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GENETICS

Polymorphic Loci of Xenobiotic Biotransformation Genes in Accumulated Mercury Pollution Liquidators and in Workers with Chronic Mercury Vapors Exposure

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The allele and genotype frequencies of the polymorphic loci *CYP1A1* (*rs1048943*), *GSTP1* (*rs1695* and *rs1138272*), *GSTM1*, and *GSTT1* genes were studied in 517 men: in 389 accumulated mercury pollution liquidators (207 firefighters of the Ministry of the Russian Federation for Civil Defence, Emergencies and Elimination of Consequences of Natural Disasters and 182 employees of the Federal Environmental Operator) and 128 former workers (82 patients in the delayed period of chronic mercury intoxication and 46 individuals contacted with mercury and had no chronic mercury intoxication). We found differences in the frequencies of *AA* and *AG* genotypes in groups of former workers (χ^2=6.96, p=0.008) for the polymorphic locus *rs1048943*, while the *AG-CYP1A1* genotype was characterized by a 5.5-fold decrease in the odds ratio for the development of chronic mercury intoxication (OR=0.18, p=0.0041). An unfavorable combination of genotypes of the studied polymorphic loci increases the risk of undesirable health effects.

Key Words: *cytochrome P4501A1; glutathione-S-transferases M1, T1, P1; genetic polymorphism; liquidators; mercury intoxication*

To date, the significant knowledge has been accumulated about the xenobiotic biotransformation system (XBS) consisting of two functionally coupled phases [1]. During phase I, xenobiotics or endogenous substrates are converted to more polar and water-soluble compounds with participation of cytochrome P450 (CYP); during phase II, their conjugation occurs, which is catalyzed, in particular, by glutathione-S-transferases (GST) [2-5]. At the same time, disruption of the coordinated functioning of xenobiotic biotransformation phases is one of the mechanisms leading to changes in the homeostasis and to the development of various pathological processes.

Previously, the feasibility of conducting a study of polymorphic variants of XBS genes in firefighters of the Ministry of the Russian Federation for Civil Defence, Emergencies and Elimination of Consequences of Natural Disasters and employees of the Federal Environmental Operator involved in mercury pollution liquidation of the environment at the industrial area of the former Usolyekhimprom LLC was justified [6]. In this context, it is interesting to compare these groups with a cohort of plant former workers who were chronically exposed to metallic mercury vapor [7].

Our aim was to study allele and genotype frequencies of polymorphic variants of the XBS genes in accumulated mercury pollution liquidators and in workers chronically exposed to mercury vapors.

MATERIALS AND METHODS

Genotyping of polymorphic loci of *CYP1A1* (*rs1048943*), *GSTP1* (*rs1695*), *GSTP1* (*rs1138272*), *GSTM1*, and *GSTT1* genes was performed in 517 men. We examined

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389 subjects: 207 firefighters of the Ministry of the Russian Federation for Civil Defence, Emergencies and Elimination of Consequences of Natural Disasters (EMERCOM group) and 182 liquidators of the Federal Environmental Operator and its affiliated organizations (FEO group). A similar examination was carried out for 128 former workers who were chronically exposed to metallic mercury vapor: 82 patients in the delayed period of chronic mercury intoxication (CMI) and 46 individuals contacted with mercury but were not diagnosed with CMI. The study was approved by the Local Biomedical Ethics Committee (Protocol No. 6 of March 10, 2020). All participants provided written informed consent to participate in the survey.

Venous blood was collected into K_3-EDTA vacutainers, aliquots of blood samples were stored at -40°C until analysis. DNA was isolated using the DNA-express-blood-plus reagent kit (Litekh). The reagent kits with TaqMan allele-specific probes (Syntol) were used for genotyping of polymorphisms of the *CYP1A1*, *GSTP1* genes, for *GSTM1* — a real-time diagnostic system for determining amplification products from Litech and a reagent kit for identifying deletions in *GSTM1* gene SibDNA, for *GSTT1* — a corresponding kit from SibDNA. Allelic discrimination was performed using C100 Thermal Cycler and CFX96 Real-Time System (Bio-Rad) equipment. Repeated analysis of randomly selected samples did not reveal any errors in genotyping.

Statistica 6.1 software (StatSoft, Inc.) was used for statistical analysis. The differences in allele and genotype frequencies between the groups were analyzed using Fisher's exact test and χ^2 test with Yates correction. The SNPStats software was used to verify the Hardy—Weinberg equilibrium (χ^2 test) and to determine the association of the studied polymorphic loci with CMI for several genetic models [8]. The results of the regression analysis were presented as odds ratio (OR), 95% confidence interval (95%CI), and exact *p* value.

RESULTS

The genotype frequency distribution for polymorphic loci *CYP1A1* (*rs1048943*) in FEO group and *GSTP1* (*rs1695* and *rs1138272*) in group of former workers without CMI did not correspond to Hardy—Weinberg equilibrium (Table 1). These results were excluded from subsequent statistical analysis.

Data analysis (Table 1) for the EMERCOM and FEO groups revealed no significant differences in allele and genotype frequency between these groups as well as compared to the group of patients with CMI. At the same time, we noted a tendency towards an increase of frequency of *AG-CYP1A1* genotype carriage in the EMERCOM group compared to the CMI group (Fisher's exact test, p=0.09, df=1). We also found a significant difference in frequencies of *AA* and *AG* genotypes carriage in groups of workers with chronic mercury vapors exposure (χ^2=6.96, Yates corrected, p=0.008) for the same polymorphic locus of *CYP1A1* gene.

The next stage of our study involved logistic regression only for the *CYP1A1* gene polymorphism in the mercury exposed groups.

We calculated only a codominant *AA*/*AG* model (comparing an *AG* heterozygote with an *AA* homozygote): OR (95%CI)=0.18 (0.05-0.63) (p=0.0041) for *CYP1A1* (*+462Ile/Val*) polymorphism due to the absence of carriers of rare *GG* homozygote in the examined groups. The *AG-CYP1A1* genotype is characterized by a 5.5-fold decrease in the odds ratio for the development of CMI.

Table 2 presents the results of analysis of haplotypes from *GST* genotypes encoding low activity of the corresponding GST or the absence of the enzyme (deletion polymorphism). We found that 30 (14%) EMERCOM employees and 11 (11%) FEO employees, for whom all four *GST* polymorphisms were studied, were carriers of combinations of three or four of the named *GST* genotypes. Carriage of all four such genotypes was detected in 4 (2%) and 3 (3%) employees and was always combined with the *AA-CYP1A1* genotype (reduced activity). At the same time, in 4 (2%) and 2 (2%) cases, carriage of the *AG-CYP1A1* genotype (increased activity) was combined with three *GST* genotypes, causing reduced activity of the conjugation reaction with glutathione.

A similar analysis for the groups of patients with CMI and former workers without CMI revealed that 9 (11%) and 8 (17%) of them, respectively, had three or four genotypes encoding low activity of GST. Carriage of all four of these genotypes was found in 3 (4%) and 2 (4%) former workers. In the CMI group, a combination with the *AA-CYP1A1* genotype (as in the EMERCOM and FEO groups) was noted for such individuals; in the group of workers without CMI, *AA-CYP1A1* was identified in one individual, and the *AG-CYP1A1* genotype was identified in another. The absence of genotype combination encoding increased *CYP1A1* activity along with low GST activity in CMI group should be noted. Whereas the combination of the *AG-CYP1A1* genotype with three *GST* genotypes was detected twice and in one case with all *GST* genotypes showing the reduced activity of the corresponding enzymes in the group of former workers without CMI.

The most significant results were obtained for the *CYP1A1* (*Ile462Val*) polymorphism. That was expressed both in the detection of differences in the frequencies of *AG* and *GG* genotypes carriage in groups of former workers, and in the inverse association of *AG* genotype with the development of CMI. Such

TABLE 1. Allele and Genotype Frequencies for XBS Gene Polymorphisms in Groups

Gene (polymorphic locus), alleles, and genotypes	EMERCOM group	FEO group	Patients with CMI	Workers without CMI
***CYP1A1* (*rs1048943*)**	***n*=207**	***n*=181**	***n*=82**	***n*=46**
A	390 (0.94)	346 (0.96)	160 (0.98)	82 (0.89)
G	24 (0.06)	16 (0.04)	4 (0.02)	10 (0.11)
AA	184 (0.89)	168 (0.92)	78 (0.95)	36 (0.78)*
AG	22 (0.11)	10 (0.06)	4 (0.05)	10 (0.22)*
GG	1 (0.00)	3 (0.02)	0 (0)	0 (0)
GSTM1	***n*=207**	***n*=182**	***n*=82**	***n*=46**
+	98 (0.47)	89 (0.49)	48 (0.58)	20 (0.43)
0/0	109 (0.53)	93 (0.51)	34 (0.42)	26 (0.56)
GSTT1	***n*=193**	***n*=104**	***n*=82**	***n*=46**
+	154 (0.80)	78 (0.75)	68 (0.83)	40 (0.87)
0/0	39 (0.20)	26 (0.25)	14 (0.17)	6 (0.13)
***GSTP1* (*rs1695*)**	***n*=207**	***n*=182**	***n*=82**	***n*=46**
A	281 (0.68)	260 (0.71)	116 (0.71)	58 (0.63)
G	133 (0.32)	104 (0.29)	48 (0.29)	34 (0.37)
AA	91 (0.44)	92 (0.50)	40 (0.49)	22 (0.48)
AG	99 (0.48)	76 (0.42)	36 (0.44)	14 (0.30)
GG	17 (0.08)	14 (0.08)	6 (0.07)	10 (0.22)
***GSTP1* (*rs1138272*)**	***n*=207**	***n*=182**	***n*=82**	***n*=46**
C	381 (0.92)	341 (0.94)	149 (0.91)	80 (0.87)
T	33 (0.08)	23 (0.06)	15 (0.09)	12 (0.13)
CC	174 (0.84)	160 (0.88)	68 (0.83)	37 (0.80)
CT	33 (0.16)	21 (0.11)	13 (0.16)	6 (0.13)
TT	0 (0)	1 (0.01)	1 (0.01)	3 (0.07)

Note. Absolute values are presented (relative frequency). *p<0.05 in comparison with the group of patients with CMI (Yates corrected χ^2).

results are somewhat unexpected since mercury is not a substrate of CYPs, but its exposure is associated with a risk of intoxication for carriers of unfavorable *GST* genotypes [9-13]. Despite the detected discrepancy in distribution, a higher frequency of carriage of mutant homozygous *GG-GSTP1* (*rs1695*) genotypes (increased GSTP1 activity) was observed in the group without CMI compared to the CMI group (0.22 and 0.07; χ^2=4.36, Yates corrected, p=0.0367). A limitation of the study is a relatively small number of former workers, which can influence the results obtained.

We should also note that the frequency distribution for *AA* and *AG* genotypes of *CYP1A1* (*Ile462Val*) polymorphism in the examined groups was comparable to the results of a survey of Russians in Eastern Siberia [14]. However, the frequency of *AG* genotype carriage in the EMERCOM group and in former workers without CMI exceeded the one in the mentioned paper (0.11 and 0.22 *vs* 0.05, p=0.055 and p=0.022 for Fisher's exact test, df=1, respectively). Overall, the frequencies of mutant homozygotes of the examined polymorphisms of *GSTM1* (0.42-0.56), *GSTT1* (0.13-0.25), *GSTP1* (*rs1695*) (0.07-0.22), and *GSTP1* (*rs1138272*) (0-0.07) genes were close to those obtained during a survey of Russians in the neighboring Republic of Buryatia [15]. The most pronounced differences were also observed in the group of former workers without CMI.

Moreover, the frequencies of *GST* genotype combinations encoding reduced GST activity were assessed in all examined groups. We focused on haplotypes consisting of three or four such *GST* genotypes in combination with the *AA*- and *AG-CYP1A1* genotypes (encoding low and increased CYP1A1 activity, respectively). We believe it is important to consider the obtained results within the relationship between the activity of both xenobiotic biotransformation phases in liquidators and former workers. Their significance for the cohort of former caustic soda production workers who were in contact with mercury for a long time

TABLE 2. Some Haplotypes from Combinations of *GST* Genotypes Encoding the Reduced Activity of GST in Groups

Combination	EMERCOM group (*n*=193)	FEO group (*n*=104)	Patients with CMI (*n*=82)	Workers without CMI (*n*=46)
Three or four *GST* genotypes	30 (0.15)	11 (0.11)	9 (0.11)	8 (0.17)
CYP1A1 genotypes with four *GST* genotypes	4 (0.02) *AA*	3 (0.03) *AA*	3 (0.04) *AA*	2 (0.04) *AA* *AG*
AG-CYP1A1 with three or four *GST* genotypes	4 (0.02)	2 (0.02)	0 (0)	3 (0.06)

Note. Absolute values are presented (relative frequency).

differs from firefighters (EMERCOM). Mercury was the most toxic compound for the first group whereas for the firefighters it had a short-term potential effect while repeated exposure to different complexes of compounds formed during the combustion of various materials [16-18]. The combination of combustion compounds determines the involvement of a wide range of CYPs (among which the CYP1 isoforms take a special place) catalyzing the biotransformation of lipophilic xenobiotics, which modification activity has an impact on the metabolism of endogenous substrates [19].

It is important to note the overlapping substrate specificity of the gene products of GST families, as well as the contribution of alternative conjugation reactions when considering the mechanisms of the toxic effect of the compounds to which the examined individuals were exposed [20]. The results of our study allow us to form a risk group of undesirable toxic effects development for firefighters (increased activity of CYP1A1 and decreased activity of GST) exposed to polycyclic aromatic hydrocarbons and dioxin-like compounds (CYP1A1 substrates) during firefighting operations. Carriers of combinations of mutant genotypes of the studied polymorphisms from the FEO group require further medical monitoring due to possible risk of developing mercury intoxication, despite the absence of differences in the distribution of mutant (adverse) genotypes in the FEO group compared to the CMI patients.

Thus, the study of polymorphic variants of XBS genes with subsequent analysis of adverse genotype combinations made it possible to identify individuals with a potential risk of negative health effects. Our findings suggest that for FEO employees, these are primarily carriers of combinations of *GST* genotypes encoding a reduced activity of the conjugation reaction with glutathione; for firefighters, these are combinations of *CYP1A1* and *GST* genotypes causing the absence of functional pairing of xenobiotic biotransformation phases.

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Conflict of interests. The author has no conflicts of interest to declare.

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