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ORIGINAL PAPER

THE GENETICS OF FOLATE METABOLISM AND MATERNAL RISKS OF BIRTH OF A CHILD WITH CONGENITAL BRAIN MALFORMATIONS

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Abstract

Introduction. According to the data of the National Registry of Republic of Moldova, the incidence of congenital brain malformations (CBM) during 2010-2022 was 1.92 per 1000 newborns, accounting for 11% of all congenital anomalies.
The objective of the study was to investigate the genetic polymorphisms involved in folate metabolism in mothers at risk of giving birth to children with congenital brain malformations.
Material and methods. The target group included 105 children with a confirmed diagnosis of CBM. 50 mothers of children with CBM underwent molecular and genetic assessment, i. e. polymerase chain reaction for detection of four polymorphisms of the folate cycle genes. Statistical data processing was performed using the Quanto program.
Results. The most common folate-dependent CBM diagnosed in heterozygous individuals after folate cycle genes was hydrocephalus, being revealed in 54 cases, polymorphisms in craniosynostosis – 15 cases,

Résumé

La génétique du métabolisme des folates et les risques maternels de naissance d'un enfant atteint de malformations cérébrales congénitales

Introduction. Selon les données du Registre National de la République de Moldova, l'incidence des malformations cérébrales congénitales (MCC) au cours de la période 2010-2022 était de 1,92 pour 1000 nouveau-nés, constituant 11% de toutes les anomalies congénitales.
L'objectif de l'étude a été d'étudier les polymorphismes génétiques impliqués dans le métabolisme des folates chez les mères à risque de donner naissance à un enfant atteint d'une malformation cérébrale congénitale.
Matériel et méthodes. Le groupe cible était composé de 105 enfants avec un diagnostic confirmé de MCC a été identifié. 50 mères d'enfants atteints de CCM ont été examinées en utilisant l'analyse génétique moléculaire, c'est-à-dire la PCR pour la détection de

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anencephaly and corpus callosum agenesis – 14 cases, encephalocele – 7 cases, meningoencephalocele – 3 and corpus callosum hypogenesis – 2 polymorphisms.
Conclusions. The genetic polymorphism assay in folate cycle metabolism allows determining the genes associated with an increased risk of having children with CBM, which allows for its effective prevention.

Keywords: congenital malformations, brain, genes, folic acid, children.

List of abbreviations

BHMT – enzyme betaine homocysteine methyltransferase
CBM – congenital brain malformations
FA – folic acid
FGCP – folyl poly-y-glutamate carboxypeptidase
FOLH1 – folate hydrolase 1
FR – folate receptor
HHcy – mild hyperhomocysteinuria
MCM – multiple congenital malformations
MTNP1 – methylenetetrahydrophosphatidyltransferase
MTRR2 – methionine synthase reductase 2
NSC – neuronal germ cells
PCR – polymerase chain reaction
RBCF – red blood cell folates
RDA – Recommended Dietary Allowance
SAM – s-adenosylmethionine
WHO – World Health Organisation

4 polymorphismes des gènes du cycle du folate. Le traitement statistique des données obtenues dans l'étude a été réalisé à l'aide du programme Quanto.
Résultats. Le MCC dépendant des folates le plus fréquemment diagnostiqué chez les personnes hétérozygotes après les gènes du cycle des folates était l'hydrocéphalie révélée dans 54 cas, chez les personnes hétérozygotes ont été révélés des polymorphismes dans la craniosynostose – 15, l'anencéphalie et l'agénésie du corps calleux – 14, l'encéphalocèle – 7, la méningo-encéphalocèle – 3 et l'hypogénèse du corps calleux – 2 polymorphismes.
Conclusions. Le dépistage génétique des polymorphismes du métabolisme du cycle des folates permet de déterminer les gènes associés à un risque élevé de naissance d'enfants atteints de MCC, ce qui permet une prophylaxie efficace.

Mots-clés: malformations congénitales, cerveau, gènes, acide folique, enfants.

Introduction

Congenital brain malformations (CBM) are an important medical and social issue, ranking second in the structure of congenital malformations in the Republic of Moldova. CBM account for about 20% of the overall structure of infant mortality and morbidity[1]. In 2010-2022, there was a prevailing incidence of multiple congenital malformations according to the structure of congenital malformations in the Republic of Moldova, as well as anomalies of the cardiovascular system, musculoskeletal system, an increase in the incidence of malformations of the central nervous system (CNS), urinary and digestive systems[2]. The data study of 2022 compared to previous years showed a twofold increase in the incidence of multiple congenital malformations (MCM), which was ranked first (20.4%), followed by cardiovascular malformations (17.5%), and CNS congenital anomalies (17.2%), which show a difficult-to explain significant increase[1,2].

In 2022, the CBM occurrence was 17.2%. The changes in the number of births of children with spina bifida in the Republic of Moldova in 2011-2022 reflect the recent increasing incidence, i. e., in 2021 – 3.32/10,000 live births, in 2022 – 3.00/10000 live births, while in 2010-2022 it was 2.06/10000, as well as in EUROCAT countries with 2.13/10000 live births for the same period[2].

Studies over the past 10-15 years suggest that genetic polymorphisms in folate metabolic encoding enzymes may lead to aberrant methylation of peri-centromeric regions of some human chromosomes, favouring the development of CBM in foetuses[3-5]. A number of studies have been oriented to investigate the role of maternal polymorphisms of folate metabolism cycle genes as potential risk factors impacting the birth of a child with CBM, resulting in some surprising data. Recent researches suggest that at least four of these polymorphisms, namely MTHFR 677C>T, MTHFR 1298 A>C, MTR 2756 A>G and MTRR 66A>G, are likely to act as maternal risk factors in delivering children with CBM, also revealing complex genetic and metabolic interactions[6].

It is known that the lack of folic acid intake by the mother in the peri- and pre-conception period

Table 1. Ratio of normal and pathological genotype of the pholate cycle genes in mothers of children with CBM (n=50).

DIAGNOSIS	MTHFR 677 C>T (A222V) C/C		MTHFR 1298 A>C (E429A) A/A		MTR 2756 A>G (D919G) A/A		MTRR 66 A>G (I22M) A/A	
	C/C standard	*Mutant*	*A/A standard*	*Mutant*	*A/A standard*	*Mutant*	*A/A standard*	*Mutant*
Agenesis of the corpus callosum	2	4	4	2	2	4	2	4
Anencephaly	3	3	4	2	3	3	0	6
Craniosynostosis	3	4	5	2	4	3	1	6
Encephalocele	2	1	0	3	3	0	0	3
Congenital hydrocephalus	11	13	14	10	14	10	3	21
Hypogenesis of the corpus callosum	1	0	0	1	1	0	0	1
Meningoencephalocele	0	1	1	0	0	1	0	1

leads to an increased risk of having a child with CBM. Recent data point to a possible development of CBM in fetuses with insufficient folic acid intake during the periconceptual period, maternal polymorphism of the folate cycle genes, and epigenetic changes due to the involvement of several genes[7, 8].

Thus, polymorphisms of the MTHFR 677C>T, MTHFR 1298 A>C, MTR 2756 A>G and MTRR 66A>G genes encodes enzymes involved in the folate metabolic pathway, also known as one-carbon metabolism, which are possible maternal risk factors for giving birth of children with CBM[9].

The objective of the study was to investigate the genetic polymorphisms involved in folate metabolism in mothers at risk of giving birth to children with CBM, aiming at reducing the birth incidence of children with such pathologies.

Materials and methods

The target group included 105 children with confirmed diagnosis of CBM. 50 mothers of children with CBM were investigated by using the molecular-genetic method, i. e. polymerase chain reaction (PCR) for detection of four polymorphisms of the folate cycle genes, i. e. MTHFR 677C>T, MTHFR 1298 A>C, MTR 2756 A>G and MTRR 66A>G. The statistical data processing was performed via the Quanto program.

Results

The study results were analysed to reveal 30 polymorphisms of the MTHFR 677 C>T gene (A222V); 21 polymorphisms of the MTHFR 1298 A>C gene (E429A); 22 polymorphisms of the MTR 2756 A>G gene (D919G) and 42 polymorphisms of the MTRR 66 A>G gene with heterozygous status, being considered as a pathological genotype, in mothers of CBM children. The analysis of these genetic polymorphisms was carried out by the clinical genetic method with subsequent interpretation of the molecular genetic results. Thus, 22 polymorphisms of gene 677 C>T (A222V) were identified in 22 cases, as well as 21 polymorphisms of gene MTHFR 1298 A>C (E429A), 22 polymorphisms of gene MTR 2756 A>G (D919G) and 42 polymorphisms of gene MTRR 66 A>G (I22M) in homozygous status. The data assessment showed that the heterozygous status of the folate cycle genes, which are considered pathological, predominated compared to the homozygous status which is considered normal in mothers with CBM children. The most common folate-dependent CBM diagnosed with heterozygous status, following the folate cycle genes, was hydrocephalus found in 54 cases, heterozygous polymorphisms followed by craniosteatosis – 15 polymorphisms, anencephaly and corpus callosum agenesis – 14 polymorphisms, encephalocele – 7 polymorphisms, meningoencephalocele – 3 polymorphisms and corpus callosum hypoigeness – 2 heterozygous polymorphisms (Table 1).

The study showed that the MTR1298 and MTRR66 mutations significantly decrease serum folate levels. Overall, mutations in the MTR1298 and MTRR66 gene with heterozygous genetic polymorphism were found in 67% of mothers of children with CBM. This genetic polymorphism is related to the folate metabolism pathway and associated with specific functional involvement and conditions, including CBM, particularly of various neural tube defects.

Only 40% of mothers of children with CBM in the prenatal and intrauterine period took folic acid

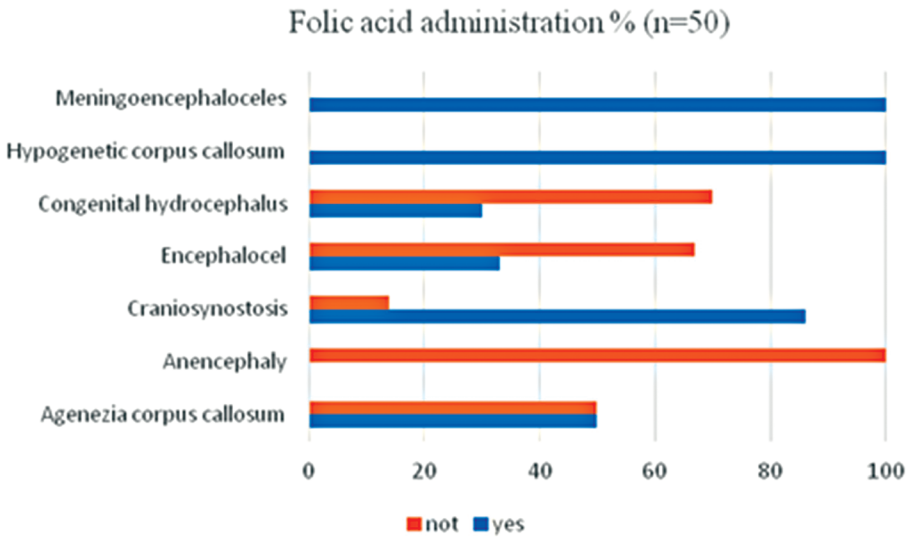

Figure 1. Data on folic acid intake in mothers, by type of CBM found in children, %.

supplements at a dose of 400 micrograms for a mean period of two months. Most women took folic acid after conception and for shorter periods of time. In 60% of cases, folic acid was not administered to mothers of children with CBM, for various reasons.

Figure 1 shows that after the clinical examination of mothers of children with CBM, the most common CBM diagnosed was anencephaly – 100%, followed by hydrocephalus – 70% and encephalocele – 65%. These CBMs were revealed based on the history collected from mothers who did not follow folic acid intake. Among other determined CBMs were agenesis of corpus callosum in 45% and craniostenosis in 15% of cases. In hypoplasia of corpus callosum and meningoencephaloceles, folic acid was administered in doses corresponding to the daily norm, though having a short mean time of administration of about one month.

Studies have shown that maternal folate deficiency can lead to the development of congenital brain anomalies, although the birth of children with brain anomalies is possible even in mothers with normal levels of folate in the blood serum. The present research found that folic acid deficiency might be a risk factor in the development of CBM in children.

Discussion

One cause of CBMs is gene dysregulation in the basic cycle of folate metabolism or methionine synthase. The incidence of heterozygous MTHFR deficiency in the population is high, the homozygous 677 TT type has been found to be a risk factor for mild hyperhomocysteinuria (HHcy), occurring in 10-15% of most populations, such as North American whites, many Europeans and East Asians[10]. Some Spanish/Southern Europeans, such as American hispanics, show a prevalence of up to 25%[11]. Various nervous system disorders occur in the homozygous MTHFR defect. Therefore, complete folic acid metabolism is modulated by several types of folate coenzymes. The basic function of a coenzyme results in the modulation of the metabolic pathway by acceptance or release of the monocarbon group[10-11].

Food folate: sources, bioavailability and requirements

Folic acid is a water-soluble B vitamin (B9), essential for the human organism, which can hardly be supplemented from food due to its low amount. Provided its very good absorption, the maximum concentration is reached in the blood in 30-60 min and no excess of folic acid is being stored in the body since it is rapidly metabolized by the liver and eliminated over 5-6 hours. Thus, foods rich in folic acid should be eaten on a daily basis to maintain the normal requirement of this vitamin. The main food sources of folate are dark green vegetables such as broccoli, spinach or lettuce, as well as pulses such as chickpeas, beans or lentils and some fruits such as avocados or oranges[19].

The folates in dietary supplements largely occur as polyglutamate, which are subsequently metabolized to monoglutamate prior to being absorbed. This folate form is used due to its stability and activity within the intestines after being reduced. Folic acid is completely oxidized, however, natural dietary folates are less stable and not completely bioavailable[11].

The enzyme responsible for this cleavage within the gut is folyl poly-y-glutamate carboxypeptidase (FGCP), which is located on the apical portion of the intestinal villi and is encoded by the glutamate

carboxypeptidase II (GCP1) gene, also known as folate hydrolase 1 (FOLH1). Folate monoglutamate is absorbed in the duodenum and upper jejunum by a high-affinity proton-coupled folate transporter PCFT1 (SLC46A1)[11].

The bioavailability of food-derived folate is estimated as ≤ 50% , whereas other studies show that folic acid ingested with food has an approximately 85% bioavailability of free folic acid, being 1.7 times higher than in natural folate[12].

The folate intake requirements were estimated based on normal plasma and erythrocyte folate concentrations maintenance. Folate supplements show a different bioavailability, compared to natural folic acid taken from food, which is quite important considering while assessing the dietary intake. Dietary folic acid equivalents are used to express folic acid recommendations and are calculated as μg of natural dietary folic acid + 1.7 times the amount of folic acid in supplements. This information is used by the Institute of Medicine of the National Academy of Sciences and other public health organizations to determine the Recommended Dietary Allowance (RDA) in some countries. In most European countries, however, this conversion factor is not applied and dietary folic acid intake is simply expressed as total folic acid in μg/day. However, recommendations may vary depending upon the country. A varied diet combined with a folic acid supplement intake of 400 μg/day is usually recommended from preconception to the end of the first trimester of pregnancy according to the World Health Organisation (WHO) recommendations [12].

Absorption, transport and metabolism

After being ingested and prior to being absorbed as dietary folate, polyglutamates must be hydrolysed to monoglutamate forms. After cleavage, they can be absorbed by a passive transport mechanism coupled with an active energy-dependent one. Transmembrane transport is dependent upon and induced by high intracellular organic anion concentrations, having a low affinity for folic acid. Monoglutamate via passive diffusion across the cell membrane occurs only in high folate concentrations[13].

Folate can be transported into the cell via three receptors in the apical membrane, α, β and γ. These folate receptors are mainly expressed within the placenta and fetal tissues. They are also found in adult tissues and in some tumours. These folate-transporting receptors are relatively slow. The receptors are significant in renal folate reabsorption, whereas α-folate receptors play an important role in embryo development and prevention of neural tube defects[13].

The intestinal metabolic rate depends upon the amount of the intracellular folic acid or folic acid intake. If the concentration is increased within the lumen, such as during supplement intake, most of the transported vitamin appears unchanged in the portal circulation since folate is methylated in the mucous membrane cells before entering the portal circulation as 5-methyl-tetrahydrofolate.

Under normal conditions, this metabolite is the primary circulating form of folate in plasma, binding to proteins, mainly albumin, which accounts for about 50% of bound folic acid. After the folate absorption into the portal circulation, almost 13-28 mg is retained in the liver and the remainder is distributed to other tissues[14]. It has been estimated that the total folate stores in the human body amount for 100 mg. The half-life of folate after entering the enterohepatic circulation is approximately 100 days. This folate cycle involves hepatic release of 5-methyl-THF into the bile via methionine synthase reductase 2 (MTRR2), which is then reabsorbed in the small intestine for being distributed to other tissues and liver, thus completing the enterohepatic cycle. The major role of this cycle is the maintenance of folate homeostasis. Minor amounts of folic acid are excreted via kidneys at normal folic acid intake.

In the cells, up to 50% of folic acid enters the mitochondria, where longer-chain glutamates normally settle. The 5-methyl-THF form of folic acid is found in the cytosol. The nucleus contains a pool of folate, the predominant form of which is used by the MTR to remethylate homocysteine to methionine. This reaction also produces THF, which can be methylated with glycine, as well as form 5,10-methylene-THF, or be catalysed to generate formate and enter the purine synthesis pathway.

Plasma folic acid concentrations are much lower than those in red blood cells and almost all red blood cell folates (RBCF) are polyglutamates of 5-methyl-THF.

Measuring total folic acid provides information about a person's folic acid status. Plasma folate levels may be affected by recent food intake[15]. Plasma folate is used to predict short-term plasma saturation, while RBC folate is an indicator of long-term folic acid status or storage. In addition, fasting plasma concentration of total homocysteine is used as a non-specific functional biomarker of folate status[15].

Epigenetic factors on spermatogenesis, a potential link to the 1 Carbon metabolic pathway

5-MTHF is known as the biologically active form of folate that enters the bloodstream and is transported to peripheral cells. 5-MTHF is the only form of folate that can cross the blood-brain barrier. During

gestation, 5-MTHF circulating in the mother binds to placental folate receptors on the chorionic surface, which is then transported to the fetus. As a result, peripheral and central organs take up 5-MTHF via the reduced folic acid, carrier (RFC) or folate receptor (FR). In peripheral cells, 5-MTHF is demethylated to THF via 5-MTHF-homocysteine methyltransferase (MTR) and vitamin B12-derived methylcobalamin as a coenzyme[15].

During the demethylation of 5-MTHF to THF, the methyl group is transferred to homocysteine to convert to methionine, linking the phenyl ring to the methionine ring in the 1-carbon pathway. A high serum homocysteine level (>100 μM) is a key biomarker of folate and vitamin B6 and B12 deficiency. Homocysteine can be methylated to methionine via the folate-independent pathway, which includes a betaine methyl group (derived from choline) and is catalyzed by the enzyme betaine homocysteine methyltransferase (BHMT)[15].

Methionine is then adenosylated to s-adenosylmethionine (SAM) , which is the universal methyl donor for methylation of DNA, RNA, proteins, phospholipids, histones, neurotransmitters, neuronal germ cells (NSC) and neuronal progenitor cells (NPC)[1]. SAM is responsible for genome, gene stability and transcription, protein localization and small molecule degradation. After donating its methyl group for DNA methylation, SAM is demethylated to S-adenosylhomocysteine (SAH) and hydrolyzed back to homocysteine by S-adenosylhomocysteine hydrolase, where it is either metabolized by a B6-dependent process to the amino acid cysteine or remethylated to methionine. The SAM:SAH ratio is an index of methylation, thus, a low ratio due to low B-vitamin intake can lead to hypomethylation of DNA and expression of pathological genes. Excessive intakes of these vitamins can increase the SAM:SAH ratio, resulting in DNA hypermethylation[2]. Therefore, the 1-carbon metabolic pathway is actually a tightly regulated metabolic chain of methyl intermediates and co-factors that lead to early epigenetic programming of life through DNA methylation and de novo nucleotide synthesis.

Some common polymorphisms (MTHFR 677C>T, MTHFR 1298 A>C, MTR 2756 A>G and MTRR 66A>G) may affect the serum folate levels. Numerous studies have shown that the MTHFR 677C>T mutation significantly lowers serum folate levels, while other studies have not confirmed such results. The association between MTHFR 1298 A>C and MTR 2756 A>G with folate levels remains controversial. The MTRR 66A>G polymorphism itself may not affect plasma folic acid levels[5]. However, these mutations may synergistically promote folate deficiency. A low folic acid level may increase the risk of hyperhomocysteinemia, which has been demonstrated in 77% of hypertensive patients. In many developed countries, i. e., US, Canada, UK, France and other Western countries, folic acid fortification has been fully implemented. This has been reported to reduce the risk of complex diseases[1].

Pathological genetic polymorphism that is linked to the folate pathway is associated with functional involvement and specific conditions, including neural tube defects (NTDs), cardiovascular disease and some cancers such as colorectal, breast and lung cancer.

The methionine synthase reductase (MTRR) enzyme plays a key role in folate-dependent homocysteine metabolism and is part of the electron transferase family. The enzyme has 3 characteristic binding sites, FMN, FAD and NADH. MTRR is responsible for the regulation of methionine synthase (MTR) by reductive methylation. Defective activity of this catalytic enzyme can lead to increased homocysteine levels. MTRR is a housekeeping gene and is located at chromosome 5 (5p15.2-p15.3)[6]. The most frequent polymorphism in the MTRR gene is the A66G substitution, which leads to a change of isoleucine to methionine at amino acid 22 (I22M). The A66G polymorphism does not alter the catalytic activity of the protein, the frequency of the 66G genotype is reported to be higher in NTD cases and their mothers than in control groups[7]. The I22M variant is located in the putative FMN-binding domain of the MTRR enzyme which is suggested to interact with MTR. Substitution of an isoleucine by a methionine in this part of the enzyme might disrupt the binding of MTRR to the MTR-cob (I) alanine complex, thereby decreasing the rate of homocysteine remethylation[16].

Malnutrition and malabsorption of folate and vitamin B12 or an inherited enzyme defect, such as MTHFR deficiency, can lead to hyperhomocysteinemia. The MTRR A66G polymorphism has also been associated with increased plasma homocysteine levels, although in some studies no effects of MTRR A66G SNP on plasma homocysteine have been reported. However, higher serum homocysteine concentration was reported as a risk factor for disorders such as neural tube defects, cardiovascular disease, Alzheimer's disease, psychiatric disorders such as schizophrenia [17], etc.

MTHFR

Methylenetetrahydrofolate reductase is an enzyme that catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, a cofactor in the remethylation of homocysteine to methionine.

In 1998, an enzyme variant termed "thermolabile" was described due to thermal instability *in*

vitro that was associated with an increased risk of coronary heart disease. This variant results from a point mutation in the MTHFR gene (C677T) and is 20% less efficient in metabolising homocysteine, which can lead to hyperhomocysteinemia, especially in people with folate deficiency. An 11% incidence in the Caucasian population of homozygous status for this mutation associated with increased risk of developing hyperhomocysteinemia and certain pregnancy complications including chromosomal abnormalities, congenital brain malformations, recurrent pregnancy loss, placental disorders and pre-eclampsia has been reported. Furthermore, in addition to the early pregnancy-related consequences in embryos or fetuses, hyperhomocysteinemia and homozygosity for the C677T mutation are also involved in the development of CBM in fetuses[18].

Patients who are homozygous for this mutation do not have hyperhomocysteinemia or an increased risk of CBMs. A mutation in the MTHFR gene has also been described that is quite common in the population, namely, A1298C. This mutation is not associated with hyperhomocysteinemia (regardless of heterozygous or homozygous status), however, combined heterozygous status for the 2 MTHFR mutations can generate clinical manifestations similar to those induced by homozygous status for the C677T mutation.

Studies have also shown that homozygosity for the C677T mutation shows a 2-3 fold increased risk for developing neural tube defects such as spina bifida and anencephaly compared to individuals without the mutation, whereas combined heterozygous status for C677T and A1298C may also be a risk factor for neural tube defects.

The mechanism by which MTHFR mutations cause pregnancy complications is poorly understood. One hypothesis is related to associated hyperhomocysteinemia, which causes damage to the vascular endothelium and leads to venous thromboembolism and placental insufficiency. However, cases of recurrent pregnancy loss associated with homozygous status for the C677T mutation have been described in which homocysteine levels were within normal limits, suggesting an independent mechanism of hyperhomocysteinemia. MTHFR mutation analysis is indicated in hyperhomocysteinemia, recurrent miscarriages, and CBMs.

Methionine synthase reductase (MTRR) polymorphism

The MTRR gene is located on chromosome 5p15.3-p15.2. Methionine synthase is a protein in methionine metabolism. The active form of methionine is involved in the biochemical transmethylation reaction. MTRR is part of the "folate" genes (such as MTR and MTHFR) and is also involved in homocysteine metabolism. Polymorphisms in folate genes cause a high risk of thrombophilia, development of atherosclerosis, recurrent thrombosis and fetal developmental defects. Polymorphism is aggravated by a vitamin B12 deficiency in the body[9].

Methylenetetrahydrophosphatidyltransferase (MTNP1*) and variant A223U (677C-> T)

The MTNP1 gene is located on chromosome 1 and is essential in folic acid metabolism. The enzyme catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate. The latter is the active form of folate required to form methionine from homocysteine and then 8-adenosylmethionine, which is essential in DNA methylation. The enzyme deficiency contributes to both teratogenic, i. e., fetal damage, as well as mutagenic DNA damage. Genotype 677C (C\C) may cause fetal neural tube defects. The presence of a heterozygous variant leads to an increase in homocysteine levels up to 30 μmol/L. In hyperhomocysteinemia, a homozygous variant of a mutation is detected in 70% of cases. A homozygous mutation of the 677T form leads to an approximately 10-fold increase in the risk of hyperhomocysteinemia. Patients with hyperhomocysteinemia often have low folic acid and vitamin B12 levels in the blood [19].

Mutations in the MTHFR and MTRR genes regulate the folate and homocysteine metabolisms, whereas their excess show higher risk of developing CBM in children. These results have also been obtained within the present study.

Conclusions

Maternal folate deficiency can lead to the development of congenital brain abnormalities, though, mothers with normal serum folate levels are likely to give birth to children with brain abnormalities, as well. The present research found that folic acid deficiency might be a risk factor of paediatric CBMs.

The study demonstrated that the MTR1298 and MTRR66 mutations significantly decrease the serum folate levels, and genetic mutations with heterozygous genetic polymorphisms were found in 67% of mothers of children with CBMs with functional involvement and specific conditions including CBM, particularly of various neural tube defects. Genetic testing for folate cycle metabolism polymorphisms may allow an effective prevention of the birth of children with CBMs.

Mutations in the MTHFR and MTRR genes regulate the folate and homocysteine metabolisms, whereas their excess shows an increased risk for the

development of CBM in children, a fact proved by the study results obtained. Thus, the study of folate cycle gene polymorphisms will contribute to a more thorough and accurate understanding of the causes of the development of CBMs in children.

The genetics of folate metabolism directly correlates with the maternal risk of delivering a child with CBMs, whereas the metabolic correction by periconceptional folic acid intake may decrease the risk of developing folate-dependent abnormalities in children.

Author Contributions:

Conceptualization, S.H.; methodology, M.S., E.V.G. and N.R.; formal analysis, O.T. and M.S.; investigation,N.L. and C.C; data curation, L.C., L.F. and O.T.; writing-original draft preparation, M.S. and O.T.; writing-review and editing, S.H. and N.R.; supervision, D.T.A.P.; project administration, S.H.; funding acquisition, S.R. and M.S. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law."

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