MTHFR rs1801133 C>T polymorphism is associated with an increased risk of tetralogy of Fallot

JIANBING HUANG, JU MEI, LIANYONG JIANG, ZHAOLEI JIANG, HAO LIU and FANGBAO DING

Department of Cardiothoracic Surgery, Xin Hua Hospital
Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, P.R. China

Received December 02, 2013; Accepted January 02, 2014

DOI: 10.3892/br.2014.222

Abstract. Abnormal folate metabolism and common variants of folate-metabolizing enzymes have been described as possible risk factors for congenital heart disease (CHD). Two important folate-metabolizing enzymes involved in the folate/homocysteine metabolic pathway are 5,10-methylenetetrahydrofolate reductase (MTHFR) and methylenetetrahydrofolate dehydrogenase 1 (MTHFD1). *MTHFR* and *MTHFD1* polymorphisms may be associated with CHD susceptibility. To evaluate the impact of *MTHFR* and *MTHFD1* single-nucleotide polymorphisms (SNPs) on CHD susceptibility, we genotyped functional *MTHFR* SNPs rs1801133 C>T, rs1801131 A>C and rs2274976 G>A, and *MTHFD* SNPs rs2236225 C>T, rs1950902 G>A and rs1076991 A>G in a hospital-based case-control study of 173 tetralogy of Fallot (TOF) cases and 207 non-CHD controls. When *MTHFR* rs1801133 CC homozygote genotype was used as the reference group, the TT genotype was associated with a significantly increased risk for TOF [TT vs. CC: odds ratio (OR)=1.67; 95% confidence interval (CI): 1.01-2.75; P=0.046]. In the recessive model, when *MTHFR* rs1801133 CC/CT genotype was used as the reference group, the TT homozygote genotype was associated with a significantly increased risk for TOF (OR=1.81, 95% CI: 1.15-2.84; P=0.010). In conclusion, our findings suggest that *MTHFR* rs1801133 C>T polymorphism may play a role in susceptibility for TOF. Large-scale studies with a more rigorous study design including diverse ethnic populations are required to confirm these findings.

Correspondence to: Professor Fangbao Ding, Department of Cardiothoracic Surgery, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Yangpu District, Shanghai 200092, P.R. China
E-mail: 40821247@qq.com

Abbreviations: CI, confidence interval; CHD, congenital heart disease; LD, linkage disequilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism

Key words: 5,10-methylenetetrahydrofolate reductase, congenital heart disease, polymorphisms, tetralogy of Fallot, molecular epidemiology

Introduction

Congenital heart disease (CHD) is currently the leading non-infectious cause of death in children and the most common type of structural malformation of the heart and large blood vessels, with a prevalence of 4-10 per 1,000 live births (1). In China, the prevalence of CHD is 73.2 per 10,000 births in high-prevalence areas.

Abnormal folate metabolism and common variants of folate-metabolizing enzymes have been previously described as possible risk factors for CHD. It has been hypothesized that the peri-conceptional supplementation of multiple vitamins (particularly folic acid) may significantly reduce the incidence of conotruncal heart defects (2). One important folate-metabolizing enzyme involved in the folate/homocysteine metabolic pathway is 5,10-methylenetetrahydrofolate reductase (MTHFR) (3). The *MTHFR* gene is located on chromosome 1p36.3 and encodes an enzyme that is essential for folate-mediated one-carbon metabolism. The common polymorphism C677T (rs1801133) in the gene that encodes MTHFR causes reduced enzymatic activity and elevated levels of homocysteine (4). Individuals who are homozygous for the 677T allele have only 30% of the MTHFR enzymatic activity measured in individuals homozygous for the wild-type C allele, whereas heterozygotes retain 65% of wild-type MTHFR enzymatic activity (5).

In a recent meta-analysis it was suggested that genotypes with *MTHFR* C677T polymorphism may be associated with risk of CHD, particularly in Caucasians (6). This risk may be due to several factors, including etiologic heterogeneity among the many and varied CHD phenotypes. Thus, additional etiologic studies that focus on subgroups of CHDs which appear to share common embryologic, genetic and epidemiologic features (e.g., conotruncal and left-sided heart defects) are necessary.

Another non-synonymous single nucleotide polymorphism (SNP) of the *MTHFR* gene, Arg594Gln (1793G>A, rs2274976), has been identified; however, its functional significance remains unknown (7). In the *methylenetetrahydrofolate dehydrogenase 1* (*MTHFD1*) gene, the rs2236225 polymorphism at nucleotide 1958 (c.1958A>G) results in an arginine→glutamate substitution at amino-acid position 653 (p.Arg653Gln), which is localized in the synthetase domain of the enzyme (8). To evaluate the impact of *MTHFR* rs1801133 C>T, rs1801131 A>C and rs2274976 G>A, and *MTHFD* rs2236225 C>T,

rs1950902 G>A and rs1076991 A>G SNPs on CHD susceptibility, we performed genotyping analyses for six SNPs in a hospital-based case-control study with 173 tetralogy of Fallot (TOF) cases and 207 controls in a Chinese population.

Materials and methods

Study population. This study included 173 TOF patients and 207 non-CHD controls. Patients were consecutively recruited from the Xinhua Hospital, Shanghai, China, between March 2009 and October 2011. The patients were ultrasonic diagnosed non-syndromic CHDs and confirmed during surgery. All the cases were identified as TOF. The controls were non-CHD patients, frequency-matched to the cases with regard to age (±3 years) and gender, recruited from the same hospital during the same time period and most of them were with trauma or infectious diseases.

All the subjects were genetically unrelated ethnic Han Chinese. After informed consent was obtained from their parents, each subject and her/his parents were personally questioned by trained interviewers using a structured questionnaire to obtain information concerning maternal diabetes mellitus (DM), teratogenic contact during pregnancy and family history of CHD in first-degree relatives (parents, siblings and children). Information regarding rubella, influenza and febrile illnesses during pregnancy was also collected. Cases with structural malformations involving another organ system or known chromosomal abnormalities were excluded. Exclusion criteria also included a positive family history of CHD in a first-degree relative (parents, siblings and children), maternal DM, phenyl ketonuria, maternal exposure to teratogens (e.g., pesticides and organic solvents), as well as rubella, influenza and febrile illnesses during pregnancy. Controls with congenital anomalies were excluded.

A 2-ml venous blood sample was collected from each subject. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Institutional Review Board and Ethics Committee of the Xin Hua Hospital Affiliated to the Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Isolation of DNA and genotyping by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-time-of-flight-MS). Samples of venous blood (2 ml) were collected from patients using vacutainer tubes. The samples were transferred to tubes containing ethylenediaminetetraacetic acid. Genomic DNA was isolated from whole blood with the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). Genotyping was undertaken by MALDI-TOF-MS as described previously (9-11). SNP genotyping was performed using the MassArray system (Sequenom, San Diego, CA, USA) by the MALDI-TOF-MS method according to the manufacturer's instructions (9-11). Completed genotyping reactions were spotted onto a 384-well SpectroChip system (Sequenom) using a MassArray Nanodispenser (Sequenom) and the genotype determined by MALDI-TOF-MS. Genotype calling was conducted in real time with MassArray RT software version 3.1 (Sequenom) and analyzed using MassArray Typer software version 4.0 (Sequenom).

Table I. Comparison of TOF patients and controls by selective characteristics.

Variable	TOF (n=173)	Controls (n=207)	P-value
Age, years (mean ± SD)	2.54±2.04	2.70±2.71	0.521
Male/female (n)	97/76	119/88	0.781
Isolated TOF, n (%)	114 (65.9)	-	-
TOF with ASD, n (%)	27 (15.6)	-	-
TOF with PDA, n (%)	11 (6.4)	-	-
TOF with ASD and PDA, n (%)	7 (4.0)	-	-
TOF with absent pulmonary valve, n (%)	3 (1.7)	-	-
TOF with pulmonary atresia, n (%)	6 (3.5)	-	-
TOF with muscular VSD, n (%)	5 (2.9)	-	-

TOF, tetralogy of Fallot; ASD, atrial septal defect; PDA, patent ductus arteriosus; VSD, ventricular septal defect.

Statistical analysis. Differences in the distributions of demographic characteristics, selected variables, the variant alleles and genotypes of the six SNPs between the cases and controls were evaluated using the Student's t-test (for continuous variables) and χ^2 test (for categorical variables). The associations between the six SNPs and risk of CHD were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analyses. All of the statistical analyses were performed with Statistical Analysis System software (version 9.1.3; SAS Institute, Inc., Cary, NC, USA).

Results

Characteristics of the study population. The demographic and clinical characteristics of all the subjects are shown in Table I. Age for the cases and controls was 2.54 (±2.04) and 2.70 (±2.71 years), respectively, (P=0.521), with 56.1 of the cases and 57.5% of the controls being males and the difference was not statistically significant (P=0.781), indicating that the frequency-matching by age and gender was adequate. Of the 173 TOF patients, 114 (65.9%) were isolated TOF, 27 (15.6%) were TOF with atrial septal defect, 11 (6.4%) were TOF with patent ductus arteriosus, 3 (1.7%) were TOF with absent pulmonary valve, 6 (3.5%) were TOF with pulmonary atresia and 5 (2.9%) were TOF with muscular ventricular septal defect. The primary information for the six genotyped SNPs is shown in Table II. The genotyping success rate ranged from 97.89 to 99.21% for the six SNPs in all 380 samples. Minor allele frequency (MAF) in our controls was similar to that for Chinese individuals in the database for all six SNPs (Table II). The observed genotype frequencies for five polymorphisms in the controls were all in Hardy-Weinberg equilibrium (HWE) instead of *MTHFR* rs1801133 C>T (P<0.050) (Table II).

Table II. Primary information for six genotyped SNPs.

Genotyped SNPs	Chr	Regulo-meDB score[a]	TFBS[b]	nsSNP	MAF for Chinese in database	MAF in our controls (n=207)	P-value for HWE test in our controls	% Genotyping value
MTHFR: rs1801133 C>T	1	4	-	Y	0.439	0.412	<0.050	97.89
MTHFR: rs1801131 A>C	1	1f	-	Y	0.198	0.160	0.712	98.95
MTHFR: rs2274976 G>A	1	4	-	Y	0.093	0.066	0.896	99.21
MTHFD: rs2236225 C>T	14	5	-	Y	0.221	0.243	0.418	98.95
MTHFD: rs1950902 G>A	14	No data	-	Y	0.268	0.274	0.599	99.21
MTHFD: rs1076991 A>G	14	4	Y	-	0.276	0.238	0.328	98.42

[a]http://www.regulomedb.org/; [b]http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm. SNPs, single nucleotide polymorphisms; Chr, chromosome; TFBS, transcription factor binding site; nsSNP, non-synonymous single-nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; *MTHFR*, 5,*10-methylenetetrahydrofolate reductase*; Y, yes; *MTHFD*, *methylenetetrahydrofolate dehydrogenase*.

MTHFR and MTHFD polymorphisms and TOF susceptibility. The *MTHFR* and *MTHFD* genotype distributions in the cases and controls were shown in Table III. In single-locus analyses, the genotype frequencies of *MTHFR* rs1801133 C>T were 37.50 (CC), 26.79 (CT) and 35.71 (TT) in TOF patients; and 41.18 (CC), 35.29 (CT) and 23.53% (TT) in the control subjects, with the difference being statistically significant (P=0.028). When the *MTHFR* rs1801133 CC homozygote genotype was used as the reference group, the CT genotype was not associated with the risk for TOF (CT vs. CC: OR=0.83, 95% CI: 0.51-1.37, P=0.471), the TT genotype was associated with a significantly increased risk for TOF (TT vs. CC: OR=1.67; 95% CI: 1.01-2.75; P=0.046). In the recessive model, when the *MTHFR* rs1801133 CC/CT genotypes were used as the reference group, the TT homozygote genotype was associated with a significantly increased risk for TOF (OR=1.81, 95% CI: 1.15-2.84, P=0.010). In the dominant model, the *MTHFR* rs1801133 CT/TT variants were not associated with the risk of TOF, compared with the *MTHFR* rs1801133 CC genotype (OR=1.17, 95% CI: 0.77-1.77, P=0.471) (Table III).

MTHFR rs1801131 A>C and rs2274976 G>A, as well as *MTHFD* rs2236225 C>T, rs1950902 G>A and rs1076991 A>G polymorphisms did not achieve a significant difference in the genotype distributions between the cases and controls. Logistic regression analyses revealed that the five SNPs were not associated with the risk of TOF (Table III).

Discussion

In this case-control study, we investigated the association of *MTHFR* rs1801133 C>T, rs1801131 A>C and rs2274976 G>A, and *MTHFD* rs2236225 C>T, rs1950902 G>A and rs1076991 A>G SNPs and risk of TOF in a Chinese population. We found that the *MTHFR* rs1801133 C>T polymorphism was associated with risk for TOF. *MTHFR* rs1801131 A>C and rs2274976 G>A, and *MTHFD* rs2236225 C>T, rs1950902 G>A and rs1076991 A>G polymorphisms may not be associated with risk for TOF.

Folate is known to play a crucial role during the development of the cardiovascular system in the embryo. Variation in the genes involved in the folate pathway plays an important part in the etiology of birth defects, including CHD (12). It has been hypothesized that genetic polymorphisms in folate-metabolizing enzymes affect global DNA methylation as well as changes in the availability of nucleotides for the synthesis and repair of DNA. Animal experiments have shown that disruption of the *MTHFR* gene results in decreased methylation capacity (13). Studies of *MTHFR* polymorphisms in humans provide evidence of an association between genotype and global DNA methylation, particularly in the presence of a low-folate diet (14). *MTHFR* C677T also causes reduced enzymatic activity and elevated concentrations of homocysteine. The results from previous meta-analyses investigating *MTHFR* C677T polymorphism and CHD risk suggested that genotypes for the *MTHFR* C677T polymorphism may be associated with CHD risk (6). The importance of the *MTHFR* C677T polymorphism has been confirmed in a large-scale, methodologically independent genome-wide association study (15).

Genetic polymorphisms often vary between ethnic groups. In this study with 207 non-CHD controls, we reported that the allele frequency of *MTHFR* rs1801133 C>T (0.412) was similar to that reported in Chinese (0.439) and Japanese (0.367) populations. However, the mutant homozygote among controls was higher compared to that reported in European (0.237) and Sub-Saharan African (0.110) controls.

Taking into consideration *MTHFR* rs1801133 C>T mutant alleles in the control group, OR, TOF samples and control samples, the power of our analysis (α=0.05) was 0.818 in 173 TOF cases and 207 controls with OR=1.81 for *MTHFR* rs1801133 C>T.

Previously reported data concerning the association between *MTHFR* C677T polymorphism and CHD risk together with the designation of the *MTHFR* C677T risk allele are contradictory and inconclusive. Junker *et al* (16) were the first to identify a significant association between the incidence of CHD and the *MTHFR* C677T polymorphism. Marinho *et al* (17) suggested that the mechanisms underlying the *MTHFR* C677T mutation may be partially responsible for TOF cardiac defects. Their results are in concordance with some of our data; the present study provides additional

Table III. Logistic regression analyses of associations between six genotyped polymorphisms and risk of TOF.

	Cases (n=173)		Controls (n=207)				
Genotype	n	%	n	%	OR (95% CI)	χ^2	P-value
MTHFR rs1801133 C>T							
CC	63	37.50	84	41.18	1.00		
CT	45	26.79	72	35.29	0.83 (0.51-1.37)	0.520	0.471
TT	60	35.71	48	23.53	**1.67 (1.01-2.75)**	**3.997**	**0.046**
CT+TT	105	62.50	120	58.82	1.17 (0.77-1.77)	0.521	0.471
CC+CT	108	64.29	156	76.47	1.00		
TT	60	35.71	48	23.53	**1.81 (1.15-2.84)**	**6.566**	**0.010**
MTHFR rs1801131 A>C							
AA	111	65.29	146	70.87	1.00		
AC	56	32.94	54	26.21	1.36 (0.87-2.14)	1.845	0.174
CC	3	1.76	6	2.91	0.66 (0.16-2.69)	0.340	0.560
AC+CC	59	34.71	60	29.13	1.29 (0.84-2.00)	1.338	0.247
AA+AC	167	98.24	200	97.09	1.00		
CC	3	1.76	6	2.91	0.60 (0.15-2.43)	0.515	0.473
MTHFR rs2274976 G>A							
GG	147	85.96	180	87.38	1.00		
GA	24	14.04	25	12.14	1.18 (0.64-2.14)	0.278	0.598
AA	0	0.00	1	0.49	-	0.0003	0.986
GA+AA	24	14.04	26	12.62	1.13 (0.62-2.05)	0.163	0.686
GG+GA	171	100.00	205	99.51	1.00		
AA	0	0.00	1	0.49	-	0.0003	0.986
MTHFD rs2236225 C>T							
CC	107	62.94	116	56.31	1.00		
CT	60	35.29	80	38.83	0.81 (0.53-1.24)	0.908	0.341
TT	3	1.76	10	4.85	0.33 (0.09-1.21)	2.796	0.095
CT+TT	63	37.06	90	43.69	0.76 (0.50-1.15)	1.694	0.193
CC+CT	167	98.24	196	95.15	1.00		
TT	3	1.76	10	4.85	0.35 (0.10-1.30)	2.452	0.117
MTHFD rs1950902 G>A							
GG	84	49.12	110	53.40	1.00		
GA	71	41.52	79	38.35	1.18 (0.77-1.81)	0.556	0.456
AA	16	9.36	17	8.25	1.23 (0.59-2.58)	0.307	0.580
GA+AA	87	50.88	96	46.60	1.19 (0.79-1.78)	0.683	0.409
GG+GA	155	90.64	189	91.75	1.00		
AA	16	9.36	17	8.25	1.15 (0.56-2.35)	0.143	0.706
MTHFD rs1076991 A>G							
AA	97	57.06	116	56.86	1.00		
AG	63	37.06	79	38.73	0.95 (0.62-1.46)	0.047	0.828
GG	10	5.88	9	4.41	1.33 (0.52-3.40)	0.351	0.553
AG+GG	73	42.94	88	43.14	0.99 (0.66-1.50)	0.0015	0.970
AA+AG	160	94.12	195	95.59	1.00		
GG	10	5.88	9	4.41	1.35 (0.54-3.41)	0.413	0.520

Bold values indicate statistically significant differences (P<0.05). TOF, tetralogy of Fallot; OR, odds ratio; CI, confidence interval; *MTHFR*, *5,10-methylenetetrahydrofolate reductase*; *MTHFD*, *methylenetetrahydrofolate dehydrogenase*.

evidence concerning the importance of the *MTHFR* 677TT genotype for conotruncal heart defects in a Han Chinese population.

Several limitations of the study need to be addressed. First, we did not obtain blood samples from the mothers to evaluate the etiological role of *MTHFR* rs1801133 C>T SNPs in CHDs.

Second, since our study was a hospital-based case-control study and selection bias could not be fully excluded, large population-based studies are required to elucidate the role of *MTHFR* rs1801133 C>T SNPs and susceptibility of TOF. Third, the sample size of this study was not large enough to evaluate the low penetrance effect of the SNPs. Fourth, there was a lack of information on the maternal folate status and maternal genotype. Investigations are required to explore the gene-environment and maternal-fetal genotype interactions. Transient elevations in serum concentrations of folate from the mother due to supplementation or dietary intake may prevent birth defects by overcoming metabolic inefficiencies or transport-related issues. Absence of information on low folate status increases the difficulty of identification of putative genotypes.

In conclusion, our findings suggest that *MTHFR* rs1801133 C>T polymorphism may play a role in susceptibility for TOF. To confirm the current findings, larger studies with more rigorous study designs that include other ethnic populations are necessary.

Acknowledgements

The study was supported by the Outstanding Young Talent Fund of Xinhua Hospital.

References

1. Botto LD, Correa A and Erickson JD: Racial and temporal variations in the prevalence of heart defects. Pediatrics 107: E32, 2001.
2. Lewis DP, Van Dyke DC, Stumbo PJ and Berg MJ: Drug and environmental factors associated with adverse pregnancy outcomes. Part II: Improvement with folic acid. Ann Pharmacother 32: 947-961, 1998.
3. Goyette P, Sumner JS, Milos R, *et al*: Human methylenetetrahydrofolate reductase: isolation of cDNA mapping and mutation identification. Nat Genet 7: 551, 1994.
4. Frosst P, Blom HJ, Milos R, *et al*: A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10: 111-113, 1995.
5. Rozen R: Molecular genetics of methylenetetrahydrofolate reductase deficiency. J Inherit Metab Dis 19: 589-594, 1996.
6. Nie Y, Gu H, Gong J, *et al*: Methylenetetrahydrofolate reductase C677T polymorphism and congenital heart disease: a meta-analysis. Clin Chem Lab Med 49: 2101-2108, 2011.
7. Bufalino A, Ribeiro Paranaiba LM, Nascimento de Aquino S, *et al*: Maternal polymorphisms in folic acid metabolic genes are associated with nonsyndromic cleft lip and/or palate in the Brazilian population. Birth Defects Res A Clin Mol Teratol 88: 980-986, 2010.
8. Etheredge AJ, Finnell RH, Carmichael SL, *et al*: Maternal and infant gene-folate interactions and the risk of neural tube defects. Am J Med Genet A 158A: 2439-2446, 2012.
9. Gong D, Gu H, Zhang Y, *et al*: Methylenetetrahydrofolate reductase C677T and reduced folate carrier 80 G>A polymorphisms are associated with an increased risk of conotruncal heart defects. Clin Chem Lab Med 50: 1455-1461, 2012.
10. Gu H, Gong D, Ding G, *et al*: A variant allele of *ADH1B* and *ALDH2*, is associated with the risk of esophageal cancer. Exp Ther Med 4: 135-140, 2012.
11. Gu H, Ding G, Zhang W, *et al*: Replication study of PLCE1 and C20orf54 polymorphism and risk of esophageal cancer in a Chinese population. Mol Biol Rep 39: 9105-9111, 2012.
12. Bailey LB and Berry RJ: Folic acid supplementation and the occurrence of congenital heart defects, orofacial clefts, multiple births, and miscarriage. Am J Clin Nutr 81: S1213-S1217, 2005.
13. Chen Z, Karaplis AC, Ackerman SL, *et al*: Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet 10: 433-443, 2001.
14. Curtin K, Slattery ML, Ulrich CM, *et al*: Genetic polymorphisms in one-carbon metabolism: associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. Carcinogenesis 28: 1672-1679, 2007.
15. Tanaka T, Scheet P, Giusti B, *et al*: Genome-wide association study of vitamin B6, vitamin B12, folate, and homocysteine blood concentrations. Am J Hum Genet 84: 477-482, 2009.
16. Junker R, Kotthoff S, Vielhaber H, *et al*: Infant methylenetetrahydrofolate reductase 677TT genotype is a risk factor for congenital heart disease. Cardiovasc Res 51: 251-254, 2001.
17. Marinho C, Alho I, Guerra A, *et al*: The methylenetetrahydrofolate reductase gene variant (C677T) as a susceptibility gene for tetralogy of Fallot. Rev Port Cardiol 28: 809-812, 2009.