MEDICAL AND BIOLOGICAL ASPECTS OF THE ASSESSMENT OF THE RISK FACTORS

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POLYMORPHISMS ASSESSMENT OF CHILD CANDIDATE GENES ASSOCIATED WITH LOW-LEVEL LONG-TERM EXPOSURE TO STRONTIUM IN DRINKING WATER

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A sequencing of the candidate genes of the pupils, exposed to strontium by the method of targeted resequencing has been performed. It is shown, that under conditions of increased revenues of strontium in drinking water the number of polymorphonuclear altered portions of candidate genes increases. As a result of the targeted resequencing in conditions of strontium exposure, the maximum polymorph modifications of the following genes are defined: sulfotransferase 1A1 (SULT1A1) and methylenetetrahydrofolate. It was shown that the structure of the mutations in conditions of the strontium exposure was characterized by the formation of defects in the gene mapping detoxification (38.5 % of all mutations) and immunoregulation (22.5 %). Analysis of the cause-effect relationships in the system "factor - the number of mutations" revealed that candidate genes reflecting strontium exposure conditions (content of strontium in drinking water is 1.3 MAC), are genes: cytochrome P450, glutathione - transaminase (detoxification); dopamine (CNS), interleukin 17 and the major histocompatibility complex (immune system), methylene-tetra-hydro-folate-reductase (reproduction).

Key words: *sequencing, strontium, candidate genes, gene polymorphism, detoxification genes.*

Actuality. Genetic testing conducted with the use of Touchdown PCR at the individual level gives solely qualitative results that require additional testing of the functional activity of the genes under study [6, 15, 16]. Combined exposure to chemical mutagens (benzo (a) pyrene, benzene, formaldehyde, chloroform, phenols, vanadium, strontium, etc.) causes genetic and epigenetic disorders the identification of which requires more subtle and deployed studies [2, 3, 4, 7, 8, 10, 11,

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12, 14]. Stable strontium is listed among the chemicals that have immunotropic and mutagenic activity (ATSDR – toxicological profiles 2004, 2005). Strontium ions are similar to calcium ions and can replace the latter in a human body which constitutes one of the main effects of the compounds of this element [1, 13, 17, 18, 20, 21]. Quantitative testing of genomic or epigenome disorders is required to confirm the implementation of the features of genetic polymorphism which can be done using the gene sequence method [1] or assessment of gene expression [19, 22, 23, 24, 25]. One of the challenges in genetic polymorphism is to detect the number of replacements in the genes in charge of the signal perception mechanisms in the immune and endocrine systems because to study them, it is impossible to use routine genotyping methods used in the detection of single-nucleotide polymorphisms (SNP) [5, 26,27]. One such method is the method of targeted resequencing using liquid biochips.

At the same time, to detect the heterogeneity of the population and the direction of the change in the genetic material affected by natural and anthropogenic environmental factors, it is necessary to use a marker that can be quantified in addition to having a high-quality performance [9].

One such marker is the amount of single-nucleotide polymorphisms (transitions, transversions), to identify which, especially under mutagenic effect of the environmental factors, it is necessary to use high-precision and advanced equipment for genotyping.

Materials and Methods. For the first time in Perm region, we were able to decode the structure of 27 human genes using the method of targeted resequencing. The study included a decoding of relevant polymorphisms of exons and regulatory regions. When performing genetic diagnostic testing, we performed a DNA sequencing of 6 children aged 7-9, permanently residing in a geochemical area characterized by elevated levels of strontium in the drinking water from underground sources. The method of resequencing allowed for simultaneous genotyping of DNA by all the genes under study. The probe library was prepared in advance by 27 genes of interest and included about 2 million oligonucleotide probes, complementary to our area of interest. We studied polymorphisms in the following genes: CYP1A2, IL17F, IL17D, IL17C, IL17B, TLR4, TERT, FAS, FOXP3, TP53, HLADRB1, MTHFR, GSTA, SULT1A1, NR3C1, VEGF, ZMPSTE, ESR1, ANKK1.

The sequencing included the following steps: isolation of human DNA from the biological material (blood), creation of a DNA library; amplification of the DNA library and hybridized DNA; conducting emulsion PCR; producing sequencing on GS Junior (Switzerland) using targeted resequencing based on live biochips.

To isolate candidate genes, we analyzed and ranged gene polymorphisms based on the functional identity by dividing them into groups: immunoregulation genes, metabolism genes, detoxification genes and somatic genes representing mainly the nervous system neurotransmitters.

Results. We detected polymorphisms of candidate genes using the targeted sequencing method in six patients under study with various levels of strontium in blood and urine (Table 1).

The maximum number of polymorph-altered regions of genes was found in the group of detoxification genes, with the highest polymorphism typical of sulfotransferase 1A1 (SULT1A1) and methylenetetrahydrofolate, and the most conservative of this functional system were superoxide dismutase (SOD) and coproporphyrinogen oxidase (CPOX).

The results of sequencing by groups of the candidate genes

Functional gene groups	Gene	Number of polymorphisms as compared to the reference sequence					
		1 p	1 p	1 p	1 p	1 p	1 p
Strontium in urine mg/dm^3	–	4,549	0,978	1,068	1,739	1,494	2,993
Strontium in blood mg/ dm^3	–	0,129	0,0574	0,0704	0,266	0,166	0,258
Detox genes	MTHFR	13	13	13	14	23	23
	ZMPSTE24	4	3	3	4	1	4
	CPOX	1	8	7	8	8	10
	GSTA4	11	12	9	15	11	15
	SOD	4	0	6	2	0	6
	CYP1A2	5	3	3	4	3	4
	SULT1A1	41	33	37	10	39	37
	7 genes	79	72	78	57	85	99
Immunoregulation and proliferation genes	IL17B	0	2	0	2	1	1
	IL17C	3	3	2	3	4	2

	IL17D	3	1	2	1	1	1
	IL17F	0	0	0	0	0	1
	VEGFA	2	4	3	2	8	5
	HLADRB1	45	19	6	0	15	10
	TLR4	0	1	1	1	2	2
	FAS	0	4	8	5	7	9
	TP53	7	7	6	6	7	6
	FOXP3	3	3	4	0	2	2
	10 genes	63	44	32	20	47	39
Metabolism genes	ACE	4	28	24	22	21	25
	APOE	3	3	3	2	2	5
	SIRT3	9	12	8	12	8	1
	PPARD	4	6	4	5	5	1
	NOS3	19	14	12	14	12	14
	TERT	9	5	14	3	6	5
	6 genes	48	68	65	58	54	51
Somatic genes	ACTA2	1	1	1	2	0	0
	CLCN6	0	1	0	0	1	1
	TH	17	16	15	8	16	18
	DRD2	19	12	16	14	19	20
	4 genes	37	30	32	24	36	39

Fewer polymorphism-related alterations were registered in the genes of the immune and nervous regulation systems. Here, HLADRB1 is mostly prone to the biggest polymorphism in this system which can be explained from the position of regulation of the immune response; many of the immune response and oncogenesis genes are rather conservative.

The structure of mutations in the conditions of strontium exposure (1,3 MPC) is characterized by the development of defects in the areas of mapping detoxification genes (38.5% of all mutations) and immunoregulation genes (22.5%).

A reliable relationship between the number of polymorphism-altered regions of the genes and strontium content in blood has been registered (Figure 1).

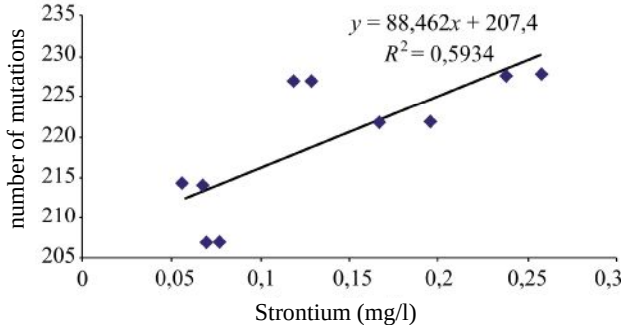

Figure 1 Relationship between the number of polymorphism-altered regions of genes and the level of strontium in blood

We observed a strong direct relationship between the strontium content in blood and the number of substitutions in the MTHFR gene, GSTA4, HLADRB1, CYP1A2, DRD2, IL17D.

The analysis of the total amount of single-nucleotide polymorphisms helped us determine the average number of mutations per person by 25 candidate genes which totaled 210 polymorphisms. At the same time, the average number of polymorphisms in the exposure group totaled 226 – by 7.1% higher than in the similar population residing outside the strontium-exposed area.

The analysis of associations in the "receptor gene" system using the method of targeted resequencing, flow cytometry, and immunofluorescence microscopy analysis allowed us to evaluate the relationship between the genes and their encoded proteins. Relationships developed between the values of nucleotide polymorphisms in a specific gene and the value of protein coded by the gene detected with the fluorescent analyzer. We found strong reliable relationships in biological logistic systems dopamine, dopamine gene, gene IL17d-IL17, gene FOXp3-CD127-, gene p53- p53. This fact correlates with the biological need to support adaptation expression of these genes and proteins at an adequate homeostatic level due to their major regulatory significance. The logistic relationships in the following systems were broken: gene GSTA4- GSTA, gene SOD- SOD, FAS-CD95 + gene, gene VEGF-VEGF, indicating physiologically unacceptable environmental conditions that change the adaptive capacity of conjugation, antioxidant protection, control of apoptosis and endothelial function.

Conclusion

Targeted resequencing used to decode the sequences in the conditions of strontium exposure

can identify the maximum polymorphic variants for the following genes: sulfotransferase 1A1 (SULT1A1) and methylenetetrahydrofolate, and the most conservative of this functional system were superoxide dismutase (SOD) and coproporphyrinogen oxidase. (CPOX).

In the result of sequencing and analysis of the "factor-number of mutations" causal relationship, we identified the following candidate genes reflecting the exposure to strontium in drinking water at the level 1.3 MAC: cytochrome P450, glutathione-transaminase (**detoxification**); dopamine (**CNS**), interleukin 17 and the major histocompatibility complex (**immune system**), methylenetetrahydrofolate reductase (**reproduction**).

Phenotyping of genetically confirmed mutations determined that strontium exposure (at 1.3 MAC) reliably realizes gene polymorphisms: dopamine (a neurotransmitter CNS), interleukin-17 gene and gene regulatory T-lymphocytes (immune system), the gene transcription factor 53 (oncosupressor).

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