CHAPTER «MEDICAL SCIENCES»

FOLATE CYCLE GENES AND ADAPTATION PROCESSES IN CHILDREN LIVING NEAR THE CHERNOBYL EXCLUSION ZONE

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Abstract. Consequences of the Chernobyl Nuclear Power Plant (ChNPP) accident, in the form of long-lived radionuclides, are currently being detected in the environment. Radioactive elements enter the bodies of local residents through food produced in contaminated areas, wild berries and mushrooms, fish, and wild game meat. The simultaneous incorporation of ^{137}Cs into vital organs causes metabolic disorders in the body, manifested as the syndrome of long-lived incorporated radionuclides (SLIR), which is based on mitochondrial damage and disruption of cellular energetics. A large number of children in the Ivankivsky and Polessky districts of the Kyiv region of Ukraine, living near the Chernobyl exclusion zone (ChEZ), have been diagnosed with hyperhomocysteinemia – an elevated level of the sulfur-containing amino acid homocysteine (H_{cy}) in the blood. The cause of hyperhomocysteinemia in these children is mitochondrial damage caused by the incorporation of ^{137}Cs into the body. It is important to determine the role of hyperhomocysteinemia caused by ^{137}Cs incorporation in the induction of pathological processes and adaptive responses. The involvement of the folate cycle (FC) and transsulfuration cycle genes should be assessed. *The aim* of the study was to determine the role of FC genes in the development of adaptive processes in the bodies of children living near the ChEZ exposed to incorporated ^{137}Cs radionuclides. *Research methods.*

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An analytical study was conducted using the results of instrumental, laboratory, and genetic testing of 1.352 children, aged 2-18 years, from the Ivankivsky and Polessky districts of the Kyiv region of Ukraine. The tests were conducted from 2013 to 2017 as part of projects by the European Commission and the Rhône-Alpes Regional Project (France). The specific activity of ^{137}Cs was determined in each child. The relationship between the analyzed parameters was assessed using Spearman's rank correlation coefficient (r_{xy}). *Results.* The study identified a link between the FC genetic system and the transsulfuration reaction cycle under conditions of ^{137}Cs radionuclide incorporation into the body. It was established that the most pronounced blocking of B_{12} methionine synthase activity occurs with a combination of environmental (radiation) and internal (FC genes) factors. In a subgroup including cases with the GMTR:2756 allele in the genome, which has a negative effect on B_{12} methionine synthase activity, associations involving vitamin B_6 were identified: an inverse association between vitamins B_{12} and B_6, and a direct association between H_{cy} and vitamin B_6. These associations indicate the utilization of H_{cy} in the transsulfuration reaction cycle, including through cystathionine β-synthase, whose coenzyme is vitamin B_6. Impaired functioning of B_{12} methionine synthase, associated with the GMTR:2756 allele in the genome, in children exposed to incorporated ^{137}Cs radionuclides, stimulates the utilization of H_{cy} in the transsulfuration reaction cycle and promotes the activation, under the influence of H_{cy}, of the antioxidant system, including cysteine and glutathione, as well as cortisol (Cortisol), triiodothyronine (T_3). *Conclusions.* The conducted analytical study revealed the involvement of FC genes in the development of adaptive responses in the bodies of children living near the ChEZ exposed to incorporated ^{137}Cs radionuclides. The negative impact of incorporated ^{137}Cs radionuclides on the mitochondrial energy system leads to disruption of FC function and an increase in H_{cy} levels in the blood of children living near the ChEZ. H_{cy} is one of the main components of the emergency system, which is activated under conditions of oxidative stress caused by exposure to incorporated ^{137}Cs. The key component of this emergency system is the transsulfuration reaction cycle, through which H_{cy} is utilized to form cysteine and glutathione, the synthesis of adrenal cortex and thyroid hormones is activated, and calcium-phosphorus metabolism is altered. Under conditions of radiation exposure

and the GMTR:2756 polymorphic allele, suppression of B_{12} methionine synthase activity, involving H_{cy}, activates a complex of antioxidant reactions aimed at restoring mitochondrial function and providing cellular energy.

1. Introduction

The ChNPP accident in 1986 contaminated a vast area of Europe with long-lived radioactive elements.

In areas near the ChNPP, these elements are still prevalent in the environment [1, p. 9; 2, pp. 120, 121].

Radioactive elements enter the bodies of local residents through food products produced in contaminated areas, wild berries and mushrooms, fish, and wild game [3, p. 250].

The simultaneous incorporation of ^{137}Cs into vital organs causes metabolic disorders in the body, known as the SLIR, which is based on mitochondrial damage and disruption of cellular energy [4, pp. 109, 110].

Radiation exposure causes the formation of reactive oxygen species in mitochondria, which can damage cellular structures [5, p. 7].

An important indicator of metabolic changes under radiation exposure is an increase in the sulfur-containing amino acid homocysteine – H_{cy} in the blood, detected in a large number of children in the Ivankivsky and Polessky districts of the Kyiv region of Ukraine living near the ChEZ [6, p. 29].

The amino acid H_{cy} occupies an intermediate position in the metabolism of the essential amino acid methionine (Met), which supplies the body with a methyl group – CH_3, which plays a crucial role in metabolic processes.

H_{cy} levels in the blood increase in cases of CH_3 deficiency due to impaired functioning of FC enzymes or decreased formation of trimethylglycine (betaine) in the mitochondria.

These processes are underpinned by a deficiency of energy carriers associated with mitochondrial damage.

Thus, H_{cy} methylation, leading to the formation of internal Met, depends on mitochondrial function.

Mitochondrial damage due to ^{137}Cs incorporation is the cause of hyperhomocysteinemia in most children living near the ChEZ.

It is important to determine the role of ^{137}Cs-induced hyperhomocysteinemia in the induction of pathological processes and

adaptive responses. Furthermore, the involvement of the FC gene system and the transsulfuration reaction cycle should be assessed.

The aim of this study was to determine the role of FC genes in the development of adaptive processes in the bodies of children living near the ChEZ exposed to incorporated ^{137}Cs radionuclides.

2. Materials and Methods

An analytical study was conducted of the results of instrumental, laboratory, and genetic testing of children from the Ivankivsky and Polessky districts of the Kyiv region of Ukraine.

The study was conducted from 2013 to 2017 as part of the European Commission's "Health and Ecological Programmes around the Chernobyl Exclusion Zone: Development, Training, and Coordination of Health-Related Projects" project and the Rhône-Alpes Regional Council (France).

During this monitoring, 1.352 children aged 2-18 years were examined repeatedly, in compliance with bioethics rules and with the consent of their parents.

The soil levels of ^{137}Cs and ^{90}Sr were assessed in the areas where the children studied lived [2, pp. 120, 121].

To continuously monitor the ^{137}Cs radionuclide levels in the bodies of children and adults living near the ChEZ, a specialized laboratory was established at the Ivankiv Central District Hospital as part of a European Commission project.

The specific activity of ^{137}Cs in the children's bodies was recorded using a 3-detector human radiation spectrometer "SICH-AKP-3" (LLC NPP "ATOMKOMPLEKSPRIBOR", Ukraine) for 10 minutes.

The children's physical development was assessed using the Rohrer weight-height index (IR) – the quotient of body weight in kilograms divided by body length in meters cubed.

Standardized anthropometric methods were used to measure the children's weight and length [7, p. 15].

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined automatically using a patient monitor (PM 9000, Penton Ltd.).

For laboratory and genetic testing, blood was drawn from the cubital vein of children in the morning, on an empty stomach.

The resulting blood samples were analyzed in a laboratory certified in accordance with European quality standards.

The children's blood was tested for H_{cy}, Cortisol, parathyroid hormone (PTH), calcium (Ca), ionized calcium (Ca^{2+}), phosphorus (P), pituitary thyroid-stimulating hormone (TSH), thyroxine (T_4), T_3, vitamin B_9, vitamin B_{12}, vitamin B_6, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) [2, p. 27; 7, p. 335; 8, p. 5].

Genetic testing of the folate cycle (FC) involved identifying the C677T and A1298C allelic variants of the MTHFR gene (associated with the enzyme methylenetetrahydrofolate reductase), A2756G of the MTR gene (associated with the enzyme B_{12} – methionine synthase), and A66G of the MTRR gene (associated with the enzyme methionine synthase reductase). Real-time PCR was used.

Statistical analysis of the obtained results was performed using IBM SPSS Statistics 22 (USA).

Student's t-test was used to compare relative indicators. The critical significance level for the null hypothesis (p) was set to 0.05.

The median (Me), interquartile range (IQR), minimum and maximum parameter values, and percentiles were calculated for the analyzed indicators. A hypothesis test regarding the distribution type was conducted (Kolmogorov-Smirnov test).

The statistical significance of the indicators was assessed by determining the p-value using a statistical program.

The Student's t-test was used to compare relative indicators.

The critical significance level for the null statistical hypothesis (p) was set to 0.05.

The relationship between the analyzed indicators was determined using Spearman's rank correlation coefficient (r_{xy}).

The strength of the correlation was assessed using a traditional scale: weak – from 0 to 0.299; moderate – from 0.3 to 0.699; strong – from 0.7 to 1.0.

3. Results and Discussion

The specific activity of ^{137}Cs was recorded in the bodies of the examined children in the range of 0.78 - 95.11 Bq/kg.

In the study group of children, serum AST activity exceeded physiological levels in 37.5% of cases, while serum ALT activity did so in 1.2% of cases, indicating damage to the mitochondria of cardiomyocytes [2, p. 28].

Furthermore, a direct correlation was found between the specific activity of ^{137}Cs in the body and serum AST values (Table 1) [2, pp. 31, 32].

Table 1

Results of the analysis of correlations between the values of specific activity of ^{137}Cs and the activity of serum AST in a group of children

Age, years	Correlation coefficient	Correlations	
		^{137}Cs (Bq/kg) – AST (U/л)	^{137}Cs (Bq/kg) – AST/ALT
12-18	Spearman's (r_{xy})	0.207**	0.187**
	Sign. (2-tailed), p	0.0001	0.0001
	N	649	649

*Note. ** – the correlation is significant at the 0.01 level (two-sided). AST – aspartate aminotransferase; ALT – alanine aminotransferase; AST/ALT – de Ritis coefficient.*

The established relationship indicates that with increasing specific activity of ^{137}Cs in the body and myocardium, mitochondrial damage increases, and higher concentrations of this radionuclide can cause cardiomyocyte damage incompatible with life.

Incorporation of ^{137}Cs leads to a decrease in myocardial contractility, as evidenced by the inverse correlation between ^{137}Cs and SBP in groups of children aged 12 and 13 years (Table 2) [8, p. 342].

At the same time, an inverse relationship was found between the specific activity of ^{137}Cs in the body and the concentration of serum calcium, indicating a disruption in the excretion of this element into the extracellular space due to a deficiency of energy carriers (Table 2) [8, p. 342].

Incorporated ^{137}Cs has a negative impact on the physical development of children, as evidenced by the inverse correlation between ^{137}Cs and the IR index, which reflects physical development (Table 2) [8, p. 342].

However, changes simultaneously occur in the body aimed at increasing the Ca content in the blood.

In most subgroups divided according to specific FC genotypes, direct correlations were found between H_{cy} and Ca^{2+}, indicating the ability of H_{cy} to increase serum Ca concentrations (Table 3) [8, p. 346].

Table 2

Results of correlation analysis in groups of boys and girls living near the Chernobyl Exclusion Zone

Age groups	Correlation coefficient	Correlations			
		^{137}Cs - Ca	^{137}Cs - IR	Ca - P	^{137}Cs - SBP
Boys 12 years old	Spearman's (r_{xy})	- 0.277**	- 0.564**	0.124	- 0.375**
	Sign. (2-tailed), p	0.009	0.0001	0.248	0.0001
	N	88	88	88	88
Girls 12 years old	Spearman's (r_{xy})	- 0.068	- 0.591**	0.231*	- 0.321**
	Sign. (2-tailed), p	0.503	0.0001	0.022	0.001
	N	98	98	98	98
Boys 13 years old	Spearman's (r_{xy})	- 0.216*	- 0.485**	0.296**	- 0.390**
	Sign. (2-tailed), p	0.030	0.0001	0.003	0.0001
	N	101	101	101	101
Girls 13 years old	Spearman's (r_{xy})	- 0.218*	- 0.578**	0.313**	0.070
	Sign. (2-tailed), p	0.028	0.0001	0.001	0.482
	N	102	102	102	102

Note. * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided); IR – Rohrer's index; Ca (mmol/l); P (mmol/l); SBP (mmHg), ^{137}Cs (Bq/kg).

The strongest H_{cy}-Ca^{2+} correlation was recorded in the subgroup with the main T/T MTHFR:677 genotype, in which the activity of methylenetetrahydrofolate reductase, the enzyme catalyzing the formation of methyltetrahydrofolate, is almost completely suppressed.

Therefore, the proportion of hyperhomocysteinemia was the highest among all genetic subgroups (Table 4).

It is most likely that H_{cy}, in hyperhomocysteinemia, affects osteoclasts, which destroy bone structures, which leads to an increase in Ca and P levels in the blood [8, p. 349].

There is a certain parity between serum Ca and P concentrations, as evidenced by the direct Ca-P correlation (Table 2) [8, p. 344].

PTH, activated by P when its concentration in the blood increases, is involved in regulating this parity. PTH, by acting on the renal tubular apparatus, promotes the reabsorption of Ca into the blood and the excretion of P in the urine.

Table 3

Results of correlation analysis in genetic subgroups of children in the Polessky districts

Genetic subgroup	Correlation coefficient	Correlations		
		H_{cy} - Ca^{2+}	H_{cy} - PTH	P - PTH
A/A MTR:2756	Spearman's (r_{xy})	0.319**	0.025	0.482**
	Sign. (2-tailed), p	0.001	0.800	0.0001
	N	104	104	104
A/G MTR:2756	Spearman's (r_{xy})	0.362*	-0.048	-0.234
	Sign. (2-tailed), p	0.014	0.755	0.121
	N	45	45	45
G/G MTR:2756	Spearman's (r_{xy})	0.201	-0.767*	0.150
	Sign. (2-tailed), p	0.604	0.016	0.700
	N	9	9	9
A/A MTHFR:1298	Spearman's (r_{xy})	0.462**	-0.020	0.280*
	Sign. (2-tailed), p	0.0001	0.858	0.011
	N	82	82	82
A/C MTHFR:1298	Spearman's (r_{xy})	0.216	0.126	0.283*
	Sign. (2-tailed), p	0.097	0.338	0.029
	N	60	60	60
C/C MTHFR:1298	Spearman's (r_{xy})	-0.192	-0.389	0.519*
	Sign. (2-tailed), p	0.476	0.137	0.039
	N	16	16	16
C/C MTHFR:677	Spearman's (r_{xy})	0.169	-0.099	0.411**
	Sign. (2-tailed), p	0.136	0.386	0.0001
	N	79	79	79
C/T MTHFR:677	Spearman's (r_{xy})	0.399**	0.073	0.115
	Sign. (2-tailed), p	0.002	0.580	0.380
	N	60	60	60
T/T MTHFR:677	Spearman's (r_{xy})	0.619**	0.326	0.394
	Sign. (2-tailed), p	0.005	0.173	0.095
	N	19	19	19
A/A MTRR:66	Spearman's (r_{xy})	0.403*	0.123	0.405**
	Sign. (2-tailed), p	0.022	0.502	0.021
	N	32	32	32
A/G MTRR:66	Spearman's (r_{xy})	0.319**	0.074	0.260*
	Sign. (2-tailed), p	0.006	0.537	0.027
	N	72	72	72
G/G MTRR:66	Spearman's (r_{xy})	0.276*	-0.067	0.270*
	Sign. (2-tailed), p	0.044	0.633	0.049
	N	54	54	54
General group	Spearman's (r_{xy})	0.314**	0.013	0.301**
	Sign. (2-tailed), p	0.0001	0.869	0.0001
	N	158	158	158

Note. * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided); H_{cy} – homocysteine; PTH – parathyroid hormone; Ca^{2+} – ionized calcium; P – phosphorus.

Table 4

The proportion of cases of hyperhomocysteinemia in genetic subgroups of children in the Polessky districts

Genetic subgroup	Boys			Girls		
	N	N^1		N	N^1	
		Abs.	%		Abs.	%
A/A MTR:2756	49	35	71.43	55	23	41.82
A/G MTR:2756	24	10	41.67	21	8	38.10
G/G MTR:2756	5	5	100.0	4	3	75.00
A/A MTHFR:1298	36	22	61.11	46	20	43.48
A/C MTHFR:1298	34	23	67.65	26	10	38.46
C/C MTHFR:1298	8	5	62.50	8	4	50.00
C/C MTHFR:677	39	20	51.28	40	14	35.00
C/T MTHFR:677	29	20	68.97	31	15	48.39
T/T MTHFR:677	10	10	100.0	9	5	55.56
A/A MTRR:66	16	8	50.00	16	4	25.00
A/G MTRR:66	35	19	54.29	37	18	48.65
G/G MTRR:66	27	23	85.19	27	12	44.44
All genotypes	78	50	64.10	80	34	42.50

Note. N is the number of cases in the subgroup; N^1 is the number of cases of hyperhomocysteinemia (H_{cy} > 10.0 μmol/l).

In the subgroups with the main genotypes A/A MTR:2756, A/A MTHFR:1298, A/C MTHFR:1298, C/C MTHFR:1298, C/C MTHFR:677, A/A MTRR:66, A/G MTRR:66, G/G MTRR:66, a direct correlation was recorded between P and PTH, reflecting the influence of P on PTH and the possibility of P excretion from the body through the kidneys in the urine (Table 3).

The P-PTH correlation was most pronounced in subgroups with the A/AMTR:2756 genotype (absence of the GMTR:2756 allele in the genome), as well as in subgroups with the C/C MTHFR:1298 and C/C MTHFR:677 genotypes (absence of the TMTHFR:677 allele in the genome).

In subgroups with the main genotypes A/G MTR:2756, G/G MTR:2756, C/T MTHFR:677, and T/T MTHFR:677, there was no P-PTH correlation, indicating a possible disruption of the regulatory processes of calcium-phosphorus homeostasis (Table 3).

In this case, the delay in P excretion from the body will create conditions for the formation of calcium-phosphorus complexes in the blood, followed by their deposition in the vessel walls and soft tissues.

In the absence of a genetic basis for slowing or blocking Hcy methylation (TMTHFR:677 and GMTR:2756 alleles in the genome), in the absence of ^{137}Cs incorporation into the body, calcium-phosphorus metabolism is restored.

Thus, the disruption of mitochondrial metabolism associated with ^{137}Cs incorporation causes hyperhomocysteinemia, leading to changes in Ca and P metabolism.

In the absence of alleles in the genome that disrupt the activity of methylenetetrahydrofolate reductase and B_{12} methionine synthase, this hyperhomocysteinemia is, to a large extent, an adaptive response to exposure to low doses of radiation.

This transient hyperhomocysteinemia contributes, in particular, to increased myocardial contractility and an increase in systolic blood pressure [8, p. 352].

Hyperhomocysteinemia following incorporation of ^{137}Cs radionuclides is an adaptive response of the body aimed at normalizing blood calcium levels and correcting metabolic processes.

A decrease in blood $H_{сн}$ levels can occur if the transsulfuration reaction cycle is activated.

Activation of the transsulfuration cycle.

The transsulfuration cycle includes a complex of reactions that utilize H_{cy} and form cysteine and glutathione [9, p. 1].

Vitamin B_6, pyridoxal 5' phosphate, is a marker for H_{cy} utilization in the transsulfuration cycle.

It is a cofactor for cystathionine β-synthase (CBS), which forms cystathionine from H_{cy} and serine.

Cystathionine γ-lyase then breaks down cystathionine, forming cysteine, alpha-ketobutyrate, and ammonia.

Cysteine is involved in the formation of glutathione, a component of the antioxidant system.

In subgroups with the GMTR:2756 allele, a direct H_{cy}-B_6 and an inverse B_{12}-B_6 correlation were found, indicating a link between FC and H_{cy} with the transsulfuration cycle (Table 5).

H_{cy} utilization in the transsulfuration cycle occurs with a decrease in the activity of B_{12}-methionine synthase, which mediates the final stage of H_{cy} methylation and the formation of internal Met.

This decrease is due to the GMTR:2756 polymorphic allele, as well as the effect of reactive oxygen species on B_{12}-methionine synthase.

Thus, impaired B_{12}-methionine synthase function caused by the GMTR:2756 allele, combined with metabolic stress caused by ^{137}Cs incorporation, are the cause of H_{cy} utilization in the transsulfuration cycle and activation of the antioxidant system.

Table 5

Correlations of vitamin B_6 in genetic subgroups of children

N	Main genotypes	Correlation coefficient	H_{cy} - B_6	B_{12} - B_6
1	2	3	4	5
1	A/A MTR:2756	Spearman's (r_{xy})	0.047	-0.021
		Sign. (2-tailed), p	0.634	0.827
		N	106	106
2	A/G MTR:2756	Spearman's (r_{xy})	-0.035	-0.365**
		Sign. (2-tailed), p	0.789	0.004
		N	61	61
3	G/G MTR:2756	Spearman's (r_{xy})	0.724*	-0.588
		Sign. (2-tailed), p	0.012	0.057
		N	11	11
4	A/A MTHFR:1298	Спирмена	-0.080	0.030
		Знач. (2-х стор.), p	0.458	0.783
		N	89	89
5	A/C MTHFR:1298	Spearman's (r_{xy})	0.127	-0.264*
		Sign. (2-tailed), p	0.263	0.018
		N	80	80
6	C/C MTHFR:1298	Spearman's (r_{xy})	0.600	-0.450
		Sign. (2-tailed), p	0.088	0.224
		N	9	9
7	C/C MTHFR:677	Spearman's (r_{xy})	0.052	-0.123
		Sign. (2-tailed), p	0.645	0.278
		N	80	80
8	C/T MTHFR:677	Spearman's (r_{xy})	-0.002	-0.117
		Sign. (2-tailed), p	0.984	0.291
		N	83	83
9	T/T MTHFR:677	Spearman's (r_{xy})	0.191	-0.170
		Sign. (2-tailed), p	0.495	0.545
		N	15	15
10	A/A MTRR:66	Spearman's (r_{xy})	0.301	-0.307
		Sign. (2-tailed), p	0.127	0.119
		N	27	27
11	A/G MTRR:66	Spearman's (r_{xy})	0.015	-0.184
		Sign. (2-tailed), p	0.883	0.078
		N	93	93

End to Table 5

1	2	3	4	5
12	G/G MTRR:66	Spearman's (r_{xy})	-0.028	0.028
		Sign. (2-tailed), p	0.836	0.836
		N	58	58
13	G/G MTR:2756+C/C MTHFR:677	Spearman's (r_{xy})	0.943**	-0.829*
		Sign. (2-tailed), p	0.005	0.042
		N	6	6
14	A/G MTR:2756+C/T MTHFR:677	Spearman's (r_{xy})	-0.103	-0.429*
		Sign. (2-tailed), p	0.608	0.026
		N	27	27
15	A/G, G/G MTR:2756+C/T, T/T MTHFR:677	Spearman's (r_{xy})	-0.026	-0.365*
		Sign. (2-tailed), p	0.880	0.026
		N	37	37
16	A/A MTR:2756+C/C MTHFR:677	Spearman's (r_{xy})	0.045	-0.069
		Sign. (2-tailed), p	0.771	0.652
		N	45	45

Note: * – correlation is significant at the 0.05 level (two-tailed); N is the group number.

Activation of Cortisol Production.

In a subgroup of girls carrying the GMTR:2756 allele, increased blood levels of Cortisol, a hormone that stabilizes metabolic processes in the body during stressful situations, were detected (Table 6) [10, p. 197].

Table 6

Statistical characteristics of Cortisol values (mcg/dL) in genetic subgroups of boys and girls

N	Main genotype	Boys		Girls	
		Me	IQR	Me	IQR
1	A/A MTR:2756	13.260	9.940-17.825	12.110	9.060-16.220
2	A/G, G/G MTR:2756	11.800	9.260-16.240	14.800	11.100-21.760*
3	A/A MTHFR:1298	13.015	9.723-16.265	13.810	9.363-17.613
4	A/C, C/C MTHFR:1298	13.410	9.470-17.745	13.070	9.550-17.038
5	C/C MTHFR:677	15.260	10.430-18.030	12.335	9.235-17.420
6	C/T, T/T MTHFR:677	11.800	9.490-16.130	13.995	10.293-16.965
7	A/A MTRR:66	12.090	8.320-17.470	12.070	9.268-17.270
8	A/G, G/G MTRR:66	13.410	10.413-17.478	13.815	13.815-17.393
General group		13.135	9.580-17.478	13.415	9.468-17.370

Note. Me – median; IQR – interquartile range; * – statistical differences between subgroups No. 1 and 2 of girls (mean rank – 36.64 and 49.00; Mann-Whitney U test – 475.000; p = 0.027).

Furthermore, a direct correlation was established between H_{cy} and Cortisol (Table 7) [10, p. 198].

The same correlation was found in a subgroup of boys with the primary genotypes, including the TMTHFR:677 allele (Table 7) [10, p. 199].

Table 7

Results of correlation analysis in genetic subgroups of children

N	Main genotype	Correlation coefficient	H_{cy} - Cortisol	
			Boys	Girls
1	A/A MTR:2756	Spearman's (r_{xy})	-0.015	0.123
		Sign. (2-tailed), p	0.916	0.369
		N	49	55
2	A/G, G/G MTR:2756	Spearman's (r_{xy})	0.079	0.503*
		Sign. (2-tailed), p	0.683	0.010
		N	29	25
3	A/A MTHFR:1298	Spearman's (r_{xy})	0.183	0.354*
		Sign. (2-tailed), p	0.285	0.016
		N	36	46
4	A/C, C/C MTHFR:1298	Spearman's (r_{xy})	-0.044	-0.002
		Sign. (2-tailed), p	0.784	0.993
		N	42	34
5	C/C MTHFR:677	Spearman's (r_{xy})	-0.041	0.181
		Sign. (2-tailed), p	0.803	0.263
		N	39	40
6	C/T, T/T MTHFR:677	Spearman's (r_{xy})	0.360*	0.270
		Sign. (2-tailed), p	0.024	0.092
		N	39	40
7	A/A MTRR:66	Spearman's (r_{xy})	0.250	0.209
		Sign. (2-tailed), p	0.350	0.438
		N	16	16
8	A/G, G/G MTRR:66	Spearman's (r_{xy})	0.034	0.243
		Sign. (2-tailed), p	0.792	0.053
		N	62	64

Note. * - correlation is significant at the 0.05 level (two-sided); N – subgroup number; H_{cy} – homocysteine; Cortisol – cortisol.

Activation of T_3 Synthesis.

In a subgroup of children with the GMTR:2756 allele, increased blood T_3 levels were recorded [11, p. 199].

Using correlational studies, we identified the ability of T_3 to increase the formation of methyltetrahydrofolate, the active form of vitamin B_9, and thereby enhance H_{cy} methylation (Fig. 1) [12, p. 65].

T_3 stimulates energy metabolism processes in mitochondria [13, p. 197],

thereby enhancing their influence on methylation processes in the body, preventing the occurrence of oxidative stress.

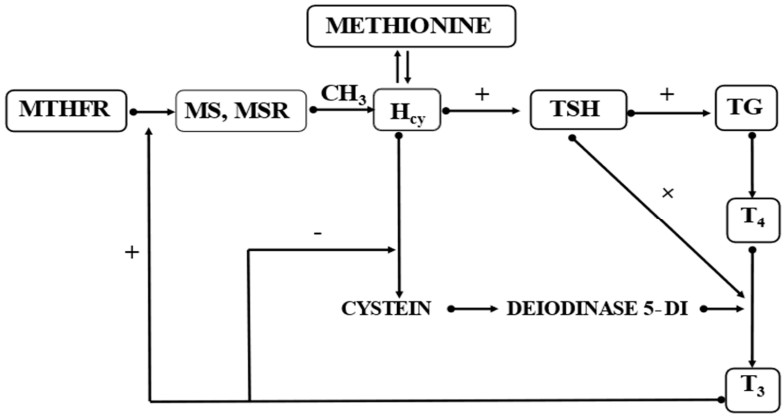

Fig. 1. The effect of T_3 on H_{cy} methylation processes.

Activation of TSH formation.

In a group of boys carrying the GMTR:2756 allele, a direct correlation was found between vitamin B_6 and TSH levels in the blood (r_{xy} = 0.244*, p = 0.023, n = 86).

TSH, in turn, activates T_4 synthesis in the thyroid gland and T_3 formation in peripheral tissues (Fig. 1).

Methylation impairment, manifested by hyperhomocysteinemia, in children living near the ChEZ depends little on the state of the FC genetic system.

Even in the absence of risk alleles analyzed by FC polymorphism in the children's genome, the proportion of cases of hyperhomocysteinemia was 66.7% (Fig. 2) [2, p. 61].

In the maternal group, the increased incidence of hyperhomocysteinemia depends on the FC genetic system, as it is associated with an increased number of FC polymorphisms with risk alleles (Fig. 3) [2, p. 62].

The study identified a link between the FC genetic system and the transsulfuration reaction cycle under conditions of ^{137}Cs radionuclide incorporation into the body.

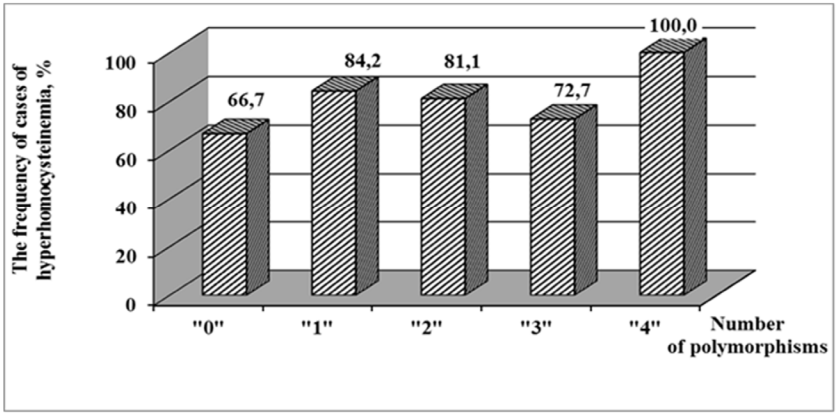

Fig. 2. The proportion of cases of hyperhomocysteinemia depending on the number of genetic polymorphisms with risk alleles in subgroups of children from the Polessky district.

Oxidative stress, which arose under conditions of radiation damage to mitochondria, caused a decrease in the formation of energy carriers, leading to disruption of the H_{cy} methylation process and hyperhomocysteinemia.

Hyperhomocysteinemia was recorded in the majority of the examined children living near the ChEZ.

Following the radioactive forest fires in the ChEZ, H_{cy} levels in these children increased significantly [14, p. 26].

Therefore, the main etiological factor of hyperhomocysteinemia should be considered the incorporation of ^{137}Cs radionuclides into the body.

However, genetic polymorphisms of FC, which affect methylenetetrahydrofolate reductase and B_{12} methionine synthase, cannot be completely ignored.

Hyperhomocysteinemia, as a consequence of impaired H_{cy} methylation, ocurs:

a) as a result of impaired function of FC enzymes and reduced methyl group delivery to the H_{cy} molecule.

This is caused by impaired formation of folic acid derivatives in the mitochondria, particularly tetrahydrofolate, and a deficiency of energy carriers.

b) due to insufficient formation of the methyl group directly in the mitochondria (the source is trimethylglycine – betaine).

c) as a result of impaired function of only B_{12} methionine synthase, due to the GMTR:2756 polymorphic allele in the genome, and exposure to reactive oxygen species.

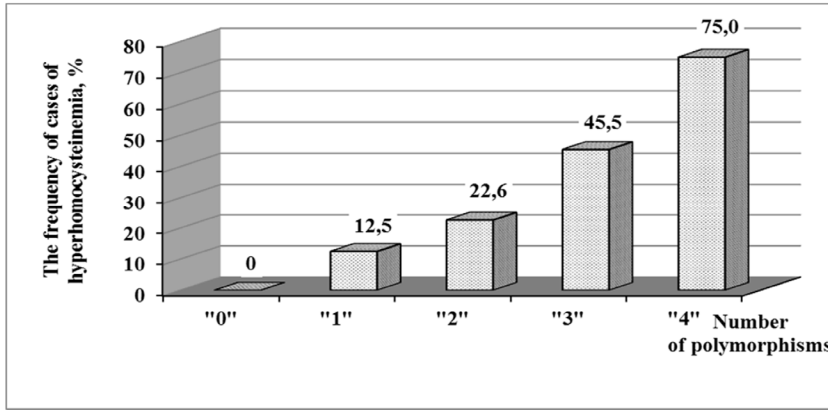

Fig. 3. The proportion of cases of hyperhomocysteinemia in subgroups with risk alleles of genetic polymorphisms in the group of mothers of examined children from the Polessky district

The most pronounced blockade of B_{12} methionine synthase activity occurs with a combination of environmental (radiation) and internal (FC genes) factors.

In a subgroup including cases with the GMTR:2756 allele in the genome, which negatively affects B_{12} methionine synthase activity, associations involving vitamin B_6 were identified: an inverse association between vitamins B_{12} and B_6, and a direct association between H_{cy} and vitamin B_6.

These associations indicate the utilization of H_{cy} in the transulfuration reaction cycle, including through cystathionine β-synthase, whose coenzyme is vitamin B_6. Thus, impaired B_{12} methionine synthase function, associated with the GMTR:2756 allele in the genome, in children exposed to incorporated ^{137}Cs radionuclides, stimulates H_{cy} utilization in the transsulfuration cycle and promotes H_{cy}-induced activation of the

antioxidant system, including cysteine and glutathione, as well as Cortisol and T_3.

When H_{cy} methylation is impaired and its concentration in the blood increases, a complex of processes is activated aimed at eliminating the cause of oxidative stress and restoring mitochondria as a source of energy carriers.

The functional relationship between B_{12} methionine synthase and the transsulfuration cycle allows for the restoration of mitochondrial metabolism disrupted by environmental radiation exposure.

This compensatory and adaptive mechanism ensures the functioning of the body with a genetic defect that suppresses the activity of the crucial FC enzyme.

Under conditions of combined exposure to external (radiation) and internal (FC gene mutations) unfavorable factors, H_{cy} is a participant in the recovery process aimed at preserving vital functions.

4. Conclusions

The conducted analytical study revealed the involvement of FC genes in the development of adaptive responses in the bodies of children living near the ChEZ exposed to incorporated ^{137}Cs radionuclides.

The negative impact of incorporated ^{137}Cs radionuclides on the mitochondrial energy system leads to disruption of FC function and an increase in H_{cy} levels in the blood of children living near the ChEZ.

H_{cy} is one of the main components of the emergency system, which is activated under conditions of oxidative stress caused by exposure to incorporated ^{137}Cs.

The key component of this emergency system is the transsulfuration reaction cycle, through which H_{cy} is utilized to form cysteine and glutathione, the synthesis of adrenal cortex and thyroid hormones is activated, and calcium-phosphorus metabolism is altered.

A significant activation of the transsulfuration reaction cycle in the analyzed group of children living near the ChEZ was detected due to impaired B_{12} methionine synthase function associated with the GMTR:2756 polymorphic allele in the genome.

Under conditions of radiation exposure and the GMTR:2756 polymorphic allele, suppression of B_{12} methionine synthase activity, involving H_{cy},

activates a complex of antioxidant reactions aimed at restoring mitochondrial function and providing cellular energy.

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