CHAPTER «MEDICAL SCIENCES»

FOLATE CYCLE GENES AND THE ENDOCRINE SYSTEM IN CHILDREN LIVING IN THE TERRITORY AFFECTED BY THE ACCIDENT AT THE CHERNOBYL NUCLEAR POWER PLANT

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Abstract. Research conducted as part of European Union projects in Ukraine in 2015 established a link between forest fires in the Chernobyl Exclusion Zone (ChEZ) and increased levels of the sulfur-containing amino acid homocysteine (H_{cy}) in the blood (a condition of hyperhomocysteinemia) in more than 70.0 % of children Polessky and Ivankovsky districts of the Kyiv region. When assessing the causes and consequences of this phenomenon, it is necessary to study the mutual participation of H_{cy}, with the hormones of the adenohypophysis, thyroid gland (TG) and adrenal cortex in the metabolic processes of the body. *The purpose of this work* was to determine the relationships between H_{cy}, thyroid-stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3) and cortisol (Cor) in groups of adolescent children (boys and girls) living near the ChEZ, taking into account the state of folate cycle (FC) genes and different levels of H_{cy} in the blood. *Research methods.* The research methodology predetermined the assessment by statistical methods of the results of laboratory and genetic examination of 379 adolescent children from populated areas located near the ChEZ. Immunochemical, genetic and statistical research methods were used. *Results.* Statistical differences in carriage of the risk allele T of the MTHFR:677 polymorphism in the

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studied genetic subgroups of boys and girls, H_{cy} content in the blood, the specific gravity of hyperhomocysteinemia levels, as well as correlations between the values of H_{cy}, TSH, T_4, T_3, Cor were studied. It has been shown that increased levels of H_{cy}, T_3 and Cor in the blood of children are associated with the risk allele T of the MTHFR:677 polymorphism. It has been established that thyroid hormoneogenesis depends on the formation of H_{cy}. At the same time, T_3, influencing the enzyme systems of FC, regulates the level of H_{cy}. An increased level of H_{cy} in the blood is a stimulating factor, not only for the production of thyroid hormones, but also for Cor. At the same time, T_3, acting as a regulator of the process of H_{cy} utilization, affects the process of Cor formation. *Conclusions.* The study allows us to formulate a hypothesis about the participation of FC in the processes of hormonogenesis of the adenohypophysis, TG and adrenal cortex, which allows us to outline a number of promising directions for preventive measures for diseases of the TG and other vital organs in people living in radioactively contaminated areas affected by the Chernobyl accident.

1. Introduction

The accident at the Chernobyl Nuclear Power Plant (ChNPP) had a negative impact on the environment of many countries [1, p. 11]. The area around the ChNPP, known as the ChEZ, was subjected to the greatest contamination with radioactive elements.

3 decades after the Chernobyl accident, the soils and forest trees of the ChEZ contain huge amounts of long-lived radionuclides ^{137}Cs – 1040.0 kBq/m^2; ^{90}Sr – 368.0 kBq/m^2; $^{238\text{-}240}Pu$ – 11.4 kBq/m^2 and ^{241}Am – 14.4 kBq/m^2 [2, p. 10], which are the cause of high background radiation [3, p. 265].

Periodically, forest fires occur in the ChEZ and then wood combustion products and radioactive elements enter the atmosphere, which spread over considerable distances with air currents. There is no doubt that adults and children living near the ChEZ become victims of these fires.

During the implementation in Ukraine of the projects of the European Commission "Health and Ecological Programs around the Chernobyl Exclusion Zone: Development, training and coordination of health-related projects" and the Regional Council of Rhone-Alpes (France), a connection was established between forest fires in the ChEZ and high levels of sulfur-containing amino acid H_{cy} in the blood of a large number of children in the Ivankovsky and Polessky districts of the Kyiv region [4, p. 30].

An increase in the level of H_{cy} in the blood, or hyperhomocysteinemia, in adults is associated with a number of serious diseases leading to death and disability [5, p. 212; 6, p. 142]. Hyperhomocysteinemia is relatively rare in children.

Thus, the registration of this condition in children living near the ChEZ, identified during European Union projects, cannot but arouse scientific and practical interest among representatives of medical science and practical healthcare.

Considering the connection of hyperhomocysteinemia with diseases of the cardiovascular system and oncopathology, it is logical to study the role of H_{cy} in the functioning of vital systems and metabolic cycles of the body.

One of the most important systems of the body is the endocrine system, which controls metabolism at all stages of human ontogenesis.

In our previous works, we investigated the connections between H_{cy} and hormones of the pituitary-thyroid axis in a group of children living near the ChEZ after radioactive forest fires in 2015, when the proportion of cases of hyperhomocysteinemia exceeded 70.0 % [2, p. 31; 7, p. 15; 8, p. 179].

It would be logical to study the connections between H_{cy} and hormones of the pituitary-thyroid axis in a group of adolescent children, with a significantly smaller number of cases of hyperhomocysteinemia, in the period preceding forest fires in the ChEZ.

The ongoing study should identify subgroups based on specific FC genotypes, taking into account blood H_{cy} levels (> 10.0 μmol/L and ≤ 10.0 μmol/L).

Considering that an increase in the level of H_{cy} in the blood is a reflection of metabolic stress [9, p. 36], an assessment should be made of the content of the adrenal hormone Cor in the body of children and its relationships with H_{cy}, TSH, T_4, T_3.

At the same time, groups of boys and girls should be separated due to puberty and the production of sex hormones, which significantly affect the metabolism of the child's developing body.

Based on this, the purpose of this work was to determine the relationships between H_{cy}, TSH, T_4, T_3 and Cor in groups of adolescent children (boys and girls) living near the ChEZ, taking into account the state of FC genes and different levels of H_{cy} in the blood.

2. Material and methods

To conduct an analytical study, the results of a genetic and laboratory examination of 379 adolescent children (12-17 years old) living in settlements in the Kyiv region of Ukraine, near the ChEZ, were used. The soils on which the children's places of residence are located were contaminated with radioactive elements as a result of the Chernobyl accident [9, p. 120, 121; 10, p. 49, 50, 51].

Blood sampling from the ulnar vein was carried out in the morning, on an empty stomach, from children attending school in the Polessky and Ivankovsky districts – 04/02/2015 (before the forest fires in the ChEZ [2, p. 10]), in the Ivankovsky district – 12/18/2015 (after forest fires in the ChEZ). All studies conducted were agreed upon with the parents.

The blood samples were examined in a laboratory certified according to European Union quality standards.

In the blood of children of both groups, the content of TSH, free T_3, free T_4, H_{cy}, Cor was determined, and the state of the FC genetic system was also studied.

The reference range of extreme values designated by the laboratory was: for TSH – 0.28-4.3 μMO/ml; for T_4 – 1.1-1.8 ng/dl; for T_3 – 2.3-5.0 pg/ml; for Cor – 6.2 – 19.4 mcg/dl.

Determination of TSH, T_3, T_4 and Cor was carried out using an immunochemical method with electrochemiluminescent detection (ECLIA). Analyzer and test system: Cobas 6000, Roche Diagnostics (Switzerland).

Determination of H_{cy} in the blood was carried out using the immunochemical method with chemiluminescent detection (ECLIA). Analyzer and test system: Architect 1000 (ABBOT Diagnostics (USA)). The level of H_{cy} in the blood of children above 10.0 μmol/l was defined as a state of hyperhomocysteinemia.

The genetic study of FC included the determination of allelic variants C677T and A1298C of the methylenetetrahydrofolate reductase (MTHFR) gene, A2756G of the gene associated with B_{12}-dependent methionine synthase (MTR), A66G of the gene associated with methionine synthase reductase (MTRR). The method used was Real-time PCR. Analyzer and test system Detection amplifier "DT-96"; "DNA-Technology" (Russia).

A comparative analysis of the content of TSH, free T_3, free T_4, H_{cy}, Cor was carried out between subgroups of one polymorphism with and without risk alleles, in particular, between subgroups No. 1 and 2, 3 and 4, 5 and 6, 7 and 8 (Table 1).

Table 1

Analyzed subgroups of children

No. subgroup	Genotype	No. subgroup	Genotype
1	A/A MTR:2756	5	C/C MTHFR:677
2	A/G, G/G MTR:2756	6	C/T, T/T MTHFR:677
3	A/A MTHFR:1298	7	A/A MTRR:66
4	A/C, C/C MTHFR:1298	8	A/G, G/G MTRR:66
9	General group		

Taking into account gender differences in adolescence, the state of metabolic processes in the studied groups of boys and girls was assessed separately.

Statistical processing of the obtained results was carried out using the IBM SPSS Statistics 22 program (USA). For the analyzed indicators, the median (Me), interquartile range (IQR), minimum and maximum parameter values, and percentiles were calculated. The hypothesis about the type of distributions was tested (Kolmogorov-Smirnov test). All studied parameters did not correspond to the law of normal distribution, and therefore the nonparametric Mann-Whitney U test was used to compare values. The statistical significance of the indicators was assessed by determining the significance level p using a statistical program.

Student's t test was used to compare relative scores. The critical level of significance of the null statistical hypothesis (p) was taken as 0.05. The relationship between the indicators H_{cy}, TSH, T_3, T_4, Cor was determined using the Spearman’s rank correlation coefficient (r_{xy}). The strength of the correlation was assessed using a traditional scale: weak – from 0 to 0.299; average – from 0.3 to 0.699; strong – from 0.7 to 1.0.

3. Results and discussion

3.1. Homocysteine, thyroxine

In the group of boys, a statistically higher level of H_{cy} in the blood was recorded in the 6th subgroup, including cases with genotypes C/T, T/T MTHFR:677, in comparison with the 5th subgroup, including cases with genotype C/C MTHFR:677, and also, in the 1st subgroup, including cases with genotype A/AMTR:2756, compared with the 2nd subgroup, including cases with genotypes A/G, G/G MTR:2756 (Tables 2a, 3).

Table 2a

Statistical characteristics of Hcy and Cor indicators in subgroups of boys in the Polessky district (04/02/2015)

No. subgroup	H_{cy}, µmol/l		Cor, mcg/dl	
	Me	IQR	Me	IQR
1	12.15	9.23 – 16.39	13.26	9.94 – 17.83
2	10.15	7.81 – 11.97	11.80	9.26 – 16.24
3	10.22	7.90 – 18.07	13.02	9.72 – 16.27
4	11.22	9.16 – 14.62	13.41	9.47 – 17.75
5	10.16	7.65 – 11.87	15.26	10.43 – 18.03
6	14.01	10.13 – 19.72	11.80	9.49 – 16.13
7	10.04	7.49 – 15.61	12.09	8.32 – 17.47
8	11.15	9.12 – 14.87	13.41	10.41 – 17.48
9	11.13	8.65 – 14.87	13.14	9.58 – 17.48

Note. Me – median; IQR – interquartile range

Higher blood H_{cy} levels are associated with the T risk allele of the MTHFR:677 genetic polymorphism. In subgroup No. 6 (cases with genotypes C/T, T/T MTHFR:677), the T allele was present in 100.0 %, in subgroup No. 1 with genotype A/A MTR:2756 – in 51.0 % of cases, in subgroup No. 2 with genotypes A/G, G/G MTR:2756 – in 48.28 % (Table 4).

The influence of the T allele on H_{cy} methylation processes led to an increase in the number of cases of hyperhomocysteinemia (H_{cy} level > 10.0 µmol/l) in subgroup No. 6 compared to subgroup No. 5 (Table 5), in which there were no cases with the T allele (Table 4).

In the group of girls, there were no statistical differences in the proportion of cases of hyperhomocysteinemia between subgroups No. 1 and 2, 3 and 4, 5 and 6, 7 and 8 (Table 6).

The level of T_3 in the blood of boys who made up subgroup No. 3 (cases with genotype A/A MTHFR:1298) was significantly higher than in subgroup No. 4, including cases with genotypes A/C, C/C MTHFR:1298 (Tables 2b, 3).

We are inclined to consider this a manifestation of the influence of the T allele of the MTHFR:677 polymorphism. In subgroup No. 3, the T allele was present in 75.0 % of cases, while in subgroup No. 4 only in 28.57 % of cases (Table 4).

Table 2b

Statistical characteristics of pituitary and thyroid hormone levels in subgroups of boys in the Polessky district (04/02/2015)

No. subgroup	TSH, µMO/ml		T_3, pg/ml		T_4, ng/dl	
	Me	IQR	Me	IQR	Me	IQR
1	2.21	1.54 – 2.74	4.45	4.09 – 4.91	1.18	1.04 – 1.27
2	2.10	1.51 – 2.76	4.55	4.24 – 4.83	1.15	1.00 – 1.26
3	2.27	1.53 – 2.68	4.66	4.27 – 5.14	1.17	1.04 – 1.27
4	2.04	1.54 – 2.82	4.40	3.95 – 4.73	1.17	1.02 – 1.27
5	2.15	1.46 – 2.86	4.42	4.24 – 4.77	1.18	1.03 – 1.29
6	2.22	1.60 – 2.63	4.60	4.03 – 5.05	1.15	1.04 – 1.26
7	1.94	1.61 – 2.46	4.58	4.31 – 5.03	1.19	1.07 – 1.32
8	2.27	1.51 – 2.85	4.43	4.13 – 4.88	1.15	1.03 – 1.26
9	2.18	1.54 – 2.71	4.49	4.14 – 4.88	1.17	1.03 – 1.27

Note. Me – median; IQR – interquartile range

Table 3

Results of statistically significant differences when comparing indicators of metabolic processes in the blood of examined boys in the Polessky district (04/02/2015)

Indicators	Groups comparisons	Number of comparison group	Average rank	U-criterion value, significance level p
H_{cy}	1	49	43.92	U = 494.000 p = 0.025
	2	29	32.03	
T_3	3	36	45.46	U = 541.500 p = 0.032
	4	42	34.39	
H_{cy}	5	39	31.18	U = 436.000 p = 0.001
	6	39	47.82	

Table 4

The proportion of cases of carriage of the T allele of the MTHFR:C677T polymorphism in the genetic subgroups of boys in the Polessky district (04/02/2015)

No. subgroup	Main genotype	N	Number of cases of carriage of the T allele of the MTHFR:C677T polymorphism	
			N	%
1	A/A MTR:2756	49	25	51.02
2	A/G, G/G MTR:2756	29	14	48.28
3	A/A MTHFR:1298	36	27	75.00
4	A/C, C/C MTHFR:1298	42	12	28.57
5	C/C MTHFR:677	39	0	0
6	C/T, T/T MTHFR:677	39	100	100.0
7	A/A MTRR:66	16	9	56.25
8	A/G, G/G MTRR:66	62	30	48.39

Note. N – number of children in the subgroup

Table 5

Proportion of cases of hyperhomocysteinemia in subgroups of boys in the Polessky district (04/02/2015)

No. subgroup	Genotype	Number of cases				
		N^1	N^2	%	N^3	%
1	A/A MTR:2756	49	35	71.43	14	28.57
2	A/G, G/G MTR:2756	29	15	51.72	14	48.28
3	A/A MTHFR:1298	36	22	61.11	14	38.89
4	A/C, C/C MTHFR:1298	42	28	66.67	14	33.33
5	C/C MTHFR:677	39	20	51.28	19	48.72
6	C/T, T/T MTHFR:677	39	30	76.92*	9	23.08
7	A/A MTRR:66	16	8	50.00	8	50.00
8	A/G, G/G MTRR:66	62	42	67.74	20	32.26
9	General group	78	50	64.10	28	35.90

Note. N^1 – number of children in the subgroup; N^2 – number of cases of $H_{cy} > 10.0$ µmol/l; N^3 – number of cases of $H_{cy} \leq 10.0$ µmol/l; * – statistical differences between subgroups No. 5 and 6 ($t = 2.45$; $p = 0.018085$)

Table 6

Proportion of cases of hyperhomocysteinemia in subgroups of girls in the Polessky district (04/02/2015)

No. subgroup	Genotype	Number of cases				
		N^1	N^2	%	N^3	%
1	A/A MTR:2756	55	23	41.82	32	58.18
2	A/G, G/G MTR:2756	25	11	44.00	14	56.00
3	A/AMTHFR:1298	46	20	43.48	26	56.52
4	A/C, C/CMTHFR:1298	34	14	41.18	20	58.82
5	C/CMTHFR:677	40	14	35.00	26	65.00
6	C/T, T/TMTHFR:677	40	20	50.00	20	50.00
7	A/AMTRR:66	16	4	25.00	12	75.00
8	A/G, G/GMTRR:66	64	30	46.88	34	53.12
9	General group	80	34	42.50	46	57.50

Note. N^1 – number of children in the subgroup; N^2 – number of cases of $H_{cy} > 10.0$ µmol/l; N^3 – number of cases of $H_{cy} \leq 10.0$ µmol/l.

3.2. Homocysteine and cortisol

In subgroup No. 2 of girls – carriers of the risk allele G of the MTR:2756 polymorphism, the level of Cor in the blood was significantly higher than in subgroup No. 1 with the absence of this allele in the genome (Table 7a).

With regard to the remaining analyzed indicators, there were no statistical differences between the subgroups (Tables 7a, 7b).

The higher level of Cor in subgroup No. 2 compared to subgroup No. 1 may be associated not only with the risk allele G of the MTR:2756 polymorphism, but also with the risk allele T of the MTHFR:677 polymorphism, the proportion of which in subgroup No. 2 is greater than in subgroup No. 1 (Table 8).

At the same time, in subgroup No. 2 a direct correlation between Hcy-Cor was determined (Table 9), indicating an interaction between the FC and the system of production of corticosteroid hormones.

The same connection was recorded in girls in the general group and subgroup No. 3 with genotype A/A MTHFR:1298 (Table 9).

Table 7a

Statistical characteristics of H_{cy} and Cor indicators of girls in the Polessky district (04/02/2015)

No. subgroup	H_{cy}, µmol/l		Cor, mcg/dl	
	Me	IQR	Me	IQR
1	9.52	8.19 – 11.43	12.11	9.06 – 16.22
2	9.44	7.89 – 10.86	14.80	11.10 – 21.76*
3	9.37	8.02 – 11.71	13.81	9.36 – 17.61
4	9.60	8.13 – 10.77	13.07	9.55 – 17.04
5	9.38	8.01 – 10.66	12.34	9.24 – 17.42
6	9.92	8.33 – 11.88	14.00	10.29 – 16.97
7	9.16	8.54 – 10.13	12.07	9.27 – 17.27
8	9.73	8.01 – 11.44	13.82	13.82 – 17.39
9	9.48	8.06 – 11.10	13.42	9.47 – 17.37

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

Table 7b

Statistical characteristics of pituitary and thyroid hormone levels of girls in the Polessky district (04/02/2015)

No. subgroup	TSH, µMO/ml		T_3, pg/ml		T_4, ng/dl	
	Me	IQR	Me	IQR	Me	IQR
1	1.59	1.24 – 2.16	3.84	3.60 – 4.04	1.20	1.11 – 1.29
2	1.87	1.32 – 2.40	3,84	3.53 – 4.17	1.14	1.06 – 1.30
3	1.53	1.13 – 2.39	3.83	3.58 – 4.07	1.20	1.07 – 1.30
4	1.80	1.50 – 2.20	3.88	3.61 – 4.06	1.17	1.12 – 1.29
5	1.67	1.25 – 2.01	3.84	3.59 – 4.03	1.22	1.12 – 1.31
6	1.70	1.34 – 2.57	3.86	3.60 – 4.16	1.15	1,06 – 1,30
7	1.89	1.70 – 2.32	3.90	3.65 – 4.10	1.24	1.12 – 1.37
8	1.58	1.17 – 2.23	3.84	3.58 – 4.06	1.18	1.09 – 1.29
9	1.67	1.29 – 2.23	3.84	3.59 – 4.06	1.18	1.11 – 1.30

Note. Me – median; IQR – interquartile range

Table 8

The proportion of cases of carriage of the T allele of the MTHFR:C677T polymorphism in the genetic subgroups of girls in the Polessky district (04/02/2015)

No. subgroup	Main genotype	N	Number of cases of carriage of the T allele of the MTHFR:C677T polymorphism	
			N	%
1	A/A MTR:2756	55	25	45.45
2	A/G, G/G MTR:2756	25	15	60.00
3	A/A MTHFR:1298	46	27	58.70
4	A/C, C/C MTHFR:1298	34	13	38.24
5	C/C MTHFR:677	40	0	0
6	C/T, T/T MTHFR:677	40	40	100.0
7	A/A MTRR:66	16	5	31.25
8	A/G, G/G MTRR:66	64	35	54.69

Note. N – number of children in the subgroup

Table 9

Correlations of metabolic parameters in genetic subgroups of girls in the Polessky district (04/02/2015)

No.	Genotype	Correlation coefficient	Parameters		
			$TSH\text{-}T_4$	$H_{cy}\text{-}Cor$	$Cor\text{-}T_4$
2	A/G, G/G MTR:2756	Spearman's		0.503*	
		Sign. (two-sided), p		0.010	
		N		25	
3	A/A MTHFR:1298	Spearman's		0.354*	
		Sign. (two-sided), p		0.016	
		N		46	
4	A/C, C/C MTHFR:1298	Spearman's			0.421*
		Sign. (two-sided), p			0.013
		N			34
8	A/G, G/G MTRR:66	Spearman's	- 0.256*		
		Sign. (two-sided), p	0.042		
		N	64		
9	General group	Spearman's		0.223*	
		Sign. (two-sided), p		0.047	
		N		80	

Note. No. – number of genetic subgroup; * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01

The direct connection of H_{cy}-Cor was also determined in the general group of boys living in the Ivankovsky district in an area with a level of ^{137}Cs in the soil > 2.0 Ku/km^2 (Table 10), as well as among boys in the Polessky district living in settlements with soil contamination with ^{137}Cs < 2.0 Ku/km^2 in subgroup No. 6 (genotypes C/T, T/T MTHFR:677), including, with different levels of H_{cy} in the blood (Table 11, 12), in subgroup No. 2 (genotypes A/G, G/G MTR:2756) with a blood level of $H_{cy} \leq 10.0$ µmol/l (Table 12).

Table 10

Correlations of metabolic indicators in groups of boys in the Ivankovsky district (04/02/2015)

Group	Correlation coefficient	Parameters	
		T_3-Cor	H_{cy}-Cor
Boys	Spearman's	- 0.549**	0.536**
	Sign. (two-sided), p	0.007	0.008
	N	23	23

Note. ** – correlation is significant at the 0.01 level (two-sided)

Table 11

Correlations of metabolic parameters in genetic subgroups of boys in the Polessky district (04/02/2015)

No.	Genotype	Correlation coefficient	Parameters		
			TSH-T_4	H_{cy}-Cor	Cor-T_4
1	A/A MTR:2756	Spearman's	- 0,308*		
		Sign. (two-sided), p	0,031		
		N	49		
4	A/C, C/C MTHFR:1298	Spearman's	- 0,420**		
		Sign. (two-sided), p	0,006		
		N	42		
5	C/C MTHFR:677	Spearman's	- 0,348*		
		Sign. (two-sided), p	0,030		
		N	39		
6	C/T, T/T MTHFR:677	Spearman's		0,360*	0,323*
		Sign. (two-sided), p		0,024	0,045
		N		39	39
8	A/G, G/G MTRR:66	Spearman's	- 0,319*		
		Sign. (two-sided), p	0,012		
		N	62		
9	General group	Spearman's	- 0,266*		
		Sign. (two-sided), p	0,019		
		N	78		

Note. No. – genetic subgroup number; * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level

Thus, the results obtained indicate the synergism of the metabolic processes of H_{cy} and Cor.

However, in the 4th subgroup of girls (genotypes A/C, C/C MTHFR:1298), with blood levels of $H_{cy} \leq 10.0$ µmol/l, the H_{cy} – Cor relationship was inverse (Table 13).

Table 12

Correlations of metabolic parameters in genetic subgroups of boys in the Polessky district with different levels of H_{cy} in the blood (04/02/2015)

No.	Genotype	H_{cy} level	Correlation coefficient	Parameters		
				H_{cy}-Cor	T_3-Cor	T_4-Cor
2	A/G, G/G MTR:2756	>10.0 µmol/l	Spearman's		0.543*	
			Sign. (two-sided), p		0.037	
			N		15	
2	A/G, G/G MTR:2756	≤10.0 µmol/l	Spearman's	0.604*		
			Sign. (two-sided), p	0.022		
			N	14		
6	C/T, T/T MTHFR:677	>10.0 µmol/l	Spearman's	0.367*	0.392*	
			Sign. (two-sided), p	0.046	0.032	
			N	30	30	
6	C/T, T/T MTHFR:677	≤10.0 µmol/l	Spearman's	0.667*		
			Sign. (two-sided), p	0.050		
			N	9		
7	A/A MTRR:66	≤10.0 µmol/l	Spearman's			- 0.747*
			Sign. (two-sided), p			0.033
			N			8

Note. No. – genetic subgroup number; * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided)

3.3. Thyroid-stimulating hormone and thyroxine

In the group of boys from the Polessky district, correlation analysis revealed a weak inverse relationship between TSH-T_4 (Table 11).

In genetic subgroups of boys No. 1, 4, 5, 8, this connection was already of medium strength (Table 11). At the same time, its formation occurred with a blood level of $H_{cy} > 10.0$ µmol/l (Table 14).

Table 13

Correlations of metabolic parameters in genetic subgroups of girls in the Polessky district with different levels of H_{cy} in the blood (04/02/2015)

No.	Genotype	Hcy level	Correlation coefficient	Parameters	
				H_{cy}-Cor	H_{cy}-T_3
4	A/C, C/C MTHFR:1298	≤ 10.0 µmol/l	Spearman's	- 0.504*	
			Sign. (two-sided), p	0.024	
			N	20	
3	A/A MTHFR:1298	≤ 10.0 µmol/l	Spearman's		- 0.458*
			Sign. (two-sided), p		0.019
			N		26
7	A/A MTRR:66	≤ 10.0 µmol/l	Spearman's		-0.699*
			Sign. (two-sided), p		0.011
			N		12

Note. No. – genetic subgroup number; * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided).

In the group of girls, an inverse weak association of TSH-T_4 was detected in subgroup No. 8, including cases with the G allele of the MTRR:66 polymorphism (Table 9).

After forest fires in the ChEZ in the spring and summer of 2015, TSH-T_4 feedback was recorded among girls in the Ivankovsky district in subgroup No. 5 with the main genotype C/C MTHFR:677, with blood levels of H_{cy} > 10.0 µmol/l, and in subgroup No. 6, including cases with the T allele of the MTHFR:677 polymorphism, with a blood H_{cy} content of ≤ 10.0 µmol/L (Table 15).

In the group of boys from the Ivankovsky district, this relationship was recorded in subgroup No. 5, with a blood level of H_{cy} > 10.0 µmol/l (Table 16).

3.4. Homocysteine and thyroxine

A direct relationship of moderate strength H_{cy}-T_4 was identified in the general group of boys in the Polessky district with a blood H_{cy} content of ≤10.0 µmol/l (Table 14). The same connection, only of greater strength, was revealed in subgroups of boys No. 1, 4, 5 and 7 with H_{cy} content in the blood ≤ 10.0 µmol/l (Table 14).

Table 14

Correlations of metabolic parameters of genetic subgroups of boys in the Polessky district with different levels of H_{cy} in the blood (04/02/2015)

No.	Genotype	H_{cy} level	Correlation coefficient	Parameters	
				H_{cy}-T_4	TSH-T_4
1	A/A MTR:2756	> 10.0 µmol/l	Spearman's		- 0.398*
			Sign. (two-sided), p		0.018
			N		35
1	A/A MTR:2756	≤10.0 µmol/l	Spearman's	0.872**	
			Sign. (two-sided), p	0.000	
			N	14	
4	A/C, C/C MTHFR:1298	>10.0 µmol/l	Spearman's		- 0.468*
			Sign. (two-sided), p		0.012
			N		28
4	A/C, C/C MTHFR:1298	≤10.0 µmol/l	Spearman's	0.568*	
			Sign. (two-sided), p	0.034	
			N	14	
5	C/C MTHFR:677	>10.0 µmol/l	Spearman's		- 0.454*
			Sign. (two-sided), p		0.044
			N		20
5	C/C MTHFR:677	≤10.0 µmol/l	Spearman's	0.620**	
			Sign. (two-sided), p	0.005	
			N	19	
7	A/A MTRR: 66	≤10.0 µmol/l	Spearman's	0.880**	
			Sign. (two-sided), p	0.004	
			N	8	
9	General group	≤10.0 µmol/l	Spearman's	0,529**	
			Sign. (two-sided), p	0.004	
			N	28	

Note. No. – genetic subgroup number; * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided)

Table 15

Correlations of metabolic parameters in genetic subgroups of girls in the Ivankovsky district with different levels of H_{cy} in the blood (12/18/2015)

No.	Genotype	H_{cy} level	Correlation coefficient	Parameters		
				H_{cy}-T_4	TSH-T_4	TSH-T_3
1	A/A MTR:2756	>10.0 µmol/l	Spearman's			0.528**
			Sign. (two-sided), p			0.001
			N			36
3	A/A MTHFR:1298	>10.0 µmol/l	Spearman's			0.419*
			Sign. (two-sided), p			0.024
			N			29
4	A/C, C/C MTHFR:1298	>10.0 µmol/l	Spearman's	0.467*		
			Sign. (two-sided), p	0.012		
			N	28		
5	C/C MTHFR:677	>10.0 µmol/l	Spearman's	0.434*	- 0.673**	
			Sign. (two-sided), p	0.005	0.001	
			N	21	21	
6	C/T, T/T MTHFR:677	≤10.0 µmol/l	Spearman's		- 0.590**	
			Sign. (two-sided), p		0.006	
			N		20	
8	A/G, G/G MTRR:66	>10.0 µmol/l	Spearman's	0.372**		0.287*
			Sign. (two-sided), p	0.008		0.046
			N	49		49

Note. No. – genetic subgroup number; * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01

Table 16

Correlations of metabolic parameters in genetic subgroups of boys in the Ivankovsky district with different levels of H_{cy} in the blood (12/18/2015)

No.	Genotype	H_{cy} level	Correlation coefficient	Parameters	
				TSH-T_4	TSH-T_3
5	C/C MTHFR:677	>10.0µmol/l	Spearman's	- 0.403*	
			Sign. (two-sided), p	0.016	
			N	35	
8	A/G, G/G MTRR:66	≤10.0µmol/l	Spearman's		- 0.636*
			Sign. (two-sided), p		0.048
			N		10

Note. No. – genetic subgroup number; * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided)

After forest fires in the ChEZ in the spring and summer of 2015, a direct connection between H_{cy}-T_4 was detected in subgroups of girls in the Ivankovsky district No. 4, 5 and 8, with Hcy in the blood >10.0 μmol/l (Table 15).

3.5. Homocysteine and triiodothyronine.

In a group of girls living in the Ivankovsky district in an area with soil contamination ^{137}Cs > 2.0 Ku/km^2, an H_{cy}-T_3 inverse relationship of moderate strength was revealed (Table 17).

The same connection was identified among girls living in the Polessky district in areas with soil contamination ^{137}Cs < 2.0 Ku/km^2, in subgroups No. 3 (genotype A/A MTHFR:1298) and 7 (genotype A/A MTRR:66), with blood H_{cy} content ≤ 10.0 μmol/l (Table 13).

It should be noted that after forest fires in the ChEZ in the spring and summer of 2015, the H_{cy}-T_3 connection in the subgroups of examined children had a direct direction [7, p. 15; 11, p. 257].

Table 17

Correlations of metabolic indicators in groups of girls in the Ivankovsky district (04/02/2015)

Group	Correlation coefficient	Parameters	
		TSH-T_3	**H_{cy}-T_3**
Girls	Spearman's	- 0.562**	- 0.588**
	Sign. (two-sided), p	0.010	0.006
	N	20	20

Note. * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided)

3.6. Thyroid-stimulating hormone and triiodothyronine

In a group of girls in the Ivankovsky district living in an area with soil contamination ^{137}Cs > 2.0 Ku/km^2, TSH-T_3 feedback was recorded (Table 17).

The same relationship was recorded after forest fires in the ChEZ, in subgroup No. 8 of boys in the Ivankovsky district living in an area with soil contamination of ^{137}Cs < 2.0 Ku/km^2, with blood levels of H_{cy} ≤ 10.0 μmol/l (Table 16).

After forest fires in the ChEZ in 2015, among girls in the Ivankovsky district living in an area with soil contamination $^{137}Cs < 2.0$ Ku/km^2, in subgroups No. 1, 3 and 8, a direct connection between TSH-T_3 was determined, with blood levels of $H_{cy} > 10.0$ µmol /l (Table 15).

3.7. Thyroid hormones and cortisol

In the group of boys from the Ivankovsky district living in an area with soil contamination $^{137}Cs > 2.0$ Ku/km^2, the T_3-Cor feedback was recorded (Table 10).

A direct relationship of medium strength T_3-Cor was determined in subgroup No. 6 of boys in the Polessky district living in areas with soil contamination $^{137}Cs < 2.0$ Ku/km^2 (Table 11), including those with blood H_{cy} levels > 10.0 µmol/l, and in subgroup No. 2, with a blood H_{cy} content > 10.0 µmol/L (Table 12).

A direct T_4-Cor connection was identified in subgroup No. 4 of girls in the Polessky district (Table 8), formed by cases with a blood level of $H_{cy} > 10.0$ µmol/L (Table 18).

In subgroup No. 7 of boys from the Ivankovsky district, with blood levels of $H_{cy} \leq 10$ µmol/l, the T_4-Cor relationship had the opposite direction (Table 12).

Table 18

Correlations of metabolic parameters in genetic subgroups of girls in the Polessky district with different levels of H_{cy} in the blood (04/02/2015)

No.	Genotype	H_{cy} level	Correlation coefficient	Parameters	
				TSH-Cor	T_4-Cor
4	A/C, C/C MTHFR:1298	> 10.0 µmol/l	Spearman's		0.854**
			Sign. (two-sided), p		0.0001
			N		14
5	C/C MTHFR:677	> 10.0 µmol/l	Spearman's	0.574*	
			Sign. (two-sided), p	0.032	
			N	14	

Note. * – correlation is significant at the 0.05 level (two-sided); ** – correlation is significant at the 0.01 level (two-sided)

3.8. Thyroid-stimulating hormone and cortisol

In the subgroup of girls in the Polessky district No. 5 (genotype C/C MTHFR:677), with blood levels of H_{cy} > 10.0 μmol/l, a direct relationship between TSH and Cor was detected (Table 18).

Studies have established that an increase in the level of H_{cy} in the blood of children living near the ChEZ is associated with the T allele of the MTHFR:677 genetic polymorphism. A similar connection was traced in relation to the hormones T_3 and Cor.

Assessment of correlations in genetic subgroups of children with different levels of H_{cy} made it possible to clarify the participation of H_{cy} in thyroid hormonogenesis.

First of all, it is worth noting the inverse correlation between TSH-T_4, which is most pronounced in genetic subgroups with H_{cy} levels > 10.0 μmol/L. This relationship reflects the process of deiodination of T_4 to form T_3 in peripheral tissues. At the same time, the content of T_4 in tissues and blood decreases.

T_3 is able to stimulate FC to increase H_{cy} methylation, as evidenced by the inverse relationship of H_{cy}-T_3 in the subgroups of girls with the main genotypes A/A MTHFR:1298 and A/A MTRR:66, with blood H_{cy} levels ≤ 10.0 μmol/L. This is a reflection of the physiological process in which T_3 stimulates FC enzymes, in the absence of serious genetic defects.

Thus, an increase in the formation of T_3 leads to a decrease in the content of T_4 and H_{cy}, which confirms the direct connection of H_{cy}-T_4 in subgroups of children with blood levels of H_{cy} ≤ 10.0 μmol/L.

Also, the resulting T_3 blocks the production of TSH in the adenohypophysis, as evidenced by the inverse relationship between TSH-T_3 in subgroups with H_{cy} levels ≤ 10.0 μmol/L.

However, after forest fires in the ChEZ, the level of H_{cy} in the blood of most children increased significantly and the H_{cy}-T_3 connection became direct.

Similar changes occurred with the TSH-T_3 relationship, given that the direct correlation between H_{cy}-TSH is well known from previous studies [9, p. 105].

Based on the above, we can conclude that an increase in the level of H_{cy} in the blood leads to an increase in the production of TSH, as well as to the activation of the trans-sulfuration cycle, which results in an increase in the process of T_4 deiodination in peripheral tissues. The resulting T_3 stimulates the activity of FC enzymes, resulting in a decrease in H_{cy} levels. At the same time, the level of T_4 in the blood also decreases. However, this occurs if the FC enzyme systems, in particular MTHFR, are able to respond to T_3.

In the case of genetic disorders of FC (allele T of the MTHFR:677 polymorphism) and exposure to environmental factors in the form of combustion products of forest trees that incorporated huge amounts of radioactive elements after the Chernobyl accident in 1986, T_3 is not able to induce the activity of FC enzyme systems and the level of H_{cy} in the blood of children remains at a high level.

A high level of H_{cy} in the blood is a constant stimulating factor for the formation of TSH in the adenohypophysis and T_3 in peripheral tissues, as evidenced by the direct connections of H_{cy}-T_3, H_{cy}-TSH, TSH-T_3.

A pathological process is formed, since the physiological cycle with the participation of H_{cy} and hormones of the pituitary-thyroid axis does not close.

Based on the above, we can conclude that thyroid hormoneogenesis depends on the formation of H_{cy}. At the same time, T_3, influencing the enzyme systems of FC, regulates the level of H_{cy}.

In the course of the studies, the connection between H_{cy} and Cor, metabolic markers of a stressful situation in the body, was assessed [12, p. 51; 13, p. 804; 14, p. 3750].

Demonstration of the connection between these two agents is the direct H_{cy}-Cor correlation, which confirms the body's response to the metabolic stress that occurs with hyperhomocysteinemia.

In this case, the influence of a genetic factor undoubtedly manifests itself in the form of the allele risk T of the MTHFR:677 polymorphism, which contributes to an increase in the H_{cy} content in the body.

In this regard, under conditions of hyperhomocysteinemia, direct connections between T_3-Cor and TSH-Cor are logical.

It should be noted that a direct TSH-Cor connection was also recorded by other researchers in apparently healthy young people [15, p. 4].

An increase in Cor levels in the blood may be a consequence of the effect of H_{cy} on the adenohypophysis. However, a direct effect of H_{cy} on the cells of the adrenal cortex cannot be excluded.

During the physiological process, T_3 is able to reduce the level of H_{cy} in the blood, thereby reducing the level of Cor, as evidenced by the corresponding inverse correlation T_3-Cor in the group of boys in the Ivankovsky district.

Thus, an increased level of H_{cy} in the blood is a stimulating factor, not only for the production of thyroid hormones, but also for Cor. At the same time, T_3, acting as a regulator of the process of H_{cy} utilization, affects the process of Cor formation.

The study allows us to formulate a hypothesis about the participation of FC in the processes of hormonogenesis of the adenohypophysis, thyroid gland and adrenal cortex, which allows us to outline a number of promising directions for preventive measures for diseases of the thyroid gland and other vital organs in people living in areas affected by the Chernobyl accident.

4. Conclusions

An increase in the level of H_{cy}, T_3, Cor in the blood of children living near the ChEZ is associated with the risk allele T of the MTHFR:677 polymorphism.

An assessment of the correlation relationships of H_{cy}, TSH, T_4, T_3 and Cor in groups of adolescent children (boys and girls) living near the ChEZ, taking into account the state of FC genes and the level of H_{cy} in the blood, allowed us to formulate a hypothesis about the participation of FC in the metabolism of hormones of the adenohypophysis, Thyroid and adrenal cortex.

The formation of correlations between H_{cy}, hormones of the pituitary gland, thyroid gland and adrenal cortex in groups of children living near the ChEZ, 3 decades after the Chernobyl accident, is associated with the state of the genetic apparatus of the FC, and also depends on the influence of external environmental factors.

In the absence of a pronounced influence of the external environment on the body and the state of the genetic apparatus of the FC is capable of effectively carrying out the processes of H_{cy} methylation, the latter participates in the processes of physiological regulation of thyroid hormoneogenesis.

At the same time, the formation of TSH in the cells of the adenohypophysis is stimulated, the cycle of trans-sulfuration reactions and the processes of T_4 deiodination are activated.

Under the influence of TSH, T_3 is formed in peripheral tissues, which has a stimulating effect on FC enzyme systems, including MTHFR, which causes increased H_{cy} methylation.

This does not happen in the case of genetic mutations of FC (risk allele T of the MTHFR:677 polymorphism, and environmental exposure in the form of combustion products of forest trees of the ChEZ, including radioactive elements.

At the same time, a pathological process develops, affecting vital organs and systems.

An increase in H_{cy} content in the body leads to an increase in the formation of Cor, a marker of metabolic stress in the body. This should be taken into account when carrying out preventive and rehabilitation measures for persons exposed to combustion products of wood containing radioactive elements, in particular during forest fires in the ChEZ.

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