРФИЗМ ГЕНОВ ГЛУТАТИОН-S-ТРАНСФЕРАЗЫ Т1, М1 И НЕСПЕЦИФИЧЕСКАЯ ГИПЕРВОСПРИИМЧИВОСТЬ БРОНХОВ ПРИ ЭОЗИНОФИЛЬНОЙ БРОНХИАЛЬНОЙ АСТМЕ У ДЕТЕЙ

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Резюме

С целью изучения взаимосвязи между наличием делеций в генах GSTT1 и GSTM1 и неспецифической гипервосприимчивостью бронхов у детей, больных эозинофильной бронхиальной астмой (БА), в условиях пульмо-аллергологического отделения областной детской клинической больницы г. Черновцы комплексно обследовано 114 пациентов школьного возраста. Эозинофильная астма (I клиническая

группа) была диагностирована у 68 больных. Вторую клиническую группу (неэозинофильная астма) сформировали 46 пациентов. Показано, что генотип T1delM1+ чаще регистрировался при эозинофильной БА, а генотип T1+M1del – у больных с нейтрофильным фенотипом заболевания. В то же время, генотип T1delM1del с одинаковой частотой регистрировался у больных с разным типом воспаления дыхательных путей, а у пациентов с эозинофильной БА ассоциировал с тяжелым течением заболевания. У больных с эозинофильной астмой, которые являются носителями дефектных аллелей генов GSTT1 и M1 в гомозиготном состоянии, регистрировалась тенденция к повышению лабильности бронхов за счет более выраженного бронхоспазма, а показатель гиперреактивности бронхов у них был достоверно выше, чем у детей с функционально полноценными аллелями этих генов.

Таким образом, генетически обусловленное отсутствие активности отдельных ферментов системы биотрансформации ксенобиотиков, в частности GSTT1 и M1, может бать причиной более высокой лабильности бронхов. Определение генотипов глутатион-S-трансфераз у больных с эозинофильной БА с учетом их гипервосприимчивости бронхов позволит прогнозировать дальнейшее течение заболевания и разработать индивидуализированные подходы к лечению.

Ключевые слова: *дети, бронхиальная астма, гены системы биотрансформации ксенобиотиков.*

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GENE POLYMORPHYSM OF GLUTATION-S-TRANSFERASE T1, M1 AND NON-SPECIFIC HYPERRESPONSIVENESS OF BRONCHUSES UNDER EOSINOPHILIC BRONCHIAL ASTHMA IN CHILDREN

L. A. Ivanova

Summary

To establish the relationship between the presence of deletions in the genes GSTM1, GSTT1 and non-specific bronchial hyperresponsiveness in, suffered from eosinophilic asthma, in pulmoallergological department of Chernivtsi Regional Clinical Hospital were examined 114 school age patients. Eosinophilic asthma (I clinical group) was diagnosed in 68 patients. 46 patients were formed the II clinical group (uneosinophilic asthma). Was established that genotype T1delM1+ often recorded in eosinophilic bronchial asthma and genotype T1+M1del + often recorded in patients with uneosinophilic phenotype of disease. However, genotype T1delM1del with equal frequency was met in patients with different types of airway inflammation, also in patients with eosinophilic asthma was associated with severe variant of disease. In patients with eosinophilic asthma, who were carriers of the defective alleles of genes GSTT1 and M1 in the homozygous state, was recorded a tendency to increase bronchial lability by more expressive bronchospasm and bronchial hyperreactivity rate was significantly higher than in children with functional full alleles of these genes.

So genetically caused lack of activity of some enzymes of xenobiotics biotransformation, including GSTT1 and M1, can cause a higher bronchial lability.

Thus, identification of genotypes of glutathione-S-transferases in patients with eosinophilic asthma considering airway hyperresponsiveness will predict the future course of the disease and develop individualized treatment approaches.

Key words: *children, bronchial asthma, genes of biotransformation system of xenobiotics.*

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