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Association of maternal folic acid supplementation and offspring *MTRR* gene polymorphism with congenital heart disease: a hospital-based case-control study in Han population

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Abstract

Background Although many studies shown that the risk of congenital heart disease (CHD) was closely related to genetic and environmental factors, the exact mechanism was still unclear. This study was to assess the association of maternal folic acid supplementation (FAS), the 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) gene polymorphisms in offspring and their interaction effects with the risk of CHD and its subtypes.

Methods A case-control study was conducted on 595 children with CHD and 605 healthy child controls. The multivariate logistic regression model was used to assess the association of maternal FAS, offspring *MTRR* gene polymorphisms and their interaction effects with CHD and its subtypes.

Results This study shown that maternal FAS was significantly associated with a reduced risk of CHD (OR = 0.55, 95%CI: 0.36–0.83) and its subtypes including ASD (OR = 0.25, 95%CI: 0.14–0.45), VSD (OR = 0.42, 95%CI: 0.27–0.64), and CTD (OR = 0.23, 95%CI: 0.09–0.59) in offspring. Offspring *MTRR* gene polymorphisms at rs162048 (GG vs. AA: OR = 2.05, 95%CI: 1.35–3.13), rs1802059 (AA vs. GG: OR = 5.13, 95%CI: 2.15–12.23; GA vs. GG: OR = 1.81, 95%CI: 1.35–2.43), rs10380 (TT vs. CC: OR = 2.27, 95%CI: 1.20–4.31) and rs1801394 (GG vs. AA: OR = 1.58, 95%CI: 1.02–2.42) were significantly associated with the risk of CHD, and similar results were also found for three subtypes of CHD. Additionally, a statistically significant interaction effect between maternal FAS and offspring *MTRR* gene polymorphism at rs1802059 was observed (OR = 0.38, 95%CI: 0.15–0.94). Among children who had a variant genotype at rs1802059, the risk of CHD was significantly decreased when their mother used folate for this pregnancy compared with mothers not using folate.

Conclusions In those of Chinese descent, maternal FAS and offspring *MTRR* gene polymorphisms are significantly associated with the risk of CHD and its three subtypes. Furthermore, maternal FAS may help to offset some of risks of

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CHD due to offspring *MTRR* genetic variants. However, more studies with prospective designs and larger samples are needed to confirm our findings.

Trial registration Registration number: ChiCTR1800016635; Registration time: 14/06/2018.

Keywords Congenital heart disease, Folic acid supplementation, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase gene, Interaction effect

Introduction

Congenital heart disease (CHD), as one of the most common birth defects, refers to structural abnormalities of the heart and/or great vessels present at birth due to abnormal cardiac development during embryonic development [1]. A recent meta-analysis showed that the global incidence of CHD was 3.81‰ [2]. Existing evidence suggests that both genetic and environmental factors contribute to the development of CHD [3].

Folic acid, also known as vitamin B9, is involved in carbon metabolism and affects the synthesis of nucleic acids and proteins, etc., and is an indispensable basic material for the human body to maintain normal physiological functions [4]. More and more studies have pointed to the important role of folic acid in preventing a variety of birth defects, including neural tube defects, cleft lip and palate, and CHD [5–8]. Previous epidemiological studies on the association between maternal folic acid supplementation (FAS) during pregnancy and the risk of CHD in offspring were conducted in the United States [9], China [10], Norway [11, 12], and Hungary [13], but the results were inconsistent. This could be caused by differences in study design, biases, racial differences, and in dietary patterns. In addition, it was proposed that the strength of the association between folic acid and CHD was heterogeneous in different subtypes [14].

As a metabolite in the folate pathway, homocysteine is an independent risk factor for CHD [15]. The 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) gene is one of the key regulatory enzymes involved in homocysteine metabolic pathway. The *MTRR* gene which is located on the short arm of chromosome 5 at position 15.31, maintains 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) in its functional state and assists in the folate/VB12 dependent conversion of Homocysteine (Hcy) to methionine. The *MTRR* gene plays an important role in folate metabolism and the maintenance of Hcy levels, and its mutation may alter the concentration of homocysteine, thereby affecting normal embryonic development [15]. A meta-analysis showed that the *MTRR* gene polymorphism at rs1801394 was significantly associated with the risk of CHD [16]. Some epidemiological studies and animal studies shown that *MTRR* gene mutations were associated with an increased risk of CHD, and even led to embryonic lethality [17–19]. However, some studies suggested no relationship [20]. Additionally, most of previous studies only focus on two genetic loci (i.e., rs1801394 and rs1532268) of *MTRR* gene [16, 21–23]. The *MTRR* gene contains other common variants within the coding sequence in addition to rs1801394 and rs1532268. Further investigation of other genetic loci in the *MTRR* gene is necessary to reveal clues related to the metabolism underlying the potential embryonic protective effects of maternal FAS during pregnancy.

Therefore, we performed a hospital-based case-control study to comprehensively assess the association of maternal FAS, multiple genetic variants of the offspring *MTRR* gene, and their interaction effects with the risk of CHD and its subtypes of Chinese descent, which will help to promote the development of primary prevention strategies to reduce the burden of CHD worldwide.

Methods

Ethics statement

The study was approved by Ethics Committee for Clinical Research of Xiangya School of Public Health of Central South University (No. XYGW-2018-36), and followed the principles of the Declaration of Helsinki. Before the participants' personal information collection and biological samples, we have obtained all mothers' written informed consent, to ensure their voluntary participation and fully understand the research purpose and procedures. The protocol of this study was registered at the Chinese Clinical Trial Registry (registration number: ChiCTR1800016635; registration time: 14/06/2018). It is available at http://www.chictr.org.cn/listbycreater.aspx.

Study design and recruitment of study participants

We performed a hospital-based case-control study from November 2017 to December 2019 using the Hunan Provincial Children's Hospital as a recruitment site. Children with CHD and their mothers from the Department of Cardiothoracic Surgery were identified as case group, and the healthy individuals were recruited from the Department of Child Healthcare during the same period. To minimize potential maternal recall bias about preconception and first-trimester related exposures, we recruited only children younger than one year of age. All cases were simple CHD that excluded malformations from other systems and were diagnosed by echocardiography as well as confirmed by surgery. All controls were

confirmed by physical examination and cardiac ultrasound that the structure and function of the heart were normal, and there were no chromosomal abnormalities and systemic malformations. In addition, to minimize predisposing factors due to genetic and cultural differences, only Han subjects were recruited, and there were no familial relationships between the case and control groups. Subjects who were unable to provide a sample or were unwilling to cooperate in filling out the questionnaire were excluded from the study.

Information and biological sample collection

In addition to CHD, this study also focused on three important CHD subtypes: arterial septal defect (ASD), ventricular septal defect (VSD), and conotruncal defects (CTD). Among them, CTD specifically includes the following four subtypes: tetralogy of Fallot, double outlet ventricle, persistent truncus arteriosus and various transposition of the great arteries. According to World Health Organization (WHO) guidelines, FAS is defined as folic acid intake of more than 0.4 mg per day on at least five days per week continuously before conception or throughout the first trimester of pregnancy. In this study, professionally trained investigators conducted face-to-face interviews to collect data. According to our previous study [24], the following covariates were used as confounders in subsequent analyses: socio-demographic characteristics (residence, maternal age, education level, child gender), family history (consanguineous marriage, birth defects), history of pregnancy complications (gestational diabetes, gestational hypertension), maternal perinatal lifestyle (active smoking, passive smoking, drinking alcohol).

After enrollment, a trained nurse in the department collected 5 ml of peripheral venous blood using an EDTA anticoagulant tube. Collected blood samples were immediately centrifuged with a low-speed centrifuge at 3500 r/min for 15 min. Subsequently, isolated plasma and blood cells were carefully packaged, labeled, and stored in a cryogenic freezer at −80 ° C. In this study, blood cells were used for single nucleotide polymorphism (SNP) detection because of their high sensitivity and reliability.

Questionnaires were conducted face-to-face between professionally trained investigators and mothers to collect information. The questionnaire used has been described in our previous published articles [24]. One of the exposures of interest was maternal FAS, which was defined as a mother who had taken folic acid in 3 months before pregnancy and/or during the first-trimester pregnancy. Based on the previous literature [24], we selected the potentially confounding factors of significance as follows: demographic characteristics of pregnant women and children (sex of child, maternal age at pregnancy onset, maternal residence location and maternal educational level); family history (consanguineous marriage and history of birth defects in family); maternal exposure in 3 months before pregnancy and/or during the first-trimester pregnancy (drinking alcohol, active smoking and passive smoking); history of pregnancy complications (gestational diabetes and gestational hypertension). In China, each pregnant woman has a perinatal care manual, which contains pregnancy and personal information. The information collected was checked for accuracy against their health manuals and related medical records, which recorded their basic demographic characteristics, illness and lots of physical and laboratory examination results every time they went to a pregnancy test.

Sequencing of *MTRR* gene SNPs

The *MTRR* gene was a candidate gene in this study. In short, SNP markers were selected using the SNPBrowserTM program (version 3.0) from Applied-Biosystems, Inc. The program allows SNP markers to be selected from the HapMap database. Sites with r^2 less than 0.8 and minimum allele frequency (MAF) less than 10% were excluded. The *MTRR* gene polymorphisms were detected using the matter-assisted laser desorption ionization time-of-flight mass spectrometry MassARRAY (Agena iPLEX assay, San Diego, CA, USA). Laboratory technicians were responsible for genotyping, retyping and double-checking samples, and recording genotype data. We specified a minimum call rate of 50% for SNP genotyping to ensure the integrity of the individual genotype data that has been called. In addition, the experimenters did not know whether the samples came from the case group or the control group to ensure the integrity of the experiment. The success rate of SNPlex detection for these eleven SNPs is greater than 90%. Finally, these loci (rs162036, rs326120, rs162048, rs1802059, rs2287779, rs10380, rs16879334, rs1801394, rs3776455, rs2303080 and rs1532268) were selected as candidate loci in this study.

Statistical analysis

Categorical variables were described using frequencies and percentages. In the univariate analysis, the Pearson chi-square test or Fisher's exact test was used to compare categorical variables; The Wilcoxon rank sum test was used for ordinal categorical variables. SNPs of the *MTRR* gene were analyzed for deviations from Hardy-Weinberg equilibrium (HWE) in the control group. A locus with a *P* value of less than 0.1 indicates an imbalance in gene frequency and is therefore eliminated. Three common genetic models were used: dominant model (Aa+AA vs. aa), recessive model (AA vs. Aa+aa), and additive model (AA vs. Aa vs. aa). Odds ratios (ORs) and their 95% confidence intervals (CIs) were used to measure the level of

Table 1 Comparison of baseline characteristics between case and control groups

Baseline characteristics	Control (*N* = 605)	Case (*N* = 595)	χ^2	*P*
Sex of child (male/female)	393/212	306/289	34.146	< 0.001
Age at pregnancy onset (< 35/≥35)	522/83	512/83	0.013	0.980
Residence (Rural/Urban)	334/217	423/172	32.507	< 0.001
Educational level (years) (≤ 9/10–12/13–16/>16) *	122/213/258/12	338/156/101/0	13.590	< 0.001
Consanguineous marriage (No/Yes)	602/3	576/19	12.128	< 0.001
History of birth defects in family (No/Yes)	603/2	561/34	29.879	< 0.001
Drinking alcohol (No/Yes)	549/56	465/130	36.319	< 0.001
Active smoking (No/Yes)	588/17	547/48	16.183	< 0.001
Passive smoking (No/Yes)	379/226	275/320	32.640	< 0.001
Gestational diabetes (No/Yes)	577/28	532/63	15.205	< 0.001
Gestational hypertension (No/Yes)	589/16	546/49	18.301	0.004

*Differences between cases and controls were tested by Wilcoxon rank-sum test

association. Unadjusted ORs were calculated by univariate logistic regression. Adjusted ORs were calculated by multivariable logistic regression. Logistic regression was used to examine the main effects and interactive effects of the gene-environment interaction effect of maternal FAS and the offspring *MTRR* gene on CHD by controlling for other influencing factors. Bioinformatics analysis included: analysis of GTEx and based on STRING protein-protein interaction network. Statistical analysis was performed using SPSS (IBM SPSS Statistics for Macintosh, Version 26.0. Armonk, NY: IBM Corp) and R software, version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria.) 4.2.2. All tests were performed significantly for a two-sided *P* value not exceeding 0.05, except where otherwise specified.

Results

Comparison of baseline characteristics across groups

From November 2017 to December 2019, a total of 595 eligible CHD infants and their mothers were recruited into the case group. Among 595 CHD cases, 126 (21.2%) were diagnosed with ASD, 428 (71.9%) with VSD, 31 (5.2%) with CTD, 125 (21.0%) with other CHD subtypes. Of note, some cases have been diagnosed with multiple subtypes of CHD. Therefore, the sum of the various subtypes was not equal to 595. During the same period, 605 eligible healthy children and their mothers were identified as the control group. Baseline characteristics were summarized in Table 1.

Overall, there were no statistically significant differences across groups for maternal pregnancy age. However, our study showed that there were statistically significant differences between two groups for the following characteristics: Sex of child, residence location, maternal education level (years), history of consanguineous marriage, history of birth defects in family, gestational hypertension, gestational diabetes during this pregnancy, and personal lifestyle and habit in the periconceptional period including drinking alcohol, active smoking and passive smoking (all *P* values < 0.05). Therefore, these factors were adjusted when accessing the association of maternal FAS, offspring *MTRR* gene polymorphisms, and their interaction effect with the risk of CHD in offspring.

Table 2 Association of maternal FAS with the risk of CHD and its subtypes in offspring

Groups	FAS proportion (%)	Unadjusted-OR (95%CI)	Adjusted-OR (95%CI) *
Control	557/605(92.07)	1.00(reference)	1.00(reference)
Total CHD	481/595(80.84)	0.36(0.25–0.52)	0.55(0.36–0.83)
ASD	96/126(76.19)	0.28(0.17–0.46)	0.25 (0.14–0.45)
VSD	363/428(84.81)	0.48(0.32–0.72)	0.42 (0.27–0.64)
CTD	23/31(74.19)	0.25(0.11–0.58)	0.23 (0.09–0.59)

* Adjusted for child sex, residence, education level (years), consanguineous marriage, history of birth defects in family, drinking alcohol, active smoking, passive smoking, gestational diabetes, gestational hypertension

Maternal FAS and risk of CHD and its subtypes in offspring

Associations of maternal FAS with the risk of CHD and its subtypes were summarized in Table 2. The proportion of mothers of cases (total CHD:19.16%; ASD: 23.81%; VSD: 15.19%; CTD: 25.81%) who did not take FAS during pregnancy was significantly higher than that of the controls (7.93%). After adjustment for baseline data, mothers taking FAS for this pregnancy, compared with those not taking FAS, were at a significantly decreased risk of total CHD (aOR = 0.55; 95% CI: 0.36–0.83) and its subtypes including ASD (aOR = 0.25, 95%CI: 0.14–0.45), VSD (aOR = 0.42, 95%CI: 0.27–0.64), and CTD (aOR = 0.23, 95%CI: 0.09–0.59) in offspring.

Offspring *MTRR* gene polymorphisms and risk of CHD and its subtypes

Genotype frequencies for each SNP of the offspring *MTRR* gene and *P* values of the HWE test in the control group were summarized in Table S1. The genotype

distributions in the control group were within HWE ($P>0.1$) except for rs162036, rs326120 and rs1532268. The results of intergroup distribution of alleles/genotypes of offspring *MTRR* locus were shown in Table S2. The genotype and allele distributions of rs162048, rs1802059, rs10380 and rs3776455 as well as the genotype distribution of rs1801394 showed statistically significant differences between the two groups (all $P<0.05$), while the genotype and allele distributions of the other loci did not show statistically significant differences. As a consequence, the association of the *MTRR* gene polymorphism at rs162048, rs1802059, rs10380, rs1801394 and rs3776455 with the risk of CHD and its subtypes was assessed in the subsequent analyses.

Associations between offspring *MTRR* gene polymorphism and the risk of CHD and its subtypes in offspring based on logistic regression analyses were summarized in Table 3. Multivariable logistic regression showed that offspring *MTRR* gene polymorphisms at rs162048, rs1802059, rs10380 and rs1801394 were significantly associated with the risk of total CHD.

For rs162048, offspring with the GG genotypes, compared with those with the AA genotype, had a significantly higher risk of total CHD (aOR=2.05; 95% CI: 1.35–3.13); in addition, the dominant model (aOR=1.53; 95% CI: 1.16–2.01), recessive model (aOR=1.75; 95% CI: 1.18–2.59) and additive model (aOR=1.40; 95% CI: 1.15–1.71) were significantly associated with an increased risk of total CHD. The results of the three subtypes were similar to those of the total CHD group. Specifically, the genotype distribution of rs162048 was statistically significant in all three subtypes, but the dominant/recessive/additive model of rs162048 in the ASD group was not statistically significant.

For rs1802059, offspring with the GA genotype (aOR=1.81; 95% CI: 1.35–2.43), and AA (aOR=5.13; 95% CI: 2.15–12.23) genotype were at a significantly increased risk of total CHD compared with those with the GG

Table 3 Associations between polymorphisms of *MTRR* gene and the risk of CHD and its subtypes

SNPs	GMS	Adjusted-OR (95%CI) *			
		Total CHD	ASD	VSD	CTD
rs162048	AA	1.00(reference)	1.00(reference)	1.00(reference)	1.00(reference)
	GA	1.32(0.98–1.76)	1.24(0.77–1.99)	1.47(1.09–1.97)	0.36(0.14–0.91)
	GG	2.05(1.35–3.13)	2.05(1.35–3.13)	2.02(1.30–3.01)	1.14(0.39–3.33)
	Dominant model	1.53(1.16–2.01)	1.25(0.79–1.96)	1.57(1.18–2.08)	0.51(0.23–1.12)
	Recessive model	1.75(1.18–2.59)	0.85(0.54–1.32)	0.82(0.62–1.02)	2.89(1.18–7.08)
	Additive model	1.40(1.15–1.71)	1.17(0.83–1.76)	1.43(1.17–1.75)	0.80(0.44–1.47)
rs1802059	GG	1.00(reference)	1.00(reference)	1.00(reference)	1.00(reference)
	GA	1.81(1.35–2.43)	1.91(1.18–3.08)	1.79(1.33–2.42)	1.36(0.73–3.68)
	AA	5.13(2.15–12.23)	2.18(0.51–9.36)	3.94(1.70–9.12)	0.000
	Dominant model	1.90(1.43–2.51)	1.93(1.21–3.07)	1.93(1.45–2.57)	1.55(0.70–3.47)
	Recessive model	4.29(1.81–10.16)	0.54(0.33–0.86)	0.59(0.44–0.80)	0.61(0.27–1.36)
	Additive model	1.94(1.06–2.49)	1.94(1.06 to 2.50)	1.77(1.170–2.68)	1.94(1.06–2.49)
rs10380	CC	1.00(reference)	1.00(reference)	1.00(reference)	1.00(reference)
	CT	1.05(0.77–1.43)	1.75(1.70–2.87)	1.14(0.83–1.55)	1.75(0.76–4.03)
	TT	2.27(1.20–4.31)	3.43(1.24–9.50)	2.51(1.33–4.75)	5.12(1.29–20.26)
	Dominant model	1.19(0.90–1.59)	1.92(1.21–3.06)	1.29(0.97–1.73)	2.09(0.97–4.52)
	Recessive model	2.25(1.19–4.24)	0.61(0.37–0.99)	0.94(0.69–1.28)	0.65(0.29–1.46)
	Additive model	1.25(0.99–1.58)	1.80(1.23–2.63)	1.34(1.06–1.70)	2.04(1.12–3.71)
rs1801394	AA	1.00(reference)	1.00(reference)	1.00(reference)	1.00(reference)
	AG	1.25(0.94–1.67)	1.78(1.10–2.87)	1.23(0.93–1.63)	0.82(0.34-2.00)
	GG	1.58(1.02–2.42)	1.22(0.5–2.890)	0.49(0.26–0.92)	2.26(0.75–6.79)
	Dominant model	1.39(0.93–2.09)	1.68(1.06–2.66)	1.10(0.84–1.44)	1.08(0.49–2.36)
	Recessive model	1.26(0.96–1.66)	0.58(0.37–0.92)	0.76(0.58-1.00)	1.43(0.61–3.36)
	Additive model	1.25(1.03–1.53)	1.33(0.94–1.87)	0.95(0.16–1.18)	1.30(0.73–2.29)
rs3776455	CC	1.00(reference)	1.00(reference)	1.00(reference)	1.00(reference)
	TC	1.04(0.73–1.44)	1.62(0.98–2.67)	1.42(1.06–1.89)	1.25(0.50–3.09)
	TT	1.70(0.54–5.30)	1.69(0.83–3.42)	1.22(0.78–1.92)	3.81(1.38–10.54)
	Dominant model	1.70(0.54–5.31)	1.64(1.02–2.63)	1.37(1.04–1.81)	1.75(0.78–3.92)
	Recessive model	1.04(0.75–1.46)	0.71(0.45–1.12)	0.74(0.56–0.97)	1.21(0.542-2.70)
	Additive model	1.10(0.81–1.50)	1.36(0.98–1.89)	1.19(0.98–1.46)	1.89(1.10–3.24)

* Adjusted for child sex, residence, education level (years), consanguineous marriage, history of birth defects in family, drinking alcohol, active smoking, passive smoking, gestational diabetes, gestational hypertension

Table 4 Interaction effects between maternal FAS and offspring *MTRR* gene polymorphisms on the risk of total CHD

SNPs interacted with FAS	Unadjusted-OR (95%CI)	*P*	Adjusted-OR (95%CI) *	*P*
rs162048	0.87(0.42–1.79)	0.695	0.84(0.38–1.88)	0.674
rs1802059	0.46(0.21–0.97)	0.042	0.38(0.15–0.94)	0.035
rs10380	0.79(0.15–4.01)	0.771	0.69(0.13–3.67)	0.661
rs1801394	1.31(0.64–2.69)	0.463	1.01(0.42–2.18)	0.914

* Adjusted for child sex, residence, education level (years), consanguineous marriage, history of birth defects in family, drinking alcohol, active smoking, passive smoking, gestational diabetes, gestational hypertension

Table 5 Genotype of offspring *MTRR* gene polymorphisms at rs1802059 by stratification of maternal FAS and risk (odds ratio) of total CHD

maternal FAS		Number of controls	Number of cases	Univariate logistic regression		Multivariable logistic regression	
				Unadjusted-OR (95%CI)	*P*	Adjusted-OR (95%CI) *	*P*
Yes	Wild genotype (GG)	400	285	1		1	
	Variant genotypes (GA + AA)	157	196	1.75(1.35–2.27)	< 0.001	1.66(1.24–2.24)	0.001
No	Wild genotype (GG)	38	57	1		1	
	Variant genotypes (GA + AA)	10	57	3.80(1.73–8.35)	0.001	4.41(1.91–10.17)	< 0.001

* Adjusted for child sex, residence, education level (years), consanguineous marriage, history of birth defects in family, drinking alcohol, active smoking, passive smoking, gestational diabetes, gestational hypertension

genotype; besides, the dominant model (aOR = 1.90; 95% CI: 1.43–2.51), recessive model (aOR = 4.29; 95% CI: 1.81–10.16) and additive model (aOR = 1.94; 95% CI: 1.06–2.49) all significantly increased the risk of total CHD. Results in the ASD and VSD groups were similar to total CHD group, while only the additive model was statistically significant in the CTD group.

For rs10380, offspring with the TT genotypes, compared with those with the CC genotype, had a significantly higher risk of total CHD (aOR = 2.27; 95% CI: 1.20–4.31); and recessive model (aOR = 2.25; 95% CI: 1.19–4.24) were significantly associated with an increased risk of total CHD; *MTRR* gene polymorphisms at rs10380 were associated with the risk of disease in the ASD (CT vs. CC; TT vs. CC; dominant model; recessive model and additive model), VSD (TT vs. CC and additive model), and CTD (TT vs. CC and additive model) groups.

For rs1801394, offspring with the GG genotypes, compared with those with the AA genotype, had a significantly higher risk of total CHD (aOR = 1.58; 95% CI: 1.02–2.42); and additive model (aOR = 1.25; 95% CI: 1.03–1.53) were significantly associated with an increased risk of total CHD; *MTRR* gene polymorphisms at rs1801394 were associated with the risk of disease in the ASD (AG vs. AA; dominant model and recessive model) and VSD groups (GG vs. AA), but not in the CTD group.

Interaction effects between maternal FAS and offspring *MTRR* gene polymorphisms

Interaction effects between maternal FAS and offspring *MTRR* gene polymorphisms (rs162048, rs1802059, rs10380 and rs1801394) on the risk of total CHD were summarized in Table 4. Multivariable logistic regression suggested that there were statistically significant interaction effects between maternal FAS and offspring *MTRR* gene polymorphisms at rs1802059 (aOR = 0.38, 95%CI: 0.15–0.94; *P* = 0.035). Additionally, we further analyzed the independent effect of offspring *MTRR* gene polymorphisms at rs1802059 on the risk of total CHD by stratification of maternal FAS (Table 5). The results suggested that the risk of total CHD was significantly decreased among offspring who had variant genotypes but maternal FAS when compared with those who had variant genotypes and no maternal FAS. Without maternal FAS, the risk of CHD for offspring with the variant genotype was 4.41 (95% CI: 1.91–10.17) for rs1802059. However, with maternal FAS, the risk of CHD for offspring with the variant genotype was 1.66 (95% CI: 1.24–2.24) for rs1802059.

Bioinformatics analysis

For the *MTRR* gene polymorphisms at rs162048, rs1802059, rs10380, rs1801394 with statistical significance, the relevant expression quantitative trait locus (eQTL) data were searched and downloaded from the GTEx database (https://www.gtexportal.org/home/), and the results were shown in Table S3. Except for rs1802059, significant associations were observed between rs162048, rs10380, rs1801394 and *MTRR* gene expression in whole blood. In addition, these sites were also statistically correlated with *MTRR* gene expression levels in other human tissues, such as brain tissue, adipose tissue, muscle tissue, and skin tissue.

Figure 1 showed the interactions between MTRR proteins and other proteins using the STRING 11.0 protein-protein interaction network database. Table 6 listed the combined scores of MTRR proteins and predicted protein interactions. From the composite scores,

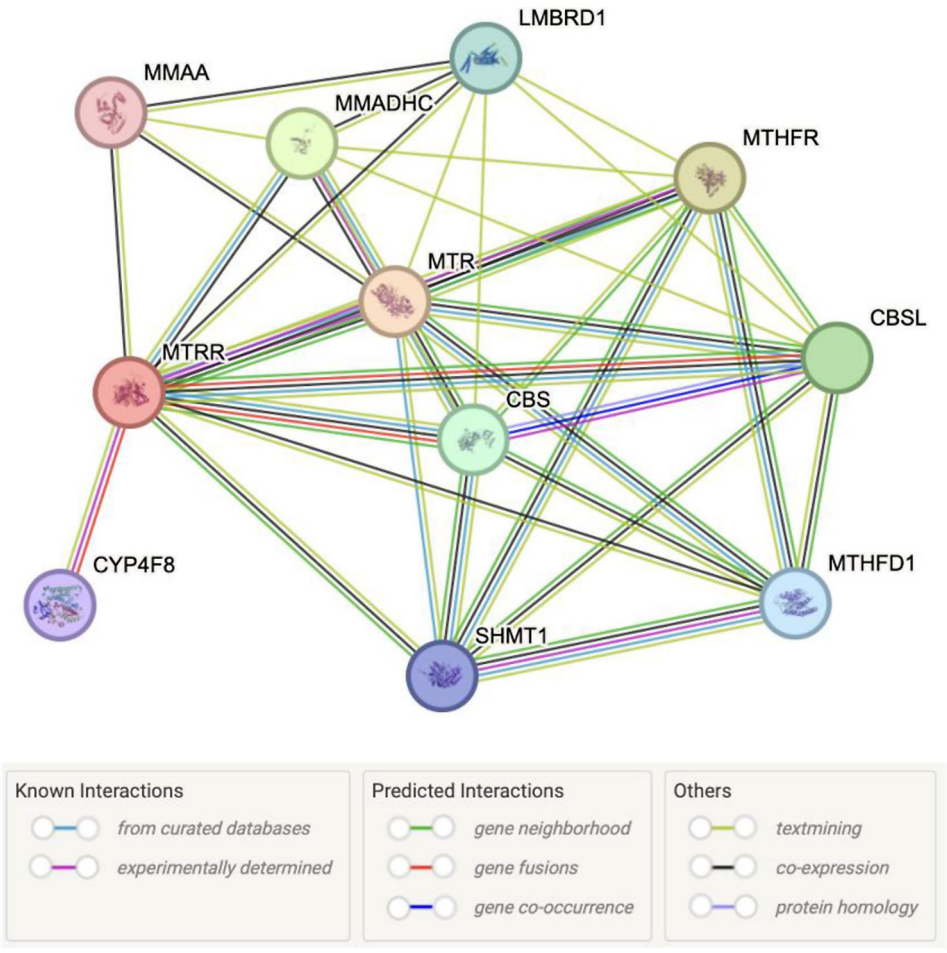

Fig. 1 Diagram of protein-protein interaction networks associated with MTRR proteins

Table 6 Composite score of MTRR proteins and predicted protein interaction

Target protein	Predicted Interacting proteins	Combined-score
MTRR	MTR	0.999
MTRR	MTHFR	0.981
MTRR	MMADHC	0.978
MTRR	CBSL	0.972
MTRR	CBS	0.97
MTRR	LMBRD1	0.945
MTRR	MTHFD1	0.941
MTRR	SHMT1	0.929
MTRR	CYP4F8	0.903
MTRR	MMAA	0.885

MTRR showed the strongest evidence of interaction with MTR, followed by Methylenetetrahydrofolate reductase (MTHFR).

Discussion

Our study showed that maternal FAS, and offspring *MTRR* gene polymorphisms were significantly associated with the risk of CHD and its subtypes of Chinese descent. Additionally, there were statistically significant interaction effects between maternal FAS and offspring *MTRR* gene polymorphisms on the risk of CHD in offspring. As far as we know, this is the first time that the association of maternal FAS, offspring *MTRR* gene polymorphisms, and their interaction effects with the risk of CHD has been exhaustively explored, which highlight the importance of periconception FAS and may help provide new clues for future etiological studies and interventions in CHD.

In this study, we observed that maternal FAS significantly reduced the risk of CHD (OR=0.55) in the offspring. The results of previous studies on this topic were consistent with our findings. For example, Wang et al. [25] analyzed the data from the birth defect surveillance system in a district of Beijing and found that periconception maternal FAS could significantly reduce the risk of CHD in their offspring. Additionally, case-control studies from the United States [26], New Zealand [27], and a birth cohort study from Gansu Province, China [28] also observed a protective effect of maternal FAS against multiple types of CHD. It is well known that maternal FAS during pregnancy has been the main preventive measure

to reduce the risk of neural tube malformations in offspring [5]. In recent years, more and more studies have shown that maternal FAS also has a preventive effect on CHD in offspring [8, 10]. Besides, there were studies claiming that folic acid can prevent birth defects such as cleft lip and palate, as well as cardiovascular diseases and cancers [6, 7]. The present study also found that the protective effect of maternal FAS on CHD subtypes such as ASD (OR=0.25), VSD (OR=0.42) and CTD (OR=0.23) was stronger than that of total CHD, and the same results were observed in the studies of Morikawa et al. [14] and Qu et al. [29]. Folic acid played an irreplaceable role in one-carbon metabolism and was essential for the synthesis of many substances such as proteins, deoxyribonucleic acid, neurotransmitters and phospholipids [30–32]. Folic acid deficiency could affect this process, which could lead to the development of a variety of diseases. Therefore, our findings emphasized that women of childbearing age should raise their awareness of FAS and ensure adequate intake of folic acid during periconception to prevent CHD in offspring.

This study also assessed the association of *MTRR* gene polymorphisms with the risk of CHD. The results suggested that polymorphisms of the *MTRR* gene at rs162048, rs1802059, rs10380 and rs1801394 were significantly associated with the risk of CHD and its subtypes. Previous studies reached the same conclusion [33–35], and proposed that *MTRR* gene polymorphism might be associated with acyanotic CHD [36]. It was worth noting that there were also some studies suggesting that the association between *MTRR* gene polymorphism and CHD was not statistically significant. For example, a case-control study from the Netherlands did not find a significant association between offspring *MTRR* rs1801394 polymorphism and the development of heart defects in children [20]. The inconsistent results may be explained by racial differences, differences in study design, and the presence of bias. In addition, several studies confirmed that *MTRR* gene polymorphisms were also associated with a variety of cancers [37–39], reproductive disorders [40] and cleft lip and palate [41]. However, most of the previous studies only focused on two loci (i.e., rs1801394 and rs1532268). Our study was the first to explore the relationship between five polymorphisms (rs162048, rs1802059, rs10380, rs1801394 and rs3776455) of *MTRR* gene and the risk of CHD and its subtypes, which provided new clues for further research on *MTRR* gene variants associated with CHD. *MTRR* gene was one of the key regulatory enzymes involved in homocysteine metabolism pathway, and homocysteine was an independent risk factor for CHD [15], so there was a high possibility that *MTRR* gene polymorphisms might be associated with CHD. *MTRR* maintained sufficient levels of activated cobalamin, which served as a cofactor for *MTR*. In the process of *MTR* catalyzed re-methylation of homocysteine to methionine, cobalamin acted as an intermediated methyl carrier between methyltetrahydrofolate and homocysteine. The cobalamin cofactor cycled between the cob (I) alamin and methyl cob (III) alamin, but the cob (I) alamin could be oxidized to the unactivated cob (II) alamin form, and for it to regain activity, cob (II) needed to be converted to the methyl cob (III) alamin form by obtaining a methyl donor for S-adenosylmethionine catalyzed by *MTRR*. This cycle ensured the activity of *MTR*, and *MTRR* acted as a "companion" that played an important role in keeping *MTR* in an active state [42]. Therefore, *MTRR* gene mutation might change the concentration of homocysteine and thus affected the normal development of embryonic heart, and studies confirmed this speculation [15]. However, the specific mechanism will still require further studies to elucidate. In addition, bioinformatics analysis showed that the evidence for the interaction between MTRR and MTR was strongest, with a combined score of 0.999 for both, followed by sub-MTHFR with a combined score of 0.981. MTR catalyzes the transfer of a methyl group from methyl-cobalamin to homocysteine, yielding enzyme-bound cob(I)alamin and methionine, while MTRR is involved in the reductive regeneration of cob(I)alamin cofactor. The utilization of methylgroups in the folate cycle is necessary, and these proteins play an important role in methylation. In addition, MTHFR also plays an important role in the folate cycle by catalyzing the conversion of 5, 10-methyltetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Therefore, we believed that MTRR had a strong interaction with MTR and MTHFR, which were closely related in folate metabolism.

In this study, we also found an interaction effect between maternal FAS and offspring *MTRR* gene polymorphisms at rs1802059 in the development of CHD. This study showed that children who carried the variant genotype and with maternal FAS had a significantly lower risk of developing CHD compared with children who carried the variant genotype but without maternal FAS. This suggested that maternal FAS might help to offset some of the risk of developing CHD due to variations in the *MTRR* gene in offspring. The interaction effect between maternal FAS and offspring *MTRR* gene polymorphisms in CHD had not been investigated before, and this study was the first to find this association. Previous literatures indicated that the insufficient activity of *MTRR* and *MTR* caused by *MTRR* gene variation will decrease the utilization rate of folic acid and increase the level of Hcy, which might eventually induce vascular endothelial injury and cardiovascular diseases [43, 44]. Additionally, insufficient maternal folic acid intake may affect the methylation of genes related to fetal growth

and development [45–47]. We speculate that this might be a mechanism of interaction between maternal FAS and offspring *MTRR* gene polymorphisms. However, the exact mechanism remained unclear and required further study. More and more evidence show that the interaction of gene and environment could affect CHD, so the influence of interaction would be one of the future research directions of CHD.

There were some limitations in this study. First, selection bias was hard to avoid. This study was a hospital-based case-control study, and our target population only included those children with CHD who were born successfully and survived. We did not know the cases of termination of pregnancy due to CHD or death after birth due to severe CHD, which may lead to a series of problems, such as the representativeness of the sample. Second, this study classified the exposure to maternal FAS as "yes" and "no", and did not investigate other ways of folic acid intake, such as dietary conditions, so that the true folic acid level of each pregnant woman during the perinatal period could not be obtained, which may affect the study results to some extent. Third, residual confounding was of concern, even though we used multivariate logistic regression to control for the effect of confounding. Fourth, previous studies shown *MTRR* gene polymorphisms at rs1532268 was associated with the risk of CHD [16]. However, in this study, the distribution of this locus in the control population did not meet the HWE test, so we did not analyze this locus. In addition, as this study included only Han Chinese, more studies in other populations need to generalize the findings. Moreover, there were no animal experimental studies on MTRR gene variants and the risk of congenital heart disease, and the functional evidence of the effects of these gene variants could not be directly obtained. These limitations suggest that future studies should conduct prospective cohort studies with larger sample sizes in different ethnic populations and experimental study.

Conclusion

In conclusion, the present study further confirms that maternal FAS is significantly associated with the risk of CHD and its subtypes in offspring of Chinese descent. This study also shows that *MTRR* gene polymorphisms at rs162048, rs1802059, rs10380 and rs1801394 are significantly associated with the risk of CHD and its subtypes. Additionally, interaction effect analyses indicate that maternal FAS can help to offset some of the risks of CHD due to genetic variants of offspring *MTRR* gene. However, the mechanism by which these factors relate to fetal heart development remained unknown, and more studies with prospective designs and larger samples as well as experimental studies need to confirm our findings.

Abbreviations

CHD	Congenital heart disease
FAS	folic acid supplementation
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase
VSD	ventricular septal defect
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase
Hcy	Homocysteine
ASD	arterial septal defect
CTD	conotruncal defects
SNPs	Single nucleotide polymorphisms
MAF	minimum allele frequency
HWE	Hardy-Weinberg equilibrium
aOR	Adjusted odds ratio
CI	Confidence interval
MTHFR	Methylenetetrahydrofolate reductase
eQTL	expression quantitative trait locus

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41043-024-00699-w.

Supplementary Material 1

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Author contributions

L L, Y C and Q C participated in Investigation; L L, J O and T W participated in Methodology; L L, Q C and Y Z participated in Software; L L participated in Formal analysis and Writing – original draft; J O participated in Conceptualization; J O, Y G and T W participated in Data curation; J O and Y G participated in Writing – review & editing; Y C participated in Resources; Q C and M L participated in Validation; M L and T W participated in Visualization; Y Z and J B participated in Funding acquisition and Supervision; Jiabi Qin participated in Project administration; All authors read and approved the final manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Xiangya School of Public Health Central South University (No. XYGW-2018-36). All mothers provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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