5.2 Hyperhomocysteinemia and the state of the genetic system of the folate cycle in Ukrainian children 35 years after the accident at the Chernobyl nuclear power plant

The accident at the Chernobyl nuclear power plant in 1986 was characterized by the release of a huge amount of short- and long-lived radioactive elements into the environment [536].

The largest amount of long-lived radionuclides is concentrated in the soil and growing forest trees within a 30-kilometer zone, called the Chernobyl exclusion zone [537]. Due to the circumstances, in Ivankovsky and Polessky districts of the Kyiv region, bordering on the specified zone, in 2020 more than 30 thousand people lived, including more than 4 thousand children [538].

During the socio-medical projects of the European Commission, carried out in 2013-2017, in the territory of these districts, significant violations of the health status of most of the examined children were revealed [539]. Of particular note is the violation of the metabolism of the essential amino acid methionine, registered in most of the examined children aged 12-17 years and manifested by an increase in the blood content of the amino acid homocysteine (H_{cy}) [540].

It is known that hyperhomocysteinemia is accompanied by impaired functioning of vital organs and systems, including cardiovascular and nervous systems, leading to oncological diseases [541, 542, 543]. This is especially important to note, since 5.6 % of children in the Ivankovsky and Polessky districts have structural changes in the thyroid gland, and 35.5 % have impaired production of thyroid hormones [539].

The analysis performed showed that the environmental factor associated with the presence of radioactive elements of Chernobyl origin in the environment played the greatest role in the induction of hyperhomocysteinemia in children.

An increase in the level of H_{cy} in the blood of children after the fires of the radioactive forest in the Chernobyl exclusion zone was registered [544]. At the same time, the role of the genetic factor, primarily the folate cycle (FC) genes, cannot be ignored as the cause of this condition. In each specific case of hyperhomocysteinemia,

it is important to determine the significance of external and internal factors, which allows you to identify the right ways to correct metabolism.

The purpose of the work is to determine the cause-and-effect relationships of the occurrence of a state of hyperhomocysteinemia in children living under conditions of constant radiation exposure, 35 years after the accident at the Chernobyl nuclear power plant.

The study was carried out with the financial support of the public organization "Children of Chernobyl" (France).

In January 2022, 217 children aged 12-17 from the Ivankovsky district of the Kyiv region were subjected to laboratory and instrumental examinations, the territory of which, to this day, remains radioactively contaminated after the accident at the Chernobyl nuclear power plant in 1986, in particular, ^{137}Cs and ^{90}Sr [545]. Blood samples were taken from children attending school on an empty stomach.

The blood samples were tested in a quality-certified laboratory and agreed with the parents.

The determination of H_{cy} in the blood was carried out using the immunochemical method with chemiluminescent detection (ECLIA). Analyzer and test system kit: 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.

In the genetic study of FC, allelic variants C677T and A1298C of the MTHFR gene (methylenetetrahydrofolate reductase), A2756G of the MTR gene (B_{12}-dependent methionine synthase), A66G of the MTRR gene (methionine synthase reductase) were determined. The method used was: PCR in Real-time mode. Analyzer and test kit DT-96 detecting cycler; "DNA-Technology" (Russia).

Genetic subgroups were formed, taking into account the genotypes of FC, 100 % representation of one specific genotype, or a combination of genotypes.

Statistical processing of the obtained results was carried out using the IBM SPSS Statistics 22 program (USA). The relationship between H_{cy} and Risk levels of the analyzed genetic polymorphisms was determined using 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. To compare the indicators, Student's t-test was used. The critical confidence level of the null hypothesis (p) was taken as 0.05.

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.

In the studied group of children, only in 5 cases (2.3 %) there were no risk alleles for all analyzed PC polymorphisms. Carriership of risk alleles of two polymorphisms occurred the most frequently. Risk alleles for all four polymorphisms were found in 20 children, or in 9.25 % of cases (Table 1).

At the same time, no relationship was established between the number of polymorphisms with defective alleles and the relative number of cases of hyperhomocysteinemia (Table 1).

The proportion of cases with a homozygous variant of the neutral allele A of the MTR polymorphism: A2756G is greater than the proportion of cases with the risk allele G of the same polymorphism.

In the case of MTHFR:C677T, MTHFR:A1298C, and MTRR:A66G polymorphisms, variants with the risk allele predominated (Table 2).

No statistical differences were found between the proportion of similar genotype variants of the studied polymorphisms in groups of children from the Ivankovsky district in 2015 and 2022 (Tables 2, 3).

Table 1.

The frequency of occurrence of genetic polymorphisms in the examined group of children, (n = 217)

Number of polymorphisms, subgroup No	Total number of children		H_{cy} >10.0, μmol/L	
	Abs. number	%	Abs. number	%
1 st –«0»	5	2.30	4	80.00
2 nd – «1»	33	15.21	21	63.64
3 rd – «2»	105	48.39	68	64.76
4 st – «3»	54	24.88	40	74.07
5 st – «4»	20	9.25	9	45.00

Table 2.

Percentage of polymorphic alleles of folate metabolism genes in the examined children from Ivankovsky district in 2022, (n = 217)

Gene, polymorphism	Genotype variants					
	«Neutral» allele Homozygous variant		«Risk allele» Heterozygous variant		«Risk allele» Homozygous variant	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	133	61.29	74	34.10	10	4.61
MTHFR:A1298C	105	48.39	92	42.40	20	9.21
MTHFR:C677T	99	45.62	100	46.08	18	8.30
MTRR:A66G	43	19.82	106	48.85	68	31.33

The proportion of cases of hyperhomocysteinemia in the currently analyzed general group of children from the Ivankovsky district was 65.44 %, while in 2015 it was 73.2 % in the general group of children from the same district [546].

At the same time, it should be noted that in 2015 in the Chernobyl exclusion zone there were fires of radioactive forest over a large area [544].

When considering individual genetic subgroups, the largest proportion of cases of hyperhomocysteinemia was registered in the subgroup with the homozygous variant T/T MTHFR:677 - 94.44 %. In other genetic subgroups, this indicator was significantly lower, however, in most of them, it exceeded the 50.0 % barrier (Table 4).

Table 3.

Percentage of polymorphic alleles of folate metabolism genes in the children from Ivankovsky district, (n = 179) [546]

Gene, polymorphism	«Neutral» allele		Risk allele «Heterozygous variant»		Risk allele «Homozygous variant»	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	106	59.2	62	34.6	11	6.2
MTHFR:A1298C	90	50.3	80	44.7	9	5.0
MTHFR:C677T	81	45.2	83	46.4	15	8.4
MTRR:A66G	27	15.1	94	52.5	58	32.4

A similar situation was also observed during the examination of children in 2015 [547].

A direct correlation between H_{cy} and the severity of the genetic risk of the MTHFR:C677T polymorphism also confirms the involvement of the risk allele T in the occurrence of hyperhomocysteinemia (Table 5). This relationship is more pronounced in the group of children with an H_{cy} level of more than 10.0 μmol/l (Table 6).

The inverse correlation between Hcy and the severity of the genetic risk of the MTHFRA1298C polymorphism is due to the fact that the number of cases with the T allele of the MTHFR:677 polymorphism progressively decreases along the line of the A/A, A/G, G/G MTHFR:1298 genotypes (Table 7).

The conducted studies indicate that the problem of elevated H_{cy} levels in the blood of children living near Chernobyl exclusion zone is relevant at the present time.

A genetic factor in the form of the T allele of the MTHFR:677 polymorphism, which negatively affects the activity of methylenetetrahydrofolate reductase, can significantly impair H_{cy} metabolism.

Table 4.

Percentage of hyperhomocysteinemia cases in subgroups of children from Ivankovsky district

Subgroup No	Polymorphisms, genotypes	Number of children in groups	Number of hyperhomocysteinemia cases	
			Abs. number	%
1	MTR:2756AA	133	92	69.17
2	MTR:2756AG	74	45	60.81
3	MTR:2756GG	10	5	50.00
4	MTHFR:1298AA	105	74	70.48
5	MTHFR:1298AC	92	57	61.96
6	MTHFR:1298CC	20	11	55.00
7	MTHFR:677CC	99	63	63.64
8	MTHFR:677CT	100	62	62.00
9	**MTHFR:677TT**	18	17	94.44
10	MTRR:66AA	43	25	58.14

11	MTRR:66AG	105	72	67.92
12	MTRR:66GG	68	45	66.18
13	MTR:2756AA+ MTHFR:677CC	67	45	67.16
14	MTR:2756AG+ MTHFR:677CT	39	22	56.41
15	MTHFR:1298AC+ MTHFR:677CT	46	28	60.87
16	Total group	217	142	65.44

Note. * - statistically significant differences between the indicator of the group and the indicator of group No. 9 ($p < 0.05$).

In particular, the homozygous variant of the T allele of the MTHFR:677 polymorphism may indeed be the main cause of hyperhomocysteinemia. However, this genotype was registered in 8.3 % of all examined children.

In most cases, an increase in the level of H_{cy} in the blood was associated with the constant exposure of the child's body to radioactive elements present in the environment.

Through food chains, long-lived radionuclides ^{137}Cs, ^{90}Sr, ^{241}Am penetrate into the body and are incorporated by the cells of vital organs [548], as a result of which the metabolism, including the sulfur-containing amino acids methionine and H_{cy}, is disturbed. The toxic effect of barium, as a decay product of ^{137}Cs, should be taken into account.

Table 5.

Correlations between H_{cy} and the genetic risk index (Risk) of analyzed polymorphisms in a group of children

Parameter	Correlation coefficient	Parameters			
		[1]Risk MTHFR: C677T	[2]Risk MTHFR: A1298C	[3]Risk MTR: A2756G	[4]Risk MTRR: A66G
H_{cy}	Spearman's	0.206**	- 0.165*	- 0.034	0.093
	Sign. (2-tailed)	0.002	0.015	0.618	0.172
	N	217	217	217	217

Note. * - Correlation is significant at the 0.05 level (2-tailed). ** – Correlation is significant at the 0.01 level (2-tailed). [1]Risk MTHFR:677 - CC, CT, TT; [2]Risk MTHFR:1298 - AA, AC, CC; [3]Risk MTR:2756 - AA, AG, GG; [4]Risk MTRR:66 - AA, AG, GG.

Table 6.

Correlations between H_{cy} and the genetic risk index (Risk) of the analyzed polymorphisms in the group of children with hyperhomocysteinemia

Parameter	Correlation coefficient	Parameters			
		[1]Risk MTHFR: C677T	[2]Risk MTHFR: A1298C	[3]Risk MTR: A2756G	[4]Risk MTRR: A66G
H_{cy}	Spearman's	0.308**	- 0.161	0.098	0.146
	Sign. (2-tailed)	0.000	0.056	0.244	0.083
	N	142	142	142	142

Note. * - Correlation is significant at the 0.05 level (2-tailed). ** – Correlation is significant at the 0.01 level (2-tailed). [1]Risk MTHFR:677 - CC, CT, TT; [2]Risk MTHFR:1298 - AA, AC, CC; [3]Risk MTR:2756 - AA, AG, GG; [4]Risk MTRR:66 - AA, AG, GG.

Table 7.

Percentage of the T allele of the MTHFR:C677T genetic polymorphism in subgroups of children in the Ivankovsky district

Sub-groups No.	Polymorphisms, genotypes	Number of children in subgroups	Number of cases with allele T MTHFR:C677T polymorphism	
			Abs. number	%
1	MTR:2756AA	133	66	49.62
2	MTR:2756AG	74	46	62.16
3	MTR:2756GG	10	6	60.00
4	MTHFR:1298AA	105	72	68.57
5	MTHFR:1298AC	92	46	50.00
6	MTHFR:1298CC	20	0	0
7	MTHFR:677CC	99	0	0
8	MTHFR:677CT	100	100	100.0
9	MTHFR:677TT	18	18	100.0
10	MTRR:66AA	43	26	60.47
11	MTRR:66AG	105	56	53.33
12	MTRR:66GG	68	36	52.94
13	MTR:2756AA+ MTHFR:677CC	67	0	0
14	MTR:2756AG+ MTHFR:677CT	39	39	100.0
15	MTHFR:1298AC + MTHFR:677CT	46	46	100.0
16	Total group	217	118	54.38

The most vulnerable are the processes of formation of 5-methyltetrahydrofolate with the help of methylenetetrahydrofolate reductase, and the transfer of the methyl group from 5-methyltetrahydrofolate to cobalamin, with the participation of B_{12}-methionine synthase.

Considering the danger for the developing organism of methionine metabolism disorders, and the resulting hyperhomocysteinemia, it is necessary to regularly assess the level of H_{cy} in the blood, as well as the state of the genetic system that controls FC in children living under conditions of constant radiation exposure. At the same time, it is mandatory to regularly monitor the content of radionuclides in the body of children and locally produced food.

Metabolic correction in most adults and children exposed to radiation can be carried out with methylated forms of folic acid and vitamin B_{12}, in particular, 5-methyltetrahydrofolate and methylcobalamin, which will prevent the accumulation of H_{cy} in the body in large quantities.

The conducted studies allow us to draw the following conclusions:

1. In the blood of 65.44 % of children aged 12-17 years from the Ivankovsky district of the Kyiv region, 35 years after the accident at the Chernobyl nuclear power plant, the H_{cy} level was above 10.0 μmol/l.

2. Carriership of the risk allele T of the MTHFR:677 polymorphism, which negatively affects the activity of methylenetetrahydrofolate reductase, predisposes to an increase in the content of H_{cy} in the blood. The largest proportion of cases of hyperhomocysteinemia was recorded in a subgroup of children with a homozygous variant of this allele.

3. In most cases, the causes of hyperhomocysteinemia were environmental factors, including radioactive elements and their decay products.

4. As preventive measures for hyperhomocysteinemia in children living in areas affected by the accident at the Chernobyl nuclear power plant, one should consider regular monitoring of the content of radionuclides in the body of children and Flocally produced food, as well as recording the level of H_{cy} in the blood and assessing the state of FC genes.

5. Correction of metabolism in case of hyperhomocysteinemia in a large number of children from the Chernobyl regions should be carried out with methylated forms of vitamins B_9 and B_{12}.