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Genetic polymorphisms and the level of blood homocysteine in children and their mothers from the areas affected by the Chernobyl nuclear power plant accident

Генетические полиморфизмы и уровень гомоцистеина в крови у детей и их матерей из районов, пострадавших в результате аварии на Чернобыльской атомной электростанции

Abstract

High rates of death because of cancer and cardiovascular disease have been recorded in the areas affected by the accident at the Chernobyl nuclear power plant. It can be caused by mutations in the genes responsible for the synthesis of enzymes of folate metabolism (FM), leading to hyperhomocysteinemia – the increase of homocysteine, the intermediate metabolite in the methionine metabolism.

The purpose of this study was to assess the level of blood homocysteine and allelic variants of the genes that regulate FM, and determine their interrelations in children and their mothers, who live continuously on the territory of Ukraine contaminated with radionuclides after the Chernobyl nuclear power plant accident.

Methods of research. Immunochemical, mathematical, and statistical.

Results. The percentage of cases with the 677CT+677TT genotype was 63.6% in the group of mothers and 47.6% ($p<0.05$) in the group of children. The percentage of cases with the 677CT/1298AC genotype was 30.3% in the group of mothers and 10.7% ($p<0.05$) in the group of children. Hyperhomocysteinemia (taking into account the established age norms) was observed in 79.8% of cases in the group of children and in 31.8% of cases ($p<0.05$) in the group of their mothers. Hyperhomocysteinemia was observed in 90.0% of cases in the group of children who are carriers of 677CT and 677TT allelic variants, and in 42.9% of cases ($p<0.05$) in the similar group of mothers. The percentage of cases of the mentioned allelic variants was 53.7% in the group of children with hyperhomocysteinemia and 85.7% ($p<0.05$) in the similar group of their mothers.

In the group of children, unlike their mothers, there were no relationships between the number of genetic polymorphisms with risk alleles and the number of cases of hyperhomocysteinemia; the level of homocysteine and the number of genetic polymorphisms with risk alleles. Instead there was a weaker correlation between the homocysteine indices and genetic risk indices.

In the examined children, unlike their mothers, the genome of FM does not regulate the process

of homocysteine metabolism. High level of blood homocysteine indicates a real threat to health of children of the second post-Chernobyl generation.
Keywords: homocysteine, hyperhomocysteinemia, genetic polymorphisms, adolescents, radiation-contaminated territories of Ukraine.

Резюме

В районах Украины, пострадавших от аварии на Чернобыльской атомной электростанции, регистрируются высокие показатели смертности населения от онкологических и сердечно-сосудистых заболеваний. Причинами этого могут быть мутации в генах, ответственных за синтез ферментов фолатного цикла (ФЦ), приводящие к гипергомоцистеинемии – увеличению в крови гомоцистеина, промежуточного метаболита в процессе обмена метионина.

Целью исследования явилась оценка уровней гомоцистеина в крови, а также аллельных вариантов генов, контролирующих ФЦ, с определением их взаимосвязей у детей и их матерей, постоянно проживающих после аварии на Чернобыльской атомной электростанции на территории Украины, загрязненной радионуклидами.

Методы исследования. Иммунохимический, математико-статистический.

Результаты. Удельный вес случаев с генотипом 677CT+677TT составил в группе матерей 63,6%, в группе детей – 47,6% ($p<0,05$). Удельный вес случаев с генотипом 677CT/1298AC составил в группе матерей – 30,3%, в группе детей – 10,7% ($p<0,05$). Состояние гипергомоцистеинемии с учетом установленных возрастных норм наблюдалось в группе детей в 79,8% случаев, в группе их матерей – в 31,8% случаев ($p<0,05$).

Гипергомоцистеинемия в группе детей – носителей аллельных вариантов 677CT и 677TT, регистрировалась в 90,0% случаев, в аналогичной группе матерей – в 42,9% случаев ($p<0,05$). В группе детей с гипергомоцистеинемией удельный вес случаев указанных аллельных вариантов составил 53,7%, в то время как в аналогичной группе их матерей – 85,7% ($p<0,05$).

В группе детей, в отличие от группы их матерей, отсутствовала зависимость между количеством генетических полиморфизмов с аллелями риска и количеством случаев гипергомоцистеинемии, а также корреляционная связь между уровнем гомоцистеина в крови и количеством генетических полиморфизмов с аллелями риска, присутствовала более слабая корреляционная связь между показателями гомоцистеина и показателями выраженности генетического риска.

У обследованных детей, в отличие от их матерей, геном фолатного цикла не регулирует процесс метаболизма гомоцистеина. Высокие уровни гомоцистеина в крови свидетельствуют о существовании реальной угрозы для здоровья детей второго постчернобыльского поколения.

Ключевые слова: гомоцистеин, гипергомоцистеинемия, генетические полиморфизмы, подростки, радиоактивно загрязненные территории Украины.

Over the past 25 years high death rates due to cardiovascular disease and cancer have been recorded in areas of Ukraine affected by the Chernobyl nuclear power plant (CNPP) accident [1]. The reason for that may be genetic polymorphisms – mutations in genes responsible for the synthesis of folate metabolism (FM) enzymes [2, 3], leading to hyperhomocysteinemia – an increase in homocysteine, an intermediate metabolite in the metabolism of methionine, an essential sulphur-containing amino acid, in the blood [4–8].

FM and methionine metabolic processes have not been studied in adults and children living in areas contaminated with radionuclides due to the CNPP accident.

■ PURPOSE OF THE STUDY

To assess homocysteine levels in the blood of and allelic variants of genes regulating folate metabolism and determine their interrelationships in children and their mothers continuously living after the CNPP accident in an area of Ukraine contaminated with radionuclides.

■ MATERIALS AND METHODS

Children and their mothers chronically living after the CNPP accident in an area of Ukraine with a ^{137}Cs soil contamination level of <2 Cu/km^2 were studied [9].

The group under study comprised 84 children, including 39 boys and 45 girls, born in 1997–2001, whose average age at the time of examination was 15.5±0.1 years (95% CI 15.4–15.7 years), and 66 mothers born in 1955–1983 whose average age at the time of examination was 42.6±0.7 years (95% CI 41.2–44.1 years). At the time of the CNPP accident the age of mothers was 12.9±0.7 years (95% CI 11.4–14.3 years). Thus, 11–13 years passed from the time of the CNPP accident (April 26, 1986) until the children were born. At the time of birth of children, the age of mothers ranged from 17–45 years old.

During the period until 1970, 15 mothers (22.7% of the total number of mothers) were born, at the time of the CNPP accident they were more than 16 years old. During the period 1970–1974, 15 mothers (22.7% of the total number of mothers) were born, at the time of the CNPP accident they were 12–16 years old. During the period 1975–1979, 30 mothers (45.5% of the total number of mothers) were born, at the time of the CNPP accident they were 7–11 years old, during the period 1980–1983, 6 mothers (9.1% of the total number of mothers) were born, at the time of the CNPP accident they were 3–6 years old.

All children at the time of examination attended school. The examined children and their mothers had blood drawn from the cubital vein on an empty stomach in the morning to determine homocysteine levels and carry out genetic analysis of FM. Blood samples were analysed in a laboratory certified under quality standards with the financial support of the Rhône-Alpes Regional Council (France).

Blood homocysteine levels were determined using an immunochemical method with chemiluminescent detection (CLIA). An analyzer and a test system: Architect 1000 (ABBOT Diagnostics (USA).

Taking into account the results of numerous studies [10, 11], in the group of children blood homocysteine levels of over 10 μmol/L were defined as hyperhomocysteinemia. In the group of mothers, hyperhomocysteinemia was identified at blood homocysteine levels of over 13.56 μmol/L (a homocysteine value which is set for adults within the laboratory which analysed blood samples). The allelic variants C677T and A1298C of the MTHFR gene (methylenetetrahydrofolate reductase), A2756G of the MTR gene (B_{12}-dependent methionine synthase) and A66G of the MTRR gene (methionine synthase reductase) were determined during genetic analysis

of FM. A real-time PCR method was used. An analyser and a test system: DT-96 detecting thermocycler, DNA-Technology (Russia).

In order to carry out correlation studies to determine the relationship between blood homocysteine levels and genetic abnormalities in FM, in a number of cases under consideration, the analysed FM genetic variants were assessed using a point system (0–3) depending on how they affect homocysteine formation and we also developed a Risk1 (R1) genetic risk score: 0 – absence of risk alleles of FM gene polymorphisms – no risk; 1 – presence of risk alleles of the following polymorphisms – A2756G of MTR gene, A66G of MTRR gene, A1298C of MTHFR gene in any combination, except C677T of MTHFR gene – low risk; 2 – presence of MTHFR 677CT genotype in any combination with polymorphisms of other genes, except MTHFR 1298AC genotype – intermediate risk; 3 – presence of 677TT or 677CT/1298AC genotypes of MTHFR gene – high risk.

A Risk2 (R2) genetic risk score represented a point-based scale (0-2) only for the MTHFR: C677T polymorphism: 0 – 677CC genotype – no risk; 1 – 677CT genotype – low risk; 2 – 677TT genotype – high risk.

The statistical processing of the obtained results was performed using the IBM SPSS Statistics 22 software (USA). The arithmetic mean (M), ± standard error of mean (SEM), 95% confidence interval (CI) for the average value, median (Me), interquartile range (IR), minimum and maximum parameter values and percentiles were calculated for the variables under analysis. The distribution hypothesis was tested (a Kolmogorov – Smirnov test). Since the most of the parameters under study did not conform to the normal distribution law, a non-parametric U Mann – Whitney test was used to compare values. The assessment of statistical significance of variables was done by determining a significance value for p using a statistical software programme. The Student's t-test was used to compare relative values. The critical level of significance for the null hypothesis (p) was set at 0.05. The relationship between blood homocysteine levels, the number of genetic polymorphisms and R1 and R2 genetic risk scores was established with the help of the Spearman's rank correlation coefficient (r_{xy}). Strength of correlation was assessed by a typical scale: weak – 0 to ±0.299; moderate – ±0.3 to ±0.699; strong – ±0.7 to ±1.0.

■ RESULTS AND DISCUSSION

The number of risk alleles of FM genetic polymorphisms in children and their mothers did not differ statistically. However, the percentage of the heterozygous variant 677CT and heterozygous association 677CT/1298AC of the MTHFR gene was higher in the group of mothers than in the group of their children. It allowed to determine a significant difference in respect of all MTHFR:C677T risk alleles in total and the 677CT/1298AC heterozygous association (Tables 1–3).

Hyperhomocysteinemia taking into account established age norms was found in 67 out of 84 examined children (79.8%), and in 21 out of 66 mothers (31.8%), $p<0.05$. At the same time, blood homocysteine levels in both groups had no statistically significant differences (Table 4).

The percentage of cases of hyperhomocysteinemia was statistically and significantly higher in the group of children than in the group of mothers with absence or presence of one or two genetic polymorphisms (Table 5).

Table 1
Percentage of polymorphic alleles of folate metabolism genes in children

Gene, polymorphism	Neutral allele		Heterozygous risk allele		Homozygous risk allele	
	Absolute number	Percentage, %	Absolute number	Percentage, %	Absolute number	Percentage, %
MTR:A2756G	57	67.9	25	29.8	2	2.3
MTHFR:A1298C	47	55.9	25	29.8	12	14.3
MTHFR:C677T	44	52.4	29	34.5	11	13.1
MTRR:A66G	17	20.2	36	42.9	31	36.9

Table 2
Percentage of polymorphic alleles of folate metabolism genes in mothers

Gene, polymorphism	Neutral allele		Heterozygous risk allele		Homozygous risk allele	
	Absolute number	Percentage, %	Absolute number	Percentage, %	Absolute number	Percentage, %
MTR:A2756G	44	66.7	21	31.8	1	1.5
MTHFR:A1298C	34	51.5	29	43.9	3	4.6
MTHFR:C677T	24	36.4	34	51.5*	8	12.1
MTRR:A66G	10	15.2	30	45.5	26	39.4

Note: * comparison between groups of children and mothers by frequency of certain types of genetic polymorphisms presented in Tables 1–2, statistically significant differences ($p<0.05$) by frequency of the heterozygous variant of the MTHFR:C677T polymorphism.

Table 3
Percentage of MTHFR genetic polymorphisms in groups

Group of examined people	677CT/1298AC genotype		677CT+677TT genotype	
	Absolute number (n)	Percentage, %	Absolute number (n)	Percentage, %
Children	9	10.7*	40	47.6*
Mothers	20	30.3	42	63.6

Note: * statistically significant differences between groups ($p<0.05$).

Table 4
Statistical characteristics of blood homocysteine values in examined children and their mothers

Group of examined people	Number, persons	Homocysteine, μmol/L		Result of comparison of two independent samples
		Me	IR	
Children	84	12.3	10.4–14.5	U=2368.50; p=0.127
Mothers	66	11.7	8.8–14.4	

The percentage of hyperhomocysteinemia cases is statistically higher in the groups of children with all genotypes under study, except MTHFR:1298AC, than in the corresponding groups of mothers. There were no statistical differences between the figures of the percentage of hyperhomocysteinemia in groups with a different genotype of one polymorphism (Table 6).

Thus, the percentage of hyperhomocysteinemia cases was 90.0% (36 out of 40) in the group of children with C677T genotype, and 42.9% (18 out of 36) $p<0.05$, in the group of mothers.

Table 5
Number of cases of hyperhomocysteinemia depending on the number of genetic polymorphisms among the examined subjects

Subgroup No.	Number of polymorphisms	Absolute number, (n)		Percentage, %	
		Children	Mothers	Children	Mothers
1	No	2	0	66.7*	0
2	One	16	1	84.2*	12.5
3	Two	30	7	81.1*	22.6
4	Three	16	10	72.7	45.5
5	Four	3	3	100	75.0**
Total		67	21	79.8	31.8

Note:
* statistically significant differences ($p<0.05$) between groups of children and mothers in subgroups No. 1–3;
** statistically significant differences ($p<0.05$) in the group of mothers between subgroups No. 3 and 5.

Table 6
Percentage of hyperhomocysteinemia cases in groups of children and mothers with polymorphisms

Polymorphisms, genotype	Children		Mothers	
	Absolute number	Percentage, %	Absolute number	Percentage, %
MTR:2756GG	2	100	0	0
MTR:2756AG	18	72.0	5	23.8
MTR:2756AA	47	82.5	16	36.4
MTHFR:1298CC	7	58.3	0	0
MTHFR:1298AC	19	76.0	15	51.7*
MTHFR:1298AA	41	87.2	6	17.7
MTHFR:677TT	11	100	4	50.0
MTHFR:677CT	25	86.2	14	41.2
MTHFR:677CC	31	70.5	3	12.5
MTRR:66GG	25	83.3	10	38.5
MTRR:66AG	29	78.4	9	30.0
MTRR:66AA	13	76.5	2	20.0
677CT/1298AC	8	88.9	13	65.0

Note: * absence of statistical differences ($p>0.05$) between groups of children and mothers.

In respect to hyperhomocysteinemia, the percentage of A1298C and C677T risk alleles of the MTHFR gene was statistically and significantly higher in the group of mothers than in the group of children due to heterozygous variants (Tables 7–8).

In the group of mothers, a direct moderate correlation was observed between blood homocysteine levels and the number of FM gene polymorphisms, homocysteine levels and R1 and R2 genetic risk scores confirmed by values of Spearman's coefficient of rank correlation.

In the group of children, a direct relationship established between homocysteine concentrations and R1 and R2 genetic risk scores was weak, and there was no association between homocysteine levels and the number of FM gene polymorphisms. In both groups, strength of relationship between the number of polymorphisms and R1 and R2 scores was moderate and correlation between R1 and R2 scores was strong (Tables 9–10).

Table 7

Percentage of polymorphic alleles of folate metabolism genes in the group of children with hyperhomocysteinemia (n=67)

Gene, polymorphism	Neutral allele		Heterozygous risk allele		Hetero- and homozygous risk alleles	
	Absolute number	Percentage, %	Absolute number	Percentage, %	Absolute number	Percentage, %
MTHFR:A1298C	41	61.2*	19	28.4*	26	38.8*
MTHFR:C677T	31	46.3*	25	37.3*	36	53.7*
MTR:A2756G	47	70.2	18	26.9	20	29.9
MTRR:A66G	13	19.4	29	43.3	54	80.6

Note: * statistically significant differences ($p<0.05$) by frequency of neutral and risk alleles of the MTHFR:C677T and MTHFR:A1298C polymorphisms between groups of children and mothers.

Table 8

Percentage of polymorphic alleles of folate metabolism genes in the group of mothers with hyperhomocysteinemia (n=21)

Gene, polymorphism	Neutral allele		Heterozygous risk allele		Hetero- and homozygous risk allele	
	Absolute number	Percentage, %	Absolute number	Percentage, %	Absolute number	Percentage, %
MTHFR:A1298C	6	28.6	15	71.4	15	71.4
MTHFR:C677T	3	14.3	14	66.7	18	85.7
MTR:A2756G	16	76.2	5	23.8	5	23.8
MTRR:A66G	2	9.5	9	42.9	19	90.5

Table 9

Results of correlation analysis between homocysteine, number of genetic polymorphisms and genetic risk scores in the group of children

Parameter	Correlation coefficient	Parameter			
		Hc	Npol	R1	R2
Hc	Spearman's	1.000	-0.060	0.239*	0.279*
	Sign. (two-sided), p	.	0.590	0.029	0.010
	N	84	84	84	84
Numpol	Spearman's	-0.060	1.000	0.525**	0.388**
	Sign. (two-sided), p	0.590	.	0.0001	0.0001
	N	84	84	84	84
R1	Spearman's	0.239*	0.525**	1.000	0.918**
	Sign. (two-sided), p	0.029	0.000	.	0.0001
	N	84	84	84	84
R2	Spearman's	0.279*	0.388**	0.918**	1.000
	Sign. (two-sided), p	0.010	0.0001	0.0001	.
	N	84	84	84	84

Note:
* a correlation is significant at the 0.05 level (two-sided);
** a correlation is significant at the 0.01 level (two-sided);
Hc – homocysteine;
Npol – number of FM gene polymorphisms;
R1 and R2 – genetic risk scores.

Table 10
Results of correlation analysis between homocysteine, number of genetic polymorphisms and genetic risk indices in the group of mothers

Parameter	Correlation coefficient	Hc	Npol	R1	R2
Hc	Spearman's	1.000	0.336**	0.501**	0.379**
	Sign. (two-sided), p	.	0.006	0.0001	0.002
	N	66	66	66	66
Numpol	Spearman's	0.336**	1.000	0.545**	0.356**
	Sign. (two-sided), p	0.006	.	0.0001	0.003
	N	66	66	66	66
R1	Spearman's	0.501**	0.545**	1.000	0.876**
	Sign. (two-sided), p	0.0001	0.0001	.	0.0001
	N	66	66	66	66
R2	Spearman's	0.379**	0.356**	0.876**	1.000
	Sign. (two-sided), p	0.002	0.003	0.0001	.
	N	66	66	66	66

Note:
** a correlation is significant at the 0.01 level (two-sided);
Hc – homocysteine;
Npol – number of folate metabolism gene polymorphisms;
R1 and R2 – genetic risk scores.

The obtained results show significant differences in the realisation of the genetic programme of FM between children and their mothers. Attention should be paid to a much larger number of cases of a heterozygous variant of the MTHFR:C677T polymorphism and the 677CT/1298AC heterozygous association in the group of mothers in comparison with the group of children.

Given the fact that the area of residence of parents of examined children is highly contaminated with radioactive elements after the CNPP accident in 1986 [9], we can assume that there can be an elimination of germ cells or developing embryos possessing C677CT and 677CT/1298AC genotypes during pregnancy.

The percentage of cases of hyperhomocysteinemia was significantly higher in the group of children than in the group of mothers (79.8% and 31.8% respectively, $p<0.05$).

The differences between groups of mothers and their children lied in the degree of expression of the MTHFR defective genome affecting methionine metabolism.

Hyperhomocysteinemia was observed in the overwhelming majority of cases (90.0% in total) in the group of children – carriers of 677CT and 677TT allelic variants, while this condition occurred more rarely (42.9% of cases) in the group of their mothers with the same genome. The percentage of cases of above allelic variants was statistically lower in the group of children with hyperhomocysteinemia than in the similar group of their mothers.The percentage of hyperhomocysteinemia cases was statistically higher in the groups of children with the studied allelic variants of FM gene polymorphisms, except MTHFR:1298AC, than in the corresponding groups of mothers. At the same time, the high percentage of hyperhomocysteinemia

cases was observed in subgroups of children with genotypes consisting only of neutral alleles of FM genetic polymorphisms.

The mother's organism continued to have genetic control over the functioning of one of the most important enzymes of FM – methylenetetrahydrofolate dehydrogenase – in the form of its polymorphism MTHFR:C677T. In cases of presence of only CC neutral allele, the percentage of hyperhomocysteinemia cases in the group of mothers was 12.5%, in heterozygous carriers of CT – 41.2% and in homozygous carriers – 50.0%.

Thus, in the group of children, unlike their mothers, there were no relationships between the number of genetic polymorphisms with risk alleles and the number of cases of hyperhomocysteinemia, and blood homocysteine levels and the number of genetic polymorphisms with risk alleles. Instead there was a weaker correlation between homocysteine values and genetic risk scores.

The absence of statistical differences between the figures of percentage of hyperhomocysteinemia in groups with different genotype of one polymorphism or with different polymorphisms indicates that the genome of FM does not regulate the process of homocysteine metabolism in the children examined.

There is an evident disruption in the regulation of methionine metabolism on the part of the genetic apparatus of FM in children of the second post-Chernobyl generation.

All of this indicates that there is an evident disruption in the regulation of methionine metabolism on the part of the genetic apparatus of FM in children of the second post-Chernobyl generation.

It should be noted that the homocysteine methylation process and methionine resynthesis depend not only on the state of the genetic apparatus of FM but on other factors, among which one should mention, first of all, folic acid and vitamin B_{12} [11].

The findings show that there is a necessity to carry out preventive measures among all children from areas affected by the CNPP accident to prevent serious diseases leading to disability and death of adults.

■ CONCLUSIONS

A higher percentage of carriership of the heterozygous variant of the MTHFR:C677T polymorphism and the 677CT/1298AC heterozygous association of this gene was detected in the group of mothers compared with the group of their children.

Hyperhomocysteinemia taking into account established age norms was observed in 79.8% of cases in the group of children and in 31.8% of cases ($p<0.05$) in the group of their mothers.

In the children examined, unlike their mothers, the genome of FM does not regulate the process of homocysteine metabolism.

High blood homocysteine levels indicate a real risk to health of children of the second post-Chernobyl generation.

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