HYPERHOMOCYSTEINEMIA AND PITUITARY-THYROID AXIS AMONG CHILDREN LIVING NEAR THE CHORNOBYL EXCLUSION ZONE

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Abstract. The prevalence of cases of hyperhomocysteinemia in the population of children living in a radioactively contaminated area due to the accident at the Chornobyl Nuclear Power Plant (ChNPP) requires an in-depth and immediate study of the aetiopathogenesis of this condition. In view of the increase in the number of cases of thyroid cancer in the period following the accident, it is advisable to determine the relationship between the level of homocysteine (H_{cy}), the state of the folate cycle (FC) and the thyroid status in children. *The aim* of the study was to determine the association of H_{cy} and pituitary-thyroid axis hormones with a different combination of genetic polymorphisms of FC in children living in areas bordering the Chornobyl Exclusion Zone (ChEZ). *The methodology* of the study is based on the evaluation by statistical methods of the results of genetic and laboratory examinations of 336 individuals aged 12-17 from the Ivankiv and Poliske districts of the Kyiv Oblast of Ukraine, obtained during the implementation of projects of the European Commission and the Regional Council of the Rhone-Alpes (France). Blood levels of pituitary thyroid stimulating hormone (TSH), free triiodothyronine (T_3), free thyroxine (T_4), H_{cy}, vitamins B_6, B_9 and B_{12} were analysed, and the status of the genetic system of FC was studied. *Results***.** It was shown that in subgroups of children from the Ivankiv district with dysfunction of the main enzymes of FC due to risk alleles of genetic polymorphisms MTR:A2756G, MTHFR:A1298C, MTHFR:C677T and MTRR:A66G, after forest fires in the ChEZ, contributing to an increase in blood H_{cy}

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content, direct correlations between H_{cy} and TSH were revealed. The formation of the analysed subgroups including the genotypes of different FC polymorphisms made it possible to detect stronger correlations between H_{cy} and TSH compared to subgroups in which only the genotypes of one polymorphism were taken into account as the main ones. At the same time, it was possible to determine the metabolic relationships of sulphur-containing amino acids and the pituitary-thyroid axis in the children's bodies, taking into account the state of the FC genes and environmental exposure, including the radiation factor. The strongest direct relationship between H_{cy} and TSH was found in the compound heterozygous association A/C MTHFR:1298 – C/T MTHFR:677 in combination with genotypes containing risk alleles of the genetic polymorphisms MTR:A2756G and AMTRR:A66G. At the same time, transsulfuration reactions using H_{cy} were activated. H_{cy}, which promotes the formation of TSH, plays an important role in the regulation of thyroid hormone production. TSH stimulates the process of deiodination of T_4 in tissues, resulting in increased production of T_3. T_3, acting on MTHFR, stimulates the formation of 5-MTHF, which is involved in the process of H_{cy} methylation. With increased formation, T_3 inhibits the activity of trans-sulfurisation reactions. *Conclusions***.** According to the results of the study, the cycle of trans-sulphurisation reactions should be considered as a metabolic mechanism linking thyroid hormone formation and the exchange of sulphur-containing amino acids. The relationship between hyperhomocysteinemia and the pituitary-thyroid axis in children of the Ivankiv district illustrates the compensatory-adaptive reaction of the developing organism aimed at increasing H_{cy} methylation under appropriate endogenous (the state of the genetic apparatus of the FC) and exogenous (radiation exposure) conditions.

1. Introduction

The problem of the long-term consequences of the Chornobyl disaster is of great national and international importance.

It should be noted that 35 years after the accident, the radioactive contamination of the area around the ChNPP remains very high.

In 2017, soil contamination levels in the ChEZ reached 100 MBq/m^2 for ^{137}Cs; 50 MBq/m^2 for ^{90}Sr, and 1 MBq/m^2 for $^{239\text{-}240}$Pu [1, p. 9]. At the same

time, the area of forests with a level of radioactive contamination higher than15.0 Ci/km^2 was 31.1 %, and with a level of radioactive contamination of 5.0-15.0 Ci/km^2 – 39.0 % [2, p. 57].

Thus, forest trees growing in the ChEZ have accumulated a significant amount of long-lived radionuclides over many years after the Chornobyl accident.

The soils and forest of the Ivankiv district, which borders the ChEZ, also contain significant amounts of ^{137}Cs and ^{90}Sr radionuclides.

In particular, in 45.0% of grain samples (wheat, rye, oats, barley) collected from the fields of Ivankiv district in 2011-2019, the concentration of ^{90}Sr exceeded the permissible level for human consumption in Ukraine [3, p. 9, 10].

Numerous forest fires in the ChEZ contribute to the spread of radioactive elements with air currents far beyond the exclusion zone.

This primarily affects the population living near the ChEZ, namely in Ivankiv and Poliske districts of Kyiv Oblast.

In the spring and summer of 2015, several large forest fires occurred in the ChEZ, during which the maximum contamination density of the territory in some areas was ^{137}Cs – 1040 kBq/m^2; ^{90}Sr – 368 kBq/m^2; $^{238-240}$Pu – 11.4 kBq/m^2 and ^{241}Am – 14.4 kBq/m^2 [1, p. 10].

After these fires, high levels of the sulphur-containing amino acid H_{cy} – an intermediate metabolite of the essential amino acid methionine (Met) – were found in the blood of more than 70.0% of the children examined in the Ivankiv and Poliske districts [4, p. 29; 1, p. 26].

This phenomenon undoubtedly warrants in-depth study, as an increase in blood H_{cy} above the physiological level, or hyperhomocysteinemia, in adults is associated with a number of serious diseases [5, p. 142; 6, p. 1087; 7, p. 212].

Taking into account the increase in the number of cases of thyroid cancer after the Chornobyl accident [8, p. 100], it is advisable to determine the relationship between the H_{cy} level, the state of the FC and the thyroid status in children living near the ChEZ.

The aim of the study was to determine the relationship between H_{cy} and pituitary-thyroid axis hormones with a different combination of FC genetic polymorphisms in children living in areas bordering the ChEZ.

2. Material and Methods

The material of the analytical study were the results of a genetic and laboratory examination of children aged 12-17 years old living near the ChEZ, on the territory of the Kyiv Oblast of Ukraine, contaminated with radioactive elements due to the Chornobyl accident [9, p. 49, 50, 51; 10, p. 120, 121].

The project was carried out with the support of the European Commission and the Rhone-Alpes Regional Council (France).

Blood samples were taken from the cubital vein in the morning, on an empty stomach, from children in Poliske district on 02.04.2015 and from children in Ivankiv district on 18.12.2015.

The blood samples were analysed in a laboratory certified in accordance with European Union quality standards and were agreed with the parents.

The pituitary TSH, T_3, T_4 and H_{cy} levels were measured in the blood of the children in both groups, and the state of the genetic system of the FC was also examined.

The reference interval of extreme values established by the laboratory was: for TSH – 0.28-4.3 μMO/ml; for T_3 – 2.3-5.0 pg/ml; for T_4 – 1.1-1.8 ng/dl. The determination of TSH, T3 and T4 was performed using an immunochemical method with electrochemiluminescence detection (ECLIA). Analyser and test system: Cobas 6000, Roche Diagnostics (Switzerland).

The determination of H_{cy} in the blood was performed by an immunochemical method with chemiluminescence detection (ECLIA). Analyser and test system: Architect 1000 (ABBOT Diagnostics (USA)). The level of H_{cy} in the blood of children over 10 μmol/l was defined as 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: PCR in Real-time mode. Analyzer and test system DT-96 detecting cycler; "DNA-Technology" (Russia).

The blood levels of vitamins B_6, B_9 and B_{12} were also measured in 178 children from Ivankiv district.

The determination of vitamin B_6 (HPLC) was performed by high performance liquid chromatography. Analyser and test system: HPLC-

System 1100, Agilent with fluoressence detection; Recipe complete kit (Germany).

The determination of vitamin B_9 (folacin) was performed using an immunochemical method with electrochemiluminescence detection (ECLIA). Analyser and test system: Cobas e411; Roche Diagnostics (Switzerland).

The determination of vitamin B12 – holotranscobalamin (active vitamin B_{12}) was performed using an immunochemical method with chemiluminescence detection (CLIA). Analyser and test system: Architect 1000 (Abbott Diagnostics), USA.

The reference interval of extreme values given by the laboratory was: for vitamin B_6 – 8.7-27.2 μg/l, for folic acid (vitamin B_9) – 4.6-18.7 ng/ml, for vitamin B_{12} – 191.0-663.0 pg/ml.

The statistical processing of the results obtained was carried out using the IBM SPSS Statistics 22 programme (USA). The median (Me), interquartile range (IQR), minimum and maximum values of the parameters and percentiles were calculated for the analysed indicators. The hypothesis of the type of distribution was tested (Kolmogorov-Smirnov criterion). All the parameters studied did not follow the law of normal distribution, so the non-parametric Mann-Whitney U test was used to compare the values. The statistical significance of the indicators was assessed by determining the significance level p using a statistical programme.

Student's t-test was used to compare relative scores. The critical confidence level of the null hypothesis (p) was set at 0.05. The relationship between the indicators of H_{cy}, TSH, T_3, T_4, B_6, B_9, B_{12}, T_3/T_4 was determined using Spearman's rank correlation coefficient (r_{xy}). The strength of the correlation was assessed according to the traditional scale: weak – from 0 to 0.299; medium – from 0.3 to 0.699; strong – from 0.7 to 1.0.

3. Results and Discussion

Previous publications have noted the role of individual polymorphisms in the occurrence of the interaction between H_{cy} and the pituitary-thyroid axis. In particular, in the general group of children in the Ivankiv district, as well as in genetic subgroups with a risk allele of a polymorphism, a weak direct correlation between H_{cy} and TSH was found [10, p. 15; 11, p. 34]. In the subgroup with the risk allele T of the MTHFR:677 polymorphism and

the C/T MTHFR:677 genotype, this association was stronger [12, p. 257]. However, in the subgroup with the main genotype C/C MTHFR:1298, the strength of the direct H_{cy}-TSH association was $r_{xy} = 0.733$ ($p = 0.025$, $n = 9$) [11, p. 34]. Thus, the emergence of a direct correlation between H_{cy} and TSH depends on the state of the genes that control one of the most important FC enzymes, MTHFR.

Thanks to its functioning, the active form of vitamin B_9, methyltetrahydrofolate (5-MTHF), is formed – a carrier of the methyl group for the reaction of conversion of H_{cy} to Met.

In view of the above, it is advisable to pay attention to the compound heterozygous association A/C MTHFR:1298 – C/T MTHFR:677, which significantly reduces the function of MTHFR. It was found in 21.35 % of cases in the group of children from Ivankiv district, and in 15.82 % in the group of children from Poliske district (Table 1).

The second important genetic association is the combination of genotypes with the risk allele G of the MTR:2756 polymorphism and the risk allele G of the MTRR:66 polymorphism, which was recorded in the group of children from Ivankiv district in more than 30.0% of cases (Table 1). These genotypes, which control the state of MTR and MTRR, disrupt the process of H_{cy} methylation (the process of transferring the methyl group from 5-MTHF to H_{cy} with the participation of cobalamin) and the formation of internal Met.

In the examined groups of children from Poliske and Ivankiv districts, the association of A/C MTHFR:1298 – C/T MTHFR:677 and A/A MTHFR:1298 – C/C MTHFR:677 genotypes was combined with AG, G/G MTR:2756 genotypes in a significantly smaller number of cases compared to AG, G/G MTRR:66 genotypes (Table 2).

The proportion of cases with the combination of genotypes A/G MTR:2756 – A/GMTRR:66 + A/C MTHFR:1298 and A/G MTR:2756 – A/GMTRR:66 + C/T MTHFR: 677 in the groups of children from both districts did not differ significantly, nor did the proportion of cases with the combination of genotypes A/A MTR:2756 – A/A MTRR:66 + A/C MTHFR:1298 and A/A MTR:2756 – A/A MTRR:66 + C/T MTHFR:677 (Table 2).

Combinations of genotypes with risk alleles of all four FC polymorphisms are of scientific and practical interest.

Table 1

Share of FC gene associations in groups of children from Poliske and Ivankiv districts

N	Genotype combinations	Groups			
		No. 1		No. 2	
		N[1]	%	N[1]	%
1	A/C MTHFR:1298 – C/T MTHFR:677	25	15.82	38	21.35
2	A/A MTHFR:1298 – C/C MTHFR:677	28	17.72	29	16.29
3	A/G, G/G MTR:2756 – A/G, G/G MTRR:66	44	27.85	58	32.58
4	A/A MTR:2756 – A/A MTRR:66	22	13.92	13	7.30
5	A/G, G/G MTR:2756 – A/CMTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	6	3.80	15	8.43
6	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	4	2.53	13	7.30
7	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – G/G MTRR:66	2	1.27	6	3.37
8	A/G, G/G MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/G, G/G MTRR:66	6	3.80	9	5.06
9	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	16	10.13	17	9.55
10	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	1	0.63	2	1.12
11	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	2	1.27	4	2.25
12	A/A MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/A MTRR:66	5	3.16	2	1.12

Note. N – subgroup number; N1 is the number of children in the subgroup. Group No. 1 – children of the Poliske district; group No. 2 – children of the Ivankiv district.

In particular, the combination of A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66 leads to the greatest blockage of the enzyme systems of FC. In the group of children from the Poliske district, this combination of genotypes was found in 3.80 % of cases, in the group of children from the Ivankiv district – in 8.43 % of cases (Table 1).

The proportion of cases of hyperhomocysteinemia in most of the analysed subgroups of children in Ivankiv district exceeded 70.0% of the benchmark and was significantly higher than in subgroups of Poliske district in subgroups 1-4 (Tables 3, 4).

Table 2

Share of FC gene associations in groups of children from Poliske and Ivankiv districts

N	Genotype combinations	Number of combinations					
		No. 1			No. 2		
		N^1	N^2	%	N^1	N^2	%
1a	A/C MTHFR:1298 – C/T MTHFR:677 + AG, G/G MTR:2756	25	7	28.0	38	17	44.7
1б	A/C MTHFR:1298 – C/T MTHFR:677 + AG, G/G MTRR:66	25	22	88.0	38	32	84.2
2a	A/A MTHFR:1298 – C/C MTHFR:677 + AG, G/G MTR:2756	28	8	28.6	29	13	44.8
2б	A/A MTHFR:1298 – C/CMTHFR:677 + AG, G/G MTRR:66	28	21	75.0	29	23	79.3
3a	A/G, G/G MTR:2756 – A/G, G/G MTRR:66 + A/C MTHFR:1298	44	17	38.6	58	29	50.0
3б	A/G, G/G MTR:2756 – A/G, G/G MTRR:66 + C/T MTHFR:677	44	21	47.7	58	26	44.8
4a	A/A MTR:2756 – A/A MTRR:66 + A/C MTHFR:1298	22	7	31.8	13	7	53.8
4б	A/A MTR:2756 – A/A MTRR:66 + C/T MTHFR:677	22	6	27.3	13	5	38.5

Note. N – subgroup number; N^1 is the number of children in the subgroup; N^2 is the number of children with a combination of genotypes. Group No. 1 – children of the Poliske district; group No. 2 – children of the Ivankiv district.

Table 3

Proportion of cases of hyperhomocysteinemia in groups of children with a combination of CFS genotypes

N	Genotype combinations	Groups					
		No. 1			No. 2		
		N^1	N^2	%	N^1	N^2	%
1	A/C MTHFR:1298 – C/T MTHFR:677	25	10	40.0	38	31	81.6
2	A/A MTHFR:1298 – C/C MTHFR:677	28	10	35.7	29	20	69.0
3	A/G, G/G MTR:2756 – A/G, G/G MTRR:66	44	22	50.0	58	45	77.6
4	A/A MTR:2756 – A/A MTRR:66	22	8	36.4	13	10	76.9
5	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	6	4	66.7	15	12	80.0

(End of Table 3)

N	Genotype combinations	Groups					
		No. 1			No. 2		
		N^1	N^2	%	N^1	N^2	%
6	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	4	2	50.0	13	11	84.6
7	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – G/G MTRR:66	2	1	50.0	6	6	100.0
8	A/G, G/GMTR:2756 – A/AMTHFR:1298 – C/CMTHFR:677- A/G, G/G MTRR:66	6	2	33.3	9	7	77.8
9	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	16	11	68.8	17	15	88.2
10	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	1	1	100.0	2	1	50.0
11	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	2	2	100.0	4	3	75.0
12	A/A MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/A MTRR:66	5	1	20.0	2	1	50.0

Note. N – subgroup number; N^1 is the number of children in the subgroup; N^2 is the number of cases of hyperhomocysteinemia. Group No. 1 – children of the Poliske district; group No. 2 – children of the Ivankiv district.

Table 4

Statistical differences in the proportion of cases of hyperhomocysteinemia between the groups of children studied

N	Genotype combinations	Student's t-test	Significance level, p
1	A/C MTHFR:1298 – C/T MTHFR:677	3.57	0.000981
2	A/A MTHFR:1298 – C/C MTHFR:677	2.67	0.012721
3	A/G, G/G MTR:2756 – A/G, G/G MTRR:66	2.96	0.004297
4	A/A MTR:2756 – A/A MTRR:66	2.60	0.019946
5	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	0.61	0.552994
6	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	1.28	0.227812
7	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – G/G MTRR:66	1.41	0.230250
8	A/G, G/G MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/G, G/G MTRR:66	1.88	0.109662

(End of Table 4)

N	Genotype combinations	Student's t-test	Significance level, p
9	A/A MTR:2756 – A/C MTHFR:1298 – C/TMTHFR:677- A/G, G/G MTRR:66	1.39	0.178404
10	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	1.41	0.154601
11	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	1.15	0.367533
12	A/A MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/A MTRR:66	0.76	0.155746

Note. N – subgroup number.

In subgroups 5-12, due to the small number of cases, no significant statistical differences were found between children from Poliske and Ivankiv districts.

Thus, a significantly higher proportion of cases of hyperhomocysteinemia in children living near the ChEZ was registered in subgroups with com-pound heterozygous associations of the genotypes A/C MTHFR:1298 – C/T MTHFR: 677, associations of genotypes with a neutral allele – A/A MTHFR:1298 -C/C MTHFR:677, an association of genotypes including the risk allele A/G, G/G MTR:2756 -A/G, G/G MTRR:66 and only the neutral allele A/A MTR:2756 – A/A MTRR:66.

The revealed statistical differences in the proportion of cases of hyperhomocysteinemia between the indicated subgroups of children in Poliske and Ivankiv districts reflect the impact of an environmental factor associated with forest fires in the ChEZ in spring and summer 2015.

Radioactive elements, together with wood combustion products, have a negative effect on metabolic processes in children's bodies, causing disturbed H_{cy} and Met metabolism [1, p. 27].

The levels of pituitary and thyroid hormones, as well as vitamins B_6, B_9 and B_{12}, in most of the children of the Ivankiv district in the analysed genetic subgroups did not exceed the reference values established by the laboratory (Tables 5, 6, 7, 8).

Correlation analysis revealed in the group of children from the Ivankovsky district a direct relationship of medium strength of H_{cy}-TSH in subgroup No. 3 with a combination of the main genotypes, including

risk alleles of MTR:A2756G and MTRR:A66G polymorphisms, which negatively affect the activity of MTR and MTRR enzymes.

Table 5

Proportion of cases with changes in the level of pituitary-thyroid hormones in subgroups of children in Ivankiv district

N	Genotype combination	Number of cases beyond the reference range					
		TSH >		T_3 >		T_4 <	
		N^1	%	N^1	%	N^1	%
1	A/C MTHFR:1298 – C/T MTHFR:677	2	5.26	7	18.42	5	13.16
2	A/A MTHFR:1298 – C/C MTHFR:677	0	0	3	10.34	6	20.69
3	A/G, G/G MTR:2756 – A/G, G/G MTRR:66	3	5.17	8	13.79	12	20.69
4	A/A MTR:2756 – A/A MTRR:66	0	0	0	0	3	23,08
5	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	2	13.33	3	20.00	2	13.33
6	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	2	15.38	2	15.38	2	15.38
7	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – G/G MTRR:66	1	16.67	1	16.67	1	16.67
8	A/G, G/G MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/G, G/G MTRR:66	0	0	1	11.11	2	22.22
9	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	0	0	4	23.53	3	17.65
10	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	0	0	0	0	0	0
11	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	0	0	0	0	0	0
12	A/A MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/A MTRR:66	0	0	0	0	0	0

Note. N – subgroup number; N^1 is the number of cases in the subgroup. > – more physiological range; < – less than the physiological range.

Table 6

Proportion of cases with changes in vitamin levels in subgroups of children in Ivankiv district

N	Genotype combination	Number of cases beyond the reference range					
		B_6 >		B_9 <		B_{12} <	
		N[1]	%	N[1]	%	N[1]	%
1	A/C MTHFR:1298 – C/T MTHFR:677	2	5.26	6	15.79	1	2.63
2	A/A MTHFR:1298 – C/C MTHFR:677	4	13.79	3	10.34	4	13.79
3	A/G, G/G MTR:2756 – A/G, G/G MTRR:66	2	3.45	12	20.69	2	3.45
4	A/A MTR:2756 – A/A MTRR:66	1	7.69	3	23.07	0	0
5	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	1	6.67	2	13.33	0	0
6	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/G, G/G MTRR:66	1	7.69	2	15.39	0	0
7	A/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – G/G MTRR:66	1	16.67	1	16.67	0	0
8	A/G, G/G MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/G, G/G MTRR:66	0	0	2	22.22	1	11.11
9	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677- A/G, G/G MTRR:66	1	5.88	2	11.76	1	5.88
10	A/G, G/G MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	0	0	1	50.00	0	0
11	A/A MTR:2756 – A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66	0	0	1	25.00	0	0
12	A/A MTR:2756 – A/A MTHFR:1298 – C/C MTHFR:677 – A/A MTRR:66	0	0	0	0	0	0

Note. N – subgroup number; N[1] is the number of cases in the subgroup. > – more physiological range; < – less than the physiological range.

Table 7

Statistical characteristics of H_{cy}, TSH, T_3, T_4 in the analysed genetic subgroups

No.	H_{cy} µmol/l		TSH µMO/ml		T_3 pg/ml		T_4 ng/dl	
	Me	IQR	Me	IQR	Me	IQR	Me	IQR
1	12.05	10.55-13.60	1.78	1.29-2.45	4.35	4.00-4.68	1.26	1.17-1.37
2	11.23	9.27-12.59	1.69	0.99-2.28	4.44	3.91-4.79	1.29	1.16-1.35
3	11.67	10.40–13.29	1.76	1.29-2.22	4.48	4.01-4.81	1.22	1.14-1.29
4	11.57	9.65–15.42	2.12	1.50-3.43	4.44	4.01-4.57	1.28	1.10-1.33

(End of Table 7)

No.	H_{cy} µmol/l		TSH µMO/ml		T_3 pg/ml		T_4 ng/dl	
	Me	IQR	Me	IQR	Me	IQR	Me	IQR
5	12.03	11.37–13.31	1.60	1.17-2.63	4.25	4.00-4.70	1.25	1.17-1.35
6	11.21	9.45–13.12	1.63	0.99-1.77	4.72	3.75-4.81	1.22	1.15-1.39
7	12.35	11.07–15.46	1.80	1.36-2.49	4.49	4.16-4.91	1.21	1.15-1.39
8	10.07	8.86–10.07	1.59	1.39-1.59	3.74	3.39-3.74	1.21	1.17-1.21
9	11.1	9.20–17.68	2.15	1.86-2.82	4.29	3.81-4.61	1.38	1.30-1.47

Note. No. – genetic subgroup number; Me – median; IQR – interquartile range.

Table 8

Statistical characteristics of vitamins B_6, B_9, B_{12} in the analysed genetic subgroups

No.	B_6 µg/l		B_9 ng/ml		B_{12} pg/ml	
	Me	IQR	Me	IQR	Me	IQR
1	18.20	14.80-22.80	6.09	4.96-7.80	342.35	252.13-431.05
2	19.30	13.60-24.90	7.15	5.48-9.08	331.50	250.20-445.75
3	17.90	13.98-20.20	6.46	4.89-8.09	284.15	238.08-377.03
4	19.90	15.60-24.80	6.33	4.37-8.41	325.10	243.90-437.50
5	18.20	13.90-19.90	6.40	5.12-8.01	318.80	238.30-463.50
6	18.20	13.35-20.65	6.40	5.06-7.87	318.80	240.70-425.40
7	18.50	16.33-24.35	6.66	4.66-7.89	297.80	232.33-419.28
8	19.20	14.95-21.0	6.02	4.01-8.93	309.10	205.50-424.10
9	18.0	14.70-24.05	6.09	5.14-7.90	334.10	290.05-408.05

Note. No. – genetic subgroup number; Me – median; IQR – interquartile range.

In subgroups No. 5 and No. 6 of the same group of children, in which genotypes containing risk alleles of all four analysed FC polymorphisms (Table 1) are presented as the main ones, the strength of the direct association H_{cy}-TSH increased significantly (Table 9a).

No correlation between H_{cy} and TSH was found in similar subgroups of children in the Poliske district.

The same pattern can be observed in relation to the B_{12}-B_6 correlation.

In subgroups No. 1 and 3, an inverse relationship of the average strength of B_{12}-B_6 was recorded (Table 9a).

In subgroups No. 5, 6, 7 with a combination of genotypes with risk alleles of all four FC polymorphisms (Table 1), this correlation was strong (Table 9a).

Table 9a

Correlations of metabolic parameters in children of Ivankiv district with a combination of FC genotypes

N	Correlation coefficient	Parameters				
		B_9-H_{cy}	B_{12} -H_{cy}	B_{12}-B_6	H_{cy}-TSH	H_{cy}-T_3
1	Spearman's	- 0.291	- 0.607**	- 0.386*	0.181	- 0.219
	Sign. (2-tailed), p	0.076	0.000	0.017	0.276	0.186
	N	38	38	38	38	38
2	Spearman's	- 0.452*	- 0.555**	0.060	- 0.025	0.415*
	Sign. (2-tailed), p	0.014	0.002	0.757	0.899	0.025
	N	29	29	29	29	29
3	Spearman's	- 0.495**	- 0.389**	- 0.400**	0.355**	0.028
	Sign. (2-tailed), p	0.0001	0.003	0.002	0.006	0.832
	N	58	58	58	58	58
4	Spearman's	- 0.555*	- 0.511	- 0.269	- 0.080	- 0.088
	Sign. (2-tailed), p	0.049	0.074	0.374	0.795	0.774
	N	13	13	13	13	13
5	Spearman's	- 0.475	- 0.446	- 0.711**	0.768**	- 0.395
	Sign. (2-tailed), p	0.074	0.095	0.003	0.001	0.145
	N	15	15	15	15	15
6	Spearman's	- 0.407	- 0.423	- 0.765**	0.736**	- 0.316
	Sign. (2-tailed), p	0.168	0.150	0.002	0.004	0.292
	N	13	13	13	13	13
7	Spearman's	- 0.143	0.371	- 0.943**	0.771	0.116
	Sign. (2-tailed), p	0.787	0.468	0.005	0.072	0.827
	N	6	6	6	6	6
8	Spearman's	- 0.150	- 0.683*	0.209	0.183	0.176
	Sign. (2-tailed), p	0.700	0.042	0.589	0.637	0.651
	N	9	9	9	9	9
9	Spearman's	- 0.157	- 0.538*	- 0.049	- 0.141	- 0.444
	Sign. (2-tailed), p	0.548	0.026	0.852	0.589	0.074
	N	17	17	17	17	17

Note. N is the genetic subgroup number; * – correlation is significant at the level of 0.05 (two-tailed); ** – correlation is significant at the level of 0.01 (two-tailed).

In subgroups No. 1, 3, 5, 6, a direct correlation between the average strength of T_3-B_9 was recorded (Table 9b).

Table 9b

Correlations of metabolic parameters in children of Ivankiv district with a combination of FC genotypes

N	Correlation coefficient	Parameters				
		T_3-B_9	T_4-TSH	B_9-TSH	B_9-T_4	B_{12}-T_4
1	Spearman's	0.429**	0.129	- 0.031	0.491**	0.119
	Sign. (2-tailed), p	0.007	0.439	0.855	0.002	0.475
	N	38	38	38	38	38
2	Spearman's	0.186	- 0.180	0.278	- 0.028	0.146
	Sign. (2-tailed), p	0.335	0.350	0.144	0.885	0.450
	N	29	29	29	29	29
3	Spearman's	0.343**	- 0.273*	- 0.273*	0.142	0.290*
	Sign. (2-tailed), p	0.008	0.038	0.038	0.289	0.027
	N	58	58	58	58	58
4	Spearman's	0.398	0.119	0.287	- 0.033	- 0.033
	Sign. (2-tailed), p	0.178	0.700	0.343	0.915	0.915
	N	13	13	13	13	13
5	Spearman's	0.649**	0.055	- 0.400	0.393	0.486
	Sign. (2-tailed), p	0.009	0.845	0.140	0.147	0.066
	N	15	15	15	15	15
6	Spearman's	0.594*	- 0.135	- 0.302	0.591*	0.616*
	Sign. (2-tailed), p	0.032	0.661	0.316	0.033	0.025
	N	13	13	13	13	13
7	Spearman's	0.406	0.429	0.200	0.257	0.600
	Sign. (2-tailed), p	0.425	0.397	0.704	0.623	0.208
	N	6	6	6	6	6
8	Spearman's	0.628	- 0.485	- 0.183	0.259	0.167
	Sign. (2-tailed), p	0.070	0.185	0.637	0.500	0.667
	N	9	9	9	9	9
9	Spearman's	0.104	0.141	0.157	0.735**	- 0.138
	Sign. (2-tailed), p	0.691	0.589	0.547	0.001	0.597
	N	17	17	17	17	17

Note. N is the genetic subgroup number; * – correlation is significant at the level of 0.05 (two-tailed); ** – correlation is significant at the level of 0.01 (two-tailed).

Thus, in subgroups 5 and 6, a combination of direct links H_{cy}-TSH and T_3-B_9 and feedback B_{12}-B_6 was observed.

In subgroups 1, 2, 3, 8, 9, B_{12}-H_{cy} feedback was recorded and in subgroups 2, 3, 4, B_9-H_{cy} feedback was recorded (Table 9a).

The results obtained indicate that when the functioning of the main enzymes of the FC, primarily MTR and MTRR, is impaired, and, as a result, there is a lack of active forms of vitamins B_{12} and B_9, the H_{cy} content increases and the enzymes of the trans-sulfurisation reaction cycle are activated, in particular, cystathionine β-synthase (CBS), in which vitamin B_6 is a coenzyme.

This increases the level of TSH, which is involved in the process of deiodination of T_4 to form T_3.

The feedback between TSH and T_4 in the subgroup of children No. 3, with a combination of the main genotypes A/G, G/G MTR:2756 – A/G, G/G MTRR:66 (Table 9b), can be considered as a reflection of the physiological mechanism of hormonal regulation of the formation of active forms of thyroid hormones (T_3) in peripheral tissues.

This is confirmed by the direct relationship TSH-T3, observed in the subgroup with the major genotype T/T MTHFR:677, in 80% of cases with the genotypes A/G, G/G MTRR:66 (Table 10), and the correlations between TSH, T_3, T_4 and T_3/T_4 index in the analysed genetic subgroups (Table 11).

Thus, TSH may be involved in the activation of deiodination in peripheral tissues, leading to an increase in T_3 production and a decrease in T_4.

The direct links B_9-T_4 (subgroups No. 1, 6, 9) and B_{12}-T_4 (subgroups No. 3, 6) indicate that vitamins B_9 and B_{12} play an important role in maintaining the correct concentration of T_4 in the blood, participating in the methylation process of H_{cy}, thereby reducing the load on the cycle of trans-sulphuration reactions and reducing the intensity of T_4 deiodination.

The T_3-B_9 relationship reflects the effect of T3 on the activity of FC enzymes, primarily MTHFR, to form active forms of vitamin B_9, in particular 5-MTHF. Thus, the body reduces the formation of H_{cy}.

The same stimulating effect on the formation of active forms of vitamin B9 can also be assumed for TSH, since in the subgroup with the main genotype A/C MTHFR:1298 – C/T MTHFR:677 – A/A MTRR:66, in the almost complete absence of genetic defects MTR and MTRR, a strong direct relationship TSH-B_9 was found. At the same time, there was no direct relationship between T_3-B_9, but there was a strong feedback of B_9-B_6, confirming the relationship between FC and the transsulfuration cycle (Table 10).

Table 10

Correlations between metabolic parameters in subgroups of children with different genetic polymorphisms

Parameters	Correlation coefficient	Subgroups		
		C/CMTHFR:1298+ 100% A/G, G/G MTRR:66	A/CMTHFR:1298- C/TMTHFR:677- A/A MTRR:66	T/TMTHFR: 677 + 80% A/G, G/G MTRR:66
H_{cy}-TSH	Spearman's	0.733*		
	Sign. (2-tailed), p	0.025		
	N	9		
B_{12}-T_4	Spearman's	0.750*		
	Sign. (2-tailed), p	0.020		
	N	9		
T_4-T_3	Spearman's	- 0.917**		
	Sign. (2-tailed), p	0.001		
	N	9		
B_9-B_6	Spearman's		- 0.886*	
	Sign. (2-tailed), p		0.019	
	N		6	
TSH-B_9	Spearman's		0.943**	
	Sign. (2-tailed), p		0.005	
	N		6	
TSH-T_3	Spearman's			0.693**
	Sign. (2-tailed), p			0.004
	N			15
B_9-H_{cy}	Spearman's			- 0.674**
	Sign. (2-tailed), p			0.006
	N			15

Note. N is the subgroup number; * – correlation is significant at the level of 0.05 (two-tailed); ** – correlation is significant at the level of 0.01 (two-tailed).

However, an examination of the correlations in subgroup No. 3 does not allow for a clear conclusion.

Table 11

Results of correlation analysis between the parameters of the pituitary-thyroid axis in children with combinations of FC genetic polymorphisms

N	Correlation coefficient	Parameters		
		TSH-T_3/T_4	T_3-T_3/T_4	T_4-T_3/T_4
1	Spearman's	-0.057	0.611**	-0.664**
	Sign. (2-tailed), p	0.732	0.0001	0.0001
	N	38	38	38
2	Spearman's	0.340	0.524**	-0.710**
	Sign. (2-tailed), p	0.071	0.003	0.0001
	N	29	29	29
3	Spearman's	0.274*	0.713**	-0.725**
	Sign. (2-tailed), p	0.037	0.0001	0.0001
	N	58	58	58
4	Spearman's	0.036	0.547	-0.869**
	Sign. (2-tailed), p	0.908	0.053	0.0001
	N	13	13	13
5	Spearman's	0.004	0.602*	-0.622*
	Sign. (2-tailed), p	0.990	0.017	0.013
	N	15	15	15
6	Spearman's	0.236	0.525	-0.580*
	Sign. (2-tailed), p	0.437	0.065	0.038
	N	13	13	13
7	Spearman's	0.086	0.319	-0.829*
	Sign. (2-tailed), p	0.872	0.538	0.042
	N	6	6	6
8	Spearman's	0.733*	0.778*	-0.586
	Sign. (2-tailed), p	0.025	0.014	0.097
	N	9	9	9
9	Spearman's	-0.118	0.575*	-0.694**
	Sign. (2-tailed), p	0.653	0.016	0.002
	N	17	17	17

Note. N is the subgroup number; * – correlation is significant at the level of 0.05 (two-tailed); ** – correlation is significant at the level of 0.01 (two-tailed).

In this subgroup with a combination of genotypes with G risk alleles of the MTR:2756 and MTRR:66 genetic polymorphisms, the TSH-B_9 association had an inverse direction and weak strength (Table 9b).

At the same time, 5-MTHF, the active form of vitamin B9, is not or very poorly utilised by cobalamin for subsequent H_{cy} methylation. The concentration of the latter increases, as indicated by the B_9-H_{cy} feedback, and there is an increase in TSH production, as indicated by the direct H_{cy}-TSH relationship. The TSH-T_4 feedback indicates the involvement of the adenohypophysis in the process of deiodination. The resulting T_3 stimulates the formation of B_9 (direct T_3-B_9 relationship).

A striking manifestation of the close interaction between FC, H_{cy} and hormones of the pituitary-thyroid axis is the subgroup in which the main genotype C/C MTHFR:1298 is combined in 100.0% of cases with genotypes containing the G allele of the MTRR:66 polymorphism and in 44.4% of cases with genotypes containing the G allele of the MTR:2756 polymorphism.

In the complete absence of genotypes with risk alleles of the T polymorphism MTHFR:677, there are strong direct relationships B_{12}-T_4 and H_{cy}-TSH, as well as a strong feedback T_4-T_3 (Table 10).

Thus, functional disorders of FC associated with mutations in the genes of several major FC enzymes lead to an increase in the H_{cy} content in the blood and stimulation of TSH production.

The correlations between H_{cy} and TSH in subgroups that included risk alleles of several FC polymorphisms were significantly stronger than in subgroups in which only one polymorphism was taken into account as a baseline.

At the same time, the transsulfuration cycle is activated, leading to an increase in the process of T4 deiodination in peripheral tissues.

On the basis of the results obtained, it can be concluded that the most pronounced activation of the cycle of transsulfuration reactions and the appearance of a link between H_{cy} and TSH occurs with the simultaneous blocking of the activity of all four FC enzymes, which is achieved by a combination of mutations in their genes and exposure to environmental factors. represented mainly by radioactive elements of Chornobyl origin.

The presence of an inverse correlation between B_{12}-B_6 indicates an important role of cobalamin in the regulation of Hcy and Met metabolism.

The activation of the reactions for the formation of cysteine from H_{cy} and serine, with the participation of vitamin B_6 as a coenzyme, occurs first of all when there is insufficient formation of methylated cobalamin.

Cysteine, after combining with selenium, participates in the processes of T_4 deiodination as a cofactor of 5-Di deiodinase. TSH plays an active role in stimulating this process.

The T_3 formed as a result of this reaction is the most active form of thyroid hormone. In certain situations, it can block the activity of the CBS, as shown by the T_3-B_6 feedback [13, p. 51].

T_3 also stimulates FC enzymes, mainly MTHFR, to form 5-MTHF, which improves Hcy methylation processes.

In the conditions created by hyperhomocysteinemia, the body uses compensatory adaptive responses to increase the processes of H_{cy} methylation, including the use of pituitary and thyroid hormones.

The presented diagram reflects the metabolic relationships in children's bodies, taking into account the state of the FC genes and environmental influences, including the radiation factor.

Despite the fact that most of these children did not show pronounced pathological changes in vital organs, it is necessary to pay close attention to the exogenous factor – in the form of long-lived radionuclides, which induces the state of hyperhomocysteinemia in children with combinations of genetic polymorphisms of FC.

This results in stimulation of TSH production, which has been identified in a number of studies as an independent predictor of thyroid cancer [14, p. 1065, 1069; 15, p. 4].

If such an environmental impact on a child's body is not eliminated, impaired metabolism of the sulphur-containing amino acids H_{cy} and Met will contribute to the formation of serious diseases at a later age, with a fatal outcome and disability.

4. Conclusions

Forest fires in the Chornobyl zone in the spring and summer of 2015 led to an increase in the number of cases of hyperhomocysteinemia in subgroups of children in Ivankiv district with different combinations of FC genetic polymorphisms in the genome.

In subgroups of children from Ivankiv district with impaired function of the main enzymes of the FC caused by risk alleles of genetic polymorphisms MTR:A2756G, MTHFR:A1298C, MTHFR:C677T and MTRR:A66G, after forest fires in the ChEZ, which contribute to an increase in the H_{cy} content in the blood, direct correlations between H_{cy} and TSH were found.

The formation of the analysed subgroups with the inclusion of genotypes of different FC polymorphisms allowed us to identify stronger correlations between H_{cy} and TSH compared to subgroups in which only one polymorphism was taken into account as the main genotypes. At the same time, it was possible to determine the metabolic interrelationships of sulfur-containing amino acids and the pituitary-thyroid axis in children's bodies, taking into account the state of the FC genes and environmental influences, including radiation.

The strongest direct relationship between H_{cy} and TSH was found in a complex heterozygous association A/C MTHFR:1298 – C/T MTHFR:677 in combination with genotypes containing risk alleles of genetic polymorphisms MTR:A2756G and AMTRR:A66G. Transsulfuration reactions using H_{cy} were activated.

H_{cy}, by promoting the formation of TSH, plays an important role in the regulation of thyroid hormone production.

TSH stimulates the process of T_4 deiodination in tissues, which leads to an increase in T_3 production.

T3, acting on MTHFR, stimulates the formation of 5-MTHF, which is involved in the process of H_{cy} methylation. With increased formation, T_3 inhibits the activity of transsulfuration reactions.

Based on the results of the study, the cycle of transsulfuration reactions should be considered as a metabolic mechanism linking thyroid hormone formation and the exchange of sulfur-containing amino acids.

The relationship between hyperhomocysteinemia and the pituitary-thyroid axis in children of Ivankiv district illustrates the compensatory and adaptive reaction of the developing organism aimed at increasing H_{cy} methylation under appropriate endogenous (state of the FC genetic apparatus) and exogenous (radiation exposure) conditions.

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