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ANALYSIS OF ILE105VAL POLYMORPHISM ASSOCIATIONS OF *GSTP1* GENE WITH IMMUNE REACTIVITY MARKERS, OXIDANT STATUS AND REGULATION OF APOPTOSIS IN THE CHILDREN'S POPULATION OF THE KRASNOYARSK REGION IN THE CONDITIONS OF BIOEXPOSURE OF ALUMINIUM

An important task in the context of environmental pollution and the accumulation of heavy metals, including aluminum, is the development of sensitive diagnostic tests, including the search of genetic markers of susceptibility, which make it possible to identify high-risk groups and justify preventive measures to reduce health damage. **The aim of the study** was to assess the role of the *Ile105Val* polymorphism of the *GSTP1* gene (rs1695) in the processes of cell death regulation in the child population under conditions of bioexposure to aluminum. **Materials and methods.** Preschool children (average age 5.34±0.10 years) were examined, 37 children permanently residing in the zone of influence of emissions from a non-ferrous metallurgy enterprise formed the observation group, and 27 children from the conditionally clean territory were included in the comparison group. Aluminum in urine was analyzed by mass spectrometry with inductively coupled plasma. Apoptosis indicators were studied by flow cytofluorometry. SNP genotyping was performed by real-time PCR. **Results.** The level of aluminum contamination in the urine of children in the observation group exceeded similar indicators in the comparison group by 1.53 times (p=0.004). A change in the ratio of immune cell populations was observed (a decrease in $CD3^+$- and $CD4^+$-lymphocytes, p=0.000-0.010) against the background of a violation of the overall oxidant-antioxidant balance (p=0.004-0.042). We identified the proapoptotic effects of the glutathione system associated with the increased frequency of *G* allele of the *GSTP1* gene (rs1695), with the balance sheet of regulatory proteins Bcl-2/Bax (decrease by 2.78 times, p<0.001) and an increase in the number of apoptotic Annexinv-$FITC^+7ADD^-$-cells by 2.59 times (p=0.016). Based on the results of the study, a hypothesis on the role of GSTP1 was formulated as an expression product *GSTP1* gene (rs1695), in the regulation of the processes of programmed cell death with an increased level of contamination of biological media with aluminum. **Conclusion.** Thus, the *G* allele (*105Val*) of the phase II detoxification gene of glutathione-S-transferase *GSTP1* can be considered as a marker of sensitivity in the examined group and can form an increased risk of immune disorders (RR=2.03; 95%CI=1.03-3.99), including dysregulation of cell death processes, under conditions of bioexposure to aluminum.

Keywords. Genetic polymorphism; *Ile105Val GSTP1;* apoptosis; oxidative stress; aluminum.

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Introduction. Aluminum is one of the most common non-essential chemical elements, making up more than 8% of the earth's crust, and the intensive development of the aluminum industry, caused by the growing consumption of the metal, leads to an increase in the level of exposure and the likelihood of developing adverse physiological effects, including diseases of the skeletal system, metabolic syndrome, neurodegenerative disorders, oncopathology [2]. The immunotropic effect of the metal is realized through general toxic properties, including prooxidant, proinflammatory, proapoptotic activity in relation to various cell lines and tissues, through the complication of cell signaling pathways, the release of cytokines, DNA damage and epigenetic changes [12].

Under conditions of a high level of accumulation of xenobiotics, the functional state of the enzyme biotransformation systems, which carry out metabolic transformations of toxic compounds, is of great importance [5]. Phase II enzymes of the xenobiotic detoxification system of glutathione-S-transferases (GST) neutralize electrophilic compounds by conjugation with glutathione, participate in the metabolism of endogenous substrates (hormones, lipids, pros-

taglandins), providing cell resistance to free radicals and DNA damage, and also participate in cellular signaling, regulation of the cell cycle [8]. The GSTP1 enzyme (π class) plays an important role in the elimination of airborne toxicants, and its participation in antioxidant protection and the relationship of individual polymorphic variants of the *GSTP1* gene are actively studied with a predisposition to various pathological conditions, including cardiovascular, atopic, lymphoproliferative diseases [1].

The aim of the study was to assess the role of the *Ile105Val* polymorphism of the *GSTP1* gene (rs1695) in the processes of cell death regulation in the pediatric population under conditions of bioexposure to aluminum.

Materials and methods. The study was conducted with the participation of preschool children (mean age 5.34±0.10 years). A total of 64 children were examined, Russians (56% boys and 44% girls), of which 37 children formed the observation group, permanently residing in the zone of influence of emissions of the non-ferrous metallurgy enterprise (Krasnoyarsk city), and 27 children were included in the comparison group from a conditionally clean territory (Divnogorsk city, Krasnoyarsk region). The formed groups were comparable in age and gender characteristics, ethnicity (p>0.05). All legal representatives of the examined children signed voluntary informed consent to participate in the study.

The content of aluminum in urine was studied by mass spectrometry with inductively coupled plasma on an Agilent 7500cx mass spectrometer (Agilent Technologies Inc., USA). Lipid hydroperoxides were studied by enzyme immunoassay using commercial test systems (Elabscience, China). Antioxidant activity of plasma was determined colorimetrically on a 5400-PE Ekros spectrophotometer (Russia). Apoptosis indices (regulatory proteins Bcl-2 and Bax, p53), lymphocyte fractions by membrane CD markers were studied by immunofluorescence using labeled monoclonal antibodies on a FACSCalibur flow cytofluorometer (Becton Dickinson, USA). Lymphocyte apoptosis was detected by binding to Annexin V-FITC and the vital dye 7-AAD (7-amino-actinomycin D) with determination of the stage of early apoptosis (AnnexinV-FITC$^+$7AAD$^-$-cells) and late apoptosis or necrosis (AnnexinV-FITC$^+$7AAD$^+$-cells) using commercial kits (Becton Dickinson, USA). The testing of the polymorphic marker *Ile105Val GSTP1* (rs1695) was performed using the SNP-screen kits (Synthol, Russia) by PCR with real-time

Table 1

Markers of immune reactivity and oxidative status in examined children with contamination of biological media with aluminum and in the comparison group

Parameter	Observation group (n=37) Me[IQR]	Comparison group (n=27) Me[IQR]	p
Aluminum [urine], mg/dm^3	0.0069[0.0054]*	0.0045[0.0036]	**0.004**
Leukocytes, 10^9/dm^3	5.5[1.9]	6.2[1.1]	**0.045**
CD3+-lymphocytes, %	67[5]	75[7]	**<0.001**
CD4+-lymphocytes, %	37[11]	41[9]	**0.010**
Plasma antioxidant activity, %	31.77[6.34]	37.39[9.55]	**0.004**
Lipid hydroperoxides, μmol/dm^3	227.5[90.2]	198.8[68.7]	**0.042**

Note: p – level of significance of intergroup differences according to the Mann-Whitney criterion. *-reference level of aluminum content in urine <0.006 mg/dm^3.

Table 2

Genetic analysis of the Ile105Val polymorphism of the *GSTP1* gene (rs1695) in examined children with contamination of biological media with aluminum and in the comparison group

Genotype, allele	Observation group (n=37), %	Comparison group (n=27), %	OR (95%CI)
AA	43.2	70.4	0.32 (0.11-0.92)
AG	45.9	25.9	2.43 (0.83-7.13)
GG	10.8	3.7	3.15 (0.33-29.93)
AA+AG	89.2	96.3	0.32 (0.03-3.01)
AG+GG	56.8	29.6	**3.12 (1.09-8.92)***
A	66.2	83.3	0.39 (0.17-0.93)
G	33.8	16.7	**2.55 (1.08-6.04)****
	RR(95%CI)=2.03(1.03-3.99)		
p^{HWE}	0.83	1.00	

Note: OR – odds ratio; RR – relative risk; CI – confidence interval; HWE – Hardy-Weinberg equilibrium. *χ^2=4,64; p=0,031, **χ^2=4,69; p=0,030.

Table 3

Features of apoptosis regulation markers in the examined children of observation group associated with the Ile105Val genotypes of the *GSTP1* gene

Parameter	*AA* (n=16) Me[IQR]	*AG+GG* (n=21) Me[IQR]	p
AnnexinV-FITC + 7AAD–-cells, %	0.64[1.07]	1.66[10.76]	**0.016**
AnnexinV-FITC+7AAD+-cells, %	2.14[3.51]	8.85[23.62]	**0.017**
Bcl-2, %	9.17[4.22]	4.0[5.7]	**0.047**
Bax, %	5.54[5.63]	10.43[9.96]	**0.036**
Bcl-2/Bax	1.50[0.69]	0.54[0.26]	**<0.001**
p53, %	9.63[6.46]	13.3[9.4]	0.478

Note: p – level of significance of intergroup differences according to the Mann-Whitney criterion.

detection on a CFX96 thermal cycler (Bio-Rad, USA).

The results of the study were processed in the Statistica 10.0 software product (Statsoft, USA). The frequencies of alleles and genotypes were calculated in the Gen-Expert online calculator. The results of the study are presented as a median and interquartile range (Me[IQR]) or frequency (%). Comparisons were made using the Mann-Whitney U-test and the chi-square test (χ^2), the odds ratio (OR) and the relative risk (RR) were calculated with the definition of the boundaries of the 95% confidence interval (95%CI). The construction of the "exposure marker – effect marker" models was performed using the method of logistic regression analysis with calculation of the Fisher criterion (F) and the determination coefficient (R^2). Differences were considered significant at $p<0.05$.

Results and discussion. The study revealed shifts in functional indicators in the group of examined children against the background of contamination of biological media with aluminum (Table 1). According to the results of chemical analysis, the level of contamination of biological media in terms of aluminum content in urine was 1.53 times higher in the observation group compared to the comparison group (p=0.004). A violation of the oxidant-antioxidant balance was revealed in children of the observation group with an increase of lipid hydroperoxide content by 14% and a decrease in the total antioxidant activity of plasma by 1.2 times according to the values in the comparison group (p=0.004-0.042). The change in the ratio of immunocompetent cell populations in the observation group was associated with a general decrease in the number of leukocytes and a decrease in the subpopulations of $CD3^+$- and $CD4^+$-lymphocytes by 11-13% relative to the comparison group (p=0.000-0.045).

The results of genetic analysis of the *Ile105Val* polymorphism of the *GSTP1* gene (Table 2) showed a lower frequency of occurrence of the homozygous *AA* genotype (by 1.63 times) and higher frequencies of homozygous and heterozygous genotypes for the allele *G* (*105Val*), by 1.92 times in the group of examined children relative to the comparison group (p=0.031). The frequency of the allele *G* exceeded the indicators in the comparison group by 2.02 times (p=0.030). The probability of the risk of negative health consequences in carriers of the *G* allele (rs1695) of *GSTP1* detoxication gene in the observation group increases by 2.03 times (RR=2.03; 95%CI=1.03-3.99).

Analysis of the distribution of apoptosis markers depending on the carriage of polymorphic variants *Ile105Val* of the *GSTP1* gene (Table 3) revealed excess levels of apoptosis in the observation group in carriers of homozygous and heterozygous genotypes for the *G* allele, relative to those with the *AA* genotype, on average by 2.59 times for the AnnexinV-$FITC^+7AAD^-$-cells indicator and by 4.14 times for the AnnexinV-$FITC^+7AAD^+$-cells indicator (p=0.016-0.017). Dysregulation of cell death processes was observed with a 2.78-fold decrease in the overall ratio of Bcl-2/Bax regulatory proteins (p<0.001), associated with carriage of the *G* allele of the *Ile105Val GSTP1* polymorphism.

Mathematical modeling showed the probability of changes in cellular homeostasis markers depending on an increase in the level of contamination of biomedia with aluminum (Table 4). Models of "exposure marker - effect marker" dependencies revealed a reliable relationship between elevation in the concentration of aluminum in the urine and a decrease in the lymphocyte fraction and total antioxidant activity of plasma (R^2=0.474-0.568; p=0.012-0.027). The probability of an increase in apoptotic AnnexinV-$FITC^+7AAD^-$-cells enhances with the level of bioexposure to aluminum (R^2=0.949; p<0.001).

Aluminum is a widespread metal with high reactivity towards cells and tissues of living organisms. Al^{3+} ions are able to influence the activity of key metabolic pathways, compete with Mg^{2+} for phosphate sites in critical biological enzymes such as ATPase, interfere with the functioning of second messenger systems; stimulate the production of reactive oxygen species (ROS), which disrupt mitochondrial metabolism, damaging the mitochondrial membrane and activating apoptosis processes, while the prooxidant effects of aluminum are associated with changes in the expression of regulatory proteins Bcl-2/Bax, a significant increase in the reactivity of Bax, caspase-3 and c-Jun N-terminal kinase (JNK), which through the regulation of transcription factors (c-Jun and ATF2, p53) affects changes in the cell cycle, repair of DNA damage and/or programmed cell death [7, 9]. Aluminum has also been shown to induce down-regulation of the Nrf2/Keap1 signaling pathway, which likely mediates the inhibitory effect of the metal on the antioxidant system and promotes ROS accumulation [6].

Glutathione-S-transferases catalyze and promote excretion of many xenobiotics, as heavy metals, including aluminum, via conjugation with glutathione. Several studies have found a significant decrease in glutathione-S-transferase activity and glutathione levels after aluminum exposure, and higher urinary aluminum concentrations were associated with lower enzyme activity [4], while differential susceptibility to heavy metals was associated with glutathione-S-transferase gene polymorphisms, enzymatic activity deficiencies, and decreased detoxification in the context of oxidative stress [11].

The *Ile105Val* polymorphism (rs1695) of the *GSTP1* gene, located on chromosome 11q13, is a missense mutation and is localized near the active center of the enzyme. The substitution of adenine (A) for guanine (G) at position 313 of exon 5 determines the exchange of the amino acid isoleucine (Ile) for valine (Val) at codon 105 and leads to a modification of the catalytic properties [14]. It is believed that the 105Val variant has low thermal sta-

Table 4

Parameters of the "exposure marker – effect marker" models in the examined children of the observation group

Exposure marker	Effect marker	Direction of change of the indicator	b_0	b_1	F	R^2	p
Aluminum [urine]	Lymphocytes	Decrease	-4.92	574.57	7.24	0.474	**0.027**
	Plasma antioxidant activity	Decrease	5.67	-804.54	10.56	0.568	**0.012**
	AnnexinV-FITC+ 7AAD–-cells	Increase	2.50	-630.84	109.41	0.949	**<0.001**

Note: b0, b1 – parameters of the mathematical model; F – Fisher criterion; R2 – determination coefficient; p – level of statistical significance of the model.

bility and reduced functional activity due to steric changes in the substrate-binding site, which can lead to a decrease in the ability to detoxify and an increase in the accumulation of toxic substances [3].

The important role of glutathione-S-transferases in the regulation of signaling pathways responsible for the stress response, cell proliferation and apoptosis is determined through interaction with stress-associated MAP kinases, when GSTP1 acts as a chaperone, forming complexes with JNK, and negatively regulates signaling that induces apoptosis (c-Jun and AP1). The *G* allele variant (*105Val*) of the *GSTP1* gene can increase JNK activity and disrupt the protective function of cells. In addition, GSTP1 modulates NF-κB-mediated regulation of transcription of target genes that mitigate the effects of oxidative stress through IκBa, when an increase in ROS promotes the dissociation of GSTP1 and the activation of signaling pathways that ensure cell survival, increased antioxidant activity and ROS elimination, which can ultimately reduce ROS-induced JNK-mediated signaling [10, 13].

Based on the results of the study, we formulate a hypothesis about the role of GSTP1, as a product of the expression of the *GSTP1* gene (rs1695), in the regulation of cell death processes at an increased level of contamination of biological media with aluminum, taking into account the features of its metabolism, the effect of the enzyme on the oxidant-antioxidant status and the regulation of intracellular signaling pathways. Further study of this issue is necessary due to the small sample size of the examined population, possible age and ethnic characteristics, intergenic interactions and epigenetic modifications affecting various aspects of the cellular homeostasis regulatory system.

Conclusion. Thus, the conducted study allowed us to establish the risk of developing apoptosis dysregulation under conditions of aluminum bioexposure and its association with the *G* allele (*105Val*) of the *GSTP1* gene (RR=2.03; 95%CI=1.03-3.99), the carriage of which can be considered as a specific indicator that allows us to differentiate high-risk groups for the purposes of optimizing treatment and preventive measures for the development of immunoregulatory disorders in children under conditions of excessive aluminum bioexposure.

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