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Association between MTHFR and GSTP1 Polymorphisms and Ventricular Septal Defect in Iranian Subjects

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Abstract

Ventricular septal defect (VSD) known as one of the most prevalent types of congenital heart defects (CHD). Both genetic and environmental risk factors are essential in its development. Methylenetetrahydrofolate reductase (MTHFR) is one of the fundamental regulatory enzymes in the homocysteine metabolic pathway, and related coding genes may play a crucial role in CHDs. GSTP1 genotypes codes Glutathione S-transferases (GSTs) play an essential role in detoxification and may increase the risk of DNA damage elicited by pesticide exposure.

This study determined the association of C677T and G1793A polymorphisms of the MTHFR gene and polymorphism of the GSTP1 gene in Iranian VSD subjects. A total of 98 children with VSDs and 89 healthy children were entered in this study. Genomic DNA was extracted from the blood samples of all the cases. The restriction fragment length polymorphism polymerase chain reaction (PCR-RFLP) method amplifies the C677T and G1793A and GSTP1 polymorphism.

The genotype frequencies of CC, CT, and TT of MTFHR C677T gene among the studied cases were 59%, 33%, and 7%, respectively, compared to 58.5%, 38%, and 3.5% controls.

The genotype frequencies of GG, GA, and AA of MTFHR G1793A gene among the studied cases were 97%, 0%, and 0%, respectively, compared to 93%, 7%, and 0% controls.

For the GSTP1 gene polymorphism, the frequencies of the genotypes of AA, AG, and GG among the cases were 42%, 51%, and 3%, respectively, while the frequencies were 40%, 49%, and 11%, respectively, among control subjects.

Significant differences were noticed ($p < 0.05$) in AA VS. AG genotype between cases and control subjects.

Keywords: MTHFR; GSTP1; polymorphism; congenital heart disease; ventricular septal defect

1. Introduction

Congenital heart defects (CHD) are reported as the most frequent type of congenital anomalies. Universal recorded CHD prevalence increased in recent years from 0.6 before 1935 to 0.9 in 1000 live delivery, and the most significant belongs to Asia, with 9.3 per 1,000 live births [1]. Currency of CHD and severe forms of it has risen by 11% and 19% after 2000, respectively [2].

The mean prevalence of CHD has been estimated at 12.30 per 1000 live births, with a yearly prevalence of 17.51 per 1000 live births in IRAN [3].

Overall ventricular septal defects account for up to 40% of all congenital cardiac malformations, and it is the most frequent of CHDs (27%) in IRAN [4,5].

The etiology of VSD is primarily unexplained. Genetic and environmental factors may have a role. Most ventricular septal defects

occur sporadically, with no apparent cause [6]. Some VSDs may have a genetic link causing heart problems to occur more frequently in certain families [7]. Familial compilation and twin research indicated susceptibility to this theory [8].

Completing the human genome project and the universal haplotype map project, converted single nucleotide polymorphisms (SNPs) to available markers to compute individuals' susceptibility to complicated illnesses, treatment efficiency, and adverse drug reactions [9-11]. This, in turn, facilitates clinicians and healthcare workers in taking proper actions. Difference methods have been affirmed for the genotyping of SNPs. Standard techniques include real-time PCR, DNA sequencing, restriction fragment length polymorphism (RFLP) analysis, and amplification refractory mutation system PCR (ARMS-PCR) [12-15].

Methylenetetrahydrofolate reductase (MTHFR) converts a molecule called 5, 10-methylenetetrahydrofolate (folic acid or vitamin B9) to 5-methyltetrahydrofolate (called levomefolic acid or active folate as well). Levomefolic acid can detoxify compounds in the body by moving its methyl group [16-17]. On the other hand, proper methylation enables the body to detoxify some potentially risky compounds generated by, or taken into, the body.

In the conversion of homocysteine to methionine, levomefolic acid or active folate is crucial. Methionine is necessary for producing glutathione, the body's main antioxidant product. Methionine is needed to produce myelin as well [18].

The body cannot produce homocysteine-derived products without this enzyme, and homocysteine upsurges in blood and other tissues. Homocysteine is mandatory for the production of cysteine, methionine, and other necessary mediators are needed for an assortment of metabolic processes like neurotransmitters dopamine, serotonin, and melatonin [19]. Raised homocysteine levels can lead to adverse affection on mental health and mood. Correlation of elevated homocysteine levels and birth defects, complex pregnancies, cardiovascular disease, high blood pressure, glaucoma, ischemic stroke, and atherosclerosis is reported [20, 21].

The MTHFR gene produces MTHFR and has been mapped to chromosome 1, region 1p36.3, and comprised 11 exons ranging in size from 102 to 432 bp [14, 15].

Several polymorphisms in the MTHFR gene have been identified. Because of researches, nearly half of the population may have an MTHFR gene mutation, and the two most problematic mutations are C677T and A1298C mutations, which denote the placement of the mutation on the gene [22].

Among them, A1298C (rs1801131) has been extensively considered, and its influence on DNA synthesis, genome stability, and sustaining proper homocysteine levels in the blood was demonstrated [23-26].

C677T is changing an alanine (A) into a valine (V) residue at codon 222 (A222 V) of the corresponding amino acid sequence, and several studies suggest this polymorphism performs an essential role in the etiology of stroke, neural tube defects, and congenital heart disease (CHD) [23 ,27-29].

Pishva and coworkers determined the association of C677T polymorphism of the MTHFR gene in Iranian VSD subjects. The frequencies for CC and CT genotypes of the MTHFR gene were 51.2% and 48.8%, respectively, in VSD patients compared to 56.8% and 43.2%, respectively, in control subjects [30]. But in Zhang T, study revealed no association between MTHFR C677T and A1298C polymorphisms and ventricular or atrial septal defect risk [31]. Kocakap BD results suggest that MTHFR 1298C allele is a risk factor for conotruncal heart disease [32].

In in-vitro experiments, Homozygosity for C677T, homozygosity for A1298C, and compound heterozygosity for A1298C and C677T are associated with a reduced enzyme activity of 45, 68, and 41%, respectively [33].

Recently, a novel polymorphic site, G1793A, in exon 11, prompting an arginine (R) to glutamine (Q) switch (R594Q) were identified [34]. Some studies repost its function on cardiovascular disease, and the theory of role-playing in CHD is concerned [35]. However, its universal distribution, especially in our country, was not adequately analyzed.

Another gene, GSTP1, codes Glutathione S-transferases (GSTs), a family of enzymes that function in xenobiotic metabolism and play an essential role in detoxification.

Studies propound the susceptibility of its relationship with childhood asthma, CAD in patients with type 2 diabetes mellitus, and malignancies as breast cancer and hepatocellular carcinoma [35- 37]. The soluble GSTs are classified into four main classes: alpha, mu, pi, and theta [38].

Fetal inherited GSTP1 Ile105Val polymorphism may modify the metabolism and excretion of xenobiotics from fetal tissue and raise the risk of congenital heart disease (CHD). Studies aimed to analyze the effects of GSTP1 genetic polymorphism (Ile105Val), and maternal environmental exposure on CHD risk revealed no significant differences in Ile105Val genotype frequencies between the children with CHD and the healthy children and no evidence of meaningful interaction between the maternal exposures and GSTP1 polymorphism [21].

No studies used a broad group of individuals to determine the frequency of the three genotypes within the general populations. In this study, we investigated the allelic frequencies of the C677T and G1793A polymorphism of the MTHFR gene and GSTP1 genotypes in 98 Iranian children.

2. Materials and methods

Our study is a case-control study that considers patients younger than 11 years old with perimembranous or muscular VSD diagnosed at the Pediatric Cardiology Unit of Taleghani children's Hospital in Gorgan, Iran, during April 2016- 2018. The appropriate local authority ethically approves it.

2.1. Subject Recruitment

A total of 187 subjects were categorized into two comparative groups, 98 VSD patients versus 89 control cases. Control subjects were selected from 89 age- and sex-matched children admitted to hospital for elective surgery, and outpatient non-cardiac patients from the same geographic area following clinical assessment included thorough history taking and complete physical evaluation.

Family history, nationality, mother's age, mother's history, birth weight, familial marriage, and extra-cardiac anomalies were evaluated in all cases. In both groups, patients, and controls, information was obtained by a questionnaire. The demographic data were showed in Table 1 in detail.

Characteristic	Patient	Control	P-value
Age (month)	17.07 ±24.59	18.13 ±19.67	P=0.124
Mother's age (year)	28.26 ±5.7	29.46 ±5.22	P= 0.127
Birth Weight(Kg)	3.05 ±0.81	3.18 ±0.56	P= 0.177

Weight(Kg)	8.43 ±5.73	9.57 ±3.98	P=0.129
Gender, male/female	44/54	41/48	P= 0.887
Familial marriage, yes/no	29/69	27/62	P= 1
Familial history, yes/no	12/86	0/89	P≤0.001
Extra Cardiac, yes/no	2/96	1/88	P= 0.373
Mother's history, yes/no	8/90	1/88	P≤0.05

Table 1.*Demographic characteristics of study subjects*

Cardiac evaluation of both groups included 12 lead electrocardiograms (ECG), chest radiography, and transthoracic echocardiography. In all cases, diagnoses were confirmed by Color-Doppler echocardiography.

All specimens were collected with informed consent from the participants' executor. DNA samples were prepared from peripheral blood samples anti-coagulated with ACD, using the standard phenol-chloroform extraction method.

Exclusion criteria were significant CHDs (such as single ventricle, double outlet RV, truncus arteriosus), distinct syndromic associations as VACTERLS, and recognized chromosomal anomaly including trisomy 18 or 21.

2.2. Genotyping assay

To determine the genotypes of MTHFR and GSTP1 genes, genomic DNA was magnified first by the respective primers using the polymerase chain reaction (PCR) technique. The PCR amplification for all the individual polymorphisms was done in a total volume of a 25 μL reaction mix consisting of 10 pmol of each primer and the Mastermix (i-DNA Biotechnology (M) Sdn Bhd, Kuchai Lama, Kuala Lumpur, Malaysia) and the template DNA. A negative control containing no genomic DNA and positive control of recognized genotype were always held in the set of reactions. All of the PCR cyclings were given to an iCycler machine (BioRad Laboratories, Hercules, CA, USA). The amplified PCR products for all the three gene polymorphisms were separated at 2%–4% agarose gel (Bioline, London, UK). The agarose gel was stained in ethidium bromide and visualized using Alpha Imager (Alpha Innotech, San Leandro, CA, USA). The PCR products of the respective genes were digested with 2–4 units of the individual restriction enzymes (Thermo Fisher Scientific, Inc, provided by Research Instruments Sdn Bhd, Petaling Jaya, Malaysia) with 10× Fast Digest Green Buffer in a final volume of 30 μL reaction mixture. Similar results were received when genotyping was performed for 15% of the specimens on two separate occasions.

2.3. Statistical Analysis

Data analysis was done via SPSS version 18.00 (SPSS Inc, South Wacker Drive, Chicago, IL, USA). Genotype and Alleles distribution were tested for deviation from the Hardy-Weinberg by a Chi-Square test. To illustrate the association, the odds ratio (OR) and its 95% confidence intervals (CI) were used and $p < 0.05$ considered in all tests to be statistically significant.

3. Results

A total of 187 abstracts that met the inclusion criteria were retrieved through history taking and physical examination, 98 VSD children, and 89 normal controls.

The mean age was 17.07 ±24.59 in the study group and 18.13 ±19.67 in the control group. The mean weight of case subjects was 8.43 ±5.73, whereas the mean weight of controls was 11.57 ±3.98. The demographic data were listed in Table 1 in detail.

In childhood patients with VSD, the genotype frequencies of the MTHFR C677T polymorphism and C & T allele were as follows: TT 7, CT 33, CC 58, C 149, and T 47. Details of the two VSD groups, muscular and peri-membranous, were represented in Table 1.

In the control group, the homozygous MTHFR 677TT genotype was present in 3 (3.5%), the CT genotype in 34 (38%), and the CC genotype in 52 (58%) individuals. The resulting OR for patients carrying the homozygous TT genotype compared to the controls was 0.47 (CI, 0.11-1.9; P=0.34). Comparing VSD patients to the controls, the frequencies of MTHFR C677T genotypes did not show statistically significant differences between study groups (VSD subtypes and control).

The merge MTHFR C677T allele frequency determined using the random-effects model was 24% in the VSD patients and was 22% in the control. These were 76% in the VSD patients and were 78% in the control respectively for MTHFR –677C allele.

Among the 187 individuals, the MTHFR G1793A genotypes GG were 94 VSD & 82 control, GA was 4 VSD & 5 control, and AA was just two in the control group. The frequency of A allele of the MTHFR G1793A varied from .02% in the VSD group to .05% in control, and all of the populations in this study were in the Hardy–Weinberg law of equilibrium ($P > 0.05$). Details of the two VSD groups, muscular and peri-membranous, were represented in Table 3.

The resulting OR for patients carrying the homozygous AA genotype compared to the controls was not significant. The frequencies of MTHFR C1793A genotypes did not show statistically significant differences between different study groups (VSD subtypes and control). Interestingly, it was noted that 94% of patients had the GG genotype, but there was no significant difference between the two groups.

The pooled MTHFR G1793A allele frequency was 2% in the VSD patients and 5% in the control group. The frequencies for MTHFR –1793G allele were 98% and 95% in the VSD patients in the control group, respectively.

Frequencies of the GSTP1 polymorphism in VSD patients were as following AA 39, AG 50, GG 9, A 128 and G 68. In muscular VSD patient frequencies were as follows: AA, n=19 (42%); AG, n=23 (51%); GG, n=3 (7%); A, n=61(68%) and G, n=29(32%).In peri-membranous VSD patients were: AA, n=21 (40%); AG, n=27 (49%); GG, n=5 (11%); A, n=69(65%) and G, n=37(35%). Results of subgroup analyses are shown in the Tables 2 and Table 3.

polymorphism	**Genotype**	**Patients(n=98)**	**Normal (n=89)**	**TEST**	**OR**	**CI 95%**	**P value**
MTHFR C677T	CC	58 (59%)	52 (58.5%)	CC vs. CT	1.149	0.6257 to 2.1105	0.757
	CT	33 (34%)	34 (38%)	CT vs. TT	0.416	0.099 to 1.747	0.314

	TT	7 (7%)	3 (3.5%)	CC vs. TT	0.478	0.1175 to 1.9450	0.341
allele	C	149(0.76)	138 (0.78)	C vs. T	0.9189	0.5680 to 1.4866	0.807
	T	47(0.24)	40 (0.22)				
MTHFR G1793A	GG	94(96%)	82 (92%)	GG vs. GA	1.4329	0.3723 to 5.5150	0.737
	GA	4(4%)	5 (6%)	GA vs. AA	-	-	NS
	AA	0(0%)	2 (2%)	GG vs. AA	-	-	NS
allele	G	192(0.98)	169 (0.95)	G vs. A	2.5562	0.7731 to 8.4515	0.157
	A	4(0.02)	9 (0.05)				
GSTP1	AA	39 (40%)	47 (53%)	AA vs. AG	0.5809	0.3170 to 1.0644	0.093
	AG	50 (51%)	35 (39%)	AG vs. GG	1.111	0.378 to 3.265	1
	GG	9 (9%)	7 (8%)	AA vs. GG	0.6454	0.2202 to 1.8912	0.587
allele	A	128(0.65)	129 (0.73)	A vs. G	0.7150	0.4599 to 1.1116	0.147
	G	68(0.35)	49 (0.27)				

Table 2. *Comparison of frequencies of MTHFR C677T and GSTP1 genotypes between study groups.*

Moreover, there were no significant differences in the prevalence of alleles and genotypes between VSD patients with and without a family history of congenital heart defects.

polymorphism	Genotype	muscular (n=45)	perimembranous (n=53)	TEST	OR	CI 95%	P-value
MTHFR C677T	CC	22 (49%)	35 (63.5%)	CC vs. CT	0.562	0.242 to 1.308	0.203
	CT	19 (42%)	16 (30%)	CT vs. TT	0.838	0.164 to 4.294	1
	TT	4 (9%)	2(6.5%)	CC vs TT	0.471	0.096 to 2.310	0.428
allele	C	63(0.7)	86(0.8)	C vs. T	0.617	0.324 to 1.174	0.189
	T	27(0.3)	20(0.2)				
MTHFR G1793A	GG	45(97%)	50(93%)	GG vs. GA	-	-	NS
	GA	0(0%)	3(7%)	GA vs. AA	-	-	NS
	AA	0(0%)	0(0%)	GG vs AA	-	-	NS
allele	G	90(1)	103(0.96)	G vs. A	-	-	NS
	A	0(0)	3(0.04)				
GSTP1	AA	19 (42%)	21 (40%)	AA vs AG	1.014	0.443 to 2.321	1
	AG	23 (51%)	27 (49%)	AG vs. GG	1.727	0.379 to 7.864	0.713
	GG	3 (7%)	5(11%)	AA vs. GG	1.704	0.383 to 7.585	0.718
allele	A	61(0.68)	69(0.65)	A vs. G	1.155	0.641 to 2.084	0.655
	G	29(0.32)	37(0.35)				

Table 3. *MTHFR C677T and GSTP1 genotypes frequencies according to VSD type*

4. Discussion

Congenital heart disease (CHD) is one of the most commonly noninfectious diseases, which compounds one-third of all congenital anomalies and is the main cause of birth defects lead in to infant mortality [39, 40]. The incidence of CHD in different studies varies from about 4/1,000 to 50/1,000 live births. The relative frequency of various significant forms of CHD also differs considerably from study to study. The prevalence of congenital cardiovascular malformations increased from 0.6 to 9.3 per 1,000 live births, and the prevalence of ventricular septal defect extended from 1.0 to 1.6 per 1,000 live births in the latest studies [1].

Ventricular septal defects are usually asymptomatic and close spontaneously [41]. There are definite multifactorial causes for CHDs, especially VSD, in which both environmental and genetic risk factors are consequential in the development of CHD [42, 43]

Mutations in the encoding gene of the homeobox transcription factor NKX2-5 were mapped to chromosome 5q35were found to cause nonsyndromic congenital heart disease and atrioventricular conduction abnormalities [44]. The significance of genetic factors in the development of CHD is also supported by data from genome-wide association studies (GWASs).it have affirmed that a zone on chromosome 4p16 adjacent to the MSX1 and STX18 genes was correlated with the risk of atrial septal defect of ostium Secundum type (ASD2) [45]. Also revealed that rs2228638 in NRP1 on 10p11 significantly increased the risk of Tetralogy of Fallot (TOF) [46]. Abdul-Sater Z and et al. have formely shown that a tandem repeat in the intrinsic region of NFATC1 is associated with ventricular septal defects. after that, they showed for the first time a potential link between a mutation in NFATC1 and tricuspid valve atresia [47]. Xuan C et al. identified HOMEZ and PLAGL1 as pathogenic genes in Chinese patients with isolated ventricular septal defects (VSDs) [48, 49].

Maternal hyperhomocysteinemia association with an increased risk of CHDs first reported in 1999 [50]. Wenstrom first noted a correlation between MTHFR gene polymorphism and susceptibility to CHD [51]. other studies supported the MTHFR -677T allele as a susceptibility cause for CHD in the Asian population and the -1298C allele role in the Caucasian pediatric population.8 Even MTHFR 677CT genotype posed as an implicating factor and as a maternal risk factor for septal defects in children with Down syndrome [52]. A study done by Zhu et al. showed that the MTHFR C677T locus variation is associated with the occurrence of the atrial septal defect (ASD) and patent ductus arteriosus (PDA) [53].

The 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene is placed on chromosome 1 at 1p36.3. The primary product of the MTHFR gene is a catalytically active 77 kDa protein that catalyzes the transformation of 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the primary circulating form of folate [8] A frequent C677T mutation (rs1801133) in the MTHFR gene has been described, which was associated with a 50% reduction of MTHFR enzyme activity, a rise in

plasma homocysteine concentration, and a decrease in plasma folic acid concentration [54]. Homozygosity (TT) for C677T polymorphism is associated with higher homocysteine levels and lower serum folate concentration than heterozygosity (CT) or wild-type genotype (CC) [55, 56]. otherwise, it was mentioned that A1298C heterozygosity and homozygosity were associated neither with higher total nor lower folate plasma concentration [57, 58].

Although many studies propound the association between MTHFR (methylenetetrahydrofolate reductase) polymorphisms and VSD/ASD risk, the results are controversial. In one study, the association of heart defects with four polymorphisms in folate-related genes was examined. The AG and 66GG genotypes were associated with decline odds ratios for heart defects. Maternal MTHFR 1298AC genotype was associated with an increased odds ratio for aortic valve stenosis. No association between SLC19A1 c.80A > G or MTHFR c.677C > T and heart defect was found [59]. Wang and coworkers carried out a meta-analysis. They reported that the infant and maternal MTHFR C667T polymorphism association might be associated with an increased occurrence of CHD.46 By contrast, Mamasoula and coworkers indicated that the MTHFR C677T polymorphism is not linked with the risk of CHD [60]. Several studies with conflicting outcomes have been published to confirm an association between MTHFR and CHD. Later studies, however, do not support the theory of MTHFR acting as a risk factor for the development of CHD [15, 61, 62]. A meta-analysis suggested that MTHFR C677T and A1298C polymorphisms are not associated with ventricular or atrial septal defect risk [16]. while the others had previously stated that they had found (for the first time!) that the embryonal MTHFR 677TT genotype was significantly associated with developing structural congenital heart malformations during early pregnancy [63].

Glutathione-S-transferases or GSTs catalyze the conjugation of many hydrophobic and electrophilic compounds and play an essential role in detoxification. The dissolved GSTs are classified into four main: alpha, mu, pi, and theta.

It is proposed that the GSTM1 (del) and GSTP1 (Ile105Val) gene polymorphisms themselves are not associated with the risk of congenital malformations (CMs) in a newborn. However, smoking may increase the risk magnitude of the GSTP1 (Ile105Val) genotypes in the formation of CMs in a child [18]. Results suggest that individuals with susceptible metabolic GSTP1 genotypes may experience an increased risk of DNA damage elicited by pesticide exposure [64]. to clear up the genetic factors causing clinical differences in children with Down syndrome and assess possible maternal risk factors; the scientists have investigated GSTM1, GSTT1, GSTP1 gene polymorphisms. Still, the data indicated no relationship between detected GST polymorphisms with the risk of having an infant with Down syndrome [20].

One study revealed an increased incidence of the GSTP1 variant genotypes among myelodysplastic syndromes (MDS) patients, providing evidence for a potential pathogenic role of the GSTP1 polymorphism on de novo MDS risk [21].

Our study aimed to assess the correlation between polymorphisms in the methylenetetrahydrofolate reductase (MTHFR), Glutathione S-Transferase Pi 1(GSTP1) genes, and the risk for VSD in Iranian subjects through a case-control study. Thus, our primary purpose was to figure out whether the MTHFR and GSTP1 gene polymorphisms are risk factors or not for the development of VSD in Iranian subjects.

5. Conclusions

This study shows no association between the MTHFR gene and VSD subjects. However, the GSTP1 gene AA polymorphisms can be considered as protective factors for VSD in Iranian subjects. More extensive cohort studies on mothers and children with distinct sub-classes are required to address risk adequately.

Declarations

Ethics approval and consent to participate: the Ethics Committee of the Gorgan University of Medical Sciences approved the study.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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