CHAPTER «MEDICAL SCIENCES»

ELEMENTS OF ETIO-PATHOGENESIS OF HYPERHOMOCYSTEINEMIA IN CHILDREN LIVING IN THE REGIONS AFFECTED FROM THE ACCIDENT AT THE CHERNOBYL NUCLEAR POWER PLANT

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DOI: https://doi.org/10.30525/978-9934-26-297-5-25

Abstract. The prevalence of hyperhomocysteinemia in the population of children living in the area affected by the accident at the Chernobyl Nuclear Power Plant (ChNPP) requires a deep study of the etio-pathogenesis of metabolic disorders of the sulfur-containing amino acids methionine (Met) and homocysteine (H_{cy}) under radiation exposure. The purpose of the study was to assess the involvement of genetic (folate cycle polymorphisms – FC) and environmental factors in the occurrence of hyperhomocysteinemia in boys and girls living in the Ivankovsky and Polessky districts of the Kyiv region of Ukraine, near the Chernobyl 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 690 children (368 girls and 322 boys), aged 8-17 years, obtained in the course of projects of the European Commission, the Regional Council of Rhone-Alpes (France) and the French public organization "Children Chernobyl". It has been shown that in most cases, the violation of H_{cy} metabolism and the occurrence of hyperhomocystinemia in children from areas affected by the Chernobyl accident are caused by the association of genotypes with risk alleles of MTHFR:C677T and MTRR:A66G polymorphisms. Given the wide

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prevalence in the population, combinations of their heterozygous forms are of the greatest importance.

The risk allele G of the MTRR:66 polymorphism also has a negative effect on the processes of H_{cy} methylation when associated with the risk allele G of the MTR:A2756G polymorphism and with compound heterozygosity A/CMTHFR:1298 – C/TMTHFR:677. In the body of boys, compared with the body of girls, combinations of risk alleles for FC polymorphisms are manifested by a more pronounced disturbance of H_{cy} metabolism. The A/AMTRR:66 genotype promotes H_{cy} utilization in the transsulfuration cycle, even if only one C allele of the MTHFR:677 polymorphism functions.

An external environmental factor in the form of radioactive agents incorporated into vital organs, undermining cellular energy, has a negative impact on the processes of cobalamin methylation. The consequence of this is the occurrence of a state of hyperhomocysteinemia in more than 50 % of boys and girls who do not have risk alleles of MTR:A2756G, MTHFR:C677T and MTRR:A66G polymorphisms in the genome. Conclusions. The main internal cause of hyperhomocysteinemia in children living near the ChEZ is the association of risk alleles for FC genetic polymorphisms.

The combined effect of endogenous (genetic mutations of FC) and exogenous (radioactive elements, their decay products, substances formed during the combustion of wood) factors leads to disruption of the H_{cy} methylation process and the emergence of a state of hyperhomocysteinemia in children living in the territory affected by the Chernobyl accident. This type of metabolic disorder can be considered a distant consequence of the Chernobyl accident. Further research should be aimed at developing measures for the prevention and treatment of hyperhomocysteinemia, as a condition associated with the occurrence of serious pathological processes.

1. Introduction

Laboratory examination of adolescent children, conducted in 2013–2017 in the Ivankovsky district of the Kyiv region of Ukraine in the course of the European Commission project "Health and Ecological Programs around the Chernobyl Exclusion Zone: Development, training and coordination

of health-related projects", with the participation of the Regional Council Rhone-Alpe (France), revealed, in more than 70 % of cases, the state of hyperhomocysteinemia (increased blood levels of homocysteine – H_{cy} > 10 μmol/l) [1, p. 30].

There is no doubt that this alarming information should serve as a reason for a deep study of the causal mechanisms of this phenomenon.

It is known from scientific information sources that an increase in the level of H_{cy} above the established physiological level is observed in 5 % of the population [2, p. 1].

The state of hyperhomocysteinemia is associated, to a greater extent, with adults, with the development of serious pathological processes in their body, including oncological, neurological and cardiovascular diseases [3, p. 1087; 4, p. 80; 5, p. 142; 6, p. 212; 7, p. 76; 8, p. 15].

There is an opinion that moderate hyperhomocysteinemia is a frequent risk factor for ischemic stroke in children [9, p. 2072].

However, hyperhomocysteinemia, which occurs in childhood, has been studied extremely insufficiently [10, p. 2645], not to mention the fact that criteria for reference H_{cy} values for healthy children of different age groups have not yet been developed [11, p. 12].

H_{cy} is a sulfur-containing amino acid, a metabolic product of the essential amino acid Met.

With the participation of these amino acids, methylation of nucleic acids is carried out, which affects the implementation of genetic traits, the aging process of the body, the occurrence of serious diseases [12, p. 12].

Met, supplied from outside the body through the food chain, is a source of methyl groups, resulting in the formation of H_{cy}, which is remethylated with the help of FC enzymes to form domestic Met.

In this process, the most significant are methylenetetrahydrofolate reductase (MTHFR), B_{12}-methionine synthase (MS), as well as methionine-synthase reductase (MSR), which restores the activity of cobalamin in the cycle of reactions of transfer of the methyl group to H_{cy} [13, p. 677].

An increase in the level of H_{cy} in the blood above physiological parameters indicates an imbalance in the functioning of a complex system of FC enzymes.

The causes of this condition in the majority of Ukrainian children living near the Chernobyl nuclear power plant (ChNPP) can be both genetic

disorders and exposure to environmental factors, including long-lived radioactive elements ^{137}Cs and ^{90}Sr, which is confirmed by the soil pollution map of Ivankovsky district, created in the course of the above project of the European Commission [14, p. 120, 121].

From a scientific and practical points of view, it is important to determine the role of genetic (endogenous) and environmental (including radioactive elements and their decay products) factors in the occurrence of H_{cy} and Met metabolism disorders in the body of children living in areas affected by the Chernobyl accident.

The purpose of the study was to assess the involvement of genetic (FC polymorphisms) and environmental factors in the occurrence of hyperhomocysteinemia in boys and girls living in the Ivankovsky and Polessky districts of the Kyiv region of Ukraine, near the ChEZ.

2. Material and methods

The results of a genetic and laboratory examination of 690 children (368 girls and 322 boys), aged 8-17 years old, living near the ChEZ in the Ivankovsky and Polessky districts of the Kyiv region of Ukraine, affected by the Chernobyl accident in 1986, were evaluated.

The project was implemented with the support of the European Commission, the Regional Council of Rhone-Alpes (France) and the French public organization "Children of Chernobyl".

In the course of the survey, agreed with the parents, blood was taken from the cubital vein in children attending school on an empty stomach in the morning.

The study of blood samples was carried out in a laboratory certified according to international quality standards.

At the same time, the content of H_{cy}, vitamins B_9, B_{12}, B_6 in the blood was determined, and the state of the FC genetic system was also assessed.

The 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 over 10 μmol/l was defined as a state of hyperhomocysteinemia.

Determination of vitamin B_9 – folacin (B_9) was carried out using the immunochemical method with electrochemiluminescent detection (ECLIA). Analyzer and test system: Cobas e411; Roche Diagnostics (Switzerland).

Determination of vitamin B_{12}, holotranscobalamin – active vitamin B_{12} (B_{12}) was carried out using an immunochemical method with chemiluminescent detection (CLIA). Analyzer and test system: Architect 1000 (Abbott Diagnostics), USA.

Determination of vitamin B6 – HPLC (B_6) was carried out using the method of high performance liquid chromatography. Analyzer and test system: HPLC-System 1100, Agilent with fluoressence detection; Recipe complete kit (Germany).

The reference values of vitamin B_9 (folacin) in the blood of children were 4.6-18.7 ng /ml, vitamin B_{12} (holotranscobalamin, or active vitamin B_{12}) – 191.0-663.0 pg /ml, vitamin B_6 – 8, 7-27.2 μg/l.

During the genetic study of FC, allelic variants of the genetic polymorphisms MTHFR:C677T and MTHFR:A1298C were determined (encode the enzyme methylenetetrahydrofolate reductase – MTHFR), MTR:A2756G (encodes the enzyme B_{12}-dependent methionine synthase – MS), MTRR:A66G (encodes the synthesis of the enzyme methionine-synthase reductase – MSR). In this case, the method was used: PCR in Real-time mode. Analyzer and test system detecting cycler "DT-96"; "DNA-Technology" (Russia).

The study compared the proportion of cases of hyperhomocysteinemia in subgroups with different combinations of FC genotypes.

Statistical processing of the obtained results was carried out using the IBM SPSS Statistics 22 program (USA).

Student's t-test was used to compare relative scores. The critical confidence level of the null hypothesis (p) was taken as 0.05.

The hypothesis about the type of distributions was tested (Kolmogorov-Smirnov criterion). All the studied parameters did not correspond to the law of normal distribution.

The relationship between the values of H_{cy}, B_9, B_{12}, B_6 was determined using the Spearman's rank correlation coefficient (r_{xy}).

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

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 its discussion
3.1. Genetic factor and hyperhomocysteinemia.
3.1.1. Boys group.

The proportion of cases of hyperhomocysteinemia was significantly more in the subgroup, which included the combination of genotypes A/GMTR:2756-G/GMTRR:66, compared with the subgroup were the combination of genotypes A/GMTR:2756-A/AMTRR:66 (Table 1a, 2), as well as, in the subgroup with a combination of genotypes G/GMTR:2756-A/GMTRR:66, compared with subgroups that include combinations of genotypes A/AMTR:2756-A/GMTRR:66 and A/GMTR:2756-A/GMTRR:66 (Tables 1a, 2).

The predominance of the proportion of cases of hyperhomocysteinemia was recorded in the subgroup with T/T MTHFR:677-A/A MTRR:66 genotypes, compared with subgroups that included combinations genotypes of C/C MTHFR:677-A/A MTRR:66 and C/T MTHFR 677-A/A MTRR:66 (Tables 1a, 2).

Table 1a

The proportion of cases of hyperhomocysteinemia with a combination of FC genotypes in a group of boys from Ivankovsky and Polessky districts

Genotypes	Polymorphism genotypes MTR:2756								
	A/AMTR:2756			A/G MTR:2756			G/G MTR:2756		
	n1	n2	%	n1	n2	%	n1	n2	%
A/AMTHFR:1298	85	63	74.12	60	43	71.67	7	6	85.71
A/CMTHFR:1298	86	66	76.74	39	29	74.36	8	5	62.50
C/CMTHFR:1298	22	12	54.55	12	7	58.33	3	2	66.67
C/C MTHFR:677	98	65	66.33	46	32	69.57	10	7	70.00
C/T MTHFR:677	83	66	79.52	52	37	71.15	6	4	66.67
T/T MTHFR:677	12	10	83.33	13	10	76.92	2	2	100.0
A/AMTRR:66	34	21	61.76	17	9	52.94	7	4	57.14
A/GMTRR:66	88	67	76.14	62	43	69.35	7	7	100.0
G/GMTRR:66	71	53	74.65	32	27	84.38	4	2	50.00

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia.

Associations of genotypes T/T MTHFR:677 – A/G MTRR:66 and C/T MTHFR:677 – A/G MTRR:66 were also accompanied by a more proportion

of cases of hyperhomocysteinemia, compared with the association C/C MTHFR:677 – A/G MTRR:66 (Tables 1b, 2).

A big proportion of cases of hyperhomocysteinemia (80.82 % and 80.49 %) was noted in subgroups where the C/T MTHFR:677 genotype was combined with the A/G MTRR:66 and G/G MTRR:66 genotypes, which is significantly more than in the subgroup with a combination of C/T MTHFR:677 and A/A MTRR:66 genotypes (Tables 1b, 2).

Table 1b

The proportion of cases of hyperhomocysteinemia with a combination of FC genotypes in a group of boys from Ivankovsky and Polessky districts

Genotypes	Polymorphism genotypes MTRR:66								
	A/AMTRR:66			A/GMTRR:66			G/GMTRR:66		
	n1	n2	%	n1	n2	%	n1	n2	%
A/AMTHFR:1298	28	17	60.71	70	53	75.71	54	42	77.78
A/CMTHFR:1298	26	15	57.69	67	54	80.60	40	31	77.50
C/CMTHFR:1298	4	2	50.00	20	10	50.00	13	9	69.23
C/C MTHFR:677	27	15	55.56	73	48	65.75	54	41	75.93
C/T MTHFR:677	24	13	54.17	73	59	80.82	41	33	80.49
T/T MTHFR:677	4	4	100.0	11	10	90.91	12	8	66.67

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia.

Table 2

Statistical differences in the proportion of cases of hyperhomocysteinemia in genetic subgroups of boys

Comparison subgroups		Student's t-test	Significance level, p
A/GMTR:2756- A/AMTRR:66	A/GMTR:2756- G/GMTRR:66	2.29	0,028304
A/AMTR:2756- A/GMTRR:66	G/GMTR:2756- A/GMTRR:66	5.26	0.000001
A/GMTR:2756- A/GMTRR:66	G/GMTR:2756- A/GMTRR:66	5.23	0.000004
C/T MTHFR:677- A/AMTRR:66	C/T MTHFR:677- A/GMTRR:66	2.39	0.019747
C/T MTHFR:677- A/AMTRR:66	C/T MTHFR:677- G/GMTRR:66	2.21	0.032425
C/C MTHFR:677- A/AMTRR:66	T/T MTHFR:677- A/AMTRR:66	4.65	0.000268
C/T MTHFR:677- A/AMTRR:66	T/T MTHFR:677- A/AMTRR:66	4.51	0.000493
C/C MTHFR:677- A/GMTRR:66	C/T MTHFR:677- A/GMTRR:66	2.09	0.039173
C/C MTHFR:677- A/GMTRR:66	T/T MTHFR:677- A/GMTRR:66	2.44	0.017757

3.1.2. Group of girls

In the group of girls, the proportion of cases of hyperhomocysteinemia in the subgroup with the combination of genotypes C/CMTHFR:1298-A/AMTR:2756 was statistically less than in the subgroups with the combination of genotypes A/CMTHFR:1298-A/AMTR:2756 and A/AMTHFR:1298 – A/AMTR:2756 (Tables 3a, 4).

The combination of G/GMTR:2756 and A/AMTRR:66 genotypes resulted in a significant decrease in the relative number of cases of hyperhomocysteinemia compared with the combination of genotypes A/AMTR:2756 and A/AMTRR:66 (Tables 3a, 4).

In the subgroup with a combination of T/T MTHFR:677 – A/AMTR:2756 genotypes, the proportion of cases of hyperhomocysteinemia was more than in subgroups with a combination of genotypes C/T MTHFR:677 – A/AMTR:2756 and C/C MTHFR:677 – A/AMTR:2756 (Tables 3a, 4).

The proportion of cases of hyperhomocysteinemia in the subgroup with a combination of T/T MTHFR:677-A/G MTRR:66 genotypes was significantly more than in subgroups with a combination of genotypes C/T MTHFR:677-A/G MTRR:66 and C/C MTHFR:677-A/G MTRR:66 (Tables 3b, 4).

Table 3a

The proportion of cases of hyperhomocysteinemia with a combination of FC genotypes in a group of girls from Ivankovsky and Polessky districts

Genotypes	Polymorphism genotypes MTR:2756								
	A/AMTR:2756			A/G MTR:2756			G/G MTR:2756		
	n 1	n 2	%	n1	n2	%	n1	n2	%
A/AMTHFR:1298	122	66	54.10	46	25	54.35	8	6	75.00
A/CMTHFR:1298	97	60	61.86	59	28	47.46	8	3	37.50
C/CMTHFR:1298	15	4	26.67	10	5	50.00	3	1	33.33
C/C MTHFR:677	106	55	51.89	52	23	44.23	8	5	62.50
C/T MTHFR:677	103	55	53.40	56	32	57.14	11	5	45.45
T/T MTHFR:677	25	20	80.00	7	3	42.86	0	0	0
A/AMTRR:66	46	30	65.22	23	10	43.48	5	1	20.00
A/GMTRR:66	107	56	52.34	54	30	55.56	10	6	60.00
G/GMTRR:66	81	44	54.32	38	18	47.37	4	3	75.00

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia.

Table 3b

The proportion of cases of hyperhomocysteinemia with a combination of FC genotypes in a group of girls from Ivankovsky and Polessky districts

Genotypes	Polymorphism genotypes MTRR:66								
	A/AMTRR:66			A/GMTRR:66			G/GMTRR:66		
	n1	n2	%	n1	n2	%	n1	n2	%
A/AMTHFR:1298	32	18	56.25	81	45	55.56	63	34	54.00
A/CMTHFR:1298	33	19	57.58	79	44	55.70	52	28	53.85
C/CMTHFR:1298	9	4	44.44	11	3	27.27	8	3	37.50
C/C MTHFR:677	35	18	51.43	73	36	49.32	58	29	50.00
C/T MTHFR:677	32	19	59.38	82	43	52.44	56	30	53.57
T/T MTHFR:677	7	4	57.14	16	13	81.25	9	6	66.67

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia.

Table 4

Statistical differences in the proportion of cases of hyperhomocysteinemia in genetic subgroups of girls

Comparison subgroups		Student's t-test	Significance level, p
A/AMTR:2756-A/AMTRR:66	G/GMTR:2756-A/AMTRR:66	2.35	0.025879
C/C MTHFR:677-A/GMTRR:66	T/T MTHFR:677-A/GMTRR:66	2.81	0.007328
C/T MTHFR:677-A/GMTRR:66	T/T MTHFR:677-A/GMTRR:66	2.57	0.013001
A/AMTHFR:1298-A/AMTR:2756	C/CMTHFR:1298-A/AMTR:2756	2.23	0.028823
A/CMTHFR:1298-A/AMTR:2756	C/CMTHFR:1298-A/AMTR:2756	2.83	0.006310
C/C MTHFR:677-A/AMTR:2756	T/T MTHFR:677-A/AMTR:2756	3.00	0.003656
C/T MTHFR:677-A/AMTR:2756	T/T MTHFR:677-A/AMTR:2756	2.83	0.005990

Thus, the presence in the genome of the risk allele G polymorphism MTRR:66 increases the likelihood of hyperhomocysteinemia in boys – carriers of risk alleles: G polymorphism MTR:2756 and T polymorphism MTHFR:677.

In girls, this dependence was observed only in relation to the homozygous variant of the T allele of the MTHFR:677 polymorphism.

Previous studies have shown a decrease in the level of Hcy in the blood of children (mostly girls) with the G/G MTR:2756 genotype, due to an increase in the activity of enzymes of the trans-sulfurization cycle [15, p. 33].

To prove the significance of the G allele of the MTRR:66 polymorphism in the processes of Hcy methylation in children permanently residing near the ChEZ, the frequency of occurrence of cases of hyperhomocysteinemia in subgroups that included a combination of risk alleles of the three genetic polymorphisms MTR:2756, MTRR:66 and MTHFR:677.

In the group of boys, the proportion of cases of hyperhomocysteinemia was significantly more in the subgroup with the combinations of genotypes A/G MTR:2756-C/T MTHFR:677 + A/G, G/G MTRR:66, than in the subgroup with the combination of genotypes A/GMTR:2756-C/TMTHFR:677 + A/AMTRR:66 (Tables 5a, 6).

In the subgroup with the combination of genotypes A/AMTR:2756-A/GMTRR:66 + C/T, T/T MTHFR:677, the proportion of cases of hyperhomocysteinemia was statistically more than in the subgroup with the combination of genotypes A/AMTR:2756-A/GMTRR:66 + C/C MTHFR:677 (Tables 5b, 6).

Table 5a

The proportion of cases of hyperhomocysteinemia with a combination of FC genotypes in a group of boys from Ivankovsky and Polessky districts

Genotypes	MTTR:A66G +								
	C/C MTHFR:677			C/T MTHFR:677			T/T MTHFR:677		
	n1	n2	%	n1	n2	%	n1	n2	%
A/AMTR:2756 + **a**	81	56	69.14	68	56	82.35	10	8	80.00
A/AMTR:2756 + **b**	17	9	52.94	15	10	66.67	2	2	100.0
A/G MTR:2756 + **a**	40	28	70.00	42	33	78.57	12	9	75.00
A/G MTR:2756 + **b**	6	4	66.67	10	4	40.00	1	1	100.0
G/G MTR:2756 + **a**	6	5	83.33	4	3	75.00	1	1	100.0
G/G MTR:2756 + **b**	4	2	50.00	2	1	50.00	1	1	100.0

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia; a – A/G+G/G MTTR:66; b – A/A MTTR:66.

In the group of girls, a significant increase in the proportion of cases of hyperhomocysteinemia was found in the subgroup with a combination of genotypes A/AMTR:2756-T/T MTHFR:677 +A/G, G/G MTRR:66, compared with subgroups that included combinations of A/AMTR:2756-C/T MTHFR:677 +A/G, G/G MTRR:66 and A/AMTR:2756-C/C MTHFR:677 +A/G, G/G MTRR:66 (Tables 7, 8).

Table 5b

The proportion of cases of hyperhomocysteinemia with a combination of FC genotypes in a group of boys from Ivankovsky and Polessky districts

Genotypes	MTHFR:C677T +								
	A/AMTTR: 66			A/GMTTR: A66			G/GMTTR: A66		
	n1	n2	%	n1	n2	%	n1	n2	%
A/AMTR:2756 + **a**	17	12	70.59	43	37	86.05	35	27	77.14
A/AMTR:2756 + **b**	17	9	52.94	45	30	66.67	36	26	72.22
A/G MTR:2756 + **a**	11	5	45.45	37	28	75.68	17	14	82.35
A/G MTR:2756 + **b**	6	4	66,67	25	15	60.00	15	13	86.67
G/G MTR:2756 + **a**	3	2	66.67	4	4	100.0	1	0	0
G/G MTR:2756 + **b**	4	2	50.00	3	3	100.0	3	2	66.67

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia; a – C/T+T/T MTHFR:C677; b – C/C MTHFR:677.

Table 6

Statistical differences in the proportion of cases of hyperhomocysteinemia in genetic subgroups of boys

Comparison subgroups		Student's t-test	Significance level, p
A/GMTR:2756-C/ TMTHFR:677 + A/G, G/G MTRR:66	A/GMTR:2756-C/ TMTHFR:677+ A/ AMTRR:66	2.30	0.027402
A/AMTR:2756- A/ GMTRR:66 + C/T, T/T MTHFR:677	A/AMTR:2756- A/ GMTRR:66 + C/C MTHFR:677	2.20	0.031108

Between the subgroups that made up the cases of combination of genotypes A/AMTR:2756-T/TMTHFR:677+A/AMTRR:66, A/AMTR:2756 – C/T MTHFR:677+A/A MTRR:66, A/AMTR: 2756-C/C MTHFR:677 + A/A MTRR:66 there were no statistical differences (Table 7).

Also, there were no statistical differences between subgroups, including associations of genotypes A/AMTR:2756-T/T MTHFR:677 +A/G, G/G MTRR:66 and A/AMTR:2756-T/T MTHFR:677+A/ A MTRR:66, A/AMTR:2756-C/T MTHFR:677 +A/G, G/G MTRR:66 and A/ AMTR:2756 -C/T MTHFR:677+A/A MTRR:66, A /AMTR:2756-C/C MTHFR:677 +A/G, G/G MTRR:66 and A/AMTR:2756-C/C MTHFR:677 + A/A MTRR:66 (Table 7).

Table 7

The proportion of cases of hyperhomocysteinemia with a combination of FC genotypes in a group of girls from Ivankovsky and Polessky districts

Genotypes	MTTR: A66G +								
	C/CMTHFR:677			C/TMTHFR:677			T/TMTHFR:677		
	n1	n2	%	n1	n2	%	n1	n2	%
A/AMTR:2756 + **a**	84	42	50.00	84	42	50.00	20	16	80.00
A/AMTR:2756 + **b**	23	13	59.09	19	13	68.42	5	4	80.00
A/G MTR:2756 + **a**	40	18	45.00	47	27	57.45	5	3	60.00
A/G MTR:2756 + **b**	12	5	41.67	9	5	55.56	2	0	0
G/G MTR:2756 + **a**	7	5	71.43	7	4	50.00	0	0	0
G/G MTR:2756 + **b**	1	0	0	4	1	25.00	0	0	0

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia; a – A/G+G/GMTTR:66; b – A/AMTTR:66.

Table 8

Statistical differences in the proportion of cases of hyperhomocysteinemia in the genetic subgroups of girls

Comparison subgroups		Student's t-test	Significance level, p
A/AMTR:2756-T/ TMTHFR:677 +A/G, G/G MTRR:66	A/AMTR:2756-C/C MTHFR:677+A/G, G/G MTRR:66	2.86	0.005914
A/AMTR:2756-T/ TMTHFR:677 +A/G, G/G MTRR:66	A/AMTR:2756-C/T MTHFR:677 +A/G, G/G MTRR:66	2.86	0.005914

In the subgroup of boys with the combination of genotypes A/CMTHFR:1298 – C/TMTHFR:677 (compound heterozygosity A/C1298-C/T677 MTHFR) + A/GMTRR:66, the proportion of cases of hyperhomocysteinemia was significantly more than in the subgroup of boys with the combination genotypes A/CMTHFR:1298 – C/TMTHFR:677 + + A/AMTRR:66 (Table 9).

In the subgroups of girls with the above combinations of genotypes, this statistical effect was not registered, while the proportion of cases of hyperhomocysteinemia in the subgroup with the combination of genotypes A/CMTHFR:1298 – C/TMTHFR:677+ A/GMTRR:66 was statistically less than in the same subgroup of boys (Table 9).

The conducted studies indicate the important role of MSR in the processes of Hcy methylation. Violation of the functioning of this enzyme due to the genetic mutation MTRR:66 leads to a significant increase in the number of cases of hyperhomocysteinemia among Ukrainian children living in areas affected by the accident at the ChNPP.

Table 9

The proportion of cases of hyperhomocysteinemia with a combination of compound heterozygosity A/CMTHFR:1298-C/TMTHFR:677 and FC genotypes in the genome of boys and girls from Ivankovsky and Polessky districts

Genotypes	A/CMTHFR:1298 – C/T MTHFR:677					
	Boys			Girls		
	n1	n2	%	n1	n2	%
A/AMTR:2756	40	31	77.50	46	29	63.04
A/GMTR:2756	20	15	75.00	32	18	56.25
G/GMTR:2756	3	2	66.67	6	2	33.33
A/AMTRR:66	12	6	50.00*	19	12	63.16
A/GMTRR:66	32	27	84.38	43	23	53.49**
G/GMTRR:66	19	15	78.95	22	14	63.64

Note. n1 – the number of cases of combination of genotypes; n2 – the number of cases of hyperhomocysteinemia. Statistical differences: * – boys with genotypes A/AMTRR:66 and A/GMTRR:66 ($t = 2.18$; $p = 0.037496$); ** – boys and girls with genotype A/GMTRR:66 ($t = 3.10$; $p = 0.003243$).

This is most pronounced in the subgroups of boys, where the G allele of the MTRR:66 polymorphism is combined with the heterozygous variant of the T allele of the MTHFR polymorphism: C677T and the heterozygous variant of the G allele of the MTR polymorphism: A2756G (Figure 1, 2, 3).

The presence of the G allele of the MTRR:66 polymorphism in children with the C/T MTHFR:677 genotype contributes to a decrease in the production of methyltetrahydrofolate (5-MTHF) and, accordingly, to a deficiency of methyl groups in the process of Hcy methylation, as evidenced by the inverse correlation between Hcy and vitamin B9 in subgroup of boys with a combination of C/TMTHFR:677 – G/GMTRR:66 genotypes, which is absent in the subgroup of boys with a combination of C/TMTHFR:677-A/A MTRR:66 genotypes (Tables 10, 11).

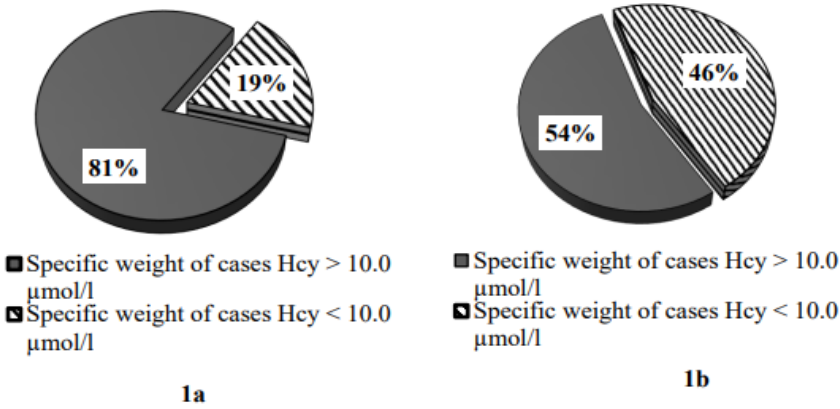


Figure 1. Proportion of cases of hyperhomocysteinemia: a) in the subgroup of boys with the combination of genotypes C/TMTHFR:677-A/GMTRR:66; b) in the subgroup of boys with the combination of genotypes C/TMTHFR:677+A/AMTRR:66

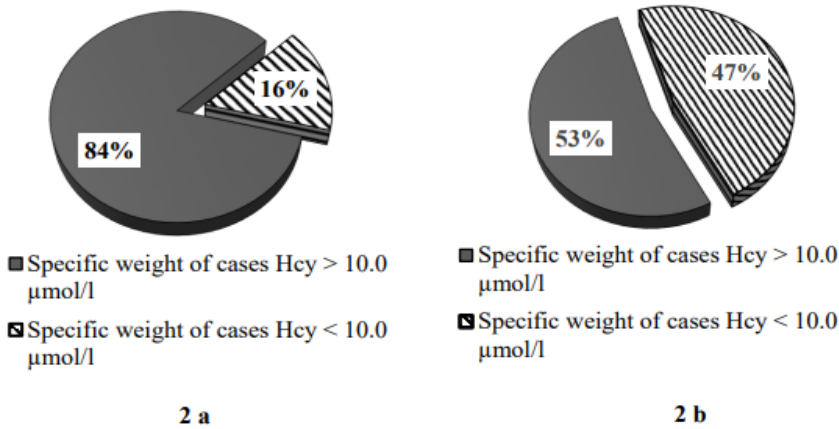


Figure 2. Proportion of cases of hyperhomocysteinemia: a) in the subgroup of boys with the combination of genotypes A/GMTR:2756-G/GMTRR:66; b) in the subgroup of boys with a combination of genotypes A/GMTR:2756-A/AMTRR:66

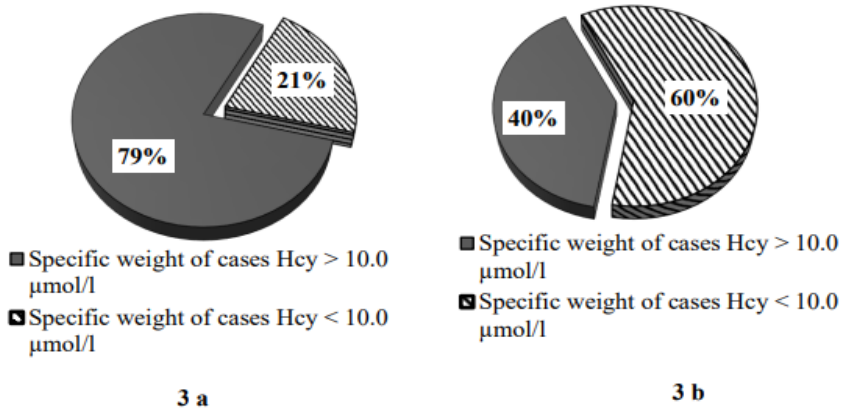


Figure 3. The proportion of cases of hyperhomocysteinemia: a) subgroup of boys with a combination of genotypes A/GMTR:2756-C/TMTHFR:677+A/G, G/GMTRR:66; b) subgroup of boys with a combination of genotypes A/GMTR:2756-C/T MTHFR:677+ A/A MTRR:66

A similar Hcy-B9 inverse correlation was registered in the subgroup of boys with the combination of A/GMTR:2756-A/GMTRR:66 genotypes ($r = -0.649$, $p = 0.0001$, $n = 27$). However, it should be taken into account that, in 17 cases (62.96 %), the T allele of the MTHFR:677 polymorphism was present in the genome.

It should be noted that the absence of the risk allele G of the MTRR:66 polymorphism in the genome of children with a decrease in the production of 5-MTHF (genotype C/TMTHFR:677) contributes to the utilization of Hcy in the cycle of transsulfurization reactions, which confirms the direct relationship of Hcy-B6 (Table 11).

Given the prevalence in the population and the impact on the process of Hcy methylation (Table 12), associations of genotypes with risk alleles of MTHFR: C677T and MTRR: A66G polymorphisms should be attributed to the main endogenous causes of hyperhomocysteinemia in children from areas affected by the Chernobyl accident.

Table 10

Correlations between H_{cy} and vitamins B_9, B_{12}, B_6 in the subgroup of boys with a combination of genotypes C/T MTHFR:677 – G/G MTRR:66

Parameter	Correlation coefficient, significance, p	Parameter			
		H_{cy}	B_6	B_9	B_{12}
H_{cy}	Spearman's (r_{xy})	1.000	- 0.245	- 0.765**	- 0.056
	Sign. (2-tailed), p	.	0.343	0.000	0.830
	N	17	17	17	17
B_6	Spearman's (r_{xy})	- 0.245	1.000	0.439	0.072
	Sign. (2-tailed), p	0.343	.	0.078	0.783
	N	17	17	17	17
B_9	Spearman's (r_{xy})	- 0.765**	0.439	1.000	0.142
	Sign. (2-tailed), p	0.000	0.078	.	0.586
	N	17	17	17	17
B_{12}	Spearman's (r_{xy})	-0.056	0.072	0.142	1.000
	Sign. (2-tailed), p	0.830	0.783	0.586	.
	N	17	17	17	17

Note. * – correlation is significant at the 0.05 level (2-tailed). ** – correlation is significant at the 0.01 level (2-tailed)

Table 11

Correlations between H_{cy} and vitamins B_9, B_{12}, B_6 in the subgroup of boys with a combination of genotypes C/T MTHFR:677-A/A MTRR:66

Parameter	Correlation coefficient, significance, p	Parameter			
		H_{cy}	B_6	B_9	B_{12}
H_{cy}	Spearman's (r_{xy})	1.000	0.810*	- 0.548	- 0.262
	Sign. (2-tailed), p	.	0.015	0.160	0.531
	N	8	8	8	8
B_6	Spearman's (r_{xy})	0.810*	1.000	- 0.262	0.071
	Sign. (2-tailed), p	0.015	.	0.531	0.867
	N	8	8	8	8
B_9	Spearman's (r_{xy})	- 0.548	- 0.262	1.000	0.524
	Sign. (2-tailed), p	0.160	0.531	.	0.183
	N	8	8	8	8
B_{12}	Spearman's (r_{xy})	- 0.262	0.071	0.524	1.000
	Sign. (2-tailed), p	0.531	0.867	0.183	.
	N	8	8	8	8

Note. * – correlation is significant at the 0.05 level (2-tailed). ** – correlation is significant at the 0.01 level (2-tailed)

3.2. Environmental factor and hyperhomocysteinemia

Among the causes of mass hyperhomocysteinemia among children living near the ChEZ, first of all, it is necessary to consider providing their bodies with vitamins B_9, B_{12}, B_6 involved in Hcy methylation reactions. The most objective will be the indicators of the active forms of these vitamins in the blood.

Table 12

The proportion of combinations of genotypes C/T,T/T MTHFR: C677T and A/G, G/G MTRR: A66G, and associated hyperhomocysteinemia, in groups of boys and girls

Groups	n	C/T, T/TMTHFR: 677 + A/G, G/G MTRR:66		H_{cy} >10.0 µmol/l	
		n	%	n	%
Boys	322	137	42.29	110	80.29
Girls	368	163	44.29	92	56.44
General	690	300	43.48	202	67.33

Note. n – the number of cases; % – proportion of cases in the group.

In the course of the studies, it was revealed that the proportion of cases of deficiency of vitamins B_9 and B_{12}, determined by laboratory methods, is significantly less than the proportion of cases of hyperhomocysteinemia (Table 13).

Table 13

The proportion of cases of vitamin content outside the reference values in the blood of boys and girls

Groups	n	H_{cy} >10.0 µmol/l		B_9 < 4.6 ng/ml		B_{12} < 191.0 pg/ml		B_6 > 27.2 µg/l	
		n	%	n	%	n	%	n	%
Boys	125	109	87.20	30	24.00	12	9.60	19	15.20
Girls	135	88	65.19	24	17.78	1	0.74	12	8.89
General	260	197	75.77	54	20.77	13	5.00	31	11.92

Note. n – the number of cases; % – proportion of cases in the group

In the group of boys with hyperhomocysteinemia, the proportion of cases of vitamin B_9 deficiency was more than in the same group of girls, and amounted to 26.61 %, vitamin B_{12} – 10.09 % (Table 14).

Table 14

The proportion of cases of vitamin content outside the reference values in groups of boys and girls with hyperhomocysteinemia (H_{cy} > 10.0 μmol/l)

Groups	n	B_9 < 4.6 ng/ml		B_{12} < 191.0 pg/ml		B_6 > 27.2 μg/l	
		n	%	n	%	n	%
Boys	109	29	26.61	11	10.09	16	14.68
Girls	88	21	23.86	1	1.14	10	11.36
General	197	50	25.38	12	6.09	26	13.20

Note. n – the number of cases; % – proportion of cases in the group.

Thus, the occurrence of hyperhomocysteinemia in most of the examined children in Ivankovsky and Polessky districts is not associated with insufficient intake of vitamins B_9 and B_{12} in their body.

A relationship has been established between the risk allele T of the MTHFR:C677T polymorphism and insufficient blood levels of vitamin B_9. The deficiency of this vitamin is most pronounced in the homozygous T/T variant of this polymorphism, with a significant blocking of MTHFR activity and 5-MTHF formation (Tables 15, 16).

Table 15

The proportion of cases of blood levels of vitamin B_9 < 4.6 ng/ml in subgroups of children with genotypes MTHFR: C677T

Subgroups	B_9 blood level < 4.6 ng/ml								
	General group			Boys			Girls		
	n1	n2	%	n1	n2	%	n1	n2	%
C/C	123	19	15.45	63	12	19.05	60	7	11.67
C/T	111	21	18.92	54	13	24.07	57	8	14.04
T/T	26	14	53.85	8	5	62.50	18	9	50.00

Note. n1 – the number of cases in the subgroup; n2 – number of cases B9 < 4.6 ng/ml; % – proportion of cases.

In the analyzed population, the proportion of cases of homozygous T/T variant was registered in the group of boys in 8.7 % of cases, in the group of girls in 8.4 % of cases. The heterozygous variant of the T allele was much more common, in the group of boys in 43.79 % of cases, in the group of girls in 46.19 % of cases [16, p. 272]. In this case, the phenotypic realization of the genetic defect will depend on the conditions in which the organism is located.

Table 16

Statistical comparisons of the proportion of blood levels of vitamin B_9 < 4.6 ng/ml in subgroups of children with MTHFR:C677T genotypes

Comparison subgroups	Genotypes MTHFR:C677T	Student's t-test	Significance level, p
General	T/T	3.72	0.000808
	C/C		
General	T/T	3.34	0.002150
	C/T		
Boys	T/T	2.44	0.028693
	C/C		
Boys	T/T	2.13	0.050585
	C/T		
Girls	T/T	3.07	0.008993
	C/C		
Girls	T/T	2.84	0.013069
	C/T		

Thus, the body of children living in areas whose ecological environment is contaminated with radioactive elements and their decay products as a result of the Chernobyl accident does not experience a significant lack of intake of vitamins B_9 and B_{12} from outside.

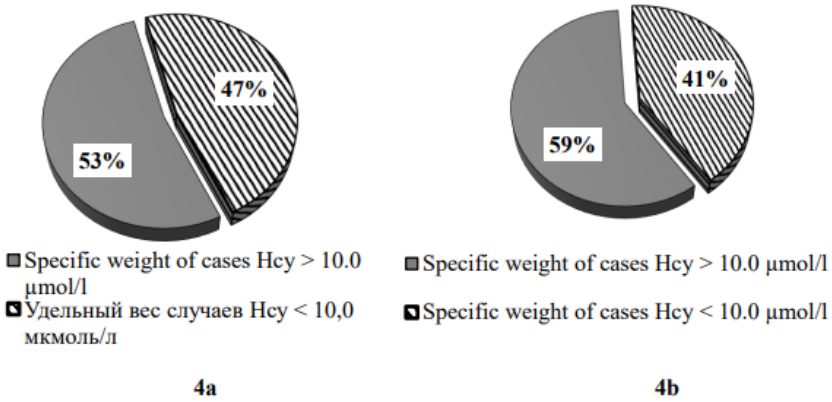
An increase in the blood content of active forms of vitamin B_6 in hyperhomocysteinemia is associated with the activation, under certain conditions, of the trans-sulfurization cycle, through which excess H_{cy} is utilized.

As an example, a strong direct relationship of Hcy-B6 with a combination of C/T MTHFR:677-A/A MTRR:66 genotypes (Table 11) and a strong inverse relationship of B_6-B_{12} ($r = -0.900$, $p = 0.037$, $n = 5$) with a combination of genotypes G/G MTR:2756 -A/A MTRR:66.

Inverse correlations between blood levels of H_{cy} and vitamins B_9, B_{12} [17, p. 41, 42] reflect the insufficient activity of the enzymes with which these vitamins are associated.

The problem of the formation and use of active forms of folic acid and cobalamin in metabolic cycles is partly associated with genetic mutations that affect the activity of FC enzymes and, thereby, contribute to an increase in the content of Hcy in the blood.

However, in more than 50 % of cases, impaired Hcy methylation occurs even in the absence of risk alleles for polymorphisms that negatively affect enzymes activity FC (Figure 4).



4a 4b

Figure 4. The proportion of cases of hyperhomocysteinemia in the subgroups of boys (a) and girls (b) with a combination of genotypes A/AMTR:2756-C/C MTHFR:677-A/A MTRR:66

Inverse correlation between H_{cy} and B_{12} ($r = -0.857$, $p = 0.014$, $n = 7$), in the absence of other relationships, in the subgroup of boys with the A/AMTR:2756-C/C MTHFR:677-A/A MTRR genotype: 66, indicates that the cause of the state of hyperhomocysteinemia is a violation of the methylation of cobalamin as a result of exposure to an environmental factor.

Children from the areas adjacent to the ChEZ are in ecologically unfavorable conditions, and the phenotypic realization of their genome occurs under constant radiation exposure.

In particular, 137Cs radionuclides, having penetrated into the body through food chains, or, as part of air masses, are incorporated simultaneously by the cellular structures of vital organs [18, p. 489], forming the syndrome of incorporated long-lived radionuclides. At the same time, the functioning of the main metabolic cycles, both in mitochondria and in the cytosol, is disrupted. There is an energy deficit, in which the activity of enzyme systems decreases [19, p. 124].

In the case of forest fires in the ChEZ, the situation is aggravated by the fact that, in addition to radioactive elements, the products of wood combustion also enter the body [20, p. 34].

Thus, the real exogenous cause of the state of hyperhomocysteinemia in children living in areas affected by the Chernobyl accident is the effect of long-lived radioactive elements and their decay products on metabolic processes, in particular, the functioning of FC enzyme systems.

The population of children living near the ChEZ is, to a greater extent, a carrier of heterozygous forms of risk alleles for polymorphisms that affect Hcy metabolism, which predetermines the vulnerability of their organism to the effects of adverse environmental agents, including radionuclides.

4. Conclusions

The prevalence of hyperhomocysteinemia in the population of children living in the area affected by the accident at the ChNPP requires a deep study of the etio-pathogenesis of metabolic disorders of the sulfur-containing amino acids Met and Hcy under radiation exposure.

Associations of genotypes with risk alleles of polymorphisms MTHFR: C677T and MTRR: A66G disrupt H_{cy} metabolism in most cases, and therefore, they should be attributed to the main endogenous causes of hyperhomocystinemia in children from areas affected by the Chernobyl accident.

Given the wide prevalence in the population, combinations of heterozygous forms of these polymorphisms are of the greatest importance.

The A/AMTRR:66 genotype promotes H_{cy} utilization in the transsulfuration cycle, even if only one C allele of the MTHFR:677 polymorphism functions and 5-MTHF production is reduced.

The risk allele G of the MTRR:66 polymorphism also has a negative effect on the processes of Hcy methylation when associated with the risk allele G of the MTR polymorphism: A2756G and in compound heterozygosity A/CMTHFR:1298 – C/TMTHFR:677.

In the body of boys, compared with the body of girls, combinations of risk alleles for FC polymorphisms are manifested by a more pronounced violation of Hcy metabolism.

Thus, associations of risk alleles of FC genetic polymorphisms are the main internal cause of hyperhomocysteinemia in children living near the ChEZ.

An external environmental factor in the form of radioactive agents incorporated into vital organs, undermining cellular energy, has a negative impact on the processes of cobalamin methylation. The consequence of this is the occurrence of a state of hyperhomocysteinemia in more than 50 % of boys and girls who do not have risk alleles of MTR polymorphisms: A2756G, MTHFR: C677T and MTRR: A66G in the genome.

The combined effect of endogenous (FC genetic mutations) and exogenous (radioactive elements, their decay products, substances formed during the combustion of wood) factors leads to disruption of the Hcy methylation process and the emergence of a state of hyperhomocysteinemia in children living in the territory affected by the Chernobyl accident.

This type of metabolic disorder can be considered a distant consequence of the Chernobyl accident.

Further research should be aimed at developing measures for the prevention and treatment of hyperhomocysteinemia, as a condition associated with the occurrence of serious pathological processes and serious diseases.

At the same time, in each specific case of Hcy metabolism disorders, individual combinations of risk alleles for FC genetic polymorphisms should be taken into account and an assessment of the environmental (radiation) impact should be carried out.

Conflicts of Interest: The authors declare no conflict of interest.

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