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FOLATE CYCLE GENES, HYPERHOMOCYSTEINEMIA, AND STRESS RESPONSE IN CHILDREN LIVING NEAR THE CHORNOBYL EXCLUSION ZONE

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- **THE AIM** of this study was to examine the relationship between homocysteine (H_{cy}) and cortisol (Cortisol), depending on the state of genetic polymorphisms of the folate cycle, in children living near the Chornobyl Exclusion Zone (ChEZ) 30 or more years after the Chornobyl accident.
- **RESEARCH METHODS.** Immunochemical, mathematical and statistical.
- **RESULTS.** A correlation analysis was conducted between the studied indicators in a group of 158 children (78 boys and 80 girls), aged 12–18 years, permanently residing in populated areas of the Polessky district of the Kyiv region. The participation of Hcy and genetic polymorphisms of folate cycle (FC) in the regulation of the content of the stress hormone Cortisol in the blood was revealed. In the group of girls, a connection was established between H_{cy}, Cortisol, and the GMTR:2756 allele, which significantly affects the process of H_{cy} methylation. In the blood of girls carriers of the GMTR:2756 allele, which negatively affects the activity of B_{12} dependent methionine synthase, the content of Cortisol in the blood is increased, in comparison with girls without this allele in the genome. The concentration of Cortisol in the blood of girls in the subgroups with the genetic alleles GMTR:2756, AMTHFR:1298, CMTHFR:677, GMTRR:66 and the general group, with the level of $H_{cy} > 10.0$ µmol/l, was statistically higher than with the level of $H_{cy} \leq 10.0$ µmol/l. At the same time, the proportion of cases with the allele GMTR:2756 was more. A direct correlation between the values of H_{cy} and Cortisol in the blood was noted in the subgroups with the genetic alleles GMTR:2756, AMTHFR:1298 and in the general group of girls, whereas among boys this relationship was recorded only in the subgroup with the TMTHFR:677 allele.
- **CONCLUSIONS.** Radiation exposure induces metabolic stress in the bodies of children living near the ChEZ, which, like mutations in the FC genes, causes an increase in the Hcy content in the blood. With moderate hyperhomocysteinemia, the antioxidant system is activated, one of the elements of which is Cortisol. In this regard, H_{cy} should be considered as a factor in stabilizing metabolic processes when exposed to incorporated ^{137}Cs.

- **KEYWORDS:** *homocysteine, Cortisol, stress response, folate cycle genes, children, Chornobyl Exclusion Zone.*

ГЕНИ ФОЛАТНОГО ЦИКЛУ, ГІПЕРГОМОЦИСТЕЇНЕМІЯ І СТРЕС-РЕАКЦІЯ У ДІТЕЙ, ЯКІ МЕШКАЮТЬ ПОБЛИЗУ ЧОРНОБИЛЬСЬКОЇ ЗОНИ ВІДЧУЖЕННЯ

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- **МЕТА ДОСЛІДЖЕННЯ:** вивчення зв'язку між гомоцистеїном (H_{cy}) та кортизолом (Cortisol) залежно від стану генетичних поліморфізмів фолатного циклу у дітей, які мешкають поблизу Чорнобильської зони відчуження через 30 і більше років після аварії на ЧАЕС.
- **Методи дослідження.** Імунохімічний, математико-статистичний.
- **РЕЗУЛЬТАТИ.** Проведено кореляційний аналіз між показниками, що вивчаються у групі 158 дітей (78 хлопчиків та 80 дівчаток), у віці 12–18 років, які постійно проживають у населених пунктах Поліського району Київської області. Виявлено участь Hсу та генетичних поліморфізмів фолатного циклу (ФЦ) у регуляції вмісту в крові стрес-гормону Cortisol. У групі дівчаток був встановлений зв'язок між H_{cy}, Cortisol, і аллелю GMTR:2756, що суттєво впливає на процес метилювання H_{cy}. У крові дівчаток-носіїв алелі GMTR:2756, що негативно впливає на активність B_{12}-метіонін синтази, збільшено вміст у крові Cortisol у порівнянні з дівчатками з відсутністю цієї алелі в геномі. Концентрація Cortisol в крові дівчаток у підгрупах з генетичними алелями GMTR:2756, AMTHFR:1298, CMTHFR:677, GMTRR:66 та загальній групі, при рівні $H_{cy} > 10,0$ мкмоль/л, була статистично більшою, ніж при рівні $H_{cy} \leq 10,0$ мкмоль/л. При цьому, питома вага випадків з алелею GMTR:2756 була більшою. Прямий кореляційний зв'язок між значеннями H_{cy} і Cortisol у крові відзначений у підгрупах з генетичними алелями GMTR:2756, AMTHFR:1298 та у загальній групі дівчаток, тоді як серед хлопчиків цей зв'язок реєструвався тільки в підгрупі з алелею TMTHFR:677.
- **ВИСНОВКИ.** Радіаційний вплив індукує в організмі дітей, які мешкають поблизу ЧЗО, метаболічний стрес, що, як і мутації генів ФЦ, є причиною збільшення вмісту у крові Hсу. При помірно вираженій

гіпергомоцистеїнемії відбувається активація антиоксидантної системи, одним із елементів якої є Cortisol цього зв'язку, слід розглядати H_{cy} як фактор стабілізації метаболічних процесів при дії інкорпорованого ^{137}Cs.

КЛЮЧОВІ СЛОВА: *гомоцистеїн, кортизол, стрес-реакція, гени фолатного циклу, діти, Чорнобильська зона відчуження.*

INTRODUCTION

Children living near the Chornobyl Exclusion Zone (ChEZ) 30 or more years after the Chornobyl Nuclear Power Plant (ChNPP) accident were found to have metabolic disorders in the cells of vital organs caused by the effect of incorporated ^{137}Cs radionuclides on mitochondrial structures [1].

The resulting energy deficit contributed to an increase in the blood content of the sulfur-containing amino acid homocysteine (H_{cy}), which affects calcium metabolism [2, 3].

This process was intensified after forest fires in the ChEZ [4].

An endogenous factor, in the form of risk alleles of genetic polymorphisms of the folate cycle (FC), also contributed to an increase in the H_{cy} content in the blood [1].

Correlation analysis revealed the involvement of H_{cy} in the formation of active forms of thyroid hormones [5].

Studies of stress reactions that occur in the body of children under the influence of incorporated radionuclides ^{137}Cs, taking into account the level of H_{cy} in the blood, are of great scientific and practical interest.

It is advisable to determine the relationship between H_{cy} and the hormone of the subrenal cortex, cortisol (Cortisol), which regulates metabolism in the body under stressful conditions, in children living near the ChEZ.

The aim of this study was to examine the relationship between H_{cy} and Cortisol, depending on the state of genetic polymorphisms of FC, in children living near the ChEZ 30 or more years after the Chornobyl accident.

MATERIAL AND METHODS

The study of the relationship between H_{cy} and Cortisol was conducted in a group of 158 children (78 boys and 80 girls), aged 12–18 years, permanently residing in settlements of the Polessky district of the Kyiv region, near the ChEZ, in the territory, the soils of which contained radioactive elements ^{137}Cs and ^{90}Sr [6].

The examination of children within the framework of the projects of the European Commission and the regional council of Rhone-Alpes (France), was agreed with their parents, and was carried out in compliance with the rules of bioethics.

In this case, in children who attended school, in the morning, on an empty stomach, blood was taken from the cubital vein in order to determine the content of H_{cy} and Cortisol in the blood, as well as to assess the genetic polymorphisms of FC.

The study of blood samples was carried out in a laboratory certified in accordance with European quality standards.

H_{cy} was determined in blood using an immunochemical method with chemiluminescent detection (ECLIA). Analyzer and test system: Architect 1000 (ABBOT Diagnostics (USA).

Exceeding the concentration of H_{cy} in the blood of 10.0 μmol/l in the examined children was recorded as a state of hyperhomocysteinemia.

Cortisol was determined using an immunochemical method with electrochemiluminescent detection (ECLIA). Analyzer and test system: Cobas 6000, Roche Diagnostics (Switzerland).

The reference interval of extreme values for Cortisol was — 6.2–19.4 μg/dl.

During the genetic study of FC, allelic variants C677T and A1298C of the MTHFR gene (association with the enzyme methylenetetrahydrofolate reductase), A2756G of the MTR gene (association with the enzyme B_{12}-dependent methionine synthase), A66G of the MTRR gene (association with the enzyme methionine synthase reductase) were determined. In this case, the method was used: PCR in Real-time mode.

Statistical processing of the obtained results was carried out using the IBM SPSS Statistics 22 program (USA). For the analyzed indicators, the median (Me), interquartile range (IQR), minimum and maximum parameter values, percentiles were calculated. A hypothesis about the type of distributions was tested (Kolmogorov-Smirnov criterion). All the studied parameters did not correspond to the normal distribution law, and therefore, the nonparametric 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 program.

To compare relative indicators, Student's *t*-test was used. The critical level of reliability of the null statistical hypothesis (p) was taken as 0.05. The relationship between the H_{cy} and Cortisol indicators was determined using the coefficient Spearman's rank correlation (r_{xy}). The strength of the correlation was assessed using the traditional scale: weak — from 0 to 0.299; average — from 0.3 to 0.699; strong — from 0.7 to 1.0.

RESULTS AND DISCUSSION

The H_{cy} level >10.0 μmol/l in the blood was detected in 84 of the 158 examined children (53.16%).

The range of H_{cy} values was 5.85–77.21 μmol/l ($n=78$) in the group of boys, 5.54–21.45 μmol/l ($n=80$) in the group of girls.

At the same time, an excess of 20.0 μmol/l was noted in 9 boys (11.54%) and in 1 girl (1.25%).

In the general group and genetic subgroups of boys with the main genotypes A/A MTR:2756, A/C, C/CMTHFR:1298, C/T, T/TMTHFR:677, A/G, G/G MTRR:66, the values of the indicators of the proportion of hyperhomocysteinemia cases were statistically more than in the general group and similar subgroups of girls (*Tables 1, 2*).

The highest proportion of hyperhomocysteinemia cases was recorded in the subgroup of boys who were carriers of the TMTHFR:677 risk allele (C/T, T/T MTHFR:677 genotypes), whereas in the subgroup of boys who did not have this allele in their genome (C/CMTHFR:677 genotype), the proportion of hyphomocysteinemia cases was significantly less ($t=2.45$; $p=0.018085$).

The blood Cortisol level exceeded the upper limit of the reference range established by the laboratory (6.2–19.4 μg/dl) in 22 children (13.92% of the number of examined children).

Among 84 children with hyperhomocysteinemia, 22 had an increase in the blood Cortisol level >19.4 μg/dl (26.19%).

Among 22 children with the Cortisol level >19.4 μg/dl, in 16 cases (72.73%) the blood H_{cy} level exceeded 10.0 μmol/l.

In the group of girls, in contrast to the group of boys, the concentration of Cortisol in the subgroup including cases with the GMTR:2756 allele (A/G MTR:2756 and G/GMTR:2756 genotypes), was significantly more than in the subgroup including cases without the GMTR:2756 allele — A/AMTR:2756 genotype (*Tables 3, 4*).

In the general group of girls, in contrast to the general group of boys, as well as in genetic subgroups of girls No. 2 (genotypes A/G MTR:2756,

Table 1. **The proportion of cases of hyperhomocysteinemia in genetic subgroups of boys and girls**

N	Genotype main	Boys			Girls		
		N_1	N_2	%	N_1	N_2	%
1	A/A MTR:2756	49	35	71.43	55	23	41.82
2	A/G, G/G MTR:2756	29	15	51.72	25	11	44.00
3	A/A MTHFR:1298	36	22	61.11	46	20	43.48
4	A/C, C/C MTHFR:1298	42	28	66.67	34	14	41.18
5	C/C MTHFR:677	39	20	51.28	40	14	35.00
6	C/T, T/T MTHFR:677	39	30	76.92	40	20	50.00
7	A/A MTRR:66	16	8	50.00	16	4	25.00
8	A/G, G/G MTRR:66	62	42	67.74	64	30	46.88
9	General group	78	50	64.10	80	34	42.50

Note: N is the subgroup number; N_1 is the number of children in the subgroup; N_2 is the number of cases $H_{cy}>10.0$.

Table 2. **Statistical differences in the proportion of hyperhomocysteinemia cases in genetic subgroups of boys and girls**

N	Genotype main	Student's *t*-test	Significance level, *p*
1	A/A MTR:2756	3.20	0.002308
2	A/G, G/G MTR:2756	0.57	0.575532
3	A/A MTHFR:1298	1.61	0.114910
4	A/C, C/C MTHFR:1298	2.29	0.027624
5	C/C MTHFR:677	1.48	0.148724
6	C/T, T/TMTHFR:677	2.59	0.012778
7	A/A MTRR:66	1.51	0.164929
8	A/G, G/G MTRR:66	2.42	0.018099
9	General group	3.17	0.002125

Note: N is the subgroup number.

Table 3. **Statistical characteristics of Cortisol values (mcg/dL) in genetic subgroups of boys and girls**

N_1	Genotype main	Boys		Girls	
		Me	IQR	Me	IQR
1	A/A MTR:2756	13.260	9.940–17.825	12.110	9.060–16.220
2	A/G, G/G MTR:2756	11.800	9.260–16.240	14.800	11.100–21.760
3	A/A MTHFR:1298	13.015	9.723–16.265	13.810	9.363–17.613
4	A/C, C/CMTHFR:1298	13.410	9.470–17.745	13.070	9.550–17.038
5	C/C MTHFR:677	15.260	10.430–18.030	12.335	9.235–17.420
6	C/T, T/T MTHFR:677	11.800	9.490–16.130	13.995	10.293–16.965
7	A/A MTRR:66	12.090	8.320–17.470	12.070	9.268–17.270
8	A /G, G/G MTRR:66	13.410	10.413–17.478	13.815	13.815–17.393
	General group	13.135	9.580–17.478	13.415	9.468–17.370

Table 4. **Results of statistically significant differences when comparing Cortisol levels in the blood of the examined girls**

Group	Subgroups comparison	Number of comparison subgroups	Average rank	*U*-criterion value, significance level *p*
Girls	1 2	55 25	36.64 49.00	$U = 475.000$ $p = 0.027$

G/G MTR:2756), No. 3 (A/A MTHFR:1298 genotype), No. 5 (C/C MTHFR:677 genotype) and No. 8 (A/G MTRR:66, G/G MTRR:66 genotypes), the concentration of Cortisol in the blood, with an H_{cy} level of >10.0 μmol/l, was statistically more than with an H_{cy} level of ≤10.0 μmol/l (*Tables 5–7*).

The TMTHFR:677 allele, which has a significant effect on the activity of methylenetetrahydrofolate

Table 5. **Statistical characteristics of Cortisol values (mcg/dL) depending on the level of H_{cy} in the blood in genetic subgroups of boys**

N_1	Genotype main	H_{cy} level, μmol/l	N_2	Indicators Cortisol	
				Me	IQR
1	A/A MTR:2756	≤10.0 >10.0	14 35	13.75 12.96	10.09–17.63 9.49–18.03
2	A/G, G/GMTR:2756	≤10.0 >10.0	14 15	14.89 11.80	11.29–16.51 10.32–16.91
3	A/A MTHFR:1298	≤10.0 >10.0	14 22	13.17 12.69	9.86–16.21 9.41–16.60
4	A/C, C/C MTHFR:1298	≤10.0 >10.0	14 28	15.65 12.03	11.37–17.96 8.95–17.93
5	C/C MTHFR:677	≤10.0 >10.0	19 20	15.62 13.05	12.08–18.90 8.78–17.93
6	C/T, T/T MTHFR:677	≤10.0 >10.0	9 30	11.45 12.11	6.94–14.88 9.58–16.60
7	A/A MTRR:66	≤10.0 >10.0	8 8	14.86 11.16	7.18–19.03 8.76–16.29
8	A /G, G/G MTRR:66	≤10.0 >10.0	20 42	14.20 12.69	11.48–16.05 9.41–17.53
	General group	≤10.0 >10.0	28 50	14.20 12.34	10.98–17.02 9.15–17.52

Note: N_1 is the genetic subgroup; N_2 is the number of cases; Me is the median; IQR is the interquartile range.

Table 6. **Statistical characteristics of Cortisol indicators (mcg/dL) depending on the level of H in the blood of genetic subgroups of girls**

N_1	Genotype main	H_{cy} level, μmol/l	N_2	Indicators Cortisol	
				Me	IQR
1	A/AMTR:2756	≤10.0 >10.0	32 23	10.75 13.71	8.99–16.18 10.34–16.80
2	A/G, G/GMTR:2756	≤10.0 >10.0	14 11	11.67 21.65	9.52–14.90 15.14–24.54
3	A/A MTHFR:1298	≤10.0 >10.0	26 20	10.53 15.26	9.00–15.96 13.27–21.89
4	A/C, C/CMTHFR:1298	≤10.0 >10.0	20 14	12.16 13.51	9.37–16.52 10.02–17.63
5	C/C MTHFR:677	≤10.0 >10.0	26 14	10.96 15.97	8.93–16.39 12.49–21.53
6	C/T, T/T MTHFR:677	≤10.0 >10.0	20 20	12.30 14.90	10.15–15.23 10.47–21.34
7	A/A MTRR:66	≤10.0 >10.0	12 4	11.00 15.10	9.03–16.07 11.00–20.83
8	A/G, G/G MTRR:66	≤10.0 >10.0	34 30	11.41 15.07	9.13–15.96 11.44–21.53
General group		≤10.0 >10.0	46 34	11.32 15.07	9.13–16.00 11.44–21.53

Note: N_1 is the genetic subgroup; N_2 is the number of cases; Me is the median; IQR is the interquartile range.

Table 7. **Results of statistically significant differences when comparing Cortisol levels in the blood of the examined girls**

N	H_{cy} level, μmol/l	Number of comparison subgroups	Average rank	*U*-criterion value, significance level *p*
2	≤10.0	14	8.71	*U* = 17.000 *p* = 0.001
	>10.0	11	18.45	
3	≤10.0	26	18.42	*U* = 128.000 *p* = 0.003
	>10.0	20	30.10	
5	≤10.0	26	17.10	*U* = 93.500 *p* = 0.012
	>10.0	14	26.82	
8	≤10.0	34	27.35	*U* = 335.000 *p* = 0.019
	>10.0	30	38.33	
General group	≤10.0	46	34.37	*U* = 500.000 *p* = 0.006
	>10.0	34	48.79	

Note: N is the genetic subgroup number.

reductase, was registered more often in the examined group of boys and girls than the GMTR:2756 allele, which has an effect on the activity of B_{12}-methionine synthase (*Tables 8, 9*).

Among girls in subgroup No. 2, the proportion of cases with the TMTHFR:677 allele was significantly more in subgroup No. 1, whereas in the group of boys these differences were absent (*Tables 8, 9*).

In the overall group and all subgroups of boys with H_{cy} levels >10.0 μmol/l, the proportion of cases with the TMTHFR: 677 allele was more than in the overall group and similar subgroups with H_{cy} levels ≤10.0 μmol/l (*Table 10*).

In the overall group and most subgroups of girls with H_{cy} levels >10 μmol/l, the proportion of cases with the TMTHFR: 677 allele was more than in the

Table 8. **The proportion of cases with TMTHFR:677 and GMTR:2756 alleles in genetic subgroups of boys**

N	**Genotype main**	**N_1**	**Risk allele TMTHFR:677**		**Risk allele GMTR:2756**	
			N_2	**%**	**N_2**	**%**
1	A/A MTR:2756	49	25	51.02	0	0
2	A/G, G/G MTR:2756	29	14	48.28	29	100
3	A/A MTHFR:1298	36	27	75.00	14	38.89
4	A/C, C/C MTHFR:1298	42	12	28.57	15	35.71
5	C/C MTHFR:677	39	0	0	15	38.46
6	C/T, T/T MTHFR:677	39	100	100	14	35.90
7	A/A MTRR:66	16	9	56.25	5	31.25
8	A/G, G/G MTRR:66	62	30	48.39	24	38.71
	General group	78	39	50.00	29	37.18

Note: N is the genetic subgroup number; N_1 is the number of children in the subgroup or group; N_2 is the number of cases with the risk allele.

Table 9. **The proportion of cases with TMTHFR:677 and GMTR:2756 alleles in genetic subgroups of girls**

N	**Genotype main**	**N_1**	**Risk allele T MTHFR:677**		**Risk allele GMTR:2756**	
			N_2	**%**	**N_2**	**%**
1	A/A MTR:2756	55	25	45.45	0	0
2	A/G, G/G MTR:2756	25	15	60.00	25	100
3	A/A MTHFR:1298	46	27	58.70	16	34.78
4	A/C, C/C MTHFR:1298	34	13	38.24	9	26.47
5	C/C MTHFR:677	40	0	0	10	25.00
6	C/T, T/T MTHFR:677	40	40	100	15	37.50
7	A/A MTRR:66	16	5	31.25	5	31.25
8	A/G, G/G MTRR:66	64	35	54.69	20	31.25
	General group	80	40	50.00	25	31.25

Note: N is the genetic subgroup number; N_1 is the number of children in the subgroup or group; N_2 is the number of cases with the risk allele.

overall group and similar subgroups with H_{cy} levels ≤10.0 μmol/l. Subgroups No. 2 and 7 were the exception (*Table 11*).

In the overall group and all subgroups of boys with H_{cy} level >10.0 μmol/l, the proportion of cases with the GMTR:2756 allele was less than in the overall group and similar subgroups with H_{cy} level ≤10.0 μmol/l (*Table 10*).

In the overall group and subgroups of girls No. 3, 5, 8 with H_{cy} level >10.0 μmol/l, the proportion of cases with the GMTR:2756 allele was more than in similar subgroups with H_{cy} level ≤10.0 μmol/l (*Table 11*).

In the general group and genetic subgroups of girls No. 2, 3, a direct correlation was found between H_{cy} and Cortisol.

In the group of boys, this relationship was recorded in subgroup No. 6 (*Table 12*).

Studies conducted in areas bordering the ChEZ indicate adverse radiation effects on the body of adolescent children.

It has been established that relatively small amounts of incorporated ^{137}Cs radionuclides cause damage to mitochondria, leading to energy deficiency [1].

Active forms of oxygen are formed in the mitochondria, in particular, superoxide and hydrogen peroxide, which have a destructive effect on cellular structures [7–9].

At the same time, mitochondrial permeability changes [10], including for ionized calcium.

Moderate hyperhomocysteinemia was recorded in children living near the ChEZ, under conditions of constant radiation exposure (in most analyzed cases, the H_{cy} level did not exceed 20.0 μmol/l).

It should be noted that the TMTHFR:677 allele plays a significant role in increasing the H_{cy} level in the blood of these children.

The proportion of cases of hyperhomocysteinemia in the overall group and individual genetic subgroups of boys was greater than in the overall group and similar genetic subgroups of girls.

Table 10. **The proportion of cases of carriage of TMTHFR: 677 and GMTR:2756 alleles, taking into account the level of H_{cy} in the blood, in genetic subgroups of boys**

N	Genotype main	A	N_1	TMTHFR: 677		GMTR:2756	
				N_1	%	N_1	%
1	A/A MTR:2756	≤10.0 >10.0	14 35	4 21	28.57 60.00	0 0	0 0
2	A/G, G/G MTR:2756	≤10.0 >10.0	14 15	5 9	35.71 60.00	14 15	100 100
3	A/A MTHFR:1298	≤10.0 >10.0	14 22	8 19	57.14 86.36	8 6	57.14 27.27
4	A/C, C/C MTHFR:1298	≤10.0 >10.0	14 28	1 11	7.14 39.29	6 9	42.86 32.14
5	C/C MTHFR:677	≤10.0 >10.0	19 20	0 0	0 0	9 6	47.37 30.00
6	C/T, T/T MTHFR:677	≤10.0 >10.0	9 30	9 30	100 100	5 9	55.56 30.00
7	A/A MTRR:66	≤10.0 >10.0	8 8	4 5	50.00 62.50	2 3	25.00 37.50
8	A/G, G/G MTRR:66	≤10.0 >10.0	20 42	5 25	25.00 59.52	12 12	60.00 28.57
	General group	≤10.0 >10.0	28 50	9 30	32.14 60.00	14 15	50.00 30.00

Note: A is the H_{cy} level, μmol/l; N is the group number; N_1 is the number of cases.

Table 11. **The proportion of cases of carriage of TMTHFR:677 and GMTR:2756 alleles, taking into account the level of H_{cy} in the blood, in genetic subgroups of girls**

N	Genotype main	A	N_1	TMTHFR:677		GMTR:2756	
				N_1	%	N_1	%
1	A/A MTR:2756	≤10.0 >10.0	32 23	11 14	34.38 60.87	0 0	0 0
2	A/G, G/G MTR:2756	≤10.0 >10.0	14 11	9 6	64.29 54.55	14 11	100 100
3	A/A MTHFR:1298	≤10.0 >10.0	26 20	14 13	53.85 65.00	8 8	30.77 40.00
4	A/C, C/C MTHFR:1298	≤10.0 >10.0	20 14	6 7	30.00 50.00	6 3	30.00 21.43
5	C/C MTHFR:677	≤10.0 >10.0	26 14	0 0	0 0	5 5	19.23 35.71
6	C/T, T/T MTHFR:677	≤10.0 >10.0	20 20	20 20	100 100	9 6	45.00 30.00
7	A/A MTRR:66	≤10.0 >10.0	12 4	4 1	33.33 25.00	4 1	33.33 25.00
8	A/G, G/G MTRR:66	≤10.0 >10.0	34 30	16 19	47.06 63.33	10 10	29.41 33.33
General group	≤10.0 >10.0	46 34	20 20	43.48 58.82	14 11	30.43 32.35	

Note: A is the H_{cy} level (μmol/l); N is the group number; N_1 is the number of cases.

Table 12. **Results of correlation analysis in genetic subgroups of children**

N	Genotype main	Correlation coefficient	H_{cy} — Cortisol	
			Boys	**Girls**
1	A/A MTR:2756	Spearman’s Sign. (2-tailed), *p* N	–0.015 0.916 49	0.123 0.369 55
2	A/G, G/G MTR:2756	Spearman’s Sign. (2-tailed), *p* N	0.079 0.683 29	0.503* 0.010 25
3	A/A MTHFR:1298	Spearman’s Sign. (2-tailed), *p* N	0.183 0.285 36	0.354* 0.016 46
4	A/C, C/C MTHFR:1298	Spearman’s Sign. (2-tailed), *p* N	–0.044 0.784 42	–0.002 0.993 34
5	C/C MTHFR:677	Spearman’s Sign. (2-tailed), *p* N	–0.041 0.803 39	0.181 0.263 40
6	C/T, T/T MTHFR:677	Spearman’s Sign. (2-tailed), *p* N	0.360* 0.024 39	0.270 0.092 40
7	A/A MTRR:66	Spearman’s Sign. (2-tailed), *p* N	0.250 0.350 16	0.209 0.438 16
8	A/G, G/G MTRR:66	Spearman’s Sign. (2-tailed), *p* N	0.034 0.792 62	0.243 0.053 64
General group		Spearman’s Sign. (2-tailed), *p* N	0.059 0.606 78	0.223* 0.047 80

Note: * — correlation is significant at the 0.05 level (two-sided); N is the subgroup number; H_{cy} is the homocysteine; Cortisol — cortisol.

Correlations between H_{cy} and thyroid hormones, ionized calcium, recorded in hyperhomocysteinemia, indicate the participation of this sulfur-containing amino acid in the regulation of metabolic processes in the body.

In the course of this study, a connection was established between H_{cy} and the adrenal cortex hormone Cortisol — an important stress biomarker, one of the main elements of the system that stabilizes the body’s condition after adverse effects [11].

It is believed that under the influence of external environmental influences, the synthesis of corticotropin-releasing hormone in the paraventricular nucleus of the hypothalamus is activated, leading to increased formation of adrenocorticotropic hormone (ACTH) in the anterior pituitary gland.

In turn, ACTH activates the formation of the glucocorticoid hormone Cortisol in the fascicular zone of the adrenal glands. H_{cy} can affect N-methyl-D-aspartate (NMDA) receptors located in various structures of the central nervous system [1], stimulating the synthesis of Cortisol.

The relationship between H_{cy} and Cortisol is most pronounced among girls.

At the same time, it should be noted that it is influenced by genetic polymorphisms of FC.

The content of Cortisol in the blood of girls with the GMTR:2756 allele in the genome was more than in the blood of girls who are not carriers of this allele.

In the subgroups of girls with the A/G, G/G MTR:2756, A/A MTHFR:1298, C/C MTHFR:677, A/G, G/G MTRR:66 genotypes and the general group, the content of Cortisol in the blood with hyperhomocysteinemia was more than with a level of $H_{cy} \leq 10.0$ μmol/l. Moreover, the proportion of cases with the GMTR:2756 allele was also more.

In the subgroups of girls with the A/G, G/G MTR:2756 and A/AMTHFR:1298 genotypes, as well as in the general group, a direct correlation between H_{cy} and Cortisol was recorded.

The occurrence of hyperhomocysteinemia and an increase in Cortisol in the blood may be associated not only with the GMTR:2756 allele.

In the group of boys, the H_{cy}–Cortisol correlation was recorded in the subgroup where the TMTHFR:677 allele was present in 100% of cases and the proportion of hyperhomocysteinemia, as well as the level of H_{cy} in the blood, were maximum.

Thus, an increase in the Cortisol content in the blood of children living near the ChEZ during the late period of the Chornobyl accident occurred in cases where their genome contained the GMTR:2756 and TMTHFR:677 alleles, which contribute to impaired H_{cy} methylation and hyperhomocysteinemia.

In the subgroup of children with a homozygous variant of the GMTR:2756 allele, which blocks the activity of B_{12} dependent methionine synthase by 80%, there was a pronounced activation of the transsulfuration reaction cycle, with the utilization of H_{cy}, which leads to the formation of glutathione, which prevents the destructive effect of active oxygen species on cellular structures [1].

It should be noted that there is information that hydrogen peroxide has a similar effect [12].

In this regard, it can be reasonably stated that stimulation of the transsulfuration reaction cycle in children living near the ChEZ occurred due to disruption of the functioning of B_{12} dependent methionine synthase induced by the GMTR:2756 allele and exposure to reactive oxygen species formed during radiation metabolic stress.

Considering the proportion of cases with GMTR:2756 and TMTHFR:677 alleles in general groups and individual genetic subgroups of boys and girls, it should be recognized that the environmental radiation factor plays a significant role in the development of hyperhomocysteinemia and increased Cortisol levels in the blood.

Environmental radiation exposure of children living near the ChEZ induced metabolic stress in their bodies, which resulted in energy deficiency, which, in combination with genetic defects of FC, led to impaired H_{cy} methylation and hyperhomocysteinemia.

Moderate hyperhomocysteinemia occurring in the bodies of these children contributed to the formation of antioxidant protection, including Cortisol, with the participation of FC genes and the transsulfuration reaction cycle.

This process is most pronounced in girls who are carriers of the GMTR:2756 allele.

CONCLUSIONS

In children living near the ChEZ, in the late period of the Chornobyl accident, a violation of H_{cy} methylation is registered, leading in most cases to moderate hyperhomocysteinemia.

The TMTHFR:677 allele plays a significant role in increasing the H_{cy} level in the blood of children.

The participation of H_{cy} and genetic polymorphisms of FC in the regulation of the content of the stress hormone Cortisol in the blood was revealed.

In the group of girls, a connection was established between H_{cy}, Cortisol, and the GMTR:2756 allele, which significantly affects the process of H_{cy} methylation.

In the blood of girls carriers of the GMTR:2756 allele, which negatively affects the activity of B12 dependent methionine synthase, the content of Cortisol in the blood is increased, in comparison with girls without this allele in the genome.

The concentration of Cortisol in the blood of girls in the subgroups with the genetic alleles GMTR:2756, AMTHFR:1298, CMTHFR:677, GMTRR:66 and the general group, with the level of $H_{cy} > 10.0$ µmol/l, was statistically higher than with the level of $H_{cy} \leq 10.0$ µmol/l. At the same time, the proportion of cases with the allele GMTR:2756 was more.

A direct correlation between the values of H_{cy} and Cortisol in the blood was noted in the subgroups with the genetic alleles GMTR:2756, AMTHFR:1298 and in the general group of girls, whereas among boys this relationship was recorded only in the subgroup with the TMTHFR:677 allele.

Radiation exposure induces metabolic stress in the bodies of children living near the ChEZ, which, like mutations in the FC genes, causes an increase in the H_{cy} content in the blood.

With moderate hyperhomocysteinemia, the antioxidant system is activated, one of the elements of which is Cortisol.

In this regard, Hcy should be considered as a factor in stabilizing metabolic processes when exposed to incorporated ^{137}Cs.

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