MTHFR 677 C→T Polymorphism and the Risk of Cardiac Septal Defects: A Pilot Study

Omneya I Youssef [1] and Ghada M El Sayed [2]

[1]Department of Pediatrics, Faculty of Medicine, Ain Shams University, Egypt
[2] Department of Clinical Pathology, National Cancer Institute, Cairo University, Egypt
[1] batata88888@yahoo.com, [2]elsayed276@yahoo.com

Abstract: Congenital heart defects (CHDs) are among the most common birth defects. The majority of CHDs are polygenic diseases affected by both genetic and environmental factors. Identification of the candidate genes in folate metabolism has suggested that the 677C→T polymorphism in the Methylenetetrahydrofolate reductase (MTHFR) gene may be particularly associated with the risk of CHDs. The objective of this study was to investigate the effect of MTHFR 677C→T locus polymorphism as a risk factor for cardiac septal defects (CSDs). Forty- two patients and 90 age and sex matched infants as control group were investigated. Eleven (26.2%) patients presented with isolated atrial septal defect, (ASD), 18 (42.9%) with isolated ventricular septal defect (VSD), 8(19%) with combined ASD+VSD, and 5 (11.9%) with atrioventicular canal (AV) canal. The investigation of MTHFR 677 C→T polymorphism was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The resulting odds ratio (OR) for patients carrying MTHFR C677T mutation was 1.8 (95% CI 0.86-3.84, x^2 =1.48, p = 0.11) compared to the control and the OR for MTHFR 677CT genotype for patients was 2.13 (95% CI 0.97-4.69, x^2 =3.63, p = 0.06) attaining only marginal significance compared with TT and CC genotype in control. Patients with AV Canal showed significantly (p = 0.007) higher percentage of CT genotype (OR 1.19 95% CI 1.0-1.4) when compared with control. Patients with combined ASD and VSD showed significantly (p =0.05) higher percentage of CT genotypes (OR 4.68 95%CI 1.03 -21.31) when compared to the control. In conclusion we suggest MTHFR 677CT genotype as a risk factor for AV canal and combined ASD + VSD, and because of the strong relation of this enzyme with folic acid we recommend preiconceptional folic acid supplementation and food fortification which might decrease CSDs in Egypt.

[Omneya I Youssef and Ghada M El Sayed. **MTHFR 677 C→T Polymorphism and the Risk of Cardiac Septal Defects: A Pilot Study.** *Life Sci J* 2012;9(4):4272-4275]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 638

Key words: Congenital heart disease; folic acid; MTHFR polymorphisms

1. Introduction

Congenital heart defects (CHDs) are common birth defects with a prevalence of confirmed defects of approximately 1:100 living births [(1).]

CHDs mainly result from incomplete development of the heart during the first 6 weeks of pregnancy. Most CHDs are thought to be of a complex multifactorial origin, with one or more alleles at a number of loci interacting with environmental factors [(2)].

The most prevalent heart disease in infants and children are atrial septal defect (ASD), ventricular septal defect (VSD), patent ductus arteriosus (PDA) and other types [(3)].

Observational studies have demonstrated an association between periconceptional use of multivitamins containing folic acid, and CHDs, both conotruncal and other heart defects. Additional support for the importance of folate in CHD risk was provided by Hernandez-Diaz *et al.,* [(4)] their study showed that periconceptional intake of medication acting as folic acid antagonists, doubled the risk for CHDs.

The protective effect of periconceptional folate on CHD has led to the search for candidate genes involved in its metabolic pathway. Methylenetetrahydrofolate reductase (MTHFR) is a promising candidate because it is a regulating key enzyme for the availability of active folate by catalyzing the reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. Reduced MTHFR activity results in a decreased availability of 5-methyltetrahydrofolate for the remethylation of homocysteine to methionine. The MTHFR 677C→T polymorphism results in a thermolabile enzyme with reduced activity [(5)].

Studies regarding MTHFR 677C→T polymorphism in relation to CHD have yielded conflicting conclusions. Junker *et al.,* [(6)] were the first to suggest an association between the incidence of CHDs and MTHFR 677C→T polymorphism. The authors observed a higher frequency of the 677TT genotype versus the combined group of CT and CC genotypes among children (n=114) with a CHD, compared with 228 controls. On the other hand, Storti *et al.,* [(7)] did not observe any association between any fetal MTHFR genotypes (n=103 versus n=200 controls) and the risk of conotruncal heart defects.

The objective of this study was to investigate the effect of MTHFR 677C→T locus polymorphism as a risk factor for cardiac septal defects (CSDs).

2. Subjects and Methods

A. Subjects

From June 2009 to June 2010 all patients presented to the outpatient clinic of Demerdash pediatric hospital, Ain Shams University with CSDs were enrolled in the study. Patients with any genetic or chromosomal disorders that may affect the heart including Down syndrome (DS), patients with any congenital cardiac abnormalities other than CSDs, infants with prenatal history of maternal diabetes, hypertension, and patients with history of exposure to chemicals irradiation or pesticides were excluded. The study population remaining for analysis was 42 patients, median age 1.5 months (range 20 days to 1 year), including 24 males (57.1%) and 18 females (42.9%). Eleven (26.2%) patients presented with isolated ASD, 18 (42.9%) with isolated VSD, 8(19%) with ASD+VSD, and 5 (11.9%) with AV canal (Table 1).

In all cases, diagnosis was confirmed by cardiovascular specialist with classical clinical manifestation and standard imaging procedure (M mode,2D ,colour ,pulsed continuous wave echocardiography using VIVIDE9Vingmed Horten, Norway).

From the same hospital 90 age and sex matched apparently healthy infants were selected as a control group. The study was approved by the ethical committee of the institute and informed consent was obtained from all mothers.

In groups, patients and controls, information on maternal use of folate or multivitamins, consanguinity, family history of CHD was obtained (Table 1).

B. Blood sampling and genotype analysis for MTHFR 677 C→T polymorphism:

Two ml blood was drawn into EDTA treated tubes for analysis of MTHFR 677 C→T polymorphism (known as c.677 C>T, rs 1801133, p.Ala 222-val). Genomic DNA was extracted using QIAampl DNA extraction kit (Cat#51104) and genotyping was performed by (PCR-RFLP) method reported by Frosst *et al.* [8].

Two primers were used for analysis; MTHFR F: 5-TGA AGG AGA AGG TGT CTG CGG GA-3 and MTHFR R: 5- AGG,ACG GTG CGG TGA GAG TG -3. One µL of genomic DNA was amplified in a 50 µl reaction volume containing 5 µl of 10X buffer containing KCl and $(NH4)_2SO_4$, pH=8.7, 1.5mM $MgCL_2$, 0.2mM each dNTPs, 0.4 µM primers, and one unit of Taq DNA polymerse enzyme (Qiagen, cat#20123). PCR reaction consisted of initial denaturation at 94°C for 150 seconds followed by 35 cycles at 94°C for 30 seconds, 57°C for 60 seconds, 72°C for 120 seconds and a final elongation step at 72°C for 3 minutes. PCR product of 198bp was analyzed on 1.5% agarose gel electrophoresis. 20 µl of PCR product was mixed with 10 units of Hinfl (Promega, Madison) enzyme and incubated at 37°C overnight incubation. The substitution creates a Hinfl recognition sequence that digests the 198bp into 175-bp and 23-bp. The MTHFR 677CC genotype had only one band: 198 bp, 677CT genotype had three bands: 198 bp, 175bp and 23 bp, and TT homozygote had two bands: 175 bp and 23 bp.

To ensure quality control, the presence of C→T polymorphism was performed with blinding to case-control status, non-template control and positive/amplification genotype control was included within each experimental run. Ten percent sample cases and controlled was genotyped twice and reproducibility was 100%.

Statistical analysis

Quantitative variables were reported as mean ± Standard Deviation (SD) number or frequency, median and range, and qualitative variables as number and percentage. The significant level was set at 0.05. Genotype frequencies in patients and controls were compared by x^2-analysis or fisher exact test. The genetic risk on CSDs was assessed by the calculation of Odds ratio (OR) with corresponding 95%-confidence intervals (CI) and correlation coefficient tests were performed. All statistical analyses were performed using Statistical Package for Social Science (SPSS) program (version (16).

3. Results

The resulting (OR) for patients carrying MTHFR 677 C→T mutation was 1.8 (95% CI: 0.86-3.84, x^2 =1.48, p = 0.11) compared to control and the OR for CT genotype for patients was 2.13 (95% CI: 0.97-4.69, x^2 =3.63, p = 0.06) attaining only marginal significance compared with TT and CC genotype in controls (Table 1).

The frequency of CT genotype was significantly (P= 0.007) higher in patients with AV canal, (OR 1.19 95% CI 1.0- 1.41) when compared with the control group. They also showed higher (P=0.33) percentage of TT genotype but they did not achieve significance (OR 0.32 95% CI 0.24 - 0.44) (Table 2).

Patients with combined ASD and VSD showed significantly (P=0.05) higher percentage of CT genotypes (OR 4.68 95%CI 1.03 - 21.31) when compared to the control but they did not showed any TT genotype (Table 2).

There was no statistical significant difference between male and female patients, history of consanguinity and family history (FH) as regards distribution of MTHFR C677T mutation (P = 0.63, 0.71 and 0.99 respectively).

None of the mothers of our patients had preiconceptional folic acid supplementation, vitamin supplements containing folic acid or eating food fortified with folic acid and Egypt is a non-folate fortified-country.

Table (1): Characteristics of patients with CSDs

		Cases	
		N	%
Sex	Male	24	57.1
	Female	18	42.9
Consanguinity (32)	-ve	21	65.6
	+ve	11	34.4
FH (31)	-ve	26	83.9
	+ve	5	16.1
MTHFR GENOTYPE	CC	22	52.4
	TT	2	4.8
	CT	18	42.9
CSD	ASD	11	26.2
	VSD	18	42.9
	ASD+VSD	8	19.0
	AV canal	5	11.9

FH, family history; CSD, cardiac septal defects;ASD, atrial septal defect; VSD ventricular septal defect; AVC, atrioventricular canal

Table 2: Distribution of MTHFR genotype in patients with different types of cardiac septal defect

MTHFR genotypes	Cardiac septal defects				Control
	ASD N=11	VSD N=18	ASD + VSD N=8	AV canal N=5	
MTHFR 677TT	0	1(5.6%)	0	1(20%)	9 (10%)
MTHFR 677CT	3(27.3%)	5(27.8%)	5(62.5%)	4(80%)	21(24.1%)
MTHFR 677CC	8(72.7%)	12(66.7%)	3(37.5%)	0	60(63.3%)
MTHFR 677 CT/TT	3(27.3%)	6(33.4%)	5(62.5%)	5(100%)	30(34.1%)

FH, family history; CSD, cardiac septal defects; ASD, atrial septal defect; VSD ventricular septal defect; AVC, atrioventricular canal

4. Discussion:

This pilot study was conducted on the basis of the hypothesis that 677TT and 677CT genotype in the MTHFR gene has an impact on emberyonal development of children and infants with CSDS. Consistent with this hypothesis, the frequency of MTHFR 677CT genotype was significantly higher in patients with AV canal and patients with combined ASD and VSD compared to healthy controls in this study. This observation was in agreement with previous studies [3, 6, 9-11].

On the other hand, some studies [7, 12-14], revealed negative results and did not observe an association for fetal and/or maternal MTHFR 677C→T polymorphism with different CHDs. However, some of these studies were heterogeneous with regard to heart defects or were performed in countries where fortification of flour and other enriched grain products with folic acid has been compulsory. In this study we have investigated the estimate risk of one type of CHD, (CSDs) for MTHFR 677CT and MTHFR 677TT genotypes in a non-folate fortified-country, Egypt.

MTHFR 677C→T homozygous genotypes (TT) and possibly the heterozygous mutant genotypes (CT) are more likely to be related to CHD risk in the absence of periconceptional folic acid supplementation. Such an interaction was found by van Beynum *et al.*, [11] for the maternal MTHFR 677C→T polymorphism in relation to CHD when the mother did not take folic acid periconceptionally. The maternal MTHFR 677CT and TT genotypes in combination with no use of periconceptional folic acid supplements were associated with, respectively, a three-fold (OR 3.3 95%CI 1.46– 7.32) and six-fold (OR 6.3 95%CI 2.32–17.27) increased risk for conotruncal heart defects in offspring [11].

Previous studies have demonstrated that mothers with the mutation could have a functional folate deficiency even if folate levels are low-normal or normal. In such patients, a superanormal plasma folate

level achievable by high dose folate supplementation might be needed to prevent birth defects [15].

Whether the detrimental effect on the developing emberyo is related to the polymorphism in the fetal or in the maternal genotype or both, in the current study, all mothers did not use preiconceptional folic acid supplementation which supports the role of gene-environment interaction in a non-folate fortified-country like Egypt and the increase in risk for having offsprings with CSDs.

To conclude, we suggest MTHFR 677CT genotype as a risk factor for AV canal and combined ASD + VSD, and because of the strong relation of this enzyme with folic acid we recommend preiconceptional folic acid supplementation and food fortification which might decrease CSDs in Egypt.

However, cardiac development is a very complicated process involving expression of many genes at different times, space and order and our conclusion is hindered by the small sample of children in each septal defect which might lower the statistical power of the study. We acknowledge that these results could be false positive and further studies with a larger cohort are warranted to enable a definite conclusion on the MTHFR 677 C→T polymorphism and CSDs. Nevertheless, we recommend periconceptional intake of multivitamin supplements that contain folic acid which may reduce the risk of congenital cardiovascular defects in offspring.

Corresponding author
Ghada M El Sayed
Department of Clinical Pathology, National Cancer Institute, Cairo University, Egypt

References

1- Maschhoff K. (2004): Elucidating the genetic cause of congenital heart defects. Neo Reviews. 5(7):283-289.
2- Van Beynum IM, den Heijer M, Blom HJ, Kapusta L. (2007): The MTHFR 677C->T polymorphism and the risk of congenital heart defects: a literature review and meta-analysis. QJM. 100(12):743-53.
3- Zhu WL, Liy, Yan L, Dao J and Lis. (2006): Maternal and offspring MTHFR gene C677T polymorphism as predictors of congenital atrial septal defect and patent ducts arteriosus. Molecular Human Reproduction 1: 51-54
4- Hernandez-Diaz 5, Werler MM, Walker AM, Mitchell AA. (2000): Folic acid antagonists during pregnancy and the risk of birth defects. N Engl J Med. 343 (22): 1608-1614.
5- Jacques P F, Bostom A G, Williams R R, Ellison R C, Eckfeldt J H, Rosenberg IH, Selhub J , Rozen R. (1996): Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation. 93: 7-9.
6- Junker R, Kotthoff S, Vielhaber H, Halimeh S, Kosch A, Koch H G, Kassenbohmer R, Heineking B, Nowak-Gottl U. (2001): Infant methylenetetrahydrofolate reductase 677TT genotype is a risk factor for congenital heart disease. Cardiovasc Res. 51: 251-254.
7- Storti S, Vittorini S, Lascone MR, Sacchelli M, Collavoli A, Ripoli A, Cocchi G, Biagini A, Cleric A. (2003): Association between 5, 10-methylenetetrahydrofolate reductase C677T and A 1298C polymorphisms and conotruncal heart defects. Clin Chern Lab Med. 41: 276-280
8- Frosst P , Blom H, Milos R, Goyette P, Sheppard CA, Matthews RG, BoersG JH, den Heijer M, Kluijtmans LAJ, van den Heuve LP, Rozen R. (1995): A Candidate Genetic Risk Factor for Vascular Disease: a Common Mutation in Methylenetetrahydrofolate Reductase. Nut Genet.10:111-113.
9- Deeparani T, Radkakrishma P and Elarazhagan T. (2009): Detection of MTHFR C677T and A1298C gene polymorphism in congenital heart disease. Middle East Journal of Scientific Research. 4(2): 127-132.
10- Lee CN, Suyn, Chen WF, Lin MT, Wang JK, Wu MH and Hsieh Fj. (2005): Association of the C677T methylene tehahydroflate neductase mutation with congenital heart disease. Acta Obstet Gynecol Scand. 4(12): 1134-1140.
11- van Beynum IM, Kapusta L, den Heijer M, Vermeulen SH, Kouwenberg M, Daniels O, Blom HJ. (2006): Maternal MTHFR 677C>T is a risk factor for congenital heart defects: effect modification bypericonceptional folate supplementation. Eur Heart J. 27:981–987.
12- McBride KL, Fernbach S, Menesses A, Molinari..L, Quay E, Pignatelli R, Towbin JA, Belmont JW. (2004): A family-based association study of congenital left-sided heart malformations and 5,10 methylenetetrahydrofolate reductase. Birth Defects. Res A Clin Mol Teratol. 70(10):825-30.
13- Hobbs CA, James 5J, Parsian A, Krakowiak PA, Jernigan 5, Greenhaw JJ, Lu Y, Cleves MA. (2006): Congenital heart defects and genetic variants in the methylenetetrahydrofolate reductase gene. J Med Genet. 43(2):162-166.
14- Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM and Lammer EJ. (1995): Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. Am J Med Genet. 59:536–545.
15- Huhta JC, Hernandez-Robles JA. (2005): Homocysteine, folate, and congenital heart defects . Fetal Pediatr Pathol. Mar-Apr.24(2):71-9.

11/2/2012