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Research article

ASSOCIATION BETWEEN *HSPA1B*, *S100B*, AND *TNF-α* GENE POLYMORPHISMS AND RISKS OF CHRONIC MERCURY POISONING

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We examined association between HSPA1B (+1267A/G, rs1061581), TNF-α (–308G/A, rs1800629), and S100B (C/T, rs9722) gene polymorphisms and chronic mercury poisoning (CMP).

PCR-RFLP analysis was used to examine a cohort consisting of 128 workers who were chronically exposed to mercury vapor; workers were distributed into two groups. The group 1 was made up of workers with long working experience who didn't have CMP (n = 46), the group 2 included patients with long-term CMP period (n = 82). In addition, we estimated frequencies of rs1061581genotypes in 298 practically healthy men from regional sub-population (group 3).

HSPA1B (+1267A/G) polymorphic variant was established to have more frequent carriage of both minor G allele (p = 0.003) and a rare GG homozygous (p = 0.005) in the group 2 against the group 1. 23.2 % patients from the group 2 turned out to have GG genotype and CMP was diagnosed in 95 % people who had it. We didn't detect any differences in genotypes distribution among people from the examined occupational cohort (groups 1 and 2) against the group 3. GG-HSP1AB (+1267A/G) homozygous genotype was shown to be associated with CMP risks (OR = 13.57, p < 0.0001, recessive model). Haplotype G–G (rs1061581–rs1800629) carriers were established to run 2.6 higher risks of CMP occurrence (p = 0.0098), and there was a significant linkage disequilibrium D' = 0.459 (p = 0.0004) between a pair of the above-mentioned polymorphic loci. These data indicate that there is genetic interaction between HSPA1B (+1267A/G) and TNF-α (–308G/A) loci in the examined cohort.

Overall, these results indicate that carriers of GG-HSPA1B (+1267A/G) genotype run high predictive risks of CMP occurrence.

Key words: *mercury, chronic exposure, chronic mercury poisoning, gene polymorphism, heat shock proteins 70, tumor necrosis factor, protein S100B, risk.*

Earlier it was reported that regular medical examinations provided for personnel employed at caustic soda factories with mercury electrolysis technology made it possible to form occupational cohort of workers who were chronically exposed to metallic mercury vapors [1]. The named cohort included patients in a long-term period of chronic mercury poisoning (CMP) with severe clinical manifestations of intoxication and its progression, and by formation of organic lesions in the brain [2]. Patients from this group had signs that neurodegenerative processes occurred in them; it stimulated our interest in heat shock proteins 70 (*HSP70*, *HSPA1* genes) that played a key role in cytoprotection in case of neurodegenerative diseases [3]. It was established that carriage of rare CC-*HSPA1A* (+190G/C) and GG-*HSPA1B* (+1267A/G) homozygotes and their combination was associated with high CMP risks [1]. Besides, carriers of GG-*HSPA1B* (+1267A/G) genotype had greater biological age as compared with carriers of other genotypes among patients with diagnosed CMP [4].

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Damage to the brain was rather specific in patients with CMP; it attracted our attention to tumor necrosis factor *TNF-α* as a key inflammatory/immune response mediator [5]. *TNF-α* gene is among the most polymorphic genes of cytokines and, just as *HSPA1* genes, is located in the locus of the major histocompatibility complex on the short arm of chromosome 6. Giacconi et al. established a significant correlation between 1267A/G *HSP70* polymorphism and TNF-α levels in blood plasma in healthy elderly people [6]. In particular, G allele carriers had elevated TNF-α level, and in authors' opinion, it indicated that this allele had anti-inflammatory properties. Upregulated *HSP70* gene expression causes repression of lipopolysaccharides-induced TNF-α and IL-6 production thus induces protection against inflammation.

A significant increase in concentration of endogenous S100B protein in blood serum taken from patients with diagnosed CMP could characterize an extent to which a pathologic process as active [7]. S100B protein belonged to S100 super-family of calcium-binding proteins which expressed in various cells of the central nervous system and its elevated concentrations were detected, in particular, in patients suffering from Alzheimer and Parkinson disease [8, 9]. Due to lack of protein specificity some researchers do not recommend to rely on its concentration when performing differential diagnostics in case a neurodegenerative disease is suspected in a patient. They also note that S100B protein has only limited utility when it comes to monitoring over disease progression [10]. A wide range of studies to assess the correlation between SNPs in the *S100B* gene and various diseases have been performed in recent years [9, 11, 12].

Our research goal was to examine the association between polymorphisms of *HSPA1B*, *S100B* and *TNF* genes with a risk that chronic mercury poisoning might occur.

Data and methods. To reveal any association between polymorphic variants of *HSPA1B* (+1267A/G, rs1061581), *TNF-α* (–308G/A, rs1800629) and *S100B* (C/T, rs9722) genes and CMP development, we examined a cohort that was created in 2016 and then expanded later; it included 128 former workers who had previously been employed at caustic production and chronically exposed to mercury vapors [1]. All the examined workers were distributed into two groups; the group 1 included workers with long working experience who had no CMP (n = 46), and the group 2 was made up of patients in a long-term CMP period (n = 82). To assess how polymorphic locus of *HSPA1B* (+1267A/G) genotype was distributed in a regional subpopulation, we additionally examined 298 male workers (group 3) who were practically healthy and had no mercury exposure at their workplaces. Long-term clinical examinations among workers, mercury contents in biological substrates and contamination detected in working areas at caustic production had been generalized earlier [1].

All workers gave their written informed consent to take part in the research. Venous blood was taken into vacutainer tubes with K_3EDTA. The samples were then aliquoted and stored at -70º C. DNA was extracted with «DNA-express-blood-plus» reagent kit («Lytech», Russia); then PCR-RFLP analysis was performed. To accomplish genotyping (rs1061581 and rs1800629), we applied primers synthesized by «Medigen» (Novosibirsk, Russia) and «Evrogen» (Moscow, Russia), and endonucleases of *Pst*I and *Taa*I restrictions produced by «Thermo Fisher Scientific» (Lithuania) accordingly. The structure of the primers and PCR conditions are described in details in the papers [13, 14]; rs9722 was examined with commercial kits produced by «Lytech» (Moscow, Russia). Electrophoresis was performed in 1.5 % agarose gel (rs1061581 and rs1800629) and 7.5 % polyacrylamide gel (rs9722), and the results were estimated in transmitting UV-light after painting with ethdium bromide staining.

We performed the exact Fischer's test (two-tailed test) to assess differences in allele and genotype frequencies between groups using «STATISTICA 6.1» software package (StatSoft, USA). We used SNPStats package

to verify with Hardy-Weinburg equilibrium (χ^2-test) and logistic regression in order to reveal a correlation between examined polymorphic loci and CMP for several genetic models [15]. Results obtained via regression analysis were presented as odds ratio (OR), 95 % confidence interval (95 % CI) and the exact significance *p*.

Results and discussion. The genotype frequency distribution for examined polymerphisms in groups corresponded to Hardy-Weinburg equilibrium. We detected some differences in alleles and genotypes frequencies between the group 1 and the group 2 only for polymorphic variant of *HSPA1B* (+1267A/G) (Table 1). It was manifested by more frequent carriage of both rare G allele ($p = 0.003$) and rare GG homozygote ($p = 0.005$) in the group 1 against the group 2. 23.2 % patients (19 out of 82) from the group 2 had GG genotype and 95 % (19 out of 82) of its carriers were diagnosed with the had CMP. We should note that our research revealed only two carriers of a rare AA homozygote rs1800629 in the group 2 and there were no carriers of TT homozygote rs9722 in both examined groups.

Table 2 contains generalized results on how genotypes of polymorphic locus +1267A/G in *HSP1AB* gene were distributed among people in the examined groups. The results indicate that significant differences between the group 1 and the group 3 were revealed only for GG genotype carriers. We should note that there were no differences

Table 1

Allele frequencies and genotype distribution of *HSP1AB*, *TNF-α* and *S100B* genes polymorphisms in groups

Gene (polymorphic locus), allele and genotype	Group 1 ($n = 46$)	Group 2 ($n = 82$)	Fisher's exact test ($df = 2$) *
HSPA1B (rs1061581)			
A	68 (0.74)	90 (0.55)	0.003
G	24 (0.26)	74 (0.45)	
AA	23 (0.5)	27 (0.33)	0.062
AG	22 (0.48)	36 (0.44)	0.714
GG	1 (0.02)	19 (0.23)	0.005
TNF-α (rs1800629)			
G	78 (0.85)	146 (0.89)	0.331
A	14 (0.15)	18 (0.11)	
AA	0 (0)	2 (0.02)	0.536
AG	14 (0.3)	14 (0.17)	0.118
GG	32 (0.7)	66 (0.8)	0.194
S100B (rs9722)			
C	83 (0.9)	143 (0.87)	0.547
T	9 (0.1)	21 (0.13)	
CC	37 (0.8)	61 (0.74)	0.518
CT	9 (0.2)	21 (0.26)	
TT	0 (0)	0 (0)	

N o t e : absolute values are presented (relative frequency); * comparison of group 1 and group 2.

Table 2

Genotype distribution of *HSPA1B* (+1267*A*>*G*) gene polymorphism in groups

Genotype	Group 1 ($n = 46$)	Group 2 ($n = 82$)	Groups 1 and 2 ($n = 128$)	Group 3 ($n = 298$)	$p_1/p_2/p_3$*
AA	23 (0.5)	27 (0.33)	50 (0.39)	109 (0.36)	0.103/0.604/0.663
AG	22 (0.48)	36 (0.44)	58 (0.45)	139 (0.47)	1.000/0.708/0.833
GG	1 (0.02)	19 (0.23)	20 (0.16)	50 (0.17)	0.006/0.197/0.887

N o t e : absolute values are presented (relative frequency); *$p_1/p_2/p_3$ is the Fischer's exact test ($df = 2$) when groups 1, 2 and 1+2 were compared with group 3 respectively.

in frequency of genotype carriage (GG included) when workers who were chronically exposed to mercury poisoning (groups 1 and 2) were compared with the male subpopulation (group 3).

In our discussion on how genotypes of the examined polymorphic loci are distributed we should note that frequency of GG- *HSP1AB* (+1267A/G) genotype was comparable both in the combined group and the group 3 (0.16 and 0.17 accordingly) with the same parameter detected for a Polish cohort (0.17) [6]. It is also interesting to note that frequency of the said genotype was somewhat lower in all the above mentioned groups than in the group 2 (0.23). As for a rare homozygote AA-*TNF-α* (–308G/A) (there were only two people who carried it among patients with CMP), its frequency was a bit higher in the group 2 (0.02) than the same parameter in an Italian and Greek selections (0.009 and 0.014) but lower than in German, French, and Polish selections (0.027, 0.029 and 0.031 respectively) [6]. Unfortunately, we did not reveal any TT-*S100B* (C/T, rs9722) homozygote carriers in the examined cohort. As it has been pointed out in literature, the named genotype is rather rare. In particular, its frequency amounted to 0.010 and 0.013 for two control cohorts in a Swedish study (421 and 372 people; 4 and 5 carriers respectively) [9]. However, frequency of this rare TT homozygote was significantly higher among patients with depression in China and amounted to 0.15 [16]. We can assume that in the latter case elevated frequency of the named genotype was caused not only by an association with the examined disease but also examined people belonging to another race, namely Mongoloid one.

Table 3 contains results obtained via logistic regression for basic genetic models. When it comes to *HSP1AB* (+1267A/G) locus, the analysis revealed that a homozygous as per rare allele GG genotype is associated with a risk of CMP development (recessive model, OR = 13.57 ($p < 0.0001$); additive model, OR = 2.32 ($p = 0.0026$)). And lower value of Akaike information criterion for the recessive model (AIC = 158.6) indicates that it is more relevant than the additive one (AIC = 162.1). We should also note that our analysis of models built for rs1800629 and rs9722 polymorphisms didn't reveal any significant associations with CMP. We analyzed only a codominant model (CC/CT) for *S100B* (C/T, rs9722) locus due to carriers of rare TT homozygote being absent in the examined groups. Genetic models that were adjusted for duration of mercury vapor exposure did not reveal more significant association with CMP.

The next stage in our study involved analyzing haplotypes for four possible allele combinations of *HSPA1B* (+1267A/G) and *TNF-α* (–308G/A) loci (Table 4). We revealed a significant 2.6 times increase in OR for CMP development only for G-G haplotype (OR = 2.6, CI 1.27–5.32, $p = 0.0098$). The significance this haplotype has seems logical since G-*HSPA1B* (+1267A/G) and G-*TNF-α* (–308G/A) alleles correlate with producing

Table 3

Association of the examined polymorphic loci of *HSP1AB*, *TNF-α* and *S100B* genes with CMP development

Gene (polymorphic locus)	Rare allele	Model	OR (95 % CI), *p*	AIC
HSPA1B (rs1061581)	G	AA/AG-GG	2.04 (0.97–4.27), 0.58	167.6
		AA-AG/GG	13.57 (1.75–105.09), 0.0004	158.6
		AA-AG-GG	2.32 (1.31–4.12), 0.0026	162.1
TNF-α (rs1800629)	A	GG/GA-AA	0.55 (0.24–1.57), 0.17	169.3
		GG-GA/AA	NA (0.00–NA), 0.18	169.4
		GG-GA-AA	0.69 (0.32–1.46), 0.43	170.2
S100B (rs9722) *	T	CC/CT	1.42 (0.59–3.42), 0.43	170.6

Note: results of logistic regression for three genetic models (top-down): dominant, recessive, and additive; NA – not defined; * codominant model; AIC is a value of Akaike information criterion.

Table 4

Haplotype frequencies of the examined *HSP1AB* and *TNF-α* polymorphisms, $n = 128$

Haplotype	*HSPA1B* (+1267A/G)	*TNF-α* (–308G/A)	Frequency	OR (95 % CI)	*p*
1	A	G	0.5447	1.00	–
2	G	G	0.3003	2.6 (1.27–5.32)	0.0098
3	G	A	0.0825	1.32 (0.45–3.89)	0.61
4	A	A	0.0425	0.38 (0.07–2.05)	0.26

elevated and reduced HSP70 and TNF-α contents respectively [6]. Besides, we detected a significant ($p = 0.0004$) linkage disequilibrium $D' = 0.459$ between polymorphic rs1061581 and rs1800629 loci. Such data indicate genetic interaction between *HSPA1B* (+1267A/G) and *TNF-α* (–308G/A) loci in the examined cohort.

We expanded our previously crated cohort via including eight patients with diagnosed CMP into it; three of them carried GG-*HSPA1B* (1267A/G) genotype. It confirmed that our conclusions made in the previous study were substantiated [1]. Besides, we established in the present work that there were no differences in genotypes distribution among the occupational cohort (groups 1 and 2) in comparison with the subpopulation (group 3). It allows us to assume that our results can hardly turn out to be false-positive in spite of our sampling of the examined workers being rather small. We should also note that we assessed polymorphic variants of *HSPA1B*, *TNF-α* and *S100B* genes in our research taking them as gene-candidates; there are no data on these genes in the latest specialized reviews that focus on genetic aspects of sensitivity to effects produced by mercury [17, 18].

By now there is general awareness that a significant role belongs to genetic variants and gene polymorphisms being able to modify mercury neurotoxicity in occupational groups running combined occupational and ecological health risks [19]. Recent studies have shown that non-organic mercury deposits in brain tissues of people who have been exposed to environmental contamination are different from those detected in people who have undergone acute or chronic exposure to high mercury concentrations at their workplaces [20]. Overall, it calls for examining workers' genetic status in order to reveal people who are hypersensitive to the toxicant and who can suffer severe damage even when they are exposed to its relatively small concentrations [19]. It seems relevant if such approaches will be implemented for liquidators involved in eliminating accumulated contamination at the industrial area belonging to the former «Usolyekhimprom» LLC where the surface mounted part of most hazardous object, namely a mercury electrolysis workshop, has just been dismantled.

Conclusions. Results obtained in the present study allow concluding that carriers of homozygote for the rare allele GG-*HSPA1B* (+1267A/G) genotype have a high prognostic CMP risk.

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