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Original Article

MTHFR c.1793G>A polymorphism is associated with congenital cardiac disease in a Chinese population

Jing Xu,[1] Xiaohan Xu,[1] Lei Xue,[1] Xiang Liu,[1] Haiyong Gu,[3] Hailong Cao,[1] Wanshan Qiu,[4] Zhibin Hu,[2] Hongbing Shen,[2] Yijiang Chen[1]

[1]*Department of Thoracic and Cardiovascular Surgery, The First Affiliated Hospital of Nanjing Medical University;* [2]*Department of Epidemiology and Biostatistics, Nanjing Medical University;* [3]*Department of Thoracic and Cardiovascular Surgery, Zhenjiang First People's Hospital;* [4]*Department of Thoracic and Cardiovascular Surgery, The Second Affiliated Hospital of Nanjing Medical University, Peoples Republic of China*

Abstract *Objectives:* To investigate whether genetic variants in methylenetetrahydrofolate reductase (*MTHFR*) and methylenetetrahydrofolate dehydrogenase (*MTHFD*) genes are associated with risk of congenital cardiac disease. *Background:* Accumulative evidence suggests that hyperhomocysteinaemia is associated with risk of congenital cardiac disease. Inherited polymorphisms in key folate metabolic pathway genes, *MTHFR* and *MTHFD*, may influence the efficiency of folate metabolism and plasma level of homocysteine. *Methods:* A two-stage case–control study of congenital cardiac disease was conducted by genotyping *MTHFR* c.1793G>A and four other variants – *MTHFR* c.677C>T, c.1298A>C, and *MTHFD* c.1958G>A, c.401C>T – in a Chinese population consisting of 1033 congenital cardiac disease patients and 1067 non-congenital cardiac disease patients. *Results:* The variant genotypes of *MTHFR* c.1793GA/AA were associated with a significantly decreased risk of congenital cardiac disease in two stages combined, with an adjusted odds ratio of 0.67 and a 95% confidence interval of 0.54–0.84 ($p = 0.0004$). In comparison with wild-type homozygote c.1793GG, the effect was significant in isolated perimembranous ventricular septal defect patients with an adjusted odds ratio of 0.60 and a 95% confidence interval of 0.43–0.83 ($p = 0.0003$). *Conclusion:* These findings indicate that *MTHFR* c.1793G>A may have a role in susceptibility to sporadic congenital cardiac disease.

Keywords: Genetics; homocysteine; malformation; variant

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Congenital cardiac disease is the malformation of the heart or the large blood vessels. The reported prevalence of congenital cardiac disease varies between 4 and 10 per 1000 live births.[1] The formation of the human heart takes place during weeks 3–9 of foetal development. The process is an intricate one that can be influenced by both genetic and environmental factors. A periconceptional supplement of multiple vitamins, especially folic acid, could significantly reduce the incidence of congenital cardiac disease.[2,3] The mechanism by which folic acid exerts its protective effect is largely unclear, and therefore, the teratogenic process that results from folate insufficiency is related to hyperhomocysteinaemia, an independent risk factor for congenital cardiac disease.[4]

Homocysteine is a type of thioalcohol amino acid, which is a comitant metabolic product of methionine demethylation and transsulphuration. An abnormality of homocysteine metabolism is shown to induce cardiac defects in developing chick embryos and folate-deficient mice models.[5,6] *MTHFR* and *MTHFD* are two important enzymes

Correspondence to: Dr Y. Chen, Department of Thoracic and Cardiovascular Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Peoples Republic of China, 210029. Tel: +86 25 85038012; Fax: +86 25 83719809; E-mail: YJChen@njmu.edu.cn

involved in the homocysteine metabolism. *MTHFR* have an important role in catalysing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which acts as a methyl group donor-induced homocysteine remethylation to methionine.[7] *MTHFD* is a trifunctional enzyme that catalyses the interconversion of three forms of one-carbon substituted tetrahydrofolate, the active form of folic acid. The one-carbon folates generated by *MTHFD* are used for the synthesis of purines, thymidylate, and serine, and to support the methylation cycle through the regeneration of methionine from homocysteine.[8,9] The *MTHFD* gene defects might lead to a decrease in enzyme activity that influences the methylation cycle through the regeneration of methionine from homocysteine and affects the supply of 10-formyltetrahydrofolate required for purine synthesis. These further affect the rate of DNA synthesis and the rate of cell doubling, which are likely to have a major impact on pregnancy and embryonic development.[10,11]

The *MTHFR* gene has at least two functional single-nucleotide polymorphisms, c.677C>T and c.1298A>C. The *MTHFR* c.677C>T allele is associated with reduced enzyme activity, decreased concentrations of folate in serum, plasma, and red blood cells, and increased plasma total homocysteine concentrations.[12] Meanwhile, *MTHFR* c.1298A>C leads to lower enzyme activity and acts as a risk factor for hyperhomocysteinaemia.[13] Rady et al[14] identified another non-synonymous single-nucleotide polymorphism of the *MTHFR* gene, c.1793G>A, but its function was unknown. Earlier studies showed inconsistent results between *MTHFR* c.677C>T and c.1298A>C polymorphisms, and congenital cardiac disease.[15–20] However, no data were available for *MTHFR* c.1793G>A genotype and risk of congenital cardiac disease. Researchers had identified several potentially functional single-nucleotide polymorphisms of *MTHFD*, such as c.1958G>A[8] and c.401C>T[21], and c.1958G>A reported the influence of folate and homocysteine levels as well as congenital cardiac disease risk.[22]

This study aimed to investigate the role of the five single-nucleotide polymorphisms of *MTHFR* and *MTHFD* in susceptibility to congenital cardiac disease in a Chinese population.

Materials and methods

Study population

The institutional review board of Nanjing Medical University, Nanjing, China approved the study. The First Affiliated Hospital of Nanjing Medical University and Nanjing Children's Hospital, Nanjing, China consecutively recruited congenital cardiac disease cases between March, 2006 and July, 2008. Surgical operations confirmed all cases that had non-syndromic congenital cardiac disease diagnosed by ultrasound. Cases with structural malformations involving another organ system or known chromosomal abnormalities were excluded. Exclusion criteria also included a positive family history of congenital cardiac disease in a first-degree relative that consists of parents, siblings, and children, maternal diabetes mellitus, phenyl ketonuria, maternal teratogen exposures, for example, pesticides and organic solvents, and maternal therapeutic drugs, including folate antagonist, and exposures during the intrauterine period. Information about regular multivitamin supplements, including regular folic acid intake during a period from 3 months before pregnancy to the first 3 months of pregnancy, rubella, influenza, and any febrile illnesses during pregnancy was also obtained. Control patients were non-congenital cardiac disease and age- and gender-matched outpatients in the same geographic area during the same time period as congenital cardiac disease patients. Most of these patients had a diagnosis of trauma or infection. The control group excluded any patients with known congenital anomalies. Both congenital cardiac disease and control patients were genetically unrelated ethnic Han Chinese. Using a structured questionnaire, trained interviewers personally interviewed patients and/or their parents after directly obtaining informed consent. After the interview, approximately 2 millilitres of venous blood were collected from each patient. This study used a cross-validated two-stage design to increase the efficiency of the comparison.

In all, 502 congenital cardiac disease cases and 527 controls were included during a period between March, 2006 and July, 2007 and grouped as stage I. The remaining 531 congenital cardiac disease cases and 540 controls were recruited between August, 2007 and July, 2008 and grouped as stage II samples.

Laboratory assays

Genomic DNA was isolated from leucocytes of venous blood by proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. The genotyping assays for the five single-nucleotide polymorphisms of *MTHFR* – c.677C>T, c.1298A>C and c.1793G>A – and *MTHFD* – c.1958G>A and c.401C>T – were previously described.[23,24] Briefly, the polymerase chain reaction primer pairs were c.677C>T F: 5′-TGAAGGAGA AGGTGTCTGCGGGA-3′, R: 5′-AGGACGGTGC GGTGAGAGTG-3′; c.1298A>C F: 5′-CTTTGGG GAGCTGAAGGACTACTAC-3′, R: 5′-CACTTTG

TGACCATTCCGGTTTG-3′; c.1793G>A F: 5′- CT CTGTGTGTGTGTGCATGTGTGCG-3′, R: 5′-GG GACAGGAGTGGCTCCAACGCAGG-3′; c.1958 G>A F: 5′-CATTCCAATGTCTGCTCCAA-3′, R: 5′-GTTTCCACAGGGCACTCC-3′; c.401C>T F: 5′-GGCGTACAAGGAATGAAAC-3′, R: 5′-GGAT GTGGATGGGTAAGTG-3′. The 15 microlitres of polymerase chain reaction mixture contained approximately 20 nanograms of genomic DNA, 12.5 picomoles of each primer, 0.1 millimolar of each dNTP, 1 × polymerase chain reaction buffer – 50 millimolars of KCl, 10 millimolars of Tris HCl, and 0.1% Triton X-100 – 1.5 millimolars of $MgCl_2$, and 1.0 unit of Taq polymerase. The polymerase chain reaction profile consisted of an initial melting step of 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 67°C for c.677C>T or 62°C for c.1298A>C or 69°C for c.1793G>A or 66°C for c.1958G>A or 55°C for c.401C>T for 40 seconds and 72°C for 45 seconds, and a final extension step of 72°C for 10 minutes. We used the restriction enzymes *Hinf*I, *Mbo*II, *Bsrb*I, *Hpa*II, and *Bsma*I (New England BioLabs, Beverly, Massachusetts, United States of America) to distinguish the c.677C>T, c.1298A>C, c.1793G>A, c.1958G>A, and c.401C>T genotypes, respectively. We also performed genotyping blindly and assayed approximately equal numbers of congenital cardiac disease cases and controls in each 96-well polymerase chain reaction plate with a positive control of a DNA sample with known heterozygous genotype. In the event of consensus on the tested genotype not being reached, two research assistants independently performed the repeated assays to achieve 100% concordance.

Statistical analyses

We evaluated differences in the distributions of selected variables and frequencies of the genotypes of *MTHFR* and *MTHFD* polymorphisms between cases and controls using the chi-square or Student's *t*-test. We also estimated the associations between the genotypes and the risk of congenital cardiac disease by adjusted odds ratio and their 95% confidence interval from logistic regression analyses, with adjustment for sex. A goodness-of-fit chi-square test to compare the observed genotype frequencies tested the Hardy–Weinberg equilibrium with the expected ones among control subjects. All of the statistical analyses were performed with Statistical Analysis System software (v.9.1.3e; SAS Institute, Cary, North Carolina, United States of America).

Results

Of the 1033 congenital cardiac disease patients, 265 (25.7%) were with cyanotic cardiac disease, 5 (0.5%) were with left-sided obstruction defects, 672 (65.0%) were with septation defects, 48 (4.6%) were with patent ductus arteriosus; Table 1 shows other classifications.

The *MTHFR* – c.677C>T, c.1298A>C, and c.1793G>A – and *MTHFD* – c.1958G>A and c.401C>T – genotype distributions in congenital cardiac disease; Table 2 shows the control patients. The observed genotype frequencies for these five polymorphisms in the controls were all in Hardy –Weinberg equilibrium. In stage I, we found the *MTHFR* c.1793GA/AA genotypes to be associated with a reduced risk of congenital cardiac disease, with

Table 1. Distributions of selected variables in congenital cardiac disease cases and controls.

	Cases		Controls		
Variable	***N***	**%**	***N***	**%**	**p-value**
Stages I and II	1033		1067		
Age (mean years)	6.50		6.69		0.21
Sex					0.75
Male	555	53.7	566	53.0	
Female	478	46.3	501	47.0	
Congenital cardiac disease classification I					
Cyanotic cardiac disease	265	25.7			
Left-sided obstruction defects	5	0.5			
Septation defects	672	65.0			
Patent ductus arteriosus	48	4.6			
Other complex abnormalities	43	4.2			
Congenital cardiac disease classification II					
Septa and valve abnormalities only	728	70.5			
Other Congenital cardiac disease abnormalities	305	29.5			
Congenital cardiac disease classification III					
Isolated congenital cardiac disease	712	69.8			
Non-isolated congenital cardiac disease	321	30.2			

an adjusted odds ratio of 0.71 and a 95% confidence interval of 0.16–0.96 (p = 0.03), compared with the c.1793GG wild-type homozygote. There were no associations between the other four variants and congenital cardiac disease risk. In stage II, a similar association was found between congenital cardiac disease risk and *MTHFR* c.1793GA/AA, compared with the c.1793GG genotype, with an adjusted odds ratio of 0.64 and a 95% confidence interval of 0.47–0.87 (p = 0.003). By considering both stages, the association between congenital cardiac disease risk and variant genotypes of *MTHFR* c.1793GA/AA, we found a 33% decreased risk of congenital cardiac disease, with an adjusted odds ratio of 0.67 and a 95% confidence interval of 0.54–0.84 for c.1793AA (p = 0.0004; Table 2).

By dividing congenital cardiac disease patients into subgroups according to the five variants in stage I,

Table 2. Logistic regression analyses of associations between *MTHFR* c.677C>T, c.1298A>C, and c.1793G>A, and *MTHFD* c.1958G>A and c.401C>T polymorphisms and risk of congenital cardiac disease.

	Cases (1033)		Controls (1067)		
Genotypes	***N***	**%**	***N***	**%**	**Odds ratio (95% confidence interval)***
Stage I	*N* = 502		*N* = 527		
MTHFR c.677C>T					
CC	162	32.3	151	28.7	1.00 (reference)
CT	244	48.6	261	49.5	0.87 (0.66–1.16)
TT	96	19.1	115	21.8	0.78 (0.55–1.11)
CT + TT	340	67.7	376	71.3	0.84 (0.65–1.10)
T allele	436	43.4	491	46.6	
MTHFR c.1298A>C					
AA	316	62.9	326	61.9	1.00 (reference)
AC	168	33.5	185	35.1	0.94 (0.72–1.22)
CC	18	3.6	16	3.0	1.17 (0.59–2.33)
AC + CC	186	37.1	201	38.1	0.96 (0.74–1.23)
C allele	204	20.3	217	20.6	
MTHFR c.1793G>A					
GG	417	83.1	409	77.6	1.00 (reference)
GA	83	16.5	110	20.9	0.74 (0.54–1.02)
AA	2	0.4	8	1.5	0.25 (0.05–1.17)
GA + AA	85	16.9	118	22.4	0.71 (0.16–0.96)
A allele	87	8.7	126	12.0	
MTHFD c.401C>T					
CC	235	46.8	250	47.5	1.00 (reference)
CT	230	45.8	231	43.8	1.24 (0.78–1.99)
TT	37	7.4	46	8.7	1.17 (0.74–1.88)
CT + TT	267	53.2	277	52.5	1.21 (0.77–1.90)
T allele	304	30.3	323	30.6	
MTHFD c.1958G>A					
GG	291	58.0	289	54.8	1.00 (reference)
GA	195	38.8	210	39.8	0.92 (0.71–1.18)
AA	16	3.2	28	5.4	0.56 (0.30–1.10)
GA + AA	211	42.0	238	45.2	0.88 (0.69–1.13)
A allele	227	22.6	266	25.2	
Stage II	531		540		
MTHFR c.1793G>A					
GG	442	83.2	412	76.3	1.00 (reference)
GA	86	16.2	115	21.3	0.69 (0.51–0.94)
AA	3	0.6	13	2.4	0.21 (0.06–0.76)
GA + AA	89	16.8	128	23.7	0.64 (0.47–0.87)
A allele	92	8.7	141	13.1	
Stages I and II					
MTHFR c.1793G>A					
GG	859	83.2	821	76.9	1.00 (reference)
GA	169	16.4	225	21.1	0.72 (0.57–0.89)
AA	5	0.4	21	2.0	0.23 (0.09–0.60)
GA + AA	174	16.8	246	23.1	0.67 (0.54–0.84)
A allele	179	8.7	267	12.9	

*Adjusted by age and sex

Table 3. Logistic regression analyses of associations between *MTHFR* c.677C>T, c.1298A>C, and c.1793G>A and *MTHFD* c.1958G>A and c.401C>T polymorphisms and risk of isolated ventricle septal defect.

Genotypes	Cases (481)		Controls (1067)		Odds ratio (95% confidence interval)*
	N	%	*N*	%	
Stage I	257		527		
MTHFR c.677C>T					
CC	83	32.3	151	28.7	1.00 (reference)
CT	130	50.6	261	49.5	0.91 (0.64–1.27)
TT	44	17.1	115	21.8	0.68 (0.44–1.06)
CT + TT	174	67.7	376	71.3	0.84 (0.61–1.16)
T allele	218	42.4	491	46.6	
MTHFR c.1298A>C					
AA	169	65.8	326	61.9	1.00 (reference)
AC	86	33.5	185	35.1	0.90 (0.65–1.23)
CC	2	0.7	16	3.0	0.24 (0.06–1.06)
AC + CC	88	34.2	201	38.1	0.85 (0.62–1.16)
C allele	90	17.5	217	20.6	
MTHFR c.1793G>A					
GG	222	86.4	409	77.6	1.00 (reference)
GA	34	13.2	110	20.9	0.56 (0.37–0.85)
AA	1	0.4	8	1.5	0.23 (0.03–1.89)
GA + AA	35	13.6	118	22.4	0.54 (0.35–0.81)
A allele	36	7.0	126	12.0	
MTHFD c.401C>T					
CC	128	49.8	250	47.5	1.00 (reference)
CT	107	41.6	231	43.8	0.90 (0.66–1.23)
TT	22	8.6	46	8.7	0.94 (0.54–1.63)
CT + TT	129	50.2	277	52.5	0.91 (0.67–1.22)
T allele	151	29.4	323	30.6	
MTHFD c.1958G>A					
GG	145	56.4	289	54.8	1.00 (reference)
GA	103	40.1	210	39.8	0.98 (0.72–1.33)
AA	9	3.5	28	5.4	0.64 (0.30–1.40)
GA + AA	112	43.6	238	45.2	0.94 (0.67–1.27)
A allele	121	23.5	266	25.2	
Stage II	224		540		
MTHFR c.1793G>A					
GG	187	83.5	412	76.3	1.00 (reference)
GA	34	15.2	115	21.3	0.62 (0.40–0.94)
AA	3	1.3	13	2.4	0.50 (0.14–1.78)
GA + AA	37	16.5	128	23.7	0.60 (0.40–0.91)
A allele	40	8.9	141	13.1	
Stages I and II					
MTHFR c.1793G>A					
GG	409	85.0	821	76.9	1.00 (reference)
GA	68	14.1	225	21.1	0.60 (0.45–0.81)
AA	4	0.9	21	2.0	0.37 (0.13–1.10)
GA + AA	72	15.0	246	23.1	0.58 (0.44–0.78)
A allele	76	7.9	267	12.9	

*Adjusted by age and sex

logistic regression analysis revealed that *MTHFR* c.1793GA/AA had a significantly protective effect in the isolated ventricle septal defect, with an adjusted odds ratio of 0.54 and a 95% confidence interval of 0.35–0.81, compared with the c.1793GG genotype, and c.1298CC variant homozygote was associated with a 3.96-fold increased congenital cardiac disease risk, compared with the c.1298AA wild-type homozygote in the isolated ostium secundum atrial septal defect, with an adjusted odds ratio of 3.96 and a 95% confidence interval of 1.20–13.05.

To further confirm the associations, we performed a replication study in stage II patients, including 224 isolated ventricle septal defect patients and 540 controls for *MTHFR* c.1793G>A, and 81 isolated ostium secundum atrial septal defect patients and 540 controls for *MTHFR* c.1298A>C. We found a similar association in *MTHFR* c.1793G>A, but

Table 4. Logistic regression analyses of associations between *MTHFR* c.677C>T, c.1298A>C, and c.1793G>A and *MTHFD* c.1958G>A and c.401C>T polymorphisms and risk of isolated ostium secundum atrial septal defect.

Genotypes	Cases (122)		Controls (1067)		Odds ratio (95% confidence interval)*
	N	%	*N*	%	
Stage I	41		527		
MTHFR c.677C>T					
CC	12	29.3	151	28.7	1.00 (reference)
CT	17	41.4	261	49.5	0.80 (0.37–1.72)
TT	12	29.3	115	21.8	1.26 (0.54–2.92)
CT + TT	29	70.7	376	71.3	0.94 (0.47–1.90)
T allele	41	50.0	491	46.6	
MTHFR c.1298A>C					
AA	21	51.2	326	61.9	1.00 (reference)
AC	16	39.0	185	35.1	1.38 (0.70–2.71)
CC	4	9.8	16	3.0	3.96 (1.20–13.05)
AC + CC	20	48.8	201	38.1	1.58 (0.83–3.00)
C allele	24	29.3	217	20.6	
MTHFR c.1793G>A					
GG	28	68.3	409	77.6	1.00 (reference)
GA	13	31.7	110	20.9	1.73 (0.86–3.46)
AA	0	0.0	8	1.5	–
GA + AA	13	31.7	118	22.4	1.61 (0.80–3.22)
A allele	13	15.9	126	12.0	
MTHFD c.401C>T					
CC	17	41.4	250	47.5	1.00 (reference)
CT	20	48.8	231	43.8	1.39 (0.68–2.63)
TT	4	9.8	46	8.7	1.36 (0.43–4.25)
CT + TT	24	58.6	277	52.5	1.38 (0.70–2.57)
T allele	28	34.1	323	30.6	
MTHFD c.1958G>A					
GG	21	51.2	289	54.8	1.00 (reference)
GA	17	41.5	210	39.8	1.16 (0.59–2.27)
AA	3	7.3	28	5.4	1.49 (0.42–5.33)
GA + AA	20	48.8	238	45.2	1.37 (0.45–3.31)
A allele	23	28.0	266	25.2	
Stage II	81		540		
MTHFR c.1298A>C					
AA	47	58.0	327	60.6	1.00 (reference)
AC	31	38.3	190	35.2	1.12 (0.68–1.85)
CC	3	3.7	23	4.2	0.75 (0.21–2.64)
AC + CC	34	42.0	213	39.4	1.08 (0.66–1.75)
C allele	37	22.8	236	21.9	
Stages I and II					
MTHFR c.1298A>C					
AA	68	55.7	653	61.2	1.00 (reference)
AC	47	38.5	375	35.1	1.22 (0.82–1.82)
CC	7	5.8	39	3.7	1.58 (0.67–3.76)
AC + CC	54	44.3	414	38.8	1.26 (0.86–1.85)
C allele	61	25.0	453	21.2	

*Adjusted by age and sex

not *MTHFR* c.1298A>C. By considering the two stages, the *MTHFR* c.1793GA/AA genotypes had a 42% reduced risk of the isolated ventricle septal defect, with an adjusted odds ratio of 0.58 and a 95% confidence interval of 0.44–0.78, whereas the *MTHFR* c.1298CC genotype had no significant effect on the isolated ostium secundum atrial septal defect, with an adjusted odds ratio of 1.58 and a 95% confidence interval of 0.67–3.76 (Tables 3 and 4). Furthermore, the stratified analysis of the isolated ventricle septal defect revealed a significant protective effect of *MTHFR* c.1793GA/AA on the isolated perimembranous ventricular septal defect, with an adjusted odds ratio of 0.60 and a 95% confidence interval of 0.43–0.83, compared with the c.1793GG genotype (Table 5).

Table 5. Logistic regression analyses of associations between *MTHFR* c.1793G>A polymorphism and risk of the subgroup of isolated ventricle septal defect.

Genotypes	Cases (481)		Controls (1067)		Odds ratio (95% confidence interval)*
	N	%	*N*	%	
MTHFR c.1793G>A isolated perimenbranous ventricle septal defect					
	384		1067		
GG	329	85.7	821	76.9	1.00 (reference)
GA	54	14.1	225	21.1	0.62 (0.45–0.86)
AA	1	0.3	21	2.0	0.12 (0.02–0.87)
GA + AA	55	14.4	246	23.1	0.60 (0.43–0.83)
A allele	56	7.3	267	12.9	
MTHFR c.1793G>A isolated subarterial ventricle septal defect					
	88		1067		
GG	73	83.0	821	76.9	1.00 (reference)
GA	12	13.6	225	21.1	0.59 (0.32–1.11)
AA	3	3.4	21	2.0	1.54 (0.45–5.30)
GA + AA	15	17.0	246	23.1	0.67 (0.38–1.20)
A allele	18	10.2	267	12.9	
MTHFR c.1793G>A isolated muscular ventricle septal defect					
	9		1067		
GG	7	77.8	821	76.9	1.00 (reference)
GA	2	22.2	225	21.1	1.05 (0.22–5.07)
AA	0	0.0	21	2.0	–
GA + AA	2	22.2	246	23.1	0.96 (0.20–4.63)
A allele	2	11.1	267	12.9	

*Adjusted by age and sex

Discussion

Goh et al[2] have reported an association between the dietary intake of folic acid and the risk of congenital cardiac disease. A striking finding was that regular periconceptional folic acid use, from 3 months before pregnancy through the first 3 months of pregnancy, could prevent approximately 25% of major cardiac defects.[3] It is known that folate provides methyl groups required for intracellular methylation reactions and *de novo* deoxynucleotide synthesis. *MTHFR* and *MTHFD* are two central regulatory enzymes in the folate metabolism and it is likely that not only folate deficiency, but also functional polymorphisms in genes associated with the folate-mediated homocysteine pathway, may contribute to congenital cardiac disease risk.[25–28]

Earlier studies have discrepancy conclusions in relation to *MTHFR* c.677C>T, c.1298A>C polymorphisms, and risk of congenital cardiac disease.[15] A meta-analysis of 13 retrospective studies showed that only five independent studies reported an association between *MTHFR* c.677C>T polymorphism and different congenital cardiac disease types and others did not find the relationship. This meta-analysis found no substantial evidence for any association between congenital cardiac disease risk and the *MTHFR* c.677C>T polymorphism.[15] Our results are consistent with the analysis. Thus far, five studies reported on the association between the *MTHFR* c.1298A>C polymorphism and congenital cardiac disease. Hobbs et al[16] reported a protective effect of the *MTHFR* c.1298 C allele on congenital cardiac disease risk. Additional independent studies showed no significant association between the *MTHFR* c.1298 A>C polymorphism and the risk of congenital cardiac disease in the other four studies.[17–20]

In this study, we investigated the associations between *MTHFR* c.677C>T, c.1298A>C, c.1793 G>A and *MTHFD* c.1958G>A, c.401C>T polymorphisms, and congenital cardiac disease risk in Chinese population. The variant genotypes of *MTHFR* c.1793G>A, but not *MTHFR* c.1298A >C, were found to be associated with a decreased risk of congenital cardiac disease, significantly in patients with isolated perimembranous ventricle septal defect.

Differences in risk estimate for *MTHFR* polymorphisms in association with congenital cardiac disease might be caused by multiple factors, including aetiologic heterogeneity between populations, geographical variations of the studied populations, different selection of controls, study design, type of cardiac defects, and lack of information on potential effect modifiers. In this study, we conducted a two-stage design to validate the findings and reveal the substantial association between *MTHFR* c.1793G>A polymorphism and

congenital cardiac disease. It is likely that *MTHFR* c.1793G>A is associated with increased enzyme activity, notwithstanding that it needs to be clarified in further studies. It is also possible that *MTHFR* c.1793G>A is not functional and might be linked with another casual variant that plays an important role in the aetiology of congenital cardiac disease.

Several limitations in this study need to be addressed. First, this study was a hospital-based case–control study and the hospitalised patients may not be representative of the general population. For example, congenital cardiac disease patients were collected from two large hospitals in Jiangsu province whose patients were prone to heavy cases, which may result in admission rate bias. Second, we failed to collect blood samples from patients' parents, and therefore it was difficult to analyse whether congenital cardiac disease was associated with parents' genetic background and folate levels during the pregnancy. Third, the functional relevance of *MTHFR* c.1793G>A is unclear, making it difficult to determine whether the polymorphism is casual loci or proxy. Finally, the sample size in this study was moderate, especially in stratifications, and therefore further studies with larger sample size are warranted to confirm the findings.

In conclusion, cardiac development is a complicated process, involving the expression of many genes at different times, spaces, and orders. The results of this study, *MTHFR* c.1793G>A, may be useful biomarkers for congenital cardiac disease, and could help to identify the risk populations for congenital cardiac disease.

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