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Study on possible role of *CYP1A1*, *GSTT1*, *GSTM1*, *GSTP1*, *NAT2* and *ADRB2* genes polymorphisms in bronchial asthma development in children

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Aim. *To study the association of polymorphisms of the enzymes genes CYP1A1 (T6235C), first phase, and NAT2 (C481T, G590A, G857A), GSTM1 («0»), GSTT1 («0») and GSTP1 (A313G), second phase of the detoxication system, as well as the ADRB2 (C79G) gene variants with the development of bronchial asthma in children.* ***Methods***. *Polymorphic variants were analyzed using PCR followed by RFLP analysis in 86 healthy individuals and in 114 patients with clinical diagnosis of bronchial asthma.* ***Results***. *The frequency of gene polymorphic variants of the enzymes of first and second phases of detoxification system as well as the ADRB2 gene was established in the children with bronchial asthma and healthy individuals.* ***Conclusions***. *Polymorphic variants of the genes NAT2 (481T), GSTP1 (313G) and ADRB2 (79G) and their combinations in geno- type were observed more frequently in the patients with bronchial asthma comparing to the control group, which indicates their invol- vement in the pathogenesis of asthma in children*

Keywords: bronchial asthma, detoxication system, β2-adrenoreceptor gene, polymorphism, combined genotype.

Introduction. Bronchial asthma (BA), a chronic inflammation of respiratory tracts, is an urgent medical and social problem in Ukraine and around the world, which is remarkable for high incidence and lethality as well as considerable economic cost, related to disability of patients with BA [1–5]. Recent epidemiologic research has demonstrated that 4–35% of population in different countries, including 5–10% of children, suffer from BA [4–8]. During recent 10–15 years the increase in environmental pollution and appearance of new chemical allergens raised the number of patients with BA, while their treatment is not always efficient, besides, the patients often have adverse drug reaction [1, 6].

Up-to-date it has been established that BA is a complex disorder, etiology and pathogenesis of which are determined by complicated interaction of genetic and environmental factors [8–10]. The research of molecular and genetic grounds of hereditary predisposition to BA is mainly concentrated on determination of the role of certain genes and enzymes,

coded by them, in BA pathogenesis as well as in the efficiency of therapy of this disease.

It was determined that due to anatomic and physiological specificities of childhood (narrow bronchial lumen, increased vascularity of respiratory tracts, insufficient rigidity of thoracic cage and elasticity of lungs, weak development of unstriated bronchial muscles, hypersecretion of viscous mucus by goblet cells) BA in children is accompanied by prevalence of edema of mucous membrane and mucus discharge into bronchial lumen over spasm of unstriated muscles [11]. The same work states that bronchial spasm in older children, like in adults, is more frequent than edema of mucous membrane. Bronchial hyperreactivity changes with age – it is more expressed in children and old people than in middle-aged people. However, BA may be without bronchial hyperreactivity. Assumed triggers of bronchoconstriction are considered to be viral infections, physical activity, and external chemical pollutants. Contrary to adults, children suffer from viral infections most frequently [11].

The genes of detoxication system enzymes are considered as possible markers of hereditary predisposition to atopy and associated diseases because their protein products participate in the metabolism of mediators of allergic inflammation as well as in the regulation of mechanisms of oxidative stress, playing an important role in BA pathogenesis in children [10, 12–17].

The specificity of enzymes of P450 cytochrome system is their high activity in main ways of xenobiotics influx into an organism, namely respiratory and food pathes.

It was also revealed that glutathione-S-transferase (GSTM1, GSTT1 and GSTP1) are involved into intracellular transport of hormones and biosynthesis of prostaglandins. Especially high concentrations of these enzymes are observed in lungs, liver, kidneys, and gastrointestinal tract [18–22]. Long deletion was described for *GSTM1* and *GSTT1* genes, due to which RNA and protein product are not synthesized at all (so called "0" allele). It was determined that in majority of populations and ethnic groups the number of individuals, homozygous by this allele, is 35–50% [17]. It was also shown that genetic polymorphism, determined by single nucleotide polymorphism A313G in *GSTP1* gene, results in the production of less active enzyme form [8, 17]. A protein product of *GSTP1* gene was found in different organs, including lungs [8, 17].

N-acetyltransferases provide for acetylation of many xenobiotics, especially drugs, containing aromatic amine or hydrazine groups. Toxic nitrosamines in tobacco smoke and pesticides also belong to NAT2 substrates. Molecular basis for the existence of "fast" and "slow acetylizers" is considered to be *NAT2* gene polymorphisms [17].

β2-adrenoreceptors are localized in bronchi, vessels of most organs as well as almost in all cells, participating in immune response. Their activation causes bronchoectasia which indicates an important role of adrenoreceptors in normal functioning of the respiratory system. Individuals with observed decreased activity of β2-receptors have a higher risk of developing the clinical picture of BA via bronchoconstriction. Another considerable evidence to the association of polymorphisms in β2-adreno-receptor gene with BA pathogenesis is the data on high efficiency of BA therapy in children using stimulation with β2-agonists [13–15]. The study on efficiency of inhalational therapy with в-agonists in patients with asthma revealed dependence between bronchodilation and definite mononucleotide polymorphisms of *ADRB2* gene [12].

Taking these data into consideration, the aim of our work was to study the association of polymorfal*CYP1A1* (T6235C) first phase and second *NAT2* (C481T, G590A, G857A), *GSTM1* («0»), *GSTT1* («0») and *GSTP1* (A313G) phase detoxication system enzymes genes polymorphisms, and also *ADRB2* (C79G) gene variants, with the development of bronchial asthma in children.

Materials and Methods. Three groups of individuals were analyzed. Two groups of children with BA were presented by unrelated individuals from two regions of Ukraine: Kyiv and Kyiv Region (observation group I) and Dniprodzerzhynsk, Dnipropetrovsk Region (observation group II). In accordance to the analytical report "Ukrainian Environment in 2009", posted at web-site of the State Statistics Committee of Ukraine, Dniprodzerzhynsk takes the 8th place among Ukrainian cities in

anthropogenic load from stationary pollution sources with 110.8 thousand tons of emissions of harmful substances [23]. The ecological state of Kyiv and Kyiv Region is considerably better. The groups were formed in 2008 and 2009. Informed consent forms for participation in the research were signed by parents of each participant. This research was approved by the Bioethics Committees of the Institute of Molecular Biology and Genetics, NAS of Ukraine, and State Enterprise "Institute of Pediatrics, Obstetrics, and Gynecology", NAMS of Ukraine.

The observation group I consisted of 52 patients, including 32 (61.5%) male and 20 (38.5%) female individuals. The observation group II consisted of 62 patients, including 43 (69.4 %) male and 19 (30.6 %) female individuals. Totally these two groups with clinical diagnosis of BA comprised 114 patients, 75 (65.8%) male and 39(34.2%) female individuals, aged from 3 to 18 years old. During several years patients from both investigated groups had defined diagnosis of BA and prior to the research they passed unified medical examination in accordance with the recommendations of the Ministry of Health of Ukraine and Global Initiative for Asthma. The results of clinical research and observed symptoms indicate persisting BA of medium degree at the stage of clinical remission in all sick children. The control group comprised 86 unrelated healthy adults from different regions of Ukraine (oocyte donors, their health condition and BA absence in anamnesis were confirmed by the results of medical examination). This group may be considered representative for estimation of DNA polymorphism frequency in autosomal genes [24, 25].

Genotyping. DNA was isolated from leukocytes of peripheral blood by standard method [26].

Polymorphic variants of genes *CYP1A1* (T6235C) [27], *GSTP* (A313G) [28], *NAT2* (C481T, G590A and G857A) [29], and homozygotes by deletions in *GSTM1* and *GSTT1* genes [29], as well as *ADRB2* (C79G) variants [30] were detected as described in corresponding works.

The data were statistically processed using MDR and OpenEpi software and Fisher's test, as well as odd ratio (OR) calculation [31, 32].

Results and Discussion. The results of molecular and genetic analysis of the polymorphic variants of the enzyme genes of first - *CYP1A1* (T6235C), and second - *NAT2* (C481T, G590A, G857A), *GSTM1* («0»), *GSTT1* («0») and *GSTP*1 (A313G) phases of the detoxication system as well as *ADRB2* (C79G) gene polymorphism in the observation groups I and II, and in the control group allowed obtaining the distribution of revealed genotypes and allele variants. The data are presented in Table 1.

It is noteworthy that there was no statistically reliable difference in the frequency of genotypes with *GSTM1*, *GSTT1* and *CYP1A1* gene polymorphisms between the observation groups I, II and the control group. The data on the absence of any association between homozygous deletions of *GSTM1* and *GSTT1* genes and the development of hereditary predisposition to the occurrence of bronchopulmonary pathologies (chronic obstructive lung disease) were received in the work of Gorovenko *et al*. [7]. On the other hand, in a number of works the authors revealed association of these polymorphisms and the risk of BA development in children [17] and in adults [10]. Besides, the research, performed in Ukraine [5], revealed the relation of homozygous deletion of *GSTT1* gene to the development of hereditary predisposition to BA in adults, while there was no determined association of homozygous deletion of *GSTM1* gene with the BA development in adults. These conflicting results testify to the necessity of extending the research and control groups, involved in the experiment. It is noteworthy that the data obtained by us regarding the absence of association of *CYP1A1* gene polymorphism, are in good agreement with the results obtained for other gene polymorphisms of P-450 family (*CYP2C19* and *CYP2E1*) [10].

It was determined that total frequency of hetero- and homozygous carriers of polymorphic allele 313G of *GSTP1* gene is reliably higher ($P < 0.05$) in both the observation group I (51.9%) and group II (56.5%) compared to the control group (33.7%). The same regularity was observed for the frequency of this allele in the corresponding groups. The results of OR indices calculation show that the risk of BA development in children is 2.5-fold increased for both hetero- and homozygous carriers of polymorphic allele 313G *GSTP1* gene (OR = 2.548, CI – 95%: 1.3–4.93).A number of investigations revealed that this enzyme was

Table 1
Distribution of genotypes and allele variants in investigated groups

Gene	Control group, *n* = 86	Observation group I, *n* = 52	Observation group II, *n* = 62	Gene	Control group, *n* = 86	ГObservation group I, *n* = 52	Observation group II, *n* = 62
CYP1A1 (T6235C)				*NAT2* (C481T)			
Genotype, *n* (%)				Allele, *n* (frequency)			
TT	72 (83,6)	44 (84,6)	46 (74,2)	F (C)	90 (0,558)	53 (0,509)	75 (0,605)
TC	12 (14)	8 (15,4)	14 (22,6)	*5 (T)	76 (0,442)	51 (0,491)	49 (0,395)
CC	2 (2,4)	0 (0)	2 (3,2)	*NAT2* (G590A)			
Allele, *n* (frequency)				Genotype, *n* (%)			
T	156 (0,907)	96 (0,923)	106 (0,855)	F/F (GG)	34 (39,5)	26 (50,0)	24 (38,7)
C	16 (0,093)	8 (0,077)	18 (0,145)	F/*6 (GA)	46 (53,5)	23 (44,2)	34 (52,8)
GSTP1 (A313G)				*6/*6 (AA)	6 (7,0)	3 (5,8)	4 (6,5)
Генотип, *n* (%)				Allele, *n* (frequency)			
AA	57 (66,3)	25 (48,1)	27 (43,5)	(G)	114 (0,663)	75 (0,721)	82 (0,661)
AG	24 (27,9)	22 (42,3)	32 (51,6)	*6 (A)	58 (0,337)	29 (0,279)	42 (0,339)
GG	5 (5,8)	5 (9,6)	3 (4,9)	*FNAT2* (G857A)			
AG + GG	29 (33,7)	27 (51,9)*	35 (56,5)*	Genotype, *n* (%)			
Allele, *n* (frequency)				F/F (GG)	79 (91,9)	46 (88,5)	60 (96,8)
A	138 (0,802)	72 (0,692)	86 (0,694)	F/*7 (GA)	7 (8,1)	6 (11,5)	2 (3,2)
G	34 (0,198)	32 (0,308)*	38 (0,306)*	*7/*7 (AA)	0 (0)	0 (0)	0 (0)
GSTM1 («0»)				Allele, *n* (frequency)			
Genotype, *n* (%)				F (G)	165 (0,959)	98 (0,942)	122 (0,984)
+/+ і +/«0»	39 (45,3)	28 (53,8)	32 (51,6)	*7 (A)	7 (0,041)	6 (0,058)	2 (0,016)
«0»/«0»	47 (54,7)	24 (46,2)	30 (48,4)	*ADRB2* (C79G)			
GSTT1 («0»)				Genotype, *n* (%)			
Genotype, *n* (%)				CC	38 (44,2)	21 (40,4)	19 (30,6)
+/+ і +/«0»	39 (45,3)	28 (53,8)	32 (51,6)	CG	36 (41,9)	20 (38,5)	36 (58,1)
«0»/«0»	47 (54,7)	24 (46,2)	30 (48,4)	GG	12 (13,9)	11 (21,1)	7 (11,3)
NAT2 (C481T)				CG + GG	48 (55,8)	31 (59,6)	43 (69,4)*
Genotype, *n* (%)				Allele, *n* (frequency)			
F/F (CC)	20 (23,3)	14 (26,9)	23 (37,1)	C	112 (0,651)	62 (0,596)	74 (0,597)
F/*5 (CT)	56 (65,1)	25 (48,1)	29 (46,8)	G	60 (0,349)	42 (0,404)	50 (0,403)
*5/*5 (TT)	10 (11,6)	13 (25,0)*	10 (16,1)				

Note. N – number of individuals; *statistically reliable difference (P < 0,05); *CYP1A1*: T – 6235T, C – 6235C; *NAT2*: F (C) – 481C, *5 (T) – 481T, F (G) – 590G, *6 (A) – 590A, F (G) – 857G, *7 (A) – 857A; *GSTM1*: «0» (deletion) – «0»/«0», + (норма) – +/+ і «0»/+; *GSTT1*: «0» (deletion) – «0»/«0», + (norm) – +/+ і «0»/+; *GSTP1*: A – 313A, G – 313G; *ADRB2*: C – 79C, G – 79G.

Table2
Distribution of genotypes and allele variants of GSTP1, NAT2, and ADRB2 genes in investigated groups

№	Genotype			Control group, *n* = 86		Observation group I, *n* = 52		Observation group II, *n* = 62	
	*NAT2*5*	*GSTP1*	ADRB2	*n*	%	*n*	%	*n*	%
1	CC	AA	CC	3	3,5	3	5,8	2	3,2
2	CT	AA	CC	19	22,1	5	9,6	4	6,5
3	CC	AA	CG	7	8,1	3	5,8	4	6,5
4	CC	AA	GG	3	3,5	2	3,8	1	1,6
5	CC	AG	CC	1	1,2	1	1,9	8	12,9
6	CC	AG	CG	3	3,5	3	5,8	4	6,5
7	CC	AG	GG	2	2,3	1	1,9	2	3,2
8	CC	GG	CC	0	0	0	0	2	3,2
9	CC	GG	CG	0	0	1	1,9	0	0
10	CC	GG	GG	1	1,2	0	0	0	0
11	CT	AA	CG	16	18,6	4	7,7	10	16,1
12	CT	AA	GG	2	2,3	1	1,9	1	1,6
13	CT	AG	CC	7	8,1	6	11,5	2	3,2
14	CT	AG	CG	6	7	2	3,8	9	14,5
15	CT	AG	GG	3	3,5	4	7,7	2	3,2
16	CT	GG	CC	2	2,3	1	1,9	0	0
17	CT	GG	CG	1	1,2	1	1,9	1	1,6
18	CT	GG	GG	0	0	1	1,9	0	0
19	TT	AA	CC	3	3,5	2	3,8	0	0
20	TT	AA	CG	3	3,5	4	7,7	5	8,1
21	TT	AA	GG	1	1,2	1	1,9	0	0
22	TT	AG	CC	2	2,3	2	3,8	1	1,6
23	TT	AG	CG	0	0	2	3,8	3	4,8
24	TT	AG	GG	0	0	1	1,9	1	1,6
25	TT	GG	CC	1	1,2	1	1,9	0	0
26	TT	GG	CG	0	0	0	0	0	0
27	TT	GG	GG	0	0	0	0	0	0
28	TT/AG + GG/CG + GG			64	74,4	44	84,6	56*	90,3
29	CC + CT/AA/CC			22	25,6	8	15,4	6	9,7

Note. N – number of individuals; *statistically reliable difference (P < 0,05); *NAT2*: C – 481C, *5 (T) – 481T; *GSTP1*: A – 313A, G – 313G; *ADRB2*: C – 79C, G – 79G.

expressed in lungs and alveoli [8, 17]. It is also found that there are several remarkable characteristics of the enzyme GSTP1 which distinguish it among other enzymes of the GST family. One of them is the absence of spatial structure, covering the catalytic centre, which makes it easily available for substrates. Another characteristic feature of this enzyme is a double nature of H-site – it is semi-hydrophobic and semi-hydrophilic [33]. The existent information allows us to assume that concentration of this enzyme in lungs is high, partly because the latter, among other organs of respiratory system, is on the border of internal and external media of the organism. So they are the first to be exposed to the harmful influence of air pollutants.

Therefore, our results and literature data suggest that the increase in frequency of carriers of polymorphic variant 313G of *GST1* gene correlates with the decrease in the activity of the enzyme in patients with this genotype, due to which the level of free radicals increases as well. The latter leads to the increase in the risk of oxidative stress occurrence in the cells of bronchopulmonary system. In its turn, it is a probable prerequisite of BA pathogenesis in children [34].

It was shown that the frequency of homozygous carriers of polymorphic allele 481T of *NAT2* gene is significantly higher ($P < 0.05$) in the observation group I (25%) compared to the control group (11.6%). The tendency of increasing frequency of individuals with this genotype was observed in the observation group II (16.1%).

The enzyme, coded by *NAT2* gene, is expressed in liver and epithelium of intestines [10]. Our study and the works of other authors demonstrated that about 50% Caucasians belong to so called "slow acetylizers" S1 (NAT2*5) and S2 (NAT2*6) [8–10, 17]. It is due to the single nucleotide polymorphisms C481T and G590A. On the other hand, allele S3 *G857A* (NAT2*7) is the most frequent among Mongoloid representatives [27].

The results of biochemical investigations demonstrated that the activity of NAT enzymes in all "slow acetylizers" was decreased by 20% on average in comparison with the norm ("fast acetylizers") [17]. Therefore, similar to 313G polymorphism of *GST1* gene, less functionally active enzyme forms of *NAT2* gene may cause the increase in oxidative stress which is one of BA agents in children [34]. The obtained results and the data of other investigators testify to the fact that "slow" acetylation is a factor of increased risk of BA development in children [8–10, 18, 19].

While studying allele polymorphism of *ADRB2* gene, it was shown that total frequency of hetero- and homozygous carriers of its polymorphism 79G is significantly higher ($P < 0.05$) in the observation group II (69.4%) compared to the control group (55.8%). The tendency of increasing frequency of these genotypes was also observed in the observation group I (59.6%). It was shown in some works that β2-adrenoreceptors play an important role in bronchial dilatation and participate in anti-inflammatory reactions [16].

The investigated polymorphism Gln>Glu (C79G) in the codon 27 of *ADRB2*-gene was shown to cause changing in spatial structure of of the receptor extracellular domain, which results in the decrease in its functional ability.

Taking into account that β2-adrenoreceptors are localized practically in all cells of the immune response, individuals with these polymorphic alleles are likely to be more sensitive to allergens and, as a result, to the development of allergy and inflammations of different kinds. This conclusion is supported by the presented data on the increase in the frequency of carriers of polymorphism 79G of *ADRB2* gene in the group of patients who live in anthropogenically polluted Dniprodzerzhynsk compared to the control group and the group of patients from Kyiv.

To analyze a possible cumulative effect of polymorphisms 313G, 481T and 79G of *GSTP1*, *NAT2*, and *ADRB2* genes, the frequency of which is significantly higher in the groups of children with BA, individuals with genotypes, containing these polymorphisms, were studied. The results of analysis of distribution of these genotypes in the observation groups and in the control group are presented in Table 2.

The frequency of individuals with genotypes, containing polymorphisms 313G, 481T and 79G of *GST1*, *NAT2*, *ADRB2* genes and their combinations, appeared to be significantly higher ($P < 0.05$, OR = 3.28, CI – 95%: 1.215–8.747) in the observation group II (90.3%) compared to the control group (74.4%). The same tendency was also observed in the observation group I (84.6%).

The data obtained suggest that due to the decrease in the activity of enzymes GSTP1 and NAT2 [8, 17, 33, 34] there may be morphofunctional changes in the tissues of bronchopulmonary system, caused by oxidative stress, which is conditioned by the excess of active forms of oxygen and free radicals. The carriers of polymorphism 79G of *ADRB2* gene may also have such pathological constituents of BA in children as modulated immune response and hypertonus of bronchopulmonary tissues [16, 33, 34].

Therefore, it may be proposed that polymorphisms 313G, 481T, and 79G of *GSTP1*, *NAT2*, *ADRB2* genes and their combinations in the genotype are the factors of increased risk of BA development in children.

The limiting trait of the present study is the absence of an additional control group consisting of healthy children who correspond to the observation groups by their age and gender. Our further investigations will be aimed at the comparative molecular and genetic study on the polymorphisms in children with BA and abovementioned control group for in-depth analysis of the regularities observed.

Investigation of allele variants and their combinations related to the increased risk of BA development in children with the consideration of environmental factors and family anamnesis will allow revealing individuals with high risk of BA development.

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Дослідження можливої ролі поліморфізму генів *CYP1A1*, *GSTT1*, *GSTM1*, *GSTP1*, *NAT2* і *ADRB2* у розвитку бронхіальної астми у дітей

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Резюме

Мета. *Дослідити зв'язок поліморфних варіантів генів ферментів першої – CYP1A1 (T6235C) та другої – NAT2 (C481T, G590A, G857A), GSTM1 («0»), GSTT1 («0») і GSTP1 (A313G) фаз системи детоксикації, а також гена ADRB2 (C79G) з розвитком бронхіальної астми (БА) у дітей.* ***Методи***. *По- ліморфні варіанти вивчали за допомогою ПЛР та ПДРФ-аналізу у 86 здорових індивідів та у 114 па- цієнтів з клінічним діагнозом БА.* ***Результати***. *Встановлено частоту поліморфних варіантів генів ферментів першої і другої фаз системи детоксикації, а також гена ADRB2 у хворих на БА дітей та у здорових індивідів.* ***Висновки***. *Поліморфні варіанти генів NAT2 (481T), GSTP1 (313G) і ADRB2 (79G) та їхні комбінації в генотипі частіше спостерігаються серед пацієнтів з БА порівняно з конт- рольною групою, що свідчить на користь залучення їх до патогенезу БА у дітей.*

Ключові слова: бронхіальна астма, система детоксикації, ген β2-адренорецептора, поліморфізм, комбінований генотип.

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Исследования возможной роли полиморфизма генов *CYP1A1*, *GSTT1*, *GSTM1*, *GSTP1*, *NAT2* и *ADRB2* в развитии бронхиальной астмы у детей

Резюме

Цель. *Исследовать ассоциацию полиморфных вариантов генов ферментов первой – CYP1A1 (T6235C) и второй – NAT2 (C481T, G590A, G857A), GSTM1 («0»), GSTT1 («0») и GSTP1 (A313G) фаз системы детоксикации, а также гена ADRB2 (C79G) с развитием бронхиальной астмы (БА) у детей.* ***Методы***. *Полиморфные варианты изучали с помощью ПЦР и ПДРФ-анализа у 86 здоровых индивидов и 114 пациентов с клиническим диагнозом БА.* ***Результаты***. *Установлена частота полиморфных вариантов генов ферментов первой и второй фаз системы детоксикации, а также гена ADRB2 у больных БА детей и здоровых индивидов.* ***Выводы***. *Полиморфные варианты генов NAT2 (481T), GSTP1 (313G) и ADRB2 79G и их комбинации в генотипе чаще наблюдаются среди пациентов с БА по сравнению с контрольной группой, что свидетельствует в пользу их вовлечения в патогенез БА у детей.*

Ключевые слова: бронхиальная астма, система детоксикации, ген β2-адренорецептора, полиморфизм, комбинированный генотип.

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