CHAPTER «MEDICAL SCIENCES»

THE COMBINATION OF FOLATE CYCLE GENOTYPES AND HYPERHOMOCYSTEINEMIA IN CHILDREN LIVING NEAR THE CHERNOBYL EXCLUSION ZONE

Yuri Bandazheuski[1]
Nataliia Dubovaya[2]

DOI: https://doi.org/10.30525/978-9934-26-255-5-2

Abstract. The state of hyperhomocysteinemia (the level of the sulfur-containing amino acid homocysteine (H_{cy}) in the blood is more than 10 μmol/l) was first detected in a large number of adolescent children living in areas located near the Chernobyl exclusion zone (ChEZ) during the implementation of the European Commission's projects "Health and environmental programs around the Chernobyl exclusion zone: development, training and coordination of projects related to health" and the Rhone-Alpes Regional Council (France). Since numerous studies have noted hyperhomocysteinemia in severe diseases in adults, it is necessary to highlight the impact of environmental factors (exogenous factor) and the state of the genetic apparatus responsible for the functioning of H_{cy} metabolic cycles (endogenous factor). *The purpose of this study* was a comparative analysis of the proportion of hyperhomocysteinemia in subgroups of boys and girls from areas bordering the ChEZ, taking into account combinations of folate cycle (FC) genotypes. *Subject and methods of research.* The analysis was based on the results of a laboratory examination of 690 children (368 girls and 322 boys) aged 8-17 years in the Ivankovsky and Polessky districts of the Kyiv region of Ukraine. Immunochemical, genetic and statistical research methods were used. *Results*. The structure was studied and the proportion of hyperhomocysteinemia was assessed in

[1] Doctor of Medical Sciences, Professor,
President of the Ecology and Health Coordination and Analytical Centre, Ukraine
[2] Candidate of Medical Sciences, Senior Researcher,
Deputy Chairman of the Board of the Ecology and Health Coordination
and Analytical Centre, Ukraine

the case of a combination of allelic variants of genetic polymorphisms of FC. Differences and features of the occurrence of hyperhomocysteinemia depending on gender were revealed. *Conclusions*. The occurrence of hyperhomocysteinemia in children living near the ChEZ can occur with a combination of homozygous variants of neutral alleles of genetic polymorphisms of FC, which indicates an external environmental impact, including a radiation factor. The presence in the genome of boys of combinations of risk alleles for FC polymorphisms, with the participation of MTHFR:677, as well as MTRR:A66G, contributes to an increase in the level of H_{cy} in the blood. Mutations in the FC genes (T/T MTHFR:677, C/T MTHFR:677 in combination with A/G MTRR:66), in which there is a violation of the activity of the enzymes of FC – methylenetetrahydrofolate reductase and methionine synthase reductase, are an internal factor contributing to the occurrence of hyperhomocysteinemia. In girls with a combination of heterozygotes and homozygotes of risk alleles for FC polymorphisms, increased amounts of H_{cy} are utilized in the cycle of trans-sulfurization reactions.

1. Introduction

The soils and forest trees of the Chernobyl Exclusion Zone (ChEZ), as well as the areas adjacent to it, contained and contain large amounts of long-lived radioactive elements, including ^{137}Cs, ^{90}Sr, ^{241}Am [1, p. 10; 2, p. 265], which penetrate the body of local residents through air and alimentary routes.

Thus, the adult and child population living near the ChEZ was and is under constant radiation exposure.

It was found that in most of the examined children living near the ChEZ, the level of the sulfur-containing amino acid homocysteine (H_{cy}) in the blood exceeds the established physiological parameters [3, p. 33; 4; 12].

This condition, defined as hyperhomocysteinemia, is recorded in adults in connection with diseases leading to mortality and disability [5, p. 4; 6, p. 1087; 7, c. 12].

Thus, it is relevant to determine the causal relationships of hyperhomocysteinemia in children living in the territory affected by the accident at the Chernobyl nuclear power plant (ChNPP).

At the same time, given the large number of cases of increased H_{cy} in the blood of children, first of all, it is necessary to highlight the impact

of environmental factors (exogenous factor) and the state of the genetic apparatus responsible for the functioning of metabolic cycles of Hcy (endogenous factor).

In this regard, a method for assessing the proportion of hyperhomocysteinemia with a combination of allelic variants of genetic polymorphisms of the folate cycle (FC) is acceptable.

The purpose of this study was a comparative analysis of the proportion of hyperhomocysteinemia in subgroups of girls and boys from areas bordering the ChEZ, taking into account combinations of FC genotypes.

2. Material and methods

The project of the European Commission "Health and Ecological Programs around the Chernobyl Exclusion Zone: Development, training and coordination of health-related projects" with the participation of the Regional Council of Rhone-Alpes (France) and the French public organization "Children of Chernobyl", made it possible to carry out a laboratory examination of 690 children (368 girls and 322 boys), aged 8-17, from Ivankovsky and Polessky districts of the Kyiv region of Ukraine, bordering the ChEZ. The examination was coordinated with the parents of the children.

In children attending school, in the morning, on an empty stomach, produced blood sampling from the cubital vein.

Blood samples were analyzed in a laboratory certified according to international quality standards.

At the same time, the content of H_{cy} in the blood was determined, as well as the state of the genetic system of FC.

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.

The genetic study of FC included the determination of allelic variants C677T and A1298C of the MTHFR gene (encodes the synthesis of the methylenetetrahydrofolate reductase enzyme), A2756G of the MTR gene (encodes the synthesis of the B_{12}-dependent methionine synthase enzyme), and A66G of the MTRR gene (encodes the synthesis of the methionine

synthase reductase enzyme). In this case, the method was used: PCR in Real-time mode. Analyzer and test system detecting cycler "DT-96"; "DNA-Technology" (Russia).

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

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

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

3. Results and its discussion

As can be seen from the previous publication [8, p. 272] there were no differences between the groups of girls and boys living near the ChEZ in terms of the proportion of polymorphic FC alleles (Tables 1, 2).

In the subgroups of boys, the proportion of cases of hyperhomocysteinemia was significantly more than in similar subgroups of girls, with the exception of subgroups with genotypes G/GMTR:2756, C/CMTHFR:1298, T/TMTHFR:677 and A/AMTRR:66, i.e. in subgroups with genotypes that include heterozygous variants of risk alleles and homozygous variants of neutral alleles of FC [8, p. 274].

Table 1

The frequency of polymorphic alleles of genes, FC in girls from Ivankovsky and Polessky districts

Gene, polymorphism	Variants of genotypes					
	Allele "neutral", homozygous variant		Allele of "risk", heterozygous variant		Allele of "risk", homozygous variant	
	Abs. number of cases	%	Abs. number of cases	%	Abs. number of cases	%
MTR:A2756G	234	63.59	115	31.25	19	5.16
MTHFR:A1298C	176	47.83	164	44.56	28	7.61
MTHFR:C677T	166	45.11	170	46.19	32	8.70
MTRR:A66G	74	20.11	171	46.47	123	33.42

Source: [8, p. 272]

Table 2

Frequency of polymorphic alleles of FC genes in boys from Ivankovsky and Polessky districts

Gene, polymorphism	Variants of genotypes					
	Allele "neutral", homozygous variant		Allele of "risk", heterozygous variant		Allele of "risk", homozygous variant	
	Abs. number of cases	%	Abs. number of cases	%	Abs. number of cases	%
MTR:A2756G	193	59.94	111	34.47	18	5.59
MTHFR:A1298C	152	47.20	133	41.30	37	11.50
MTHFR:C677T	154	47.83	141	43.79	27	8.38
MTRR:A66G	58	18.01	157	48.76	107	33.23

Source: [8, p. 272]

In the group of girls, the proportion of cases of hyperhomocysteinemia in the subgroup with the T/T MTHFR:677 genotype is statistically more than in the subgroups with the C/C MTHFR:677 and C/T MTHFR:677 genotypes (Tables 3, 4).

In the group of boys, the proportion of cases of hyperhomocysteinemia in the subgroup with the A/A MTRR:66 genotype was significantly less than in the subgroups with the A/G MTRR:66 and G/G MTRR:66 genotypes (Tables 5, 6).

In addition to the analysis of subgroups with single main genotypes, it is important to determine the proportion of hyperhomocysteinemia in the case of combinations of genotypes of FC polymorphisms with different allelic variants.

The proportion of cases of hyperhomocysteinemia with a combination of genotypes that included only neutral alleles of FC polymorphisms was statistically more in boys than in girls in subgroups C/CMTHFR:677 – A/AMTR:2756 and A/AMTR:2756 – A/AMTHFR:1298 (Tables 7, 8, 11).

The proportion of cases of hyperhomocysteinemia with a combination of heterozygotes of FC polymorphisms was statistically more in boys than in girls in subgroups C/TMTHFR:677 – A/GMTRR:66, C/TMTHFR:677 – A/CMTHFR:1298, A/GMTR:2756 – A/CMTHFR:1298, A/GMTRR:66 – A/CMTHFR:1298, C/TMTHFR:677 – A/CMTHFR:1298 – A/G MTRR:66 and A/GMTR:2756 – A/CMTHFR:1298 – A/G MTRR:66 (Tables 9, 10, 11).

Table 3

The proportion of cases of hyperhomocysteinemia (H_{cy} > 10 µmol/l) in subgroups of girls with FC genotypes

Gene, polymorphism	H_{cy} > 10 µmol/l					
	Allele "neutral", homozygous variant		Allele of "risk", heterozygous variant		Allele of "risk", homozygous variant	
	n	%	n	%	n	%
MTR:A2756G	130	55.55	58	50.43	10	52.63
MTHFR:A1298C	97	55.11	91	55.49	10	35.71
MTHFR:C677T	83	50.00	92	54.12	23	71.87
MTRR:A66G	41	55.40	92	53.80	65	52.84

Table 4

Statistical differences in the proportion of cases of hyperhomocysteinemia in subgroups of girls with FC genotypes

Gene, polymorphism	Comparison indicators of the proportion of cases of hyperhomocysteinemia in subgroups					
	1 – 2		1 – 3		2 – 3	
	t	p	t	p	t	p
MTR:A2756G	0.90	0.367727	0.25	0.805919	0.18	0.859304
MTHFR:A1298C	0.07	0.943934	1.98	0.050304	2.01	0.047309
MTHFR:C677T	0.76	0.450281	2.47	0.015021	2.01	0.046488
MTRR:A66G	0.23	0.816464	0.35	0.727443	0.16	0.872211

Note. Student's t-test; p – significance level. Subgroups: "1" – allele "neutral", homozygous variant; "2" – "risk" allele, heterozygous variant; "3" – "risk" allele, homozygous variant.

Table 5

The proportion of cases of hyperhomocysteinemia in subgroups of boys with FC genotypes

Gene, polymorphism	H_{cy} > 10 µmol/l					
	Allele "neutral", homozygous variant		Allele of "risk", heterozygous variant		Allele of "risk", homozygous variant	
	n	%	n	%	n	%
MTR:A2756G	141	73.06	79	71.17	13	72.22
MTHFR:A1298C	112	73.68	100	75.19	21	56.76
MTHFR:C677T	104	67.53	107	75.89	22	81.48
MTRR:A66G	34	58.62	117	74.52	82	76.63

Table 6

Statistical differences in the proportion of cases of hyperhomocysteinemia in subgroups of boys with FC genotypes

Gene, polymorphism	Comparison indicators of the proportion of cases of hyperhomocysteinemia in subgroups					
	1 – 2		1 – 3		2 – 3	
	t	p	t	p	t	p
MTR:A2756G	0.35	0.724429	0.08	0.939403	0.09	0.926834
MTHFR:A1298C	0.29	0.770850	1.90	0.059174	2.06	0.041949
MTHFR:C677T	1.60	0.110285	1.67	0.098378	0.67	0.501930
MTRR:A66G	2.16	0.032046	2.35	0.020288	0.39	0.693438

Note. Student's t-test; p – significance level. Subgroups: "1" – allele "neutral", homozygous variant; "2" – "risk" allele, heterozygous variant; "3" – "risk" allele, homozygous variant.

In the group of girls, there were no statistical differences in the proportion of cases of hyperhomocysteinemia between subgroups of the same polymorphisms with genotypes consisting of homozygotes of neutral alleles and heterozygotes of risk alleles, as well as of homozygotes of neutral alleles and homozygotes of risk alleles (Tables 9, 13).

In the boys group, the proportion of cases of hyperhomocysteinemia was statistically more in the C/TMTHFR:677-A/GMTRR:66 subgroup compared to the C/CMTHFR:677 – A/AMTRR:66 subgroup, in the C/TMTHFR:677 – A/CMTHFR:1298 – A/G MTRR:66 compared to subgroup C/CMTHFR:677 – A/AMTHFR:1298 -A/A MTRR:66, and also, in subgroup T/TMTHFR:677 – G/GMTR:2756 compared to subgroup C/CMTHFR:677 – A/AMTR:2756 (Tables 10, 12).

Between all similar subgroups of girls and boys with combinations of homozygotes of risk alleles of the studied FC polymorphisms, statistical differences were not determined (Tables 13, 14).

The conducted studies indicate the occurrence of hyperhomocysteinemia in children – carriers of neutral alleles of FC polymorphisms.

The proportion of hyperhomocysteinemia in most subgroups with various combinations of neutral alleles of the studied FC polymorphisms was in the range of 50.0-65.2 % in girls, and in the range of 44.44-74.70 % in boys. At the same time, a statistically significant predominance of the proportion of cases of hyperhomocysteinemia in boys, compared with girls, was observed only in two genetic subgroups.

Table 7

Combinations of genotypes with neutral alleles of FC, and associated hyperhomocysteinemia, in the group of girls

Genotypes	Manifestation in a group		H_{cy} > 10 µmol/l	
	Abs. number of cases	%	Abs. number of cases	%
C/CMTHFR:677-A/AMTR:2756	106	28.80	55	51.89
C/CMTHFR:677-A/AMTRR:66	35	9.51	18	51.43
C/CMTHFR:677-A/AMTHFR:1298	58	15.76	31	53.45
A/AMTR:2756-A/AMTRR:66	46	12.5	30	65.22
A/AMTR:2756-A/AMTHFR:1298	122	33.15	66	54.10
A/AMTRR:66-A/AMTHFR:1298	32	8.69	18	56.25
C/CMTHFR:677-A/AMTR:2756-A/A MTRR:66	22	5.98	13	59.09
C/CMTHFR:677-A/AMTR:2756-A/AMTHFR:1298	40	10.87	20	50.00
C/CMTHFR:677-A/AMTHFR:1298-A/A MTRR:66	12	3.26	7	58.33
A/AMTR:2756 – A/AMTHFR:1298-A/A MTRR:66	21	5.72	13	61.90
A/AMTR:2756 – A/AMTHFR:1298-A/A MTRR:66-C/CMTHFR:677	10	2.72	6	60.00

Thus, the absence of risk alleles for several FC polymorphisms in the genome of children living near the ChEZ did not guarantee the physiological level of the H_{cy} remethylation process with the formation of internal methionine.

Table 8

Combinations of genotypes with neutral alleles of FC, and associated hyperhomocysteinemia, in the group of boys

Genotypes	Manifestation in a group		H_{cy} > 10 µmol/l	
	Abs. number of cases	%	Abs. number of cases	%
C/CMTHFR:677-A/AMTR:2756	98	30.43	65	66.33
C/CMTHFR:677-A/AMTRR:66	27	8.38	15	55.55
C/CMTHFR:677-A/AMTHFR:1298	47	14.60	31	65.96
A/AMTR:2756-A/AMTRR:66	32	9.10	20	62.50
A/AMTR:2756-A/AMTHFR:1298	83	25.77	62	74.70
A/AMTRR:66-A/AMTHFR:1298	25	7.76	15	60.00
C/CMTHFR:677-A/AMTR:2756-A/A MTRR:66	17	5.28	9	52.94
C/CMTHFR:677-A/AMTR:2756-A/AMTHFR:1298	30	9.32	18	60.00
C/CMTHFR:677-A/AMTHFR:1298-A/A MTRR:66	9	2.79	4	44.44
A/AMTR:2756-A/AMTHFR:1298-A/A MTRR:66	13	4.04	6	46.15
A/AMTR:2756-A/AMTHFR:1298-A/A MTRR:66-C/CMTHFR:677	5	1.55	0	0

We attribute this to the effect on the FC enzyme systems of long-lived radioactive elements ^{137}Cs, ^{90}Sr, ^{241}Am, which are widespread in the environment after the Chernobyl accident [9, p. 79].

With the appearance of risk alleles for FC polymorphisms in the genome of children, the proportion of cases of hyperhomocysteinemia in the group of boys increased.

Table 9

Combinations of genotypes with heterozygous variants of risk alleles of FC and associated hyperhomocysteinemia in the group of girls

Genotypes	Manifestation in a group		H_{cy} > 10 µmol/l	
	Abs. number of cases	%	Abs. number of cases	%
C/TMTHFR:677-A/GMTR:2756	56	15.22	32	57.14
C/TMTHFR:677-A/GMTRR:66	82	22.28	43	52.44
C/TMTHFR:677-A/CMTHFR:1298	84	22.83	49	58.33
A/GMTR:2756-A/GMTRR:66	54	14.67	30	55.56
A/AGMTR:2756-A/CMTHFR:1298	59	16.03	28	47.46
A/GMTRR:66-A/CMTHFR:1298	79	21.47	44	55.70
C/TMTHFR:677-A/GMTR:2756-A/G MTRR:66	29	7.78	17	58.62
C/TMTHFR:677-A/GMTR:2756-A/CMTHFR:1298	32	8.70	18	56.25
C/TMTHFR:677-A/CMTHFR:1298-A/GMTRR:66	43	11.69	23	53,49
A/GMTR:2756-A/CMTHFR:1298-A/G MTRR:66	32	8.70	17	53.13
A/GMTR:2756-A/CMTHFR:1298-A/G MTRR:66-C/TMTHFR:677	9	2.45	6	66.67

In most cases, statistical differences between subgroups of boys with heterozygotes of risk alleles and homozygotes of neutral alleles and differences in the same type of heterozygous subgroups of boys and girls were associated with C/T MTHFR:677 and A/GMTRR:66 polymorphisms, both in combination with each other and with compounding. – heterozygous

C/TMTHFR:677 – A/CMTHFR:1298. There were also differences between homozygotes of neutral alleles and risk alleles of the MTHFR:C677T polymorphism.

Table 10

Combinations of genotypes with heterozygous variants of risk alleles of FC, and associated hyperhomocysteinemia, in the group of boys

Genotypes	Manifestation in a group		H_{cy} > 10 µmol/l	
	Abs. number of cases	%	Abs. number of cases	%
C/TMTHFR:677-A/GMTR:2756	52	16.15	37	71.15
C/TMTHFR:677-A/GMTRR:66	73	22.67	59	80.82
C/TMTHFR:677-A/CMTHFR:1298	63	19.57	48	76.19
A/GMTR:2756-A/GMTRR:66	62	19.25	43	69.35
A/AGMTR:2756- A/CMTHFR:1298	39	12.11	29	74.36
A/GMTRR:66-A/CMTHFR:1298	67	20.81	54	80.60
C/TMTHFR:677-A/GMTR:2756-A/G MTRR:66	29	9.01	21	72.41
C/TMTHFR:677-A/GMTR:2756-A/CMTHFR:1298	20	6.21	15	75.00
C/TMTHFR:677-A/CMTHFR:1298-A/GMTRR:66	32	9.94	27	84.38
A/GMTR:2756-A/CMTHFR:1298-A/G MTRR:66	20	6.21	16	80.00
A/GMTR:2756-A/CMTHFR:1298-A/G MTRR:66-C/TMTHFR:677	10	3.11	8	80.00

Table 11

Statistical comparisons of the proportion of cases of hyperhomocysteinemia between similar subgroups of girls and boys

Comparison subgroups	Combination of genotypes	Student's t – test	Significance level, p
Girls&Boys	C/CMTHFR:677-A/AMTR:2756	2.12	0.035886
Girls&Boys	A/AMTR:2756-A/AMTHFR:1298	3.23	0.001576
Girls&Boys	C/TMTHFR:677-A/GMTR:2756	0.92	0.359693
Girls&Boys	C/TMTHFR:677-A/GMTRR:66	3.95	0.000146
Girls&Boys	C/TMTHFR:677-A/CMTHFR:1298	2.35	0.020888
Girls&Boys	A/GMTR:2756-A/GMTRR:66	1.54	0.127726
Girls&Boys	A/GMTR:2756-A/CMTHFR:1298	2.82	0.006735
Girls&Boys	A/GMTRR:66-A/CMTHFR:1298	3.37	0.001086
Girls&Boys	C/TMTHFR:677-A/GMTR:2756-A/GMTRR:66	1.12	0.271912
Girls&Boys	C/TMTHFR:677-A/GMTR:2756-A/CMTHFR:1298	1.44	0.161500
Girls&Boys	C/TMTHFR:677-A/CMTHFR:1298-A/G MTRR:66	3.10	0.003243
Girls&Boys	A/GMTR:2756 – A/CMTHFR:1298 - A/G MTRR:66	2.14	0.040642
Girls&Boys	A/GMTR:2756 – A/CMTHFR:1298-A/G MTRR:66-C/TMTHFR:677	0.66	0.522287

Genotypes that include the risk allele T of the MTHFR:677 polymorphism cause disruption of the functioning of methylenetetrahydrofolate reductase, the main enzyme of FC, resulting in a decrease in the formation of

5-methyltetrahydrofolate – the main supplier of the methyl group for the Hcy remethylation reaction.

Table 12

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

Comparison subgroups		Student's t – test	Significance level, p
C/CMTHFR:677-A/AMTRR:66	C/TMTHFR:677-A/GMTRR:66	2.38	0.020001
C/CMTHFR:677-A/AMTHFR:1298-A/A MTRR:66	C/TMTHFR:677-A/CMTHFR:1298-A/G MTRR:66	2.25	0.032582
C/CMTHFR:677-A/AMTR:2756	T/TMTHFR:677-G/GMTR:2756	7.06	0.00001

The risk allele G of the MTRR:66 polymorphism disrupts the functioning of methionine synthase reductase, which is involved in the restoration of the activity of B12-dependent methionine synthase, an enzyme that promotes the transfer of the methyl group to Hcy.

Thus, the appearance in the genome of boys living near the ChEZ of risk alleles of MTHFR:677 and MTRR:66 polymorphisms negatively affects the processes of Hcy remethylation and leads to hyperhomocysteinemia.

In the subgroups of girls with heterozygous and homozygous variants of the risk allele T of the MTHFR:C677T polymorphism, the proportion of cases of hyperhomocysteinemia is statistically greater than in the subgroup with the homozygous variant of the neutral allele C. However, when several polymorphisms are combined with risk alleles, there is no significant increase compared to the same type homozygous variants of the neutral allele.

The absence of differences, in girls, between the groups of the same name with combined variants of several polymorphisms of homozygotes of neutral alleles and combined variants of heterozygotes, as well as homozygotes of risk alleles, can be explained by the utilization of Hcy in the trans-sulfurization cycle. As previous studies have shown, this cycle of reactions functions more intensively in the body of girls, in comparison with the body of boys [8, p. 280]. Ultimately, during these reactions,

Table 13

Combinations of genotypes with homozygous variants of FC risk alleles and associated hyperhomocysteinemia in the group of girls

Genotypes	Manifestation in a group		H_{cy} > 10 µmol/l	
	Abs. number of cases	%	Abs. number of cases	%
T/TMTHFR:677-G/GMTR:2756	0	0	0	0
T/TMTHFR:677-G/GMTRR:66	9	2.45	6	66.67
T/TMTHFR:677-C/CMTHFR:1298	0	0	0	0
G/GMTR:2756-G/GMTRR:66	4	1.09	3	75.00
G/GMTR:2756-C/CMTHFR:1298	3	0.81	1	33.33
G/GMTRR:66-C/CMTHFR:1298	8	2.17	3	37.50
T/TMTHFR:677-G/GMTR:2756-G/G MTRR:66	0	0	0	0
T/TMTHFR:677-G/GMTR:2756-C/CMTHFR:1298	0	0	0	0
T/TMTHFR:677-C/CMTHFR:1298-G/GMTRR:66	0	0	0	0
G/GMTR:2756-C/CMTHFR:1298-G/G MTRR:66	0	0	0	0
G/GMTR:2756-C/CMTHFR:1298-G/G MTRR:66-T/TMTHFR:677	0	0	0	0

reduced glutathione is formed, which provides antioxidant protection for cells [10, p. 580].

Based on the results of the studies, it can be concluded that external and internal (FC genes) factors are involved in the occurrence of H_{cy}

Table 14

Combinations of genotypes with homozygous variants of FC risk alleles, and associated hyperhomocysteinemia, in the group of boys

Genotypes	Manifestation in a group		H_{cy} > 10 µmol/l	
	Abs. number of cases	%	Abs. number of cases	%
T/TMTHFR:677-G/GMTR:2756	2	0.62	2	100.0
T/TMTHFR:677-G/GMTRR:66	12	3.73	8	66.67
T/TMTHFR:677-C/CMTHFR:1298	0	0	0	0
G/GMTR:2756-G/GMTRR:66	4	1.24	2	50.00
G/GMTR:2756-C/CMTHFR:1298	3	0.93	2	66.67
G/GMTRR:66-C/CMTHFR:1298	13	4.04	9	69.23
T/TMTHFR:677-G/GMTR:2756-G/G MTRR:66	0	0	0	0
T/TMTHFR:677-G/GMTR:2756-C/CMTHFR:1298	0	0	0	0
T/TMTHFR:677-C/CMTHFR:1298-G/GMTRR:66	0	0	0	0
G/GMTR:2756-C/CMTHFR:1298-G/G MTRR:66	2	0.62	1	50.00
G/GMTR:2756-C/CMTHFR:1298-G/G MTRR:66-T/TMTHFR:677	0	0	0	0

remethylation disorders with the formation of internal methionine in children living near the ChEZ.

The more proportion of cases of hyperhomocysteinemia in the subgroups of boys and girls living near the ChEZ, with combinations of homozygotes of neutral alleles of the investigated FC polymorphisms, indicates an

environmental impact on the enzyme systems involved in the metabolic transformations of H_{cy}.

Taking into account the results of studies of the level of H_{cy} in the blood of the same children before and after fires of forest trees in the ChEZ, it can be argued that the radiation factor has a negative effect on FC enzymes [1, p. 25; 11, p. 24].

An increase in the proportion of cases of hyperhomocysteinemia occurred in groups of boys with the appearance in their genome of risk alleles for FC polymorphisms, mainly MTHFR:C677T, to a greater extent in combination with MTRR:A66G.

An increase in the Hcy content in the blood in the overwhelming majority of cases occurs with the T/T MTHFR:C677T genotype, while in the case of the heterozygous C/T MTHFR:C677T variant, this can occur with disruption of the functioning of enzymes that provide the final stages of H_{cy} methylation.

Thus, mutations in the FC genes (T/T MTHFR:677, C/T MTHFR:677 in combination with A/G MTRR:66), causing disruption of the activity of the FC enzymes methylenetetrahydrofolate reductase and methionine synthase reductase, are an internal factor contributing to the occurrence hyperhomocysteinemia.

The results of the conducted studies can be used in the development of methods for the prevention and treatment of conditions associated with impaired metabolism of sulfur-containing amino acids.

4. Conclusions

The occurrence of hyperhomocysteinemia in children living near the ChEZ can occur with a combination of homozygous variants of neutral alleles of genetic polymorphisms of FC, which indicates an external environmental impact, including a radiation factor.

The presence in the genome of boys of combinations of risk alleles for FC polymorphisms, with the participation of MTHFR:677, as well as MTRR:A66G, contributes to an increase in the level of H_{cy} in the blood.

Mutations in the FC genes (T/T MTHFR:677, C/T MTHFR:677 in combination with A/G MTRR:66), in which there is a violation of the activity of the enzymes of FC – methylenetetrahydrofolate reductase and methionine synthase reductase, are an internal factor contributing to the occurrence of hyperhomocysteinemia.

In girls with a combination of heterozygotes and homozygotes of risk alleles for FC polymorphisms, increased amounts of Hcy are utilized in the cycle of trans-sulfurization reactions.

References:

1. Bandazhevsky Yu. I., Dubovaya N. F. (2021) Forest fires in the Chernobyl exclusion zone and children's health. Ivankov: PI Coordination and Analytical Center «Ecology and health». Kyiv: "Aliant" LLC, 44 p.

2. Bandazhevsky Yu. I., Dubova N. F. (2022) Hyperhomocysteinemia in Ukrainian children living near the Chernobyl Exclusion Zone: International scientific conference "New trends and unsolved issues in medicine": conference proceedings (July 29-30, 2022. Riga, the Republic of Latvia). Riga, Latvia: "Baltija Publishing", pp. 263–266. DOI: https://doi.org/10.30525/978-9934-26-226-5-69

3. Bandazhevsky Yu. I., Dubovaya N. F. (2022) Chernobyl catastrophe and childrens health. 35 years of world tragedy. Ivankov: PI Coordination and Analytical Center "Ecology and health". Kyiv: "Alyant" LLC, 158 p.

4. Bandazhevskyi Yu. I., Dubova N. F. (2022) Genetic control of homocysteine metabolism in children living nearthe Chernobyl exclusion zone. *Environment & Health,* no. 2 (103), pp. 10–15. DOI: https://doi.org/10.32402/dovkil2022.02.010

5. Lai W.K., Kan M.Y. (2015) Homocysteine-Induced Endothelial Dysfunction. *Ann Nutr Metab,* 67(1): 1–12. DOI: https://doi.org/10.1159/000437098. Epub 2015 Jul 18. PMID: 26201664.

6. Keshteli A., Baracos V., Madsen K. (2015) Hyperhomocysteinemia as a potential contributor of colorectal cancer development in inflammatory bowel diseases: A review. *World J Gastroenterol,* 21(4): 1081–1090. DOI: https://doi.org/10.3748/wjg.v21.i4.1081

7. Moretti, R., Caruso, P. (2019) The Controversial Role of Homocysteine in Neurology: From Labs to Clinical Practice. *Int. J. Mol. Sci.*, 20, 231. DOI: https://doi.org/10.3390/ijms20010231

8. Bandazheuski Yu., Dubovaya N. (2022) Hyperhomocysteinemia and folate genes in girls and boys living near the Chernobyl Exclusion Zone. *Traditional and innovative approaches to scientific research: theory, methodology, practice: Scientific monograph.* Riga, Latvia: Baltija Publishing, pp. 268–282. DOI: https://doi.org/10.30525/978-9934-26-241-8-12

9. Chernobyil 25 let: inkorporirovannyie radionuklidyi Cs-137 i zdorove lyudey (2011) / Pod red. prof. Yu. I. Bandazhevskogo. Kyiv: Koordinatsionnyiy analiticheskiy tsentr "Ekologiya i zdorove", 156 p. (in Russian)

10. Dimitrova K. R., DeGroot K., Myers A. K., Kim Y. D. (2002, February) Estrogen and homocysteine. *Cardiovascular Research*, vol. 53, issue 3, pp. 577–588. DOI: https://doi.org/10.1016/S0008-6363(01)00462-X

11. Bandazhevskyi Yu. I., Dubovaya N. F. (2020) Hyperhomocysteinemia in children and forest fires in the Chornobyl exclusion zone. *Environment & Health*, no. 4(97), pp. 21–26.