PPN

Personalized Psychiatry and Neurology

Article

Prediction of Congenital Malformations in The Fetus Based on The Carriage of Single Nucleotide Variants of Folate Cycle Genes by a Mother with Epilepsy

Albina V. Yakunina[1], Anastasiya S. Chesnokova[1], Natalia N. Savelieva[1]

[1] Samara State Medical University, 443099 Samara, Russia;
* Correspondence: ayakunina@bk.ru (A.V.Y.)

Abstract: The prediction and prevention of congenital malformations in the fetus of women with epilepsy is an urgent problem due to the need for long-term use of antiepileptic drugs. **The aim** was to study the frequency of carriage of single-nucleotide variants (SNVs) rs1801133 and rs1801131 of the *MTHFR* gene; rs1801394 of the *MTRR* gene, rs1805087 of the *MTR* gene and rs1051266 of the *SLC19A1* gene in women with epilepsy and to evaluate their associations with congenital malformations of the fetus (CMF). **Materials and methods.** The study included 61 patients with epilepsy who had a history of one or more pregnancies with a known outcome due to the presence of CMF in the child. The patients were divided into two groups: 20 patients had various CMF (the main group), 41 patients had children who were born without CMF (the comparison group). DNA was isolated from the blood, and genotyping of five DNA sequences in four genes was performed by polymerase chain reaction. The frequencies of genotypes and alleles in the mothers of the main group and the comparison group were determined, the differences were assessed using Pearson's chi-square criterion ($\chi 2$) and Fisher's exact criterion**.** **Results.** There were no statistically significant differences in the frequencies of genotypes and alleles for all analyzed SNVs between the main group and the comparison group ($p > 0.05$). There were no statistically significant differences in the frequencies of genotypes and alleles of SNVs of the studied genes in mothers of children with CMF (n = 14) and without CMF (n = 22) taking valproic acid ($p > 0.05$). A statistically significant relationship has been revealed between the carrier of a certain haplogroup of the mother and the formation of CMF. **Conclusion.** The development of VPD in a child is a multifactorial phenomenon in which genetic factors with a small effect size can play a role only in the case of certain unfavorable combinations.

Keywords: *folate cycle genes, single nucleotide variant, epilepsy, pregnancy, congenital malformation.*

Citation: Yakunina, A.V.; Chesnokova, A.S.; Savelieva, N.N. Prediction of Congenital Malformations in The Fetus Based on The Carriage of Single Nucleotide Variants of Folate Cycle Genes by a Mother with Epilepsy. *Personalized Psychiatry and Neurology* **2025**, *5 (2)*: 19-25. https://doi.org/10.52667/2712-9179-2025-5-2-19-25

Chief Editor: Nikolaj G. Neznanov, DMedSci, Professor

Received: 05 May 2025
Accepted: 11 June 2025
Published: 15 June 2025

Publisher's Note: V.M. Bekhterev NMRC PN stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. INTRODUCTION

The prognosis and prevention of congenital malformations in the fetus (CMF) in children from women with epilepsy is an urgent problem due to the need for long-term use of antiepileptic drugs (AEDs) [1-3]. It has been proven that the teratogenic risk increases with the use of polytherapy, high daily dosages, as well as individual AEDs, which include valproates and topiramate [4-6]. The lack of alternative and less aggressive treatment options for epilepsy (especially in the case of its pharmacoresistance) makes it necessary to actively search for other possible modifiable maternal factors for the occurrence

of CMF. Currently, folate cycle disorders with folate deficiency and hypercysteinemia are considered to be one of the significant teratogenic predictors [4, 7, 8]. Folate cycle products are used for cellular processes such as methionine reduction, synthesis of purine and pyrimidine nucleotides, and DNA methylation [7]. More than ten enzymes are involved in the folate cycle, the main of which are MTHFR (methylenetetrahydrofolate reductase), MTR (methionine synthase), MTRR (methionine synthase reductase), and the folate transporter protein SLC19A1. Significant single nucleotide variants (SNVs) have been described for the genes encoding these proteins, which lead to a change in enzymatic activity [9]. In turn, impaired folate metabolism in women of fertile age leads to a greater susceptibility to the effects of teratogenic and mutagenic environmental factors on the fetus, including xenobiotics, which include AEP [10].

The greatest attention is currently being paid to the SNVs rs1801133 and rs1801131 of the *MTHFR* gene; rs1801394 of the *MTRR* gene, rs1805087 of the *MTR* gene and rs1051266 of the *SLC19A1* gene, as there is evidence of their significant role in the occurrence of neural tube failure and other organs and systems [9]. Meanwhile, studies on the development of folate cycle genes in the population of women with epilepsy, including on the background of taking AEP, are few, and the results are ambiguous. In this regard, it is an urgent task to determine the genomic architecture of the folate cycle in women with epilepsy, which predisposes to the occurrence of CMF.

Aim – to study the frequency of SNVs rs1801133 and rs1801131 of the *MTHFR* gene; rs1801394 of the *MTRR* gene, rs1805087 of the *MTR* gene and rs1051266 of the *SLC19A1* gene in women with epilepsy and to evaluate their associations with major CMFs.

2. MATERIAL AND METHODS

The study included 61 women with epilepsy who had a history of one or more pregnancies that did not terminate in the first trimester, with a known outcome due to the presence of CMF in the child. The patients were divided into two groups: 20 patients had various CMFs (the main group), 41 patients had children who were born without CMF (the comparison group). The groups were comparable in terms of the age of the patients and the forms of epilepsy.

Treatment of epilepsy during pregnancy: 8/61 (13.1%) women did not receive PEP, 37/61 (60.7%) women took one PEP, 16/61 (26.2%) received polytherapy. 23/37 (62.2%) patients received monotherapy with valproic acid, while 13/16 (81.3%) patients received polytherapy with valproic acid. Fetal HPV was reported in 14/36 (38.9%) cases in children born to women who took valproic acid during pregnancy, as well as in 6/26 (24.0%) cases in children born to women who did not receive valproic acid.

Genomic DNA was isolated from venous blood samples. Then, the carriage of SNVs rs1801133 and rs1801131 of the *MTHFR* gene, rs1805087 of the *MTR* gene; rs1801394 of the MTRR gene and rs1051266 of the *SLC19A1* gene was determined using the method of allele-specific amplification in Tertsik amplifiers (DNA technology) followed by electrophoresis with ethidium bromide in 3% agarose gel.

Table 1. The frequency of alleles and genotypes of the SNVs rs1801133 and rs1801131 of the *MTHFR* gene; rs1805087 of the *MTR* gene; rs1801394 of the *MTRR* gene and rs1051266 of the *SLC19A1* gene

Genotype/ Alleles	**CMF "yes" (main group)**		**CMF "no" (comparison group))**		**p**	**OR**	
	n	**%**	**n**	**%**		**n**	**95% CI**
rs 1801133 of the *MTHFR* gene							
Genotype							
CC	12	60	24	59	0.89	1.1	0.32 - 3.7
CT	8	40	17	42			
TT	0	0	0	0			
Total	20	100	41	100			
HWL, p		0.34		0.11			
Allele							
C	32	80	65	79	0.91	1.0	0.38 – 3.1
T	8	20	17	21			
Total	40		82	100			
rs 1801133 of the *MTHFR* gene							
Genotype							
AA	6	30	20	49	0.35	-	-
AC	11	55	16	39			
CC	3	15	5	12			
Total	20	100	41	100			
HWL, p		0,84		0,34			
Allele							
C	23	57	56	68	0.71	1.6	0.7 – 3.7
A	17	43	26	32			
Total	40		82				
rs1801394 of the *MTRR* gene							
Genotype							
AA	3	15	6	15	0.44	-	-
AG	12	60	18	44			
GG	5	25	17	41			
Total	20	100	41	100			
HWL, p		0.52		0.21			
Allele							
A	18	57	30	68	0.93	1.4	0.6 – 3.3
G	22	43	52	32			
Total	40	100	82	100			
rs1805087 of the *MTR* gene							
Genotype							
AA	12	60	27	66	0.66	-	-

AG	7	35	10	24			
GG	1	5	4	10			
Total	20	100	41	100			
HWL, p		0.76		0.78			
Allele							
A	31	77	64	78	0.91	1.0	0.6 – 2.8
G	9	23	18	22			
Total	40	100	82	100			
rs1051266 of the *SLC19A1* gene							
Genotype							
AA	5	60	17	66	0,66	-	-
AG	9	35	11	24			
GG	6	5	13	10			
Total	20	100	41	100			
HWL, p		0.52		0.002			
Allele							
A	19	48	45	55	0.51	1.3	0.6 – 3.1
G	21	52	37	45			
Total	40	100	82	100			

To determine the statistical significance of the differences between qualitative characteristics, we applied Pearson's chi-square criterion ($\chi 2$) and Fisher's exact criterion. To assess the risk factors associated with the development of CMFs, the odds ratio (OR, 95% CI) was evaluated. Intergroup differences were recognized as statistically significant at a value of $p < 0.05$. To identify the pattern of similarity and the formation of internally homogeneous blocks, the original author's program SANCT – structural analysis of contingency tables (N.N. Khromov-Borisov, T.B.L. Kist, G.B. Lazzarotto) was used.

3. RESULTS

The distribution of genotypes in the main group and the comparison group corresponded to the Hardy-Weinberg law (HWL), which indicates the representativeness of the sample and the ability to extrapolate the results of the study to the entire population. The results of the frequency of carriage of alleles and genotypes of the studied TB are presented in Table 1. The frequency of carriage of alleles and genotypes of rs1801133 and rs1801131 of the *MTHFR* gene, rs1805087 of the MTR gene, rs1801394 of the *MTRR* gene and rs1051266 of the *SLC19A1* gene did not differ in women with children with CMF and women without CMF in offspring (Table 1).

The frequency of alleles and genotypes rs1801133 and rs1801131 of the *MTHFR* gene, rs1801394 of the *MTRR* gene, rs1805087 of the *MTR* gene and rs1051266 of the *SLC19A1* gene was evaluated in women receiving valproic acid during pregnancy, depending on

the observation group: CMF "yes" (n1 = 14) and CMF "no" (n2 = 22). There were no statistically significant intergroup differences in the frequency of alleles and genotypes of the folate cycle genes studied in women with and without children with CMFs.

Table 2. The frequency of occurrence of SNV haplogroups rs1801133 and rs1801131 of the *MTHFR* gene, rs1801394 of the *MTRR* gene, rs1805087 of the *MTR* gene and rs1051266 of the *SLC19A1* gene in mothers of children with and without congenital malformation of fetus (CMF)

Haplogroups	**CMF**		**Total**
	"yes" (n = 40)	**"no" (n = 82)**	
Block 1 *TAAAA, CAGAG, CAGGA, TAGAG, CAGGG, CCGAG, TAAAG ,CAAAG, CCGAA, CCAAG, CCAGA ,CAAGG, CAGAA*	39 97.5%	68 82.9%	107 87.7%
Block 2 *TAAGA, TAGAA, CAAAA, CAAGA, CAGAA, CCGGA, CCGGG*	1 2.5%	14 17.1%	15 12.3%
All	40 (100.0%)	82 (100.0%)	122 (100.0%)
p	0.022		

As a result of processing using the SANCT program, two internally homogeneous blocks of SNV haplogroups rs1801133 and rs1801131 of the *MTHFR* gene were isolated; rs1801394 of the *MTRR* gene, rs1805087 of the *MTR* gene and rs1051266 of the *SLC19A1* gene with statistically significant differences (p = 0.01). In addition, a statistically significant relationship was demonstrated between the carriage of a certain haplogroup of the mother and the formation of congenital malformations of the fetus (Table 2).

There is evidence of a combined effect on the occurrence of CMFs of the carrier of folate cycle genes and the intake of AEPs by the mother during pregnancy [15]. The high teratogenic potential of valproic acid is well known [16, 17], so we identified mothers who received this drug during pregnancy in a separate group. However, no statistically significant differences were found in the frequency of alleles of the genotypes of the folate cycle genes studied in mothers of children with and without CMFs, depending on the fact of valproic acid therapy.

Many authors admit that there are no single (defined) candidate genes for predicting the risk of developing CMFs that affect management during high-risk pregnancy. An assessment of polygenic risk is necessary when the genetic risk is summed up, depending on the contribution of several SNVs associated with a particular phenotype [19-20]. Using the SANCT program allowed us to identify two blocks of homogeneous haplogroups with statistically significant differences between each other. We have shown that the combined carriage of haplogroups TAAAA, CAGE, CAGE, TAG, CAG, CCGAG, TAAAG, CAAAG, CCGAA, CCAGA, CAAGG, CAGAA, CCAAG in a mother with epilepsy can be regarded

as a risk factor for the development of VPD in her child (OR 8.029 [95% CI 1.017-63.418], p = 0.022). Haplogroups TAIGA, TAIGA, CAAAA, CAAGA, CAGAA, CCGGA, CCGGG can be considered as a protective factor that reduces the risk of developing CVD (OR 0.026 [95% CI 0.016-0.984] p = 0.022).

4. LIMITATION

It should be recognized that the present study includes a small sample size (women with epilepsy), so the results should be interpreted with caution, especially when used in real clinical practice.

5. CONCLUSION

Thus, the development of AEP-induced CMFs is a multifactorial phenomenon in which genetic factors with a small effect size can play a significant role only in the case of certain unfavorable combinations.

Author Contributions: A.V.Ya., A.S.Ch. – scientific data collection, its systematization and analysis, wrote the first draft of the manuscript. N.N.S. – managed the development of the study concept, detailed manuscript editing and revision, statistical data processing. A.V.Ya. - manuscript editing and revision. All authors have read and agreed to the published version of the manuscript.

Funding: The study was the authors' initiative without external funding.

Institutional Review Board Statement: The present study was approved by the Ethics Committee of Samara State Medical University.

Informed Consent Statement: All female patients in the study signed a voluntary informed consent to participate in this study and genetic testing.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES:

1. Meador K.J. Effects of maternal use of antiseizure medications on child development. *Neurol Clin*. **2022**;40(4):755-768. doi: 10.1016/j.ncl.2022.03.006
2. Holmes L.B., Quinn M., Conant S., et al. Ascertainment of malformations in pregnancy registries: Lessons learned in the North American AED Pregnancy Registry. *Birth Defects Res*. **2023**;115(14):1274-1283. doi: 10.1002/bdr2.2188
3. Lagana A.S., Triolo O., D'Amico V., et al. Management of women with epilepsy: from preconception to postpartum. *Arch Gynecol Obstet*. **2016**;293(3):493-503. doi: 10.1007/s00404-015-3968-7
4. Dmitrenko D.V., Shnayder N.A., Egorova A.T. *Epilepsy and pregnancy*. Moscow, 2014. (In Russ.). ISBN 978-5-98495-025-1
5. Keni R.R., Jose M., Sarma P.S., Thomas S.V. Kerala Registry of Epilepsy and Pregnancy Study Group. Teratogenicity of antiepileptic dual therapy: Dose-dependent, drug-specific, or both? *Neurology*. **2018**;90(9):e790-e796. doi: 10.1212/WNL.0000000000005031
6. Cohen J.M., Alvestad S., Cesta C.E., et al. Comparative safety of antiseizure medication monotherapy for major malformations. *Ann Neurol*. **2023**;93(3):551-562. doi: 10.1002/ana.26561

7. Shengelia M.O., Bespalova O.N., Shengelia N.D., et al. Folate-dependent congenital malformations of the fetus. *Womens health and reproduction*. **2022**;1(52):49-57. (In Russ.).
8. Sijilmassi O., Del Río Sevilla A., Maldonado Bautista E., Barrio Asensio M.D.C. Gestational folic acid deficiency alters embryonic eye development: Possible role of basement membrane proteins in eye malformations. *Nutrition*. **2021**;90:111250. doi: 10.1016/j.nut.2021.111250
9. Almekkawi A.K., Al Jardali M.W., Daadaa H.M., et al. Folate pathway gene single nucleotide polymorphisms and neural tube defects: A systematic review and meta-analysis. *J Pers Med.* **2022**;12(10):1609. doi: 10.3390/jpm12101609
10. Liew S.C., Gupta E.D. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. *European Journal of Medical Genetics*. **2015**;58(1):1-10. doi: 10.1016/j.ejmg.2014.10.004
11. Taiwo E.T., Cao X., Cabrera R.M., et al. Approaches to studying the genomic architecture of complex birth defects. *Prenat Diagn*. **2020**;40(9):1047-1055. doi: 10.1002/pd.5760
12. Dewelle W.K., Melka D.S., Aklilu A.T., et al. Polymorphisms in maternal selected folate metabolism-related genes in neural tube defect-affected pregnancy. *Adv Biomed Res*. **2023;**12:160. doi: 10.4103/abr.abr_103_22
13. Godbole K., Gayathri P., Ghule S., et al. Maternal one-carbon metabolism, MTHFR and TCN2 genotypes and neural tube defects in India. *Birth Defects Res A Clin Mol Teratol*. **2011**;91:848–56. doi: 10.1002/bdra.20841
14. Ouyang S., Li Y., Liu Z., et al. Association between MTR A2756G and MTRR A66G polymorphisms and maternal risk for neural tube defects: A meta-analysis. *Gene.* **2013**;515:308–12. doi: 10.1016/j.gene.2012.11.070
15. Dmitrenko D.V., Shnayder N.A., Strotskaya I.G., et al. Mechanisms of valproate-induced teratogenesis. *Neurology, Neuropsychiatry, Psychosomatics*. **2017**;1S:89–96. doi: 10.14412/2074-2711-2017-1S-89-96
16. Shnayder N.A., Grechkina V.V., Khasanova A.K., et al. Therapeutic and toxic effects of valproic acid metabolites. *Metabolites.* **2023**;13(1):134. doi: 10.3390/metabo13010134
17. Shnayder N.A., Grechkina V.V., Trefilova VV, et al. Valproate-induced metabolic syndrome. *Biomedicines.* **2023**;11(5):1499. doi: 10.3390/biomedicines11051499
18. Boyle E.A., Li Y.I., Pritchard J.K. An expanded view of complex traits: from polygenic to omnigenic. *Cell.* **2017;**169(7):1177–1186. doi: 10.1016/j.cell.2017.05.038
19. Finnell R.H., Caiaffa C.D., Kim S.E., et al. Gene environment interactions in the etiology of neural tube defects. *Front Genet.* 2021;10;12:659612. doi: 10.3389/fgene.2021.659612
20. Choi S.W., Mak T.S., O'Reilly P.F. Tutorial: a guide to performing polygenic risk score analyses. *Nat. Protoc.* **2020**;15(9):2759–2772. doi: 10.1038 /s41596-020-0353-1