

Impact of *MTHFR* Gene Polymorphisms C677T and A1298C on Congenital Atrial Septal Defect Risk in an Iranian Cohort

Noor Mohammad Noori^{1,2}, Saeedeh Yaghoubi², Ali Aghighi³, Mohsen Taheri⁴,
Gholamreza Bahari*^{1,3}

Abstract

Background: Congenital heart defects (CHD) are recognized as the most common heart abnormalities amongst newborns and children, and atrial septal defect (ASD) is recognized as one of the most frequent forms of CHD. Prior studies indicated that the methylenetetrahydrofolate reductase (*MTHFR*) gene contributes to the etiology of CHD. Therefore, we designed a case-control study to assess the possible role of the *MTHFR* gene, specifically the C677T (rs1801133) and A1298C (rs1801131) polymorphisms within the Iranian ASD population sample.

Method: A total of 166 subjects (81 children diagnosed with ASD and 85 control participants) were enrolled in this research. Samples genotyped for *MTHFR* rs1801133 and rs1801131 polymorphisms using the PCR-RFLP and ARMS-PCR approaches.

Results: Our results indicated that rs1801131 variant reduced the risk of ASD in codominant (OR [95%CI]: 0.41[0.21-0.83], P=0.012), dominant (OR[95%CI]: 0.48 [0.25-0.93], p=0.028) and overdominant (OR[95%CI]: 0.44 [0.23-0.81], P=0.009) models. Moreover, rs1801133 variant increased the risk of ASD in codominant (OR[95%CI]: 2.68[1.39-5.16], P = 0.003), dominant (OR [95% CI]: 2.72 [1.43–5.14], P = 0.002), overdominant (OR [95% CI]: 2.50 [1.31–4.78], P = 0.005), and allelic (OR [95% CI]: 2.16 [1.27–3.69], P = 0.004) models.

Conclusion: Our findings suggest that *MTHFR* rs1801133 and rs1801131 variants may potentially affect the onset of ASD.

Keywords: Atrial Septal Defect, Congenital Heart Defects, Folate Metabolism, Genetic Polymorphism, Methylenetetrahydrofolate Reductase.

Introduction

Congenital heart diseases or defects (CHD) are the most common heart malformations among newborns and children (1). This condition occurs due to inadequate heart development during the first six weeks of pregnancy (2). Even though in recent years, the ability to diagnose and treat CHDs has advanced significantly, the disorder still imposes a significant burden in many

countries. Compared to other childhood disorders, the treatment of CHDs remains more costly and complex (3, 4).

Nowadays, it is commonly acknowledged that CHD is a multifactorial disorder that includes environmental and genetic components (5, 6). Based on some epidemiological findings, one-third of individuals with CHD are affected by genetic factors (7). In addition, the interaction

1: Children and Adolescent Health Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran.

2: Department of Pediatric, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.

3: Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.

4: Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

*Corresponding author: Gholamreza Bahari; Tel: +98 5433295791; E-mail: ghbahari@zaums.ac.ir.

Received: 19 Aug, 2024; Accepted: 26 Dec, 2024

between genes and environment is involved in the etiology of CHD, highlighting the complexity of the disorder (8, 9). Atrial septal defect (ASD), ventricular septal defect (VSD), and atrioventricular septal defects (AVSD) are three main forms of CHD (10). ASD, as one of the more prevalent forms of CHD, has a prevalence of around 100 per 100,000 live births (11) and is responsible for 10-15% of CHD cases worldwide (12-14).

Numerous investigations have shown that maternal periconceptional intake of multivitamins or folic acid may decrease the risk of CHD in offspring of females with mutations in genes involved in folate metabolism (15). Nevertheless, research is still ongoing on the mechanism of this effect. It has been shown that homocysteine concentration is affected by folate and vitamin B12 (2). Previous research has demonstrated that the methylenetetrahydrofolate reductase (*MTHFR*) gene (NM_005957.5) contributes to CHD etiology (16). It acts as a major regulatory protein in the metabolism of folate (17). The conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate is catalyzed by this enzyme, which is essential for the remethylation process of homocysteine (Hcy) to methionine (18). The *MTHFR* gene contributes to folate metabolism, and mutations in this gene prevent the removal of Hcy, an independent risk factor for CHD occurrence (19, 20).

The prior studies indicated that two well-studied SNPs in the *MTHFR* gene, C677T (rs1801133, Ala222Val) and A1298C (rs1801131), are correlated with reduced enzyme activity and elevated plasma homocysteine levels (21). Numerous studies have assessed the role of *MTHFR* gene polymorphisms in various disorders, such as childhood acute lymphoblastic leukemia (22), Alzheimer disease (23), breast cancer (24), pregnancy complications (25), and ischemic stroke (26). In addition, several studies investigated the possible effect of *MTHFR* gene polymorphisms on CHD in different

ethnicities (1, 27-33); however, the outcomes have been inconsistent. Therefore, we conducted the current research for the first time among ASD patients from an Iranian population sample.

Materials and Methods

In current case-control research, 81 children diagnosed with ASD and 85 healthy individuals were recruited in southeast Iran. Both groups were paired based on age and gender. The methodology and enrollment process has been previously elucidated (22).

The Local Ethics Committee of Zahedan University of Medical Sciences authorized the research protocol (IR.ZAUMS.REC.1402.360), and parents of both patients and controls provided informed consent. In our study, we included all children under the age of 18 with a confirmed diagnosis of ASD who were referred to the pediatric cardiology clinics at Ali Asghar and Ali Ibn Abi Talib Hospitals in Zahedan, or who were admitted to these facilities. Exclusion criteria included individuals with other cardiac abnormalities, such as tetralogy of Fallot, complex septal defects, single ventricle, or a history of exposure to teratogenic substances. As a control group, we enrolled healthy children attending the Ali Asghar Clinic for routine annual examinations, provided they had no known underlying diseases.

We employed the salting-out approach to extract DNA from peripheral whole blood (34). The primers were synthesized by SinaClon, Iran. To genotype of *MTHFR* C677T polymorphism, the PCR-RFLP approach was utilized. Each PCR reaction comprised 1 µl of DNA, 1 µl of both forward and reverse primers, 10 µl of Premix (2X Prime Taq, Genet Bio, South Korea), and 7 µl of water. The PCR products were treated with restriction enzyme *HinfI* (Thermo Scientific, USA). Digestion of the T allele resulted in 222-bp and 167-bp fragments, whereas the undigested C allele produced a 389-bp fragment. Genotyping of the *MTHFR* A1298C variant was performed by using the ARMS-PCR approach. The A or C allele generated a 317-bp fragment, with the internal control producing a 574-bp fragment.

The primers for amplifying both loci were reported in our previous publication (22).

Statistical Analysis

An independent sample t-test and χ^2 test were employed for data analysis using SPSS software (version 20). The correlation between the MTHFR C677T (rs1801133) and A1298C (rs1801131) variants and ASD risk was examined through logistic regression analyses to calculate the OR and 95% CI. We also used SNPStats software for haplotype analysis. A p-value below 0.05 was considered statistical significance.

Results

The research population consisted of eighty-

one ASD cases (36 males, 45 females; age: 6.01 ± 3.98 years) and eighty-five healthy children (43 males, 42 females; age: 5.74 ± 2.44 years). There were no statistically significant differences between the groups regarding gender or age ($p = 0.428$ and $p = 0.588$, respectively).

Table 1 summarizes the genotype and allelic distribution of MTHFR gene variants. The results showed that the rs1801131 (A1298C) variant was associated with a decreased risk of ASD in codominant (OR[95%CI]: 0.41[0.21-0.83], $p=0.012$, AC vs AA), and dominant (OR[95%CI]: 0.48[0.25-0.93], $p=0.028$, AC+CC vs. AA) and overdominant (OR[95%CI]: 0.44[0.23-0.81], $P=0.009$, AC vs. AA+CC).

Table 1. Genotypic and allelic frequencies of MTHFR gene polymorphisms in childhood ASD and control subjects.

Polymorphisms	ASD n (%)	Control n (%)	OR (95%CI)	P-value
rs1801131				
<i>Codominant</i>				
AA	33(40.7)	21(24.7)	1	-
AC	35(43.3)	54(63.5)	0.41(0.21- 0.83)	0.012
CC	13(16.0)	10(11.8)	0.83(0.31- 2.23)	0.707
<i>Dominant</i>				
AA	33(40.7)	21(24.7)	1	-
AC+CC	48(59.3)	64(75.3)	0.48(0.25- 0.93)	0.028
<i>Recessive</i>				
AA+AC	68(84.0)	75(88.2)	1	-
CC	13(16.0)	10(11.8)	1.43(0.59- 3.48)	0.425
<i>Overdominant</i>				
AA+CC	46(56.7)	31(36.5)	1	-
AC	35(43.3)	54(63.5)	0.44(0.23- 0.81)	0.009
<i>Alleles</i>				
A	101(62.3)	96(56.5)	1	-
C	61(37.7)	74(43.5)	0.78(0.505- 1.22)	0.276
rs1801133				
<i>Codominant</i>				
CC	38 (46.9)	60 (70.6)	1	-
CT	39 (48.2)	23 (27.1)	2.68(1.39- 5.16)	0.003
TT	4 (4.9)	2 (2.3)	3.16(0.55- 18.09)	0.197
<i>Dominant</i>				
CC	38 (46.9)	60 (70.6)	1	-
CT+TT	43 (53.1)	25 (29.4)	2.72(1.43- 5.14)	0.002
<i>Recessive</i>				
CC+CT	77 (95.1)	83 (97.7)	1	-
TT	4 (4.9)	2 (2.3)	2.16(0.38- 12.11)	0.373
<i>Overdominant</i>				
CC+TT	42(51.8)	62(72.9)	1	-
CT	39 (48.2)	23 (27.1)	2.50(1.31- 4.78)	0.005
<i>Alleles</i>				
C	115(71.0)	143 (84.1)	1	-
T	47 (29.0)	27 (15.9)	2.16(1.27- 3.69)	0.004

OR: Odds Ratio, CI: Confidence Interval, Significant p-values are shown in bold ($p < 0.05$).

Conversely, the rs1801133 variant (C677T) was found to increase the risk of ASD across all assessed genetic models. Specifically, in the codominant (OR[95%CI]: 2.68[1.39-5.16], $p=0.003$, CT vs. CC), dominant (OR[95%CI]: 2.72[1.43-5.14], $p=0.002$, CT+TT vs. CC), overdominant (OR[95%CI]: 2.50[1.31-4.78], $P=0.005$, CT vs. CC+TT) and allele (OR [95%CI]: 2.16 [1.27- 3.69], $p=0.004$, T vs. C) models.

Furthermore, we also examined the

interaction of *MTHFR* polymorphisms on ASD risk (Table 2). Compared to the reference *MTHFR* rs1801131AA/ rs1801133 CC, the AA/CT genotypes were associated with an elevated risk of ASD (OR[95%CI]: 5.56[1.50-20.53], $p=0.007$). Table 3 presents the results of haplotype analysis. The results revealed that TA haplotypes were associated with an increased risk of ASD compared to CA haplotypes (rs1801133C/ rs1801131A), (OR [95%CI]: 3.64 [1.25-10.63], $P= 0.02$).

Table 2. Interaction of *MTHFR* rs1801131 and rs1801133 polymorphisms on ASD risk.

rs1801131	rs1801133	ASD n (%)	Control n (%)	OR (95%CI)	P-value
AA	CC	13(16.1)	17(20.0)	1.00	-
AA	CT	17(21.0)	4(4.7)	5.56(1.50- 20.53)	0.007
AA	TT	3(3.7)	0(0.0)	-	-
AC	CC	18(22.2)	35(41.1)	0.67(0.27- 1.69)	0.396
AC	CT	16(19.8)	17(20.0)	1.23(0.46- 3.32)	0.682
AC	TT	1(1.2)	2(2.4)	0.65(0.05- 8.02)	0.738
CC	CC	7(8.6)	8(9.4)	1.14(0.33- 3.97)	0.832
CC	CT	6(7.4)	2(2.4)	3.92(0.68- 22.71)	0.111
CC	TT	0(0.0)	0(0.0)	-	-

OR: Odds Ratio, CI: Confidence Interval, Significant p-values are shown in bold ($p < 0.05$).

Table 3. Haplotype frequencies of *MTHFR* polymorphisms in ASD risk.

Haplotype _{rs1801131, rs1801133}	ASD n (%)	Control n (%)	OR (95%CI)	p-value
AC	0.4081	0.4917	1.00	-
CC	0.3018	0.3494	1.00(0.53- 1.90)	0.99
AT	0.2153	0.0731	3.64(1.25- 10.63)	0.02
CT	0.0748	0.0859	1.12(0.37- 3.41)	0.84

OR: Odds Ratio, CI: Confidence Interval, Significant p-values are shown in bold ($p < 0.05$).

Discussion

This study demonstrates that the *MTHFR* gene polymorphisms rs1801133 and rs1801131 are significantly associated with congenital atrial septal defect (ASD) susceptibility in an Iranian cohort. Specifically, the rs1801133 variant was associated with an increased risk of ASD, whereas the rs1801131 variant was found to be associated with a decreased risk. These findings are consistent with previous studies indicating the importance of *MTHFR*

polymorphisms in congenital heart defects (CHD).

It is well-established that the genetic and environmental aspects are potential contributing factors for CHD. However, numerous genetic investigations have indicated that genetic factors are one of the key causes of CHD susceptibility. Twin studies revealed that monozygotic twins had a 63% higher risk of CHD compared with dizygotic twins. In addition, whole exome sequencing research showed an 8.5%

diagnostic rate for pathogenic genetic variations in fetuses exhibiting structural cardiac anomalies and a 15.4% ratio in those with multisystemic anomalies. Genetic factors are also identified as the main agents of pathogenesis in ASD, the most common congenital heart defect, in both familial and sporadic patients. Thus, finding the involved genes and related mutations in the onset of ASD is essential for disease prevention and prenatal diagnosis (35).

Various metabolic pathways regulate the neural tube formation and cardiac development throughout the embryogenesis period. Among them, the folate-homocysteine metabolic pathway has a significant role in controlling the level of folate and homocysteine, two important factors significantly contributing to the development of CHD (2). Therefore, the genes modulating the folate-homocysteine metabolic pathway, as well as mutations within their sequences, are likely crucial risk factors for CHD and ASD disorders. For this reason, in the current study, we selected two common exonic single nucleotide polymorphisms (SNPs) (rs1801133 and rs1801131) of the *MTHFR* gene to evaluate the correlation of *MTHFR* gene variations with the risk of ASD.

The rs1801133 variant is located in the fourth exon of *MTHFR* at the 677th nucleotide position (C667T). This mutation leads to the amino acid substitution of alanine to valine (Ala222Val). It results in a reduction in *MTHFR* activity and consequent metabolic impairment of Hcy, which slightly elevate plasma Hcy levels slightly (36). Furthermore, the rs1801133 missense variant, localizing at 1298 position (A1298C), resulting in glutamine to alanine change (Glu429Ala), which significantly decreases the activity of *MTHFR* enzyme (37). Our results demonstrated that rs1801133 increased the ASD risk in four genetic models (allelic, codominant, dominant, and overdominant). In comparison, rs1801131 was associated with a decreased risk of ASD in three studied models (codominant, dominant, and overdominant).

Our finding was consistent with the meta-analysis report regarding the association between rs1801133 SNP and CHD by Liu et al (2020). They detected a significant link between this polymorphism and CHD occurrence under the recessive model (OR[95% CI]: 1.35[1.06-1.71]) for the entire population, including 15 eligible studies. Subgroup analyses by ethnicity showed similar correlations in Asians but not for Caucasians (38). Similar data were obtained from the next meta-analysis (2022) by pooling the results of 26 eligible studies. A statistically significant association between rs1801133 and CHD was identified under all applied genetic models. This correlation was also seen in Asian populations but not in others (2). However, Ali et al. (2024) reported a lack of a significant association between rs1801133 and congenital septal defects in Pakistan, similar to our data, they observed that heterozygous mutant (CT) was the more prevalent genotype in CHD patients in comparison with controls (39). In another study by Shivkar et al. (2022), indicative hyperhomocysteinemia in carriers of T allele for rs1801133 genotype was discovered in young coronary artery disease (CAD) patients. The authors proposed that the link between hyperhomocysteinemia and CAD depended on typical cardiovascular risk factors. Thus, the risk of hyperhomocysteinemia and young CAD could be assessed through *MTHFR* polymorphism analysis, followed by measuring serum levels of homocysteine, folate, and vitamin B12 (40). Moreover, the maternal rs1801133 variant was detected as a CHD risk factor for their offspring, possibly attributable to hyperhomocysteinemia resulting from abnormal metabolism of Hcy (41).

There is limited data on the influential role of rs1801131 on CHD risk. Sun et al. (2021) found that rs1801131 was significantly associated with the risk of CHD in the homozygote comparisons in the Chinese population (42); their result was in concordance with our finding. Furthermore,

Zidan *et al.* reported the correlation of rs1801131 CC genotype as well as a higher level of homocysteine with a raised risk of CHD in the Egyptian population. They also observed that the frequencies of rs1801131 AC and CC genotypes and C allele were remarkably raised among mothers with children affected by CHD (43). Moreover, the meta-analysis of 18 studies revealed a significant correlation between the rs1801131 and myocardial infarction risk (44). Song *et al.*, nonetheless, found no relationship between rs1801131 and coronary artery disease development in Qingdao, China (45).

In conclusion, our data revealed the potential role of *MTHFR* rs1801133 and rs1801131 polymorphisms on ASD susceptibility. More genetic association studies in larger groups, various ethnicities, and functional studies are suggested to strengthen data validity.

References

1. Zhu WL, Li Y, Yan L, Dao J, Li S. Maternal and offspring MTHFR gene C677T polymorphism as predictors of congenital atrial septal defect and patent ductus arteriosus.. *Mol Hum Reprod* 2006;12(1):51-4.
2. Raina JK, Panjaliya RK, Dogra V, Sharma S, Anupriya, Kumar P. Association of MTHFR and MS/MTR gene polymorphisms with congenital heart defects in North Indian population (Jammu and Kashmir): a case-control study encompassing meta-analysis and trial sequential analysis. *BMC Pediatr.* 2022;22(1):223.
3. GBD 2017 Congenital Heart Disease Collaborators. Global, regional, and national burden of congenital heart disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Child Adolesc Health.* 2020;4(3):185-200.
4. 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.
5. Vecoli C, Pulignani S, Foffa I, Andreassi MG. Congenital heart disease: the crossroads of

Acknowledgment

The authors would like to thank the study participants in this research.

Ethical Approval

The Local Ethics Committee of Zahedan University of Medical Sciences authorized the research protocol (IR.ZAUMS.REC.1402.360). The written consent was obtained prior the research from all participants.

Funding

This study was supported by a research grant (No. 9917) from Zahedan University of Medical Sciences.

Competing Interest

The authors declare that they have no competing interests

genetics, epigenetics and environment. *Curr Genomics.* 2014;15(5):390-9.

6. Muntean I, Togănel R, Benedek T. Genetics of Congenital Heart Disease: Past and Present. *Biochem Genet.* 2017;55(2):105-23.

7. Szot JO, Cuny H, Blue GM, Humphreys DT, Ip E, Harrison K, *et al.* A Screening Approach to Identify Clinically Actionable Variants Causing Congenital Heart Disease in Exome Data. *Circ Genom Precis Med.* 2018;11(3):e001978.

8. Hobbs CA, James SJ, Jernigan S, Melnyk S, Lu Y, Malik S, Cleves MA. Congenital heart defects, maternal homocysteine, smoking, and the 677 C>T polymorphism in the methylenetetrahydrofolate reductase gene: evaluating gene-environment interactions. *Am J Obstet Gynecol.* 2006;194(1):218-24.

9. Krauss RS, Hong M. Gene-Environment Interactions and the Etiology of Birth Defects. *Curr Top Dev Biol.* 2016;116:569-80.

10. Chaithra S, Agarwala S, Ramachandra NB. High-risk genes involved in common septal defects of congenital heart disease. *Gene.* 2022;840:146745.

11. Geva T, Martins JD, Wald RM. Atrial septal defects. *Lancet.* 2014;383(9932):1921-32.

12. Schwedler G, Lindinger A, Lange PE, Sax U, Olchvary J, Peters B, et al. Frequency and spectrum of congenital heart defects among live births in Germany : a study of the Competence Network for Congenital Heart Defects. *Clin Res Cardiol.* 2011;100(12):1111-7.
13. Reller MD, Strickland MJ, Riehle-Colarusso T, Mahle WT, Correa A. Prevalence of congenital heart defects in metropolitan Atlanta, 1998-2005. *J Pediatr.* 2008;153(6):807-13.
14. Wu MH, Chen HC, Lu CW, Wang JK, Huang SC, Huang SK. Prevalence of congenital heart disease at live birth in Taiwan. *J Pediatr.* 2010;156(5):782-5.
15. Shi H, Yang S, Liu Y, Huang P, Lin N, Sun X, et al. Study on Environmental Causes and SNPs of MTHFR, MS and CBS Genes Related to Congenital Heart Disease. *PloS one.* 2015;10(6):e0128646.
16. Zhong T, Song X, Liu Y, Sun M, Zhang S, Chen L, et al. Association of methylenetetrahydrofolate reductase gene polymorphisms and maternal folic acid use with the risk of congenital heart disease. *Front Pediatr.* 2022;10:939119.
17. Poodineh M, Saravani R, Mirhosseini M, Sargazi S. Association of two methylenetetrahydrofolate reductase polymorphisms (rs1801133, rs1801131) with the risk of type 2 diabetes in South-East of Iran. *Rep Biochem Mol Biol.* 2019;8(2):178.
18. Majstorović D, Barišić A, Božović IB, Čače IB, Čače N, Štifanić M, Vraneković J. DNMT3B rs2424913 as a Risk Factor for Congenital Heart Defects in Down Syndrome. *Genes.* 2023;14(3).
19. Sahin-Uysal N, Gulumser C, Kocaman E, Varan B, Bayraktar N, Yanik F. Maternal and cord blood homocysteine, vitamin B12, folate, and B-type natriuretic peptide levels at term for predicting congenital heart disease of the neonate: A case-control study. *J Matern Fetal Neonatal Med.* 2020;33(15):2649-56.
20. Elizabeth KE, Praveen SL, Preethi NR, Jissa VT, Pillai MR. Folate, vitamin B12, homocysteine and polymorphisms in folate metabolizing genes in children with congenital heart disease and their mothers. *Eur J Clin Nutr.* 2017;71(12):1437-41.
21. Castiglia P, Sanna V, Azara A, De Miglio MR, Murgia L, Pira G, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms in breast cancer: a Sardinian preliminary case-control study. *Int J Med Sci* 2019;16(8):1089-95.
22. Bahari G, Hashemi M, Naderi M, Taheri M. Association between Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphisms and Susceptibility to Childhood Acute Lymphoblastic Leukemia in an Iranian Population. *Int J Hematol Oncol Stem Cell Res.* 2016;10(3):130-7.
23. Bouguerra K, Tazir M, Melouli H, Khelil M. The methylenetetrahydrofolate reductase C677T and A1298C genetic polymorphisms and plasma homocysteine in Alzheimer's disease in an Algerian population. *Int J Neurosci.* 2024;134(8):918-923.
24. Gimenez-Martins APD, Castanhole-Nunes MMU, Nascimento-Filho C, Santos SPD, Galbiatti-Dias ALS, Fernandes GMM, et al. Association between folate metabolism polymorphisms and breast cancer: a case-control study. *Genet Mol Biol.* 2021;44(4):e20200485.
25. Mohammadpour-Gharehbagh A, Teimoori B, Narooei-Nejad M, Mehrabani M, Saravani R, Salimi S. The association of the placental MTHFR 3'-UTR polymorphisms, promoter methylation, and MTHFR expression with preeclampsia. *J Cell Biochem.* 2018;119(2):1346-54.
26. Jin M, Wang N, Li X, Zhang H, Zhou J, Cong M, et al. Relationship between MTHFR C677T, homocysteine, and ischemic stroke in a large sample of the Han Chinese population. *Medicine.* 2022;101(38):e30562.
27. 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.
28. Noori N, Miri-Moghaddam E, Dejkam A, Garmie Y, Bazi A. Are polymorphisms in MTRR A66G and MTHFR C677T genes associated with congenital heart diseases in Iranian population? *Caspian J Intern Med.* 2017;8(2):83-90.
29. van Driel LM, Verkleij-Hagoort AC, de Jonge R, Uitterlinden AG, Steegers EA, van Duijn CM, Steegers-Theunissen RP. Two MTHFR polymorphisms, maternal B-vitamin intake, and

- CHDs. *Birth Defects Res A Clin Mol Teratol.* 2008;82(6):474-81.
30. van Beynum IM, Kapusta L, den Heijer M, Vermeulen SH, Kouwenberg M, Daniëls O, Blom HJ. Maternal MTHFR 677C>T is a risk factor for congenital heart defects: effect modification by periconceptional folate supplementation. *Eur Heart J.* 2006;27(8):981-7.
31. Zidan HE, Rezk NA, Mohammed D. MTHFR C677T and A1298C gene polymorphisms and their relation to homocysteine level in Egyptian children with congenital heart diseases. *Gene.* 2013;529(1):119-24.
32. Obermann-Borst SA, van Driel LM, Helbing WA, de Jonge R, Wildhagen MF, Steegers EA, Steegers-Theunissen RP. Congenital heart defects and biomarkers of methylation in children: a case-control study. *Eur J Clin Invest.* 2011;41(2):143-50.
33. Sahiner UM, Alanay Y, Alehan D, Tuncbilek E, Alikasifoglu M. Methylene tetrahydrofolate reductase polymorphisms and homocysteine level in heart defects. *Pediatr Int.* 2014;56(2):167-72.
34. Hashemi M, Hanafi Bojd H, Eskandari Nasab E, Bahari A, Hashemzahi NA, Shafieipour S, et al. Association of Adiponectin rs1501299 and rs266729 Gene Polymorphisms With Nonalcoholic Fatty Liver Disease. *Hepat Mon.* 2013;13(5):e9527.
35. Jin Y, Zhao M, Guo Q, Zhao W, Lei M, Zhang Y, et al. Association study of FLT4 and HYDIN single nucleotide polymorphisms with atrial septal defect susceptibility in the Han Chinese population of Southwest China. *Ital J Pediatr.* 2024;50(1):62.
36. Mohieldeen WA, Ahmed A, Elmosaad YM, Suliman RS, Alfahed A, Hjazi A, et al. Detection of Methylene Tetrahydrofolate Reductase (MTHFR C677T) Mutation among Acute Lymphoblastic Leukemia in Sudanese Patients. *Rep Biochem Mol Biol.* 2023;12(3):458.
37. Fan Y, Wu L, Zhuang W. Methylene tetrahydrofolate Reductase Gene rs1801133 and rs1801131 Polymorphisms and Essential Hypertension Risk: A Comprehensive Analysis. *Cardiovasc Ther.* 2022;2022:2144443.
38. Liu P-F, Ding B, Zhang J-Y, Mei X-F, Li F, Wu P, et al. Association between MTHFR C677T polymorphism and congenital heart disease a prisma-compliant meta-analysis. *Int Heart J.* 2020;61(3):553-61.
39. Ali SI, Khan OY, Naveed N, Ahmad H, Patel N, Arif A. Congenital septal defects in Karachi, Pakistan: an update of mutational screening by high-resolution melting (HRM) analysis of MTHFR C677T. *Hum Genomics.* 2024;18(1):6.
40. Shivkar RR, Gawade GC, Padwal MK, Diwan AG, Mahajan SA, Kadam CY. Association of MTHFR C677T (rs1801133) and A1298C (rs1801131) polymorphisms with serum homocysteine, folate and vitamin B12 in patients with young coronary artery disease. *Indian J Clin Biochem.* 2022;37(2):224-31.
41. Shi H, Yang S, Lin N, Huang P, Yu R, Chen M, et al. Study on Maternal SNPs of MTHFR Gene and HCY Level Related to Congenital Heart Diseases. *Pediatr Cardiol.* 2021;42(1):42-46.
42. 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.
43. Zidan HE, Rezk NA, Mohammed D. MTHFR C677T and A1298C gene polymorphisms and their relation to homocysteine level in Egyptian children with congenital heart diseases. *Gene.* 2013;529(1):119-24.
44. Samii A, Aslani S, Imani D, Razi B, Samaneh Tabae S, Jamialahmadi T, Sahebkar A. MTHFR gene polymorphisms and susceptibility to myocardial infarction: Evidence from meta-analysis and trial sequential analysis. *Int J Cardiol Heart Vasc.* 2023;49:101293.
45. Song H, Zhao Z, Liu S, Li C, Zhou Y, Xin Y. Methylene tetrahydrofolate Reductase Gene rs1801131 and rs1801133 Polymorphisms were Associated with Susceptibility to Coronary Artery Disease and Nonalcoholic Fatty Liver Disease. *Gene Expr.* 2023;22(2):102-8.