FOLATE METABOLISM GENE POLYMORPHISMS AND HOMOCYSTEINEMIA IN CHILDREN FROM FAMILIES CONTINUOUSLY LIVING IN AN AREA AFFECTED BY THE CHERNOBYL NUCLEAR POWER PLANT ACCIDENT

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A high mortality rate associated to a greater extent with cancer and cardiovascular diseases is recorded in areas affected by the Chernobyl nuclear power plant accident [1].

It is extremely important practically and scientifically to find causes of this phenomenon. In this regard, it is reasonable to carry out studies to assess blood concentrations of homocysteine - an amino acid formed during transmethylation of methionine - a sulfur-containing amino acid essential for the human body.

During metabolic reactions, homocysteine is converted into cysteine or transformed back into methionine. The resynthesis of methionine from homocysteine is carried out during the metabolism of folic acid by transfer of the methyl group involving methylenetetrahydrofolate reductase, B_{12}-dependent methionine synthase and methionine synthase reductase.

Elevated levels of homocysteine in the body may be caused by folate metabolism disturbances, including genetic defects of above enzymes and may lead to serious consequences in the form of a higher risk of developing cardiovascular diseases, cancer, diabetes, chronic miscarriage, congenital abnormalities and other diseases [2].

The purpose of this study was to assess blood homocysteine concentrations and genetic system of enzymes associated with folate metabolism in children from families continuously residing in an area affected by the Chernobyl nuclear power plant accident.

In the conducted studies, blood homocysteine concentrations were measured in 201 children (101 boys and 100 girls) aged 13-17 years from families permanently living in Polesie and Ivankov districts of Kiev region after the Chernobyl accident. In addition there were used the immunochemical method with

chemiluminescent detection (CLIA), the analyzer and the test system: Architect 1000 (ABBOT Diagnostics (USA)).

The genetic system responsible for folate metabolism was analysed in all examined children. In particular, there were determined allelic variants C677T and A1298C of the MTHFR gene (methylenetetrahydrofolate reductase), an allelic variant A2756G of the MTR gene (B_{12}-dependent methionine synthase) and an allelic variant A66G of the MTRR gene (methionine synthase reductase). The real-time PCR method was used for this purpose, as well as the analyzer and the test system: detecting thermocycler DT-96; DNA-Technology (Russia).

These tests were performed in a laboratory certified by quality standards and were funded by the Rhône-Alpes Regional Council (France). Normative values for each test, as well as sex and age of a child at the time of taking blood from a vein were taken into account when assessing the obtained results.

The assessment of results of measurement of children's blood homocysteine levels was carried out on the basis of results of previous studies of the metabolite by other researchers [3]. We used the value of 10 mcmol/L as the criterion of deviation of the homocysteine level from physiological parameters, exceeding this value was defined as homocysteinemia.

Systematization of the material and primary mathematical processing were performed using Microsoft Excel 2010 spreadsheets.

Obtained results and their discussion.

The study showed the presence of polymorphisms in genes under discussion in 195 children (97.0 %). No risk alleles were found only in 6 cases (3.0 % of the number of examined children).

The risk alleles for one of the stated polymorphisms were observed in 39 cases (19.4 %), for 2 polymorphisms - in 86 cases (42.8 %), for 3 polymorphisms –

in 63 cases (31.3 %), for 4 polymorphisms – in 7 cases (3.5 %). The absence of risk alleles for these polymorphisms was recorded in the group of boys in 2 cases (2.0 % of the number of examined boys), in the group of girls - in 4 cases (4.0 % of the number of examined girls). The risk allele for one polymorphism was detected in the group of boys in 19 cases (19.0 %), in the group of girls - in 20 cases (20.0 %). The combination of risk alleles of 2 polymorphisms was seen in the group of boys in 47 cases (46.5 %), in the group of girls - in 39 cases (39.0 %). The combination of risk alleles for 3 polymorphisms was found in the group of boys in 30 cases (29.7 %), in the group of girls - in 33 cases (33.0 %). The combination of risk alleles for 4 polymorphisms was noticed in the group of boys in 3 cases (3.0 %), in the group of girls - in 4 cases (4.0 %).

The risk alleles of the MTRR gene were the most frequently observed risk alleles in the group of children under study. The number of polymorphisms C677T and A1298C of the MTHFR gene was smaller. The MTR gene was characterized by the highest incidence of the normal genotype composed from wild-type alleles (Tables 1, 2). The same tendency was noted in the group of boys and girls. Heterozygous mutations in all studied folate metabolism genes were found more often than homozygous ones (Tables 3, 4).

Table 1

The presence of folate metabolism gene polymorphic alleles in examined children

Gene, polymorphism	"Neutral" allele		Risk allele	
	Absolute number (n)	Ratio, %	Absolute number (n)	Ratio, %
MTR:A2756G	133	66.2	68	33.8
MTHFR:A1298C	99	49.3	102	50.8
MTHFR:C677T	103	51.2	98	48.8
MTRR:A66G	42	20.9	159	79.1

Table 2

The frequency of folate metabolism gene polymorphic alleles in examined children

Gene, polymorphism	"Neutral" allele		"Heterozygous" risk allele		"Homozygous" risk allele	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	133	66.2	57	28.4	11	5.4
MTHFR:A1298C	99	49.3	80	39.8	22	10.9
MTHFR:C677T	103	51.2	78	38.8	20	10.0
MTRR:A66G	42	20.9	91	45.3	68	33.8

Table 3

The frequency of folate metabolism gene polymorphic alleles in examined children (boys)

Gene, polymorphism	"Neutral" allele		"Heterozygous" risk allele		"Homozygous" risk allele	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	67	66.3	28	27.7	6	5.9
MTHFR:A1298C	46	45.5	42	41.6	13	12.9
MTHFR:C677T	54	53.5	36	35.6	11	10.9
MTRR:A66G	22	21.8	46	45.5	33	32.7

Table 4

The frequency of folate metabolism gene polymorphic alleles in examined children (girls)

Gene, polymorphism	"Neutral" allele		"Heterozygous" risk allele		"Homozygous" risk allele	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	66	66.0	29	29.0	5	5.0
MTHFR:A1298C	53	53.0	38	38.0	9	9.0
MTHFR:C677T	49	49.0	42	42.0	9	9.0
MTRR:A66G	20	20.0	45	45.0	35	35.0

During the research, ***homocysteinemia*** was found in 98 cases (48.8 % of the number of all examined children), while in 4 cases homocysteine levels ranged from 30-100 mcmol/L. This group of children included 59 boys and 39 girls (respectively 60.2 % and 39.8 %, or 58.4 % of the number of examined boys and 39.0 % of the number of examined girls). The polymorphisms under study were recorded in children with homocysteinemia in 97 cases (99.0 %). At the same time, homocysteinemia was diagnosed in 49.7 % of cases among children with the above polymorphisms (n=195). An increase in the homocysteine level above 10 mcmol/L was found in one case (16.7 %) among the children with only normal alleles of all folate metabolism genes under consideration (n=6). 13 cases of homocysteinemia (33.3 %) were identified in the group of children with one polymorphism (n=39), 46 cases (53.5 %) - in the group of children with two polymorphisms (n=86), 34 cases (54.0 %) - in the group of children with three polymorphisms (n=63), 4 cases (57.1 %) - in the group of children with four polymorphisms (n=7).

No homocysteinemia was observed in the group of boys with normal alleles of all genes under study (n=2), 8 cases of homocysteinemia (42.1 %) were found in the group of boys with one polymorphism (n=19), with two polymorphisms (n=47) - 29 cases of homocysteinemia (61.7 %), with three polymorphisms (n=30) - 19 cases of homocysteinemia (63.3 %), with four polymorphisms (n=3) - 3 cases of homocysteinemia (100 %).

One case of homocysteinemia (25.0 %) was noted in the group of girls with normal alleles of all genes under study (n=4), 5 cases of homocysteinemia (25.0 %) were found in the group of girls with one polymorphism (n=20), with two polymorphisms (n=39) - 17 cases of homocysteinemia (43.6 %), with three polymorphisms (n=33) - 15 cases of homocysteinemia (45.5 %), with four polymorphisms (n=4) - one case of homocysteinemia (25.0 %).

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Thus, the number of children with homocysteinemia increases with increase of the number of polymorphic risk alleles. This relationship manifests itself most clearly in the group of boys.

The greatest differences between the groups of children with homocysteinemia and the homocysteine level below 10 mcmol/L were observed on the part of the MTHFR C677T polymorphism.

The frequency of heterozygous and homozygous C677T mutations among children with homocysteinemia was higher than among children with the homocysteine level below 10 mcmol/L (Tables 5, 6), including in the groups of boys and girls. Moreover, the frequency of homozygous mutations in the group of boys was higher than in the group of girls.

The ratio of the risk allele was higher than 50 % (Tables 7-10).

The frequency of heterozygous and homozygous forms of the MTHFR A1298C polymorphism in the groups of children with and without homocysteinemia had no significant differences (Tables 5, 6).

The frequency of the homozygous form of the MTRR A66G polymorphism among the children with homocysteinemia was significantly higher than among the children with the homocysteine level below 10 mcmol/L (Tables 5, 6), including in the group of boys. At the same time, the ratio of the risk allele was higher than 50 % (Tables 7-10).

The frequency of the homozygous form of the MTR A2756C polymorphism among the children with homocysteinemia was higher than among the children with the homocysteine level below 10 mcmol/L (Tables 5, 6), including in the groups of boys and girls. However, the ratio of the risk allele (heterozygous and homozygous forms in total) did not exceed 50 % (Tables 7-10).

The literature provides information on the special role of the homozygous variant of the C677T polymorphism (T/T genotype), that can lead to reduction of activity of the enzyme methylenetetrahydrofolate reductase by 70 %, at the same time the blood homocysteine level increases. Compound heterozygosity for the 677T and 1298C alleles of this gene is accompanied by a decrease in the enzyme activity by 40 – 50 %, an increase in the concentration of homocysteine, as well as in case of homozygous carriership of the 677T allele [4, 5].

Table 5

The frequency of folate metabolism gene polymorphic alleles in children with a blood homocysteine level of more than 10 mcmol/L

Gene, polymorphism	"Neutral" allele		Risk allele, heterozygous mutation		Risk allele, homozygous mutation	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	69	70.4	21	21.4	8	8.2
MTHFR:A1298C	47	48.0	41	41.8	10	10.2
MTHFR:C677T	40	40.8	42	42.9	16	16.3
MTRR:A66G	13	13.3	45	45.9	40	40.8

Table 6

The frequency of folate metabolism gene polymorphic alleles in children with a blood homocysteine level of less than 10 mcmol/L

Gene, polymorphism	"Neutral" allele		Risk allele, heterozygous mutation		Risk allele, homozygous mutation	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	64	62.1	36	35.0	3	2.9
MTHFR:A1298C	52	50.5	39	37.9	12	11.7
MTHFR:C677T	63	61.2	36	35.0	4	3.9
MTRR:A66G	29	28.2	46	44.7	28	27.2

Table 7

The frequency of folate metabolism gene polymorphic alleles in boys with a blood homocysteine level of more than 10 mcmol/L

Gene, polymorphism	"Neutral" allele		Risk allele, heterozygous mutation		Risk allele, homozygous mutation	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	42	71.2	12	20.3	5	8.5
MTHFR:A1298C	25	42.3	28	47.5	6	10.2
MTHFR:C677T	25	42.4	23	39.0	11	18.6
MTRR:A66G	9	15.3	24	40.6	26	44.1

Table 8

The frequency of folate metabolism gene polymorphic alleles in girls with a blood homocysteine level of more than 10 mcmol/L

Gene, polymorphism	"Neutral" allele		Risk allele, heterozygous mutation		Risk allele, homozygous mutation	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	27	69.2	9	23.1	3	7.7
MTHFR:A1298C	22	56.4	13	33.3	4	10.3
MTHFR:C677T	15	38.5	19	48.7	5	12.8
MTRR:A66G	4	10.3	21	53.9	14	35.9

Therefore, we assessed the frequency of homocysteinemia in groups of children with above mutations.

Elevated homocysteine concentrations were recorded in 16 out of 20 children (80.0 %) with a homozygous variant of 677T, including in 11 out of 11 boys (100 %) and in 5 out of 9 girls (55.6 %). A heterozygous variant of 677T was combined with a heterozygous variant of 1298C (compound heterozygosity for the 677T and

1298C alleles) in 34 cases (16.9 % of the number of examined children), including in 14 boys and 20 girls.

Table 9

The frequency of folate metabolism gene polymorphic alleles in boys with a blood homocysteine level of less than 10 mcmol/L

Gene, polymorphism	"Neutral" allele		Risk allele, heterozygous mutation		Risk allele, homozygous mutation	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	25	59.5	16	38.1	1	2.4
MTHFR:A1298C	21	50.0	14	33.3	7	16.7
MTHFR:C677T	29	69.0	13	31.0	0	0
MTRR:A66G	13	31.0	22	52.4	7	16.6

Table 10

The frequency of folate metabolism gene polymorphic alleles in girls with a blood homocysteine level of less than 10 mcmol/L

Gene, polymorphism	"Neutral" allele		Risk allele, heterozygous mutation		Risk allele, homozygous mutation	
	Absolute number	Ratio, %	Absolute number	Ratio, %	Absolute number	Ratio, %
MTR:A2756G	39	63.9	20	32.8	2	3.3
MTHFR:A1298C	31	50.8	25	41.0	5	8.2
MTHFR:C677T	34	55.7	23	37.7	4	6.6
MTRR:A66G	16	26.2	24	39.3	21	34.5

An increased concentration of homocysteine in the blood was observed in 22 children (64.7 % of the number of children with compound heterozygosity for two alleles 677T and 1298C), including in 13 boys (92.9 % of the number of boys with

the above association of genes) and in 9 girls (45.0 % of the number of girls with the above association of genes).

A homozygous allelic variant of 677T and compound heterozygosity for the 677T and 1298C alleles were found in 54 cases (26.9 % of the number of all examined children), including in 25 boys and in 29 girls. In this group of children, an elevated concentration of homocysteine in the blood was noted in 38 cases (70.4 %), including in 24 boys (96.0 %) and in 14 girls (48.3 %).

Thus, among adolescent boys with the above combination of folate metabolism genes, there is a high probability of the increase in the blood homocysteine level above physiological norms compared with the girls of the same age.

Conclusions.

1. During a genetic study of children from families continuously living in Polesie and Ivankov districts after the Chernobyl nuclear power plant accident, polymorphic allelic variants (risk alleles) of the MTHFR, MTR and MTRR genes controlling folate metabolism were found in 97.0 % of cases; moreover, carriership of two, three or four risk alleles was recorded in 77.6 % of cases.

2. There were no significant differences in the frequency of risk alleles under study between the group of boys and the group of girls.

3. An increased homocysteine level in the blood above 10 mcmol/L – homocysteinemia- was observed in 48.8 % of cases among examined children. Among the children with homocysteinemia, boys made up 60.2 %, girls – 39.8 %.

4. With increase of polymorphic alleles of the MTHFR, MTR and MTRR genes the number of children with homocysteinemia increases. This relationship most clearly manifests itself in the group of boys.

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5. The MTHFR: C677T and MTRR: A66G polymorphisms were most commonly recorded in the group of children with homocysteinemia.

6. The frequency of the 677T allele in the group of children with homocysteinemia was 59,2 %, including the homozygous variant – 80.0 % (in the group of boys – 100 %, in the group of girls - 55.6 %), while in the children with a blood homocysteine level of less than 10 mcmol/L – 38.8 %.

7. In the group of children with a homozygous allelic variant of 677T and compound heterozygosity for the 677T and 1298C alleles of the MTHFR gene homocysteinemia was observed in 70.4 % of cases (in the group of boys – in 96.0 % of cases, in the group of girls – in 48.3 % of cases).

8. Carriership of the MTHFR gene polymorphisms responsible for the synthesis of the enzyme methylenetetrahydrofolate reductase may result in homocysteinemia most probably in adolescent boys rather than in girls of the same age.

9. Taking into account the obtained results, it is necessary to develop a complex of preventive measures aimed at preventing manifestations of genetic abnormalities of folate metabolism starting from the adolescence period on the basis of sex and age of a child.

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