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Association between 5,10-Methylenetetrahydrofolate Reductase C677T Polymorphisms and conotruncal heart defects in Chinese children

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Abstract: Objective To explore the role of 5, 10-methylenetetrahydrofolate reductase (MTHER) C677T polymorphisms in Chinese children with conotruncal heart defects (CTD). **Methods** A total of 97 children with CTD and 118 healthy controls were recruited into the study. MTHER genetic C677T polymorphisms were determined by PCR-RFLP. The 677TT genotype was compared between the two groups. **Results** The frequency of the TT genotype and T allele in CTD patients was 24.7% and 52.6%, respectively, which was significantly higher than that of controls (13.6% and 42.8%) ($P = 0.036$, $P = 0.043$, respectively). In patients with tetralogy of Fallot, coarctation of aorta or interruption of aortic arch, the frequency of the TT genotype varied between 29.7% and 40.0%. **Conclusions** MTHFR gene is associated with CTD, and 677TT genotype might be a risk factor for congenital heart malformations.

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Key words: Heart defects, congenital; Conotruncus; Methylenetetrahydrofolate reductase; Polymorphism, genetic; Child

中国心脏圆锥动脉干缺损患儿 MTHFR 基因 C677T 突变研究

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[**摘 要**] **目的** 心脏圆锥动脉干缺损(CTD)是一组严重的、复杂的青紫型先天性心脏病。N5,10-亚甲基四氢叶酸还原酶(MTHFR)是参与甲硫氨酸-叶酸代谢的关键酶,其基因 677 位点 C→T 错义突变可造成此酶活性降低,导致高同型半胱氨酸血症,现已证明高同型半胱氨酸是诱发胎儿出生缺陷和心血管疾病的一个独立危险因素。本研究旨在了解中国 CTD 患儿 MTHFR 基因 C677T 位点突变的情况。**方法** 用多聚酶链反应-限制性内切酶片段长度多态性技术(PCR-RFLP)检测 97 例 CTD 患儿和 118 例正常健康人 MTHFR 基因 677 位点 C→T 突变多态性,并比较两组 677TT 的纯合突变率。**结果** CTD 患儿中 TT 基因型频率为 24.7%(24/97),显著高于对照组 13.6%,$\chi^2 = 4.40$, $P = 0.036$,OR 值 2.1(95% CI:1.04~4.22);CTD 患儿组 T 等位基因频率为 52.6%,显著高于对照组 42.8%,$\chi^2 = 4.09$, $P = 0.043$,OR 值 1.5(95% CI:1.01~2.17)。其中法洛氏四联症、主动脉缩窄、主动脉弓离断的 TT 基因型频率为 29.7%~40.0%。**结论** MTHFR 基因与心脏圆锥动脉干缺损有一定关系,其 677TT 基因型可能是引起先天性心脏畸形的危险因素之一。

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[**关 键 词**] 心脏缺损,先天性;圆锥动脉干;四氢叶酸还原酶;多态性,基因;儿童

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Most of congenital heart diseases result from the interaction between genetic predisposition and environmental factors. Conotruncal heart defects (CTD) are a group of severe, complex congenital heart disease with cyanosis and hypoxemia, which include tetralogy of Fallot (TOF), double-outlet right ventricle (DORV), persistent truncus arteriosus (PTA), transposition of the great arteries (TGA) and aortic arch anomalies. Up to now, the cause and pathogenesis of CTD in neonates have not been very clear.

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Use of folic acid to supplement periconceptional maternal diet is known to reduce the risk of occurrence of congenital malformation which contains neural tube defects, urinary tract defects and cardiovascular malformations[1-3]. Folic acid is involved in the homocysteine metabolism, in which the methylenetetrahydrofolate reducatase (MTHFR) is a key enzyme. The MTHFR gene is located on the short (p) arm of chromosome 1 at position 36.3. The major product of the MHTFR gene in humans is a catalytically active 74.6KD protein, catalyzing the reduction of 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. The MTHFR 677C→T polymorphism results in conversion of an alanine to a valine, resulting in a "thermolabile" variant of the enzyme. Individuals who are homozygous for the thermolabile variant of MTHFR (TT) have an increased risk of hyperhomocysteinemia and lower levels of folate in plasma and red blood cells[4,5]. It has been demonstrated that hyperhomocysteinaemia is an independent risk factor for inducing congenital defect and cardiovascular diseases[6,7]. This experiment aimed to study the relation between MTHFR gene C677T mutation and CTD in Chinese children.

Subjects and methods

Subjects

A total of 97 Chinese children with CTD admitted to Xinhua Hospital and the Children's Medical Center of Shanghai Second Medical University (61 males and 36 females aged 1 month -15 years, with a mean age of 3.3 ± 3.8 years) were enrolled in this study. CTD was confirmed by clinical findings, echocardiography, heart catheterization and operation. A total of 118 healthy children from the same geographic area were used as controls.

Methods

White blood cells were isolated from EDTA-treated venous blood using routine measurements of hematology. The genomic DNA was phenol-chloroform extracted, anhydrous alcohol precipitated, dissolved in TE buffer, then quantitated by ultraviolet spectrophotometer. The genotypes were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and were PCR amplified using p1 primer 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and p2 primer 5′-AGG ACG GTG CGG TGA GAG TG-3′[6]. Each reaction contained 1 μL DNA template, 2.5 μL 10 × reaction buffer, 1 μL 10 mM dNTPs, Taq polymerase (3 U/μL), 1 μL p1 and p2 primers (12.5 pmol/μL) respectively and double distilled water to a final volume of 25 μL. PCR was performed as follows: denaturation at 94℃ for 5 minutes followed by 35 cycles of denaturation at 94℃ (30 seconds), annealing at 62℃ (45 seconds) and extension at 72℃ (45 seconds), the final extension was at 72℃ for 10 minutes. The PCR products were 198 bp and were digested with HinfI for 16 hours at 37℃. PCR fragments were electrophoresis on a 10% polyacrylamide gels for 2 hours, then stained with silver.

Statistical analysis

Using SPSS version 10.0 statistical software, genotype frequencies in patients and controls were compared by χ^2 analysis or Fisher′s exact test. An odds ratios (OR) and 95% confidence intervals (CI) were calculated.

Results

MTHFR gene polymorphism

PCR products were analyzed by electrophoresis in a 2% agarose gel. A clear strip of 198bp was observed. After digestion with HinfI restriction enzyme, products were analyzed by electrophoresis in a 10% polyacrylamide gel. Three kinds of genotype could be classified: homozygotic mutation TT genotype with one fragment of 175bp, normal CC genotype with one fragment of 198bp and heterozygotic mutation CT genotype with two fragments of 198bp and 175bp (Figure 1).

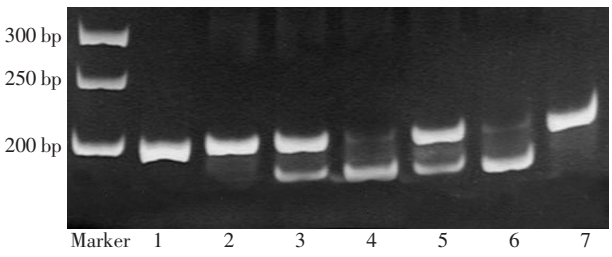

Figure 1 Result of polyacrylamide gel electrophoresis
1 Negative control; 2, 7 CC normal homozygote; 3, 5 CT heterozygote; 4, 6 TT homozygosis mutation

Table 1 MTHFR gene polymorphism test for Hardy-Weinberg equilibrium in the Control group (n = 118)

	Genotype		
	CC	CT	TT
Expected value	39	58	22
Observed value	33	69	16

Hardy-Weinberg equilibrium test

Genotype distribution of MTHFR-677 in the

Control group is shown in Table 1. Statistical analysis confirmed its accordance with Hardy-Weinberg law of genetic equilibrium ($x^2 = 2.40, P > 0.05$), which indicated that cases were sampled from a same population of genetic equilibrium.

Genotype distribution in the CTD group and Control group were also in accordance with the Hardy-Weinberg law of genetic equilibrium ($\chi^2 = 5.20, P > 0.05$), suggesting that cases were sampled from a same population.

Table 2 MTHFR genotype and allele frequencies in the CTD and Control groups

Group	*n*	Genotype			Alleles	
		CC(%)	CT(%)	TT(%)	C(%)	T(%)
Control	118	33(27.9)	69(58.5)	16(13.6)	135(57.2)	101(42.8)
CTD	97	19(19.6)	54(55.7)	24(24.7)	92(47.4)	102(52.6)

Allele frequency of C/T in MTHFR-677

Compared with the Control group, the CTD group had a higher TT homozygotic mutation frequency (24.7% vs 13.6%, $\chi^2 = 4.40, P = 0.036$, OR = 2.1, 95% CI 1.04-4.22) and a higher T allele frequency (52.6% vs 42.8%, $\chi^2 = 4.09, P = 0.043$, OR = 1.5, 95% CI 1.01-2.17) (Table 2).

Comparison of TT genotype frequency on CTD categories

CTD was classified into subgroups in order to analyze the TT genotype frequency in different CTD. It was noticed that in most subgroups of CTD, the MTHFR-677 TT genotype frequency was higher than that of the Control group. The MTHFR-677 TT genotype frequencies were relatively higher in patients with interruption of aortic arch, coarctation of aorta and tetralogy of Fallot (40.0%, 37.5% and 29.7% respectively) as well as the OR value of relative risk (4.3, 3.8 and 2.7 respectively). A significant difference was observed between the cases of tetralogy of Fallot and the Control group ($P = 0.024$). See Table 3.

Table 3 Comparison of the MTHFR-677 TT genotype frequency in the control group and the subgroups of CTD

Group	*n*	677TT(%)	OR(CI)	*P*
Control	118	16(13.6)	-	-
CTD	97	24(24.7)	2.1(1.04-4.22)	0.036
Tetralogy of Fallot	37	11(29.7)	2.7(1.12-6.50)	0.024
Double-outlet right ventricle	17	4(23.5)	2.0(0.57-6.77)	>0.05
Transposition of the great arteries	15	3(20.0)	1.6(0.41-6.28)	>0.05
Coarctation of aorta	8	3(37.5)	3.8(0.83-17.58)	>0.05
Interruption of aortic arch	5	2(40.0)	4.3(0.66-27.44)	>0.05
Ventricular septal defect (sub-PA)	7	1(14.3)	1.1(0.12-9.41)	>0.05
Persistent truncus arteriosus	2	0	-	-
Pulmonary artery atresia/ intact ventricular septum	3	0	-	-
Malposition of great artery	1	0	-	-
Other	2	0	-	-

Discussion

Since the first report of the association between congenital neural tube defects and MTHFR gene C677T homozygotic mutation by van der Put[8] in 1995, successive studies have indicated that the MTHFR 677TT genotype is a risk factor for coronary heart disease, hypertension, stroke and diabetes mellitus. However, it is controversial whether the MTHFR C677T mutation is related to the development of congenital heart diseases.

Wenstrom and colleagues[9] reported that the amniotic fluid homocysteic acid concentration in the mother of a fetus with congenital heart disease was higher than that of normal controls and the MTHFR C677T mutation frequency in amniotic fluid cells was also significantly increased (35% vs 13%). Junker[10] reported that in 114 children with congenital heart diseases, MTHFR gene 677TT homozygotic mutation was detected in 18.4% of the cases; while in 228 normal children it was only 9.2% ($P < 0.05$). They found that the 677TT genotype mutation frequency was as high as 38%-67% in cases with pulmonary artery stenosis, hypoplastic left heart syndrome and coarctation of aorta. A study performed by YAN and colleagues[11] suggested that the MTHFR 677 polymorphism was related to the development of patent ductus arteriosus, atrial septal defect in the Chinese population. However, there are contradictory opinions. McBride[12] reported that the MTHFR C677T mutation was not related to congenital left ventricular outflow obstructive diseases such as aortic valve stenosis, co-

arctation of aorta and hypoplastic left heart syndrome. Storti[13] reported that the parental MTHFR gene polymorphism was unrelated to CTD in children in the Italian population. They also found that the 677 T allele frequency in the Italian population was higher than that in other European countries. It was reported that the MTHFR gene 677 C-T mutation frequency varied between different races, countries, and regions[14].

Embryonic development of the conotruncal heart, which originated from a locus connect to the neural tube develop site, is related to neural crest cells[15]. Currently, the MTHFR gene 677TT genotype is considered as one of the pathogenesis factors of congenital neural tube defects, therefore it is worthy to study whether this mutation is related to the development of the conotruncal heart. The current study was performed to elucidate the MTHFR gene C677T mutation distribution in Chinese CTD patients. The results showed that the allele frequency distribution of normal controls is in accordance to the Hardy-Weinberg law of genetic equilibrium, suggesting that the population studied possesses a genetic equilibrium background and is representative. The comparison analysis indicated that the MTHFR 677TT homozygotic mutation frequency as well as T allele frequency in CTD patients were significantly higher than that of controls. These results suggested that the MTHFR 677TT genotype was more frequent in CTD patients and there might be a potential relationship between the genotype and CTD. A MTHFR 677TT homozygotic mutation may cause hyperhomocysteinaemia, which may be related to the development of CTD. The subgrouping analysis indicated that the MTHFR 677TT genotype frequency in most of the CTD patients was apparently higher than that of controls, especially in interruption of aortic arch, coarctation of aorta and tetralogy of Fallot subgroups. As relatively higher OR values were also observed in these subgroups, which was similar to the findings of Junker[10]. It was observed in this study that the 677TT genotype frequency in patients with tetralogy of Fallot was significantly different from that of the Control group, while not in other CTD subgroups, which may be attributed to the limited sample number for other groups. Further studies are needed to address the question whether the CTD patients with 677TT genotype are complicated by hyperhomocysteinaemia.

Results of this study indicated that the MTHFR gene is related to the development of CTD, and that the 677TT genotype may be one of the risk factors of congenital heart malformation. Screening of fetuses in high-risk group, early acidum folicum supplement and interference of multiple vitamins may be helpful to prevent or reduce the development of CTD.

[References]

[1] Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation [J]. N Engl J Med, 1992, 327(26): 1832-1835.

[2] Li DK, Daling JR, Mueller BA, Hickok DE, Fantel AG, Weiss NS. Periconceptional multivitamin use in relation to the risk of congenital urinary tract anomalies [J]. Epidemiology, 1995, 6(3): 212-218.

[3] Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring [J]. Am J Med Gent, 1995, 59(4): 536-545.

[4] Fodinger M, Horl WH, Sunder-Plassmann G. Molecular biology of 5,10-methylenetetrahydrofolate reductase [J]. J Nephrol, 2000, 13(1): 20-33.

[5] Rosenblatt DS. Methylenetetrahydrofolate reductase [J]. Clin Invest Med, 2001, 24(1): 56-59.

[6] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase [J]. Nat Genet, 1995, 10(1): 111-113.

[7] Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy [J]. Am J Clin Nutr, 2000, 71(5 Suppl): 1295S-1303S.

[8] van der Put NM, Steegers-Theunissen RP, Frosst P, Trijbels FJ, Eskes TK, van den Heuvel LP, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. [J]. Lancet, 1995, 346(8982): 1070-1071.

[9] Wenstrom KD, Johanning GL, Johnston KE, DuBard M. Association of the C677T methylenetetrahydrofolate reductase mutation and elevated homocysteine levels with congenital cardiac malformations [J]. Am J Obstet Gynecol, 2001, 184(5): 806-812.

[10] Junker R, Kotthoff S, Vielhaber H, Halimeh S, Kosch A, Koch HG, et al. Infant methylenetetrahydrofolate reductase 677TT genotype is a risk factor for congenital heart disease [J]. Cardiovasc Res, 2001, 51(2): 251-254.

[11] YAN L-Y, LI S-Q, ZHAO H-J, ZHAO R-B, DAO J-J, ZHU W-L, et al. Effect of 5,10-methylenetetrahydrofolate reductase genotypes in parents on the risk of congenital heart disease in offspring (in Chinese) [J]. Chin J Dis Control P Rev, 2003, 7(2): 94-97.

[12] McBride KL, Fernbach S, Menesses A, Molinari L, Quay E, Pignatelli R, et al. A family-based association study of congenital left-sided heart malformations and 5,10 methylenetetrahydrofolate reductase [J]. Birth Defects Res A Clin Mol Teratol, 2004, 70(10): 825-830.

[13] Storti S, Vittorini S, Lascone MR, Sacchelli M, Collavoli A, Ripoli A, et al. Association between 5,10-methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and conotruncal heart defects[J]. Clin Chem Lab Med, 2003, 41(3): 276-280.

[14] Schneider JA, Rees DC, Liu YT, Clegg JB. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation [J]. Am J Hum Genet, 1998, 62(8): 1258-1260.

[15] Kirby ML, Waldo KL. Neural crest and cardiovascular patterning [J]. Circ Res, 1995, 77(2): 211-215.

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