

Detection of *MTHFR* C677T Genotyping for Spina Bifida Defects using ARMS-PCR Technique

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Abstract

Neural tube defects (NTDs) are complex structural deformities in newborn infants. These defects are caused by a blend of genetic and environmental factors in the abnormal phenotype. The current study aims to determine the genetic variation polymorphism for methylenetetrahydrofolate reductase (*MTHFR*) C677T rs180133 genotyping and to detect single nucleotide polymorphisms (SNPs) in a DNA sample of Spinal Bifida (SB) and compare it with control newborn children. Blood samples were collected from newborns (50 children under one year old) (25 cases of spinal bifida (SB) and 25 controls). The DNA was extracted and amplified by the tetra ARMS-PCR technique (Amplification Refractory Mutation System Polymerase Chain Reaction). ARMS-PCR technique consists of four primers (two outer and two inner) to amplify target DNA sequences for different genotypes for the distribution of the genetic variation of *MTHFR* genotyping of different genotypes (wild-type (CC) and mutant-type (TT) alleles). The results indicate that the genetic mutation of the enzyme tetrahydrofolate reductase was associated with spina bifida in newborns and could serve as a biomarker to detect the development of spina bifida in newborns.

Keywords: Genotyping, Methylenetetrahydrofolatereductase, Neural tube defect, Spine bifida, Tetra primer ARMS PCR technique.

Introduction

Neural tube defect is a general term encompassing congenital malformations of the central nervous system (CNS). Neural tube defects (NTDs) are a type of birth defect that occurs when the neural tube fails to close during early gestation. It is the result of the incomplete closure of the neural tube and incidence worldwide ranges from 1.0 to 10.0 per 1,000 births^{1,2}. NTDs can be categorized as "open" or "closed" based on whether the neural tissue is covered. This condition can lead to anencephaly, spina bifida, and other congenital abnormalities³. Spina bifida (SB), a type of NTD, occurs when the development of the fetus's spine is

incomplete during the first month of pregnancy⁴. The mortality rate for spina bifida is 7%, whereas it is 46% for encephalocele and 100% for anencephaly⁵. The severity of the condition can vary, ranging from mild with no noticeable symptoms to severe with nerve damage. Spina bifida is usually evident at birth and often occurs within the first 28 days of pregnancy before a woman even realizes she is pregnant^{6,7}. Infants with anencephaly typically survive only a few days after birth⁸. Folate metabolism genes have a function in the folate metabolism cycle recent research has revealed that the incidence rate of SB is

approximately 3.63 per 10,000 live births in the United States (U.S.) and 18.6 per 10,000 worldwide. Although the mortality rate for liveborn infants with SB has decreased to around 8%, it remains more than 10 times higher than the national average for all U.S. births. Survivors often experience motor impairment, bowel and bladder dysfunction, and neurological complications. Notably, the presence of Arnold-Chiari II malformations, characterized by the downward displacement of the cerebellar vermis and tonsils, is associated with SB, and can significantly impact motor, cranial nerve, and cognitive functions, affecting the quality of life for SB patients and their families. Both genetic and environmental factors play a role in the development of spina bifida. While the genetic component is estimated to contribute to 60-70% of cases, non-genetic risk factors like decreased folate consumption, maternal anticonvulsant therapy, diabetes mellitus, and obesity also play a role⁹. Genes of the methionine cycle involved in the folate metabolism cycle have been studied as potential risk factors for SB. The candidate genes of the methionine cycle to evaluate the association with NTD risk (i.e., DHFR, MTHFR, MTHFD1, MTR, MTRR, and TYMS and methyltransferases¹⁰. The methionine cycle, which is crucial for translating genes and involves the conversion of 5-MTHF and Homocysteine (Hcy) to methionine, S-adenosyl-methionine (SAM), and S-adenosyl Hcy, is particularly relevant^{11,12}. One specific

candidate gene in this pathway is the methylenetetrahydrofolate reductase MTHFR gene. This enzyme plays a role in the processing of amino acids and it is important for a chemical reaction involving forms of folic acid vitamin (vitamin B9), which undergoes a mutation called Single nucleotide polymorphisms (SNP) resulting in a C to T transition at nucleotide 677¹³. This mutation has been associated with an increased risk of developing NTDs in certain populations. The MTHFR gene is located on chromosome 1 p 36.3¹⁴. The MTHFR enzyme plays a significant role in processing amino acids and it is essential for the conversion of folic acid into 5-methyltetrahydrofolate^{15,16}. This conversion is necessary for the multi-step process that transforms the amino acid homocysteine into methionine, a vital component for protein production and other essential compounds in the body¹⁷. Variations in the MTHFR gene can impact the processing capacity of methylenetetrahydrofolate reductase folate and potentially increase the risk of NTDs^{18,19}. First-degree relatives, such as siblings and children, have an increased risk of developing spina bifida compared to the general population²⁰. Therefore, the current study aimed to investigate the role of C677T MTHFR gene polymorphism, in children with spina bifida within the Iraqi population and serve as a biomarker for the detection of the MTHFR genotype risk factor and development of SBs in the Iraqi population

Materials and Methods

Sampling

The study was designed as a case-control prospective study dependent on this research on 50 Iraqi infants (25 with cases of SBs and 25 healthy control) in the age range 0– 2 years both sexes in the period from August 2022 to October 2022 from hospitals in Iraq. Two mL of venous blood samples in an EDTA tube were collected for the genotyping study. All the participants were evaluated by a qualified physician neurosurgical in the department of hospitals in Hillah and Baghdad cities after obtaining the Institutional Ethical Committee approval and written informed consent from study subjects after clinical examination for everyone.

Genomic DNA Extraction

The Genomic DNA samples were isolated from 2ml whole blood using a DNA Extraction and Purification Kit (Bioneer, Korea) following the instructions of the manufacturer protocol. The concentration of DNA and purity were measured using a nano-drop and quality was checked by electrophoresis using a 1% agarose gel.

Genetic Analysis of *MTHFR* C677T

In the current study, primers were designed based on an online tool in a National Center for Biotechnology Information (NCBI) database repository which is available for free to the public use, following the optimal standard criteria. Then synthesis was performed by Bioneer, Korea. The primer sequences are as follows:

Table 1. Specific Tetra Primer sequences for C677T *MTHFR* gene.

SNP Primer	Primer Sequence	Length (bp)
<i>MTHFR</i>		
C677T		
IFP (T allele)	5'AAGGTGTCTGCGGGCGT3'	165
IRP (C allele)	5'AAAGCTGCGTGATGATGAAATAA G3'	181
OFP	5'CCAGTCCCTGTGGTCTCTTC3'	305
ORP	5'AGGGAGCTTATGGGCTCTCCT3'	305

IFP: Inner forward primer, ORP: Outer reverse primer, OFP: Outer forward primer, IRP: Inner reverse primer.

Inner forward primer IFP (T allele), Inner reverse primer IRP (C allele), Outer forward primer OFP and Outer reverse primer ORP. Genotyping for the *MTHFR* (rs180133, C677T) variant was carried out through ARMS-PCR. The ARMS-PCR product reaction volume was 25µl containing 1µl IFP, 1µl IRP, 1µl OFP, 1µl ORP, 12.5 µl of Green Master Mix, and 3µl of gDNA, and 5.5µl of Nuclease free water. The conditions of the ARMS-PCR were performed on a thermal cycler (Bioneer, Korea) and included an initial pre-denature at 95°C for 10 minutes (min.) followed by

35 cycles under the following conditions: denaturation at 94°C for 2 min. annealing at 57°C for 1min, extension at 72°C for 1min, and a final extension cycle of 72°C was for 5 mins. The ARMS-PCR products were visible on 2% agarose gel, stained with ethidium bromide, and visualized by UV light using a DNA ladder (100 bp). The distribution of the *MTHFR* genotype in the control mother was found to be in Hardy-Weinberg equilibrium (HWE) as shown below: HWE: ($p^2 + 2pq + q^2 = 1$) Where p is defined as the frequency of the dominant allele and q as the frequency of the recessive allele for a trait controlled by a pair of alleles (A and a).

Statistical analysis

The Statistical Analysis System ²¹ program was utilized to assess the impact of various factors on study parameters. Hardy-Weinberg equilibrium (HWE) was examined for a genetic epidemiology study, with a significance level set at $P < 0.05$. Logistic regression analysis was used to calculate all odds ratios (ORs) and 95% confidence intervals (CIs) to demonstrate the degree of association.

Results and Discussion

The results of the current study show that the distribution of sex was 12 (48%) males, 13 (52%) females for the case group 14 (56%) males, and 11 (44%) females for the control group. These results show there were no significant differences between both study groups for the sexes. A similar result there were no significant differences between both study groups for the sexes was reported by Behere *et al* ²². The gDNA produced a sharp intense band product with high integrity and purity when electrophoresed on a 1% agarose as shown in Fig. 1. The concentration of gDNA was counted by nano-drop spectrophotometer ranging from 80–120 ng /µl and the 260/A280 ratio provided ranged between 1.8 and 1.9 in purity.



Figure 1. Genomic DNA electrophoresed on 1% agarose, 5V/cm. Lane 1-13 refers to cases and Lane 14-19 refers to controlling children.

The result of the *MTHFR* C677T genotyping product used for ARMS-PCR was electrophoresed in agarose gel (Fig. 2 and Fig. 3) for both studied groups giving three polymorphisms, including the wild-type homozygous CC genotype having a single band with length of 305bp, the mutant homozygous TT genotype having a single band with length of 165bp, while the heterozygous CT genotype having 2 bands with length of 181bp and 305bp.

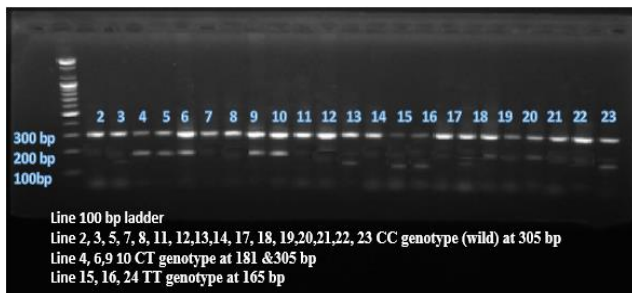


Figure 2: Electrophoresis of ARMS-PCR products for *MTHFR* C677T polymorphism cases' group.

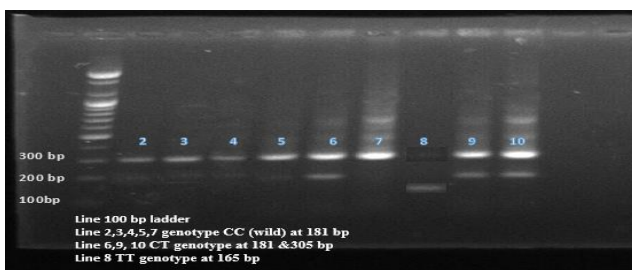


Figure 3: Electrophoresis of ARMS-PCR products for *MTHFR* C677T polymorphism of control group.

The results of *MTHFR* gene polymorphism as shown in Table 2 provided the genotype of the wild-type homogenous CC 18 (72%) and 22 (88%) for the cases and controls, respectively, with a P-value of 0.527 and a reference Ref¹. On one hand, the results of the mutant homogenous TT were 2 (16%) and 0 (0%) for the cases and controls, respectively, with a P-value of 0.289 and an OR of 0.052 (0.02-0.27). These results revealed there were no significant differences between homogenous CC and TT genotypes for the *MTHFR* C677T. On the other hand, the results of the *MTHFR* gene polymorphism for the heterogeneous CT genotype were 4 (16%) for the cases and 3 (12%) for the controls, with a P-value of 0.705 and an OR of 0.613 (0.32-1.150). These results of the CT genotype were not significantly different between the two studied groups of children. Moreover, the results of *MTHFR* genotyping of C allele frequency were 0.8 and 0.94 for cases and controls, respectively, with an OR of 0.219 (0.10-0.74). In addition, the results of *MTHFR* genotyping of T allele frequency were 0.2 and 0.6 for cases and controls, respectively, with an OR of 0.307 (0.14-0.91). These results explain that there were no risk factors for the SB case. The results indicate the distribution of *MTHFR* gene polymorphisms (CC, TT, and CT genotypes) and

allele frequencies in cases and control for no significant differences between the two groups for the CC genotype, and for the TT genotype, the OR suggests a potential protective effect, as do the results for the CT genotype²³. The C allele frequency analysis shows no significance in cases compared to controls, with an OR indicating a potential protective effect. In addition, the T allele frequency shows no significant difference in cases compared to controls, indicating a potential protective effect. was associated with neural tube defect risk in patients and it might be counted as a molecular marker for evaluating the susceptibility of NTDs. A similar result showed that the presence of a mutant allele in homozygous or heterozygous conditions for both SNPs had increased the risk associated with NTDs²⁴.

Table 2. *MTHFR* C677T Genotype distribution and allele frequency in both study groups.

<i>MTHFR</i> C677T Genotyping N=50	Cases N=25	Controls N=25	P-value	OR (95%) of SBs
CC	18 (72%)	22 (88%)	0.527 NS*	Ref.**=1
CT	4 (16%)	3 (12%)	0.705 NS*	0.613 (0.32-1.150)
TT	3 (12%)	0 (0%)	0.289 NS*	0.052 (0.02-0.27)
Allele Frequency C	0.8	0.94	0.453 NS*	0.219 (0.10-0.74)
Allele Frequency T	0.2	0.06	0.052 NS*	0.307 (0.14-0.91)

NS*: Non-Significant, Ref.**: Reference=1

Based on the current results, it seems that the observed and expected allele frequencies for the CC, TT, and CT genotypes in both cases and controls were compared to assess deviations from HWE as shown in Table 3. The results, according to HWE, produced each of the observed and expected results as follows: In these cases, the CC allele frequency produced 18 and 16 (64%) as observed and expected, respectively. On the one hand, the TT allele frequency produced 3 and 1 (4%) as observed and expected, respectively. On the other hand, the results of CT allele frequency were 4 and 8 (32%) for observed and expected, respectively. These results were significant between the CT allele frequency observed and expected, with a p-value of

0.0001. Moreover, in controls, the CC allele frequency produced 22 and 22.09 (88.36%) as observed and expected, respectively. On the one hand, the TT allele frequency produced 0 and 0.09 (0.36%) as observed and expected, respectively. On the other hand, the results of CT allele frequency were 4 and 2.82 (11.28%) for observed and expected, respectively. These results were significant between CT allele frequency observed and expected, with a p-value of 0.0001. In the case and control groups, the observed and expected frequencies for the CC, TT, and CT genotypes were compared. The significant difference between the observed and expected CT allele frequency suggests a deviation from HWE for this genotype in the case and control groups. These deviations from HWE could be indicative of various factors such as non-random mating, genetic drift, mutation, selection, or population substructure within the studied population. The Hardy-Weinberg equilibrium is used in genetic populations to describe the expected and predicted frequencies of genotypes under certain conditions under various factors such as non-random mating, genetic drift, mutation, selection, or population substructure. Several causes, including non-random mating, genetic drift, mutation, selection, and population substructure, can be indicated by deviations from HWE²⁵. Similar results reported that there is an association between the CT heterozygote variant of the MTHFR C677T gene and SB risk factors that is significantly correlated with the genotype (CT/TT) of the MTHFR C677T gene polymorphism²⁶.

Table 3. MTHFR C677T Genotype distribution according to Hardy–Weinberg equilibrium in both study groups.

MTHFR Genotyping N=50		Observed No	Expected No (%)	P-value of Exp.
Cases	CC	18	16 (64%)	0.0001 **
	CT	4	8 (32%)	
	TT	3	1 (4%)	
Controls	CC	22	22.09 (88.36%)	0.0001 **
	CT	3	2.82 (11.28%)	
	TT	0	0.09 (0.36%)	

** (P≤0.01).

The amino acid alanine is converted to valine via the MTHFR SNP mutation, which occurs at position 677

within exon 4 and includes a transition from cytosine (C) to thymine (T). DNA, RNA, and proteins need one carbon (a methyl group) for methylation and re-methylation, usually catalyzed by MTHFR. MTHFR also catalyzes the conversion of homocysteine into methionine²⁷. These associations may be influenced by various factors such as nutrition, environment, and genetics^{28,29}. Exposure to environmental factors such as physical and chemical mutagenic agents. The mutation and the risk of developing SB differences in the findings could be linked to ethnicity, country of origin, or demographic factors, as reported by³⁰. Others have hypothesized that folate has a role in DNA methylation, which results in the over-expression of some genes involved in autoimmunity associated with the progression of NTDs^{31,32}. Previous studies have reported an association between maternal MTHFR C677T genetic genotyping and adverse birth outcomes, particularly in pregnant women with insufficient folate intake³³. The TT genotype of the MTHFR C677T allele has been associated with an increased risk of preterm birth and low birth weight under various genetic models^{34,35}. The pore supplementation of folic acid during early pregnancy is necessary to prevent potential pathological conditions caused by folate deficiency^{36,37}. Inadequate folate intake may be due to poor dietary intake of green vegetables, fruits, legumes, and animal-origin food products or a decreased bioavailability of folate influenced by genetic factors, such as carriers of the MTHFR 677CT/TT polymorphism^{38,39}. Many studies showed the closely related genetic factors (SNPs) interactions with the risk of congenital heart disease (CHD). They indicated genetic polymorphisms of the maternal MTHFR gene were significantly associated with the risk of fetal CHD in the Chinese population⁴⁰. While the current study has several strengths, including examining the relationship between MTHFR gene genotyping and SBs, there are also a few limitations. These include a small sample size, a semi-quantitative assessment of maternal dietary folate intake derived from questionnaires, and a lack of information regarding the fetal MTHFR genotype, which makes it impossible to determine the relative contribution of the fetal genotype to the observed effects. Studies in different ethnic populations and with a larger sample are required to confirm these findings.

Conclusion

The MTHFR C677T genetic mutation has been associated with certain health in newborns, but its contribution to the development of most SB is not considered significant. Although the presence of the

MTHFR mutation can be a biomarker and risk factor for SBs in certain populations, such as the Iraqi population, more research is necessary to fully comprehend its influence on neural tube development.

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Authors' Declaration

Conflicts of Interest: None to declare.

Ethical Clearance:

We confirm that this manuscript's data, tables, and figures are ours. The authors signed ethical consideration approval.

Ethical clearance: The local ethical committee at the University of Baghdad approved the project.

- No animal studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.
- Ethical Clearance: The local ethical committee at the University of Baghdad approved the project.

Authors' Contribution Statement

This work was a collaborative effort among all authors H.H.H was responsible for sample collection, while H.K.A and Z.F.A authors contributed to the research

experiment and writing of the manuscript. The final manuscript was revised and approved by all authors.

References

1. Sanz C M, Chmait RH, Lapa DA, Belfort M A, Carreras E, Miller J L. *et al.* Experience of 300 cases of prenatal fetoscopic open spina bifida repair: report of the International Fetoscopic Neural Tube Defect Repair Consortium. *Am J Obstet Gynecol.* 2021; 225(6): 678.e1-678.e11. <https://doi.org/10.1016/j.ajog.2021.05.044>
2. Panigrahi DD, Patel S, Rajbhar S, Padhi P, Shah S, Nanda R. *et al.* Association of Methylenetetrahydrofolate Reductase Gene Polymorphism in Mothers with Adverse Clinical Outcomes in Neonates. *Cureus*, 2023; 15(4):e38001. <https://doi.org/10.7759/cureus.38001>
3. Aguilar-Lacasaña S, López-Flores I, González-Alzaga B, Giménez-Asensio MJ, Carmona FD, Hernández AF. Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphism and Infant's Anthropometry at Birth. *Nutrients.* 2021; 13(3): 831-846. <https://doi.org/10.3390/nu13030831>
4. Bulloch RE, Wall CR, McCowan LME, Taylor RS, Roberts CT, Thompson JMD. The Effect of Interactions between Folic Acid Supplementation and One Carbon Metabolism Gene Variants on Small-for-Gestational-Age Births in the Screening for Pregnancy Endpoints (SCOPE) Cohort Study. *Nutrients.* 2020; 12(6): 1677-1681. <https://doi.org/10.3390/nu12061677>
5. Goyal A, Kumawat M, Vashisth M, Gill PS, Sing I, Dhoulakhandi DB. Study of C677T Methylene Tetrahydrofolate Reductase Gene Polymorphism as a Risk Factor for Neural Tube Defects. *Asian J Neurosurg.* 2021; 16(3): 554-561. https://doi.org/10.4103/ajns.AJNS_372_20
6. Kwon BN, Lee NR, Kim HJ, Kang YD, Kim JS, Park JW, et al. Folate metabolizing gene polymorphisms and genetic vulnerability to preterm birth in Korean women. *Genes Genomics.* 2021; 43(8): 937-945. <https://doi.org/10.1007/s13258-021-01082-3>

7. Ledowsky CJ, Schloss J, Steel A. Variations in folate prescriptions for patients with the MTHFR genetic polymorphisms: A case series study. *Explor Res Clin Soc Pharm.* 2023; 10: 100277. <https://doi.org/10.1016/j.rcsop.2023.100277>.
8. Agrawal S, Al-Refai A, Abbasi N, Kulkarni AV, Pruthi V, Drake J, *et al.* Correlation of fetal ventricular size and need for postnatal cerebrospinal fluid diversion surgery in open spina bifida. *Ultrasound Obstet Gynecol.* 2022; 59(6): 799-803. <https://doi.org/10.1002/uog.24767>
9. Hassan AS, Du YL, Lee SY, Wang A, Farmer DL. Spina Bifida: A Review of the Genetics, Pathophysiology and Emerging Cellular Therapies. *J Dev Biol.* 2022; 10(2): 22-37. <https://doi.org/10.3390/jdb10020022>
10. Lapa DA, Chmait RH, Gielchinsky Y, Yamamoto M, Persico N, Santorum M., *et al.* Percutaneous fetoscopic spina bifida repair: effect on ambulation and need for postnatal cerebrospinal fluid diversion and bladder catheterization. *Ultrasound Obstet Gynecol.* 2021; 58(4): 582-589. <https://doi.org/10.1002/uog.23658>
11. Froese DS, Fowler B, Baumgartner MR. Vitamin B₁₂, folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation. *J Inherit Metab Dis.* 2019; 42(4): 673-685. <https://doi.org/10.1002/jimd.12009>
12. Bajic Z, Sobot T, Skrbic R, Stojiljkovic MP, Ponorac N, Matavulj. Homocysteine, Vitamins B6 and Folic Acid in Experimental Models of Myocardial Infarction and Heart Failure-How Strong Is That Link?. *Biomolecules.* 2022; 12(4): 536-543 <https://doi.org/10.3390/biom12040536>
13. Raghubeer S, Matsha TE. Methylenetetrahydrofolate (MTHFR), the One-Carbon Cycle, and Cardiovascular Risks. *Nutrients.* 2021; 13(12): 4562-4577 <https://doi.org/10.3390/nu13124562>
14. Cernera G, Comegna M, Gelzo M, Savoia M, Bruzzese D, Mormile M. Molecular Analysis of Prothrombotic Gene Variants in Patients with Acute Ischemic Stroke and with Transient Ischemic Attack. *Medicina (Kaunas).* 2021; 57(7): 723-802. <https://doi.org/10.3390/medicina57070723>
15. Feng W, Zhang Y, Pan Y, Zhang Y, Liu M, Huang Y. *et al.* Association of three missense mutations in the homocysteine-related MTHFR and MTRR gene with risk of polycystic ovary syndrome in Southern Chinese women. *Reprod Biol Endocrinol.* 2021; 19(1): 5-12. <https://doi.org/10.1186/s12958-020-00688-8>
16. Nasri K, Midani F, Kallel A, Ben Jemaa N, Aloui M, Boulares M, *et al.* Association of MTHFR C677T, MTHFR A1298C, and MTRR A66G Polymorphisms with Neural Tube Defects in Tunisian Parents. *Pathobiol.* 2019; 86(4): 190-200. <https://doi.org/10.1159/000499498>
17. Ji D, Luo C, Liu J, Cao Y, Wu J, Yan W. *et al.* Insufficient S-Sulfhydration of Methylenetetrahydrofolate Reductase Contributes to the Progress of Hyperhomocysteinemia. *Antioxid Redox Signal.* 2022; 36(1-3): 1-14. <https://doi.org/10.1089/ars.2021.0029>
18. Caiaffa CD, Gene-environment interactions underlying the etiology of neural tube defects. *Curr Top Dev Biol.* 2023; 152: 193-220. <https://doi.org/10.1016/bs.ctdb.2022.10.007>
19. Isaković J, Šimunić I, Jagečić D, Hribljan V, Mitrečić D. Overview of Neural Tube Defects: Gene-Environment Interactions, Preventative Approaches and Future Perspectives. *Biomedicines.* 2022; 10(5): 965-990 <https://doi.org/10.3390/biomedicines10050965>
20. Beierwaltes P, Munoz S, Wilhelmy J. Integument: Guidelines for the care of people with spina bifida. *J Pediatr Rehabil Med.* 2020; 13(4): 543-548. <https://doi.org/10.3233/PRM-200723>
21. SAS Innovate. Statistical Analysis System, User's Guide. Version 9.6th ed. SAS. 2018; Inst. Inc. Cary. N.C. USA. ISBN 1-59047-243-8.
22. Behere RV, Deshmukh AS, Otiv S, Gupte MD, Yajnik CS. Maternal Vitamin B12 Status During Pregnancy and Its Association With Outcomes of Pregnancy and Health of the Offspring: A Systematic Review and Implications for Policy in India. *Front Endocrinol (Lausanne).* 2021; 12: 619176. <https://doi.org/10.3389/fendo.2021.619176>
23. Omran MH, Fotouh BE, Shousha WG, Ismail A, Ibrahim NE, Ramadan SS. Strong Correlation of MTHFR Gene Polymorphisms with Breast Cancer and its Prognostic Clinical Factors among Egyptian Females. *Asian Pac J Cancer Prev.* 2021; 22(2): 617-626. <https://doi.org/10.31557/APJCP.2021.22.2.617>
24. Kumari R, Kumar S, Thakur VK, Singh K, Kumar U. MTHFR C677T and MTR A2756G Gene Polymorphism in Neural Tube Defect Patients and Its Association with Red Blood Cell Folate Level in Eastern Indian Population. *J Indian Assoc Pediatr Surg.* 2022; 27(6): 699-706. https://doi.org/10.4103/jiaps.jiaps_29_22
25. Guan D, Ji Y, Lu X, Feng W, Ma W. Associations of MTHFR gene polymorphism with lipid metabolism and risk of cerebral infarction in the Northwest Han Chinese population. *Front Neurol.* 2023; 14: 1152351. <https://doi.org/10.3389/fneur.2023.1152351>

26. Raina JK, Sharma M, Panjaliya RK, Dogra V, Bakaya A, Kumar P. Association of ESR1 (rs2234693 and rs9340799), CETP (rs708272), MTHFR (rs1801133 and rs2274976) and MS (rs185087) polymorphisms with Coronary Artery Disease (CAD). *BMC Cardiovasc Disord.* 2020; 20(1): 340-370. <https://doi.org/10.1186/s12872-020-01618-7>
27. Raigani M, Lakpour N, Soleimani M, Johari B, Sadeghi MR. A Association of MTHFR C677T and MTRR A66G Gene Polymorphisms with Iranian Male Infertility and Its Effect on Seminal Folate and Vitamin B12. *Int J Fertil Steril.* 2021; 15(1): 20-25. <https://doi.org/10.22074/ijfs.2021.6155>
28. Fituri S, Akbar Z, Ganji V. Impact of metformin treatment on cobalamin status in persons with type 2 diabetes. *Nutr Rev.* 2023; 82(4): 553-560. <https://doi.org/10.1093/nutrit/nuad045>
29. Sarwar S, Shabana Tahir A, Liaqat Z, Naseer S, Seme RS. Study of variants associated with ventricular septal defects (VSDs) highlights the unique genetic structure of the Pakistani population. *Ital J Pediatr.* 2022; 48(1): 124. <https://doi.org/10.1186/s13052-022-01323-5>
30. Mouhoub-Terrab R, Chibane AA, Khelil M. No association between MTHFR gene C677T/A1298C polymorphisms, serum folate, vitamin B12, homocysteine levels, and prostate cancer in an Algerian population. *Mol Genet Genomic Med.* 2023; 11(9): e2194. <https://doi.org/10.1002/mgg3.2194>
31. Liu Y, Xu C, Wang Y, Yang C, Pu G, Zhang L. *et al.* Association analysis of MTHFR (rs1801133 and rs1801131) and MTRR (rs1801394) gene polymorphisms towards the development of hypertension in the Bai population from Yunnan, China. *Clin Exp Hypertens.* 2023; 45(1): 2206066. <https://doi.org/10.1080/10641963.2023.2206066>
32. Hari K K, Uma M B, Michelle P, Chithra S, Vasanthi J, Karthik K. *et al.* The Association between Methylenetetrahydrofolate Reductase (MTHFR) Mutations and Serum Biomarkers of Cardiac Health. *Open J Prev Med.* 2023; 13(4): 87-107. <https://doi.org/10.4236/ojpm.2023.134007>
33. Wei J, Wang T, Song X, Liu Y, Shu J, Sun M. *et al.* Association of maternal methionine synthase reductase gene polymorphisms with the risk of congenital heart disease in offspring: a hospital-based case-control study. *J Matern Fetal Neonatal Med.* 2023; 36(1): 2211201. <https://doi.org/10.1080/14767058.2023.2211201>
34. Tsukamoto M, Hishida A, Tamura T, Nagayoshi M, Okada R, Kubo Y. *et al.* GWAS of folate metabolism with gene-environment interaction analysis revealed the possible role of lifestyles in the control of blood folate metabolites in Japanese - the J-MICC Study. *J Epidemiol.* 2023. <https://doi.org/10.2188/jea.JE20220341>
35. White M, Arif-Pardy J, Connor KL. Identification of novel nutrient-sensitive gene regulatory networks in amniocytes from fetuses with spina bifida. *Reprod Toxicol.* 2023; 116: 108333. <https://doi.org/10.1016/j.reprotox.2022.12.010>
36. Tamkeen N, Farooqui A, Alam A, Najma Tazyeen S, Ahmad MM. Identification of common candidate genes and pathways for Spina Bifida and Wilm's Tumor using an integrative bioinformatics analysis. *J Biomol Struct Dyn.* 2024; 42(2): 977-992. <https://doi.org/10.1080/07391102.2023.2199080>
37. Papadakis I, Papadomanolaki M, Vougiouklakis G, Petrakis E, Mazokopakis E. Influence of the methylene tetrahydrofolate reductase (mthfr) gene polymorphisms on serum folate, cobalamin and homocysteine levels in healthy greek adults. *J Hypertension.* 2023 Jun 1;41(Suppl 3):e143-4.. <https://doi.org/10.1097/01.hjh.0000940260.55102.76>
38. Ji D, Luo C, Liu J, Cao Y, Wu J, Yan W, *et al.* Insufficient S-Sulfhydration of Methylenetetrahydrofolate Reductase Contributes to the Progress of Hyperhomocysteinemia. *Antioxid Redox Signal.* 2022; 36(1-3): 1-14. <https://doi.org/10.1089/ars.2021.0029>
39. Alset D, Kubyshkina DV, Butenko EV, Pokudina IO, Shkurat TP. Association of C677T and A1298C genetic polymorphisms in MTHFR gene with fetal growth restriction, small for gestational age and low birth weight. *Human gene.* 2023; 37. <https://doi.org/10.1016/j.humgen.2023.201190>
40. Sun M, Wang T, Huang P, Diao J, Zhang S, Li J, *et al.* Association analysis of maternal MTHFR gene polymorphisms and the occurrence of congenital heart disease in offspring. *BMC Cardiovasc Disord.* 2021; 21(1): 298. <https://doi.org/10.1186/s12872-021-02117-1>

الكشف عن التنميط الجيني لانزيم الميثيلين تتراهيدروفولات المختزل C677T لعيوب السنسنة المشقوقة باستخدام تقنية سلسلة البلمرة بنظام تضخيم الطفرة الحرارية رباعي البوادي

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الخلاصة

عيوب الأنبوب العصبي هي تشوهات هيكلية معقدة عند الأطفال حديثي الولادة. تنجم هذه العيوب عن مزيج من العوامل الوراثية والبيئية في النمط الظاهري غير الطبيعي. تهدف الدراسة الحالية إلى تحديد تعدد أشكال التباين الوراثي في التنميط الجيني لانزيم ميثيلين تتراهيدروفولات المختزل والكشف عن تعدد أشكال النوكليوتيدات المفردة في عينة الحمض النووي لمرضى السنسنة المشقوقة ومقارنتها مع الأطفال الأصحاء حديثي الولادة. تم جمع عينات الدم من الأطفال حديثي الولادة (50 طفلاً أقل من سنة واحدة) (25 حالة من حالات انشقاق العمود الفقري و25 حالة من الأصحاء). تم استخراج الحمض النووي وتضخيمه بواسطة تقنية تفاعل البلمرة المتسلسل لنظام تضخيم الطفرة الحرارية (تفاعل البلمرة المتسلسل لنظام الطفرة المقاومة للحرارة). تتكون تقنية تفاعل البلمرة الرباعي المضخم من أربعة بادئات (اثنتان خارجيان واثنتان داخليان) لتضخيم تسلسل الحمض النووي المستهدف للأنماط الجينية المختلفة (أليلات النوع البري والنوع الطافر). تشير النتائج إلى أن الطفرة الجينية لانزيم تتراهيدروفولات المختزل كانت مرتبطة بالسنسنة المشقوقة عند الأطفال حديثي الولادة ويمكن أن يكون كمؤشر حيوي للكشف عن تطور أمراض السنسنة المشقوقة للأطفال حديثي الولادة.

الكلمات المفتاحية: عيب الأنبوب العصبي، انشقاق العمود الفقري، إنزيم ميثيلين رباعي هيدروفولات اختزال، التنميط الجيني، سلسلة البلمرة بنظام تضخيم الطفرة الحرارية رباعي البوادي