Int J Clin Exp Med 2017;10(12):15809-15824
www.ijcem.com /ISSN:1940-5901/IJCEM0051704

Review Article

Association of *methylenetetrahydrofolate reductase* rs1801133 C>T polymorphism and congenital heart disease: a meta-analysis of 12,523 subjects

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Received January 22, 2017; Accepted September 4, 2017; Epub December 15, 2017; Published December 30, 2017

Abstract: Objective: The potentially functional *MTHFR* rs1801133 C>T polymorphism was predicted to be related to risk of congenital heart disease (CHD). The aim of this meta-analysis was to assess the relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk in three groups: Group 1: CHD patients *vs.* healthy controls; Group 2: mothers with CHD offspring *vs.* mother controls with healthy offspring and Group 3: father with CHD offspring *vs.* father controls with healthy offspring. Methods: All case-control studies up date to June 12, 2016 on the relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk were identified by retrieving EMBASE and PubMed databases. The association of this polymorphism with CHD risk was estimated by odds ratios with 95% confidence intervals. Results: A total of 44 case-control studies from 24 articles were collected in our meta-analysis. Overall, *MTHFR* rs1801133 TT genotype and T allele increased CHD risk significantly in Group 1 and Group 3. In Group 1, based on a stratified analysis by ethnicity, rs1801133 TT genotype and T allele caused raised CHD incidence in Asians; however, the rs1801133 TT genotype reduced CHD risk in Caucasus. Additionally, based on a stratified analysis by the type of CHD, we found that the *MTHFR* rs1801133 C>T was associated with conotruncal heart disease, patent ductusarteriosus, transposition of great artery, coarctation of the aorta, and other CHD type. In Group 2, *MTHFR* rs1801133 TT genotype of Asians and Caucasus might increase offspring CHD morbidity. Conclusions: *MTHFR* rs1801133 C>T polymorphism is related to CHD risk from three respects of children, mother and father.

Keywords: MTHFR, polymorphism, CHD risk, meta-analysis

Introduction

Congenital heart disease (CHD) is the most prevalent defect with 1% incidence worldwide and contributes to non-infectious cause of mortality and morbidity in newborns [1]. CHD is a multifactorial disease and its aetiology is not clearly figured out. Epidemiological studies reveal some momentous environmental contributions to the nosogenesis of CHD [2, 3]. Except for certain CHD caused by a single gene mutation, most of CHD are polygenic diseases influenced by both environmental and genetic factors [4]. Familial aggregation and twin studies have demonstrated the existence of genetic factors for the risk of this appearance [5-7]. The materiality of genetic factors in the occurrence and development of CHD is also sustained by recent information from genome wide association study (GWAS) [8]. Chromosomal abnormalities and genetic mutations account for about 28% of congenital defects among affected individuals [9].

Folate is the general term for vitamin B9, a water-soluble B vitamin, which is naturally fou-

nd in the following foods: strawberries, green leafy vegetables, kiwis, liver, some citric fruits, beans, cereals, and egg yolks [10]. With higher bioavailability and similar structure to natural folate, folic acid is a synthetic compound which is applied in supplements and fortified foods [11]. The main form of plasma circulating folate is 5-methyltetrahydrofolate (5-methyl THF). It can be intransit to the cells by way of folate receptors and carriers [12]. The folate pathway is essential for the synthesis of nucleic acid. THF, caused by the reaction catalyzed by methionine synthase (MTR), can be directly transformed into 5,10-methylene THF through the action catalyzed serine hydroxymethyltransferase (SHMT). According to cellular demands, 5,10-methylene THF can be applied to thymidylate synthesis, to purine synthesis, or to the production of 5-methyl THF required for homocysteine (Hcy) remethylation reactions. In turn, 5,10-methylene THF can be reduced to 5-methyl THF catalyzed by methylene tetra hydrofolate reductase (MTHFR) which is important to regulate available folate derivatives for DNA methylation and Hcy remethylation [13].

Some studies indicated the relationship of CHD risk with the preconceptional use multivitamin which could lead to 40%-60% decrease of CHD incidence [14, 15]. Maternal folic acid supplement has been proved to decrease the CHD morbidity [16]. In a randomized controlled trial, it was found that there was a significant reduction of CHD after multivitamins supplementation [17, 18] and similar reduction was found in a cohort controlled trial [19]. The merging results of these two intervention trials indicated a 43% reduction in the incidence for CHD. High doses of folic acid during the critical period of cardiovascular form (*i.e.*, the second and third months of gestation) significantly reduced the birth morbidity of CHD [20, 21]. Folic acid antagonist drugs, inhibiting dihydrofolatereductase which is necessary to DNA synthesis, increased CHD incidence in the children of pregnant women [22].

Abnormal folic acid metabolism and common variants of the enzymes in folic acid metabolism have been previously depicted as possible risk factors of CHD. One important enzyme involved in the folic acid metabolism is MTHFR. *MTHFR* gene exists in 1p 36.3. MTHFR is a 77 kDa protein and catalyses 5,10-methylenetetrahydrofolate into 5-methyl THF which is a major circulating form of folic acid and crucial precursor in methylation reactions. Alteration in MTHFR activity has many influences on some metabolic pathways, such as DNA and RNA synthesis, nucleotide balance, epigenetics in DNA, and DNA repair. One of important single nucleotide polymorphisms (SNPs) in *MTHFR* gene is rs1801133 C>T (C677T) which results in the amino acid transformation of alanine to valine at 226 position of MTHFR protein. This mutation leads to a 50% MTHFR enzyme activity reduction, an increase of plasma Hcy concentration and a decrease of plasma folic acid concentration.

Since Wenstrom *et al.* first verified the relationship between *MTHFR* gene polymorphisms and CHD risk [23], many studies have been conducted to replicate this study. Some recent case-control studies have ascertained that *MTHFR* rs1801133 C>T polymorphism was a risk factor of CHD in Asians, particularly in Chinese Han population [8, 24, 25]. However, all of these case-control studies have yielded contradictory results. Herein, we performed this updated meta-analysis of all published case-control studies to expound the relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk in three groups: Group 1, CHD patients vs. healthy controls; Group 2, mothers with CHD offspring vs. mother controls with healthy offspring; and Group 3, father with CHD offspring vs. father controls with healthy offspring.

Materials and methods

Search strategy

Articles focusing on the association of CHD risk with *MTHFR* rs1801133 C>T polymorphism were identified by comprehensively searching the related literatures up date to June 12, 2016 in the PubMed and EMBASE database. The following terms were used for searching: 'congenital heart disease' or 'congenital anomalies' or 'birth defect' or 'heart defect' or 'CHD' and 'polymorphism' or 'mutation' or 'variant' or 'SNP' and 'Methylenetetrahydrofolatereductase' or 'MTHFR'. The publication language was restricted to English and Chinese, and all studies were only in regard to human subjects. The update and most complete results were adopted when multiple articles were

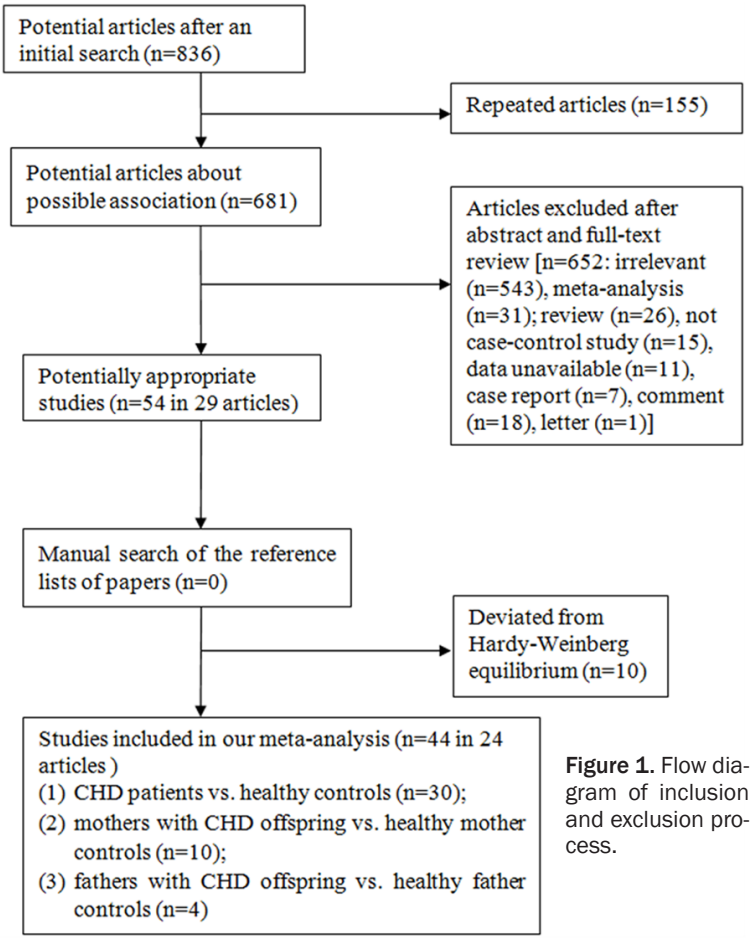

Figure 1. Flow diagram of inclusion and exclusion process.

derived from the same study group. All references in these eligible studies or reviews were also manually searched of to supply the electronic retrieval results.

Inclusion and exclusion criteria

The major selection criteria were: (a) studies focusing on the association between *MTHFR* rs1801133 C>T polymorphism and CHD; (b) case-cohort or case-control studies; (c) original data; (d) proper CHD diagnosis criteria. Accordingly, the major exclusion criteria were: (a) not associated to CHD risk and *MTHFR* rs1801133 C>T polymorphism; (b) no available data; (c) not case-control or cohort study; (d) conference papers, comments, reviews and letters; (e) repeated studies.

Data extraction

Two authors (Y. Wang and J. Xie) extracted the data independently, and a third investigator (W. Tang) reviewed the results. For each study, the following information was extracted: first author's name, publication year, ethnicity of study population, the number of cases and controls in each study, genotype information, genotyping methods, the type of CHD (conotruncal heart disease; patent ductusarteriosus, PDA; transposition of great artery, TGA; ventricular septal defect, VSD; atrial septal defect, ASD; coarctation of the aorta, CoA; and others), source of control and Hardy-Weinberg equilibrium (HWE) in controls. If any information essential to the meta-analysis was not available from a study, we did our best to get in touch with the authors to obtain the missing data. If conflicting evaluations were encountered, unanimity was reached through a comprehensive discussion.

Quality score

We harnessed the Newcastle-Ottawa Scale (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) to evaluate the quality score of the eligible studies [34]. Each included studies were assessed by 8 items of 3 aspects. When quality score was ≥ 7 stars, it was considered as high-quality study.

Statistical analysis

The crude odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated to assess the relationship between MTHFR rs1801133 C>T polymorphism and CHD risk for an allele model (T vs. C), a homozygote model (TT vs. CC), a dominant model (TT+CT vs. CC), and a recessive model (TT vs. CT+CC). The HWE in control groups was calculated by an online test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) and $P<0.05$ was regarded as the statistical significance [26, 27]. The X^2-based Q test and the I^2 test were conducted to analyze heterogeneity between the studies. When $P<0.1$ or $I^2<50\%$, the random-effect model was applied [28]. Otherwise, the fixed-effect model was

Table 1. Studies characteristics in the meta-analysis

Study	Year	Ethnicity	No. of cases/ controls	Source of case	Genotype method	Objective of study
Group 1: CHD patients vs. healthy controls						
Wang et al.	2016	Asian	147/168	Hospital-based	HRM	Group 1
Li et al.	2015	Asian	150/150	Hospital-based	RFLP-PCR	Group 1
Koshy et al.	2015	Asian	96/100	Population-based	ABI 3730 automated sequencer	Group 1
Sayin et al.	2014	Caucasus	79/99	Hospital-based	RFLP-PCR	Group 1
Chao et al.	2014	Asian	17/34	Hospital-based	RFLP-PCR	Group 1
Sahiner et al.	2014	Caucasus	117/93	Hospital-based	RFLP-PCR	Group 1
Sahiner et al.	2014	Caucasus	45/93	Hospital-based	RFLP-PCR	Group 1
Wang et al.	2013	Asian	160/188	Hospital-based	SNaPShot	Group 1
Kotby et al.	2012	Caucasus	30/30	Hospital-based	RFLP-PCR	Group 1
Gong et al.	2012	Asian	120/136	Hospital-based	MALDI-ToF-M	Group 1
Gong et al.	2012	Asian	124/136	Hospital-based	MALDI-ToF-M	Group 1
Xu et al.	2010	Asian	502/527	Hospital-based	RFLP-PCR	Group 1
Xu et al.	2010	Asian	257/527	Hospital-based	RFLP-PCR	Group 1
Xu et al.	2010	Asian	41/527	Hospital-based	RFLP-PCR	Group 1
Kuehl et al.	2010	Caucasus	64/477	Hospital-based	Multilocus allele-specific hybridization assay	Group 1
Li et al.	2009	Asian	104/208	Hospital-based	RFLP-PCR	Group 1
Van et al.	2008	Caucasus	229/251	Hospital-based	RFLP-PCR	Group 1
Galdieri et al.	2007	Caucasus	58/38	Hospital-based	RFLP-PCR	Group 1
Zhu et al.	2006	Asian	22/104	Population-based	Taq-Man	Group 1
Zhu et al.	2006	Asian	35/104	Population-based	Taq-Man	Group 1
Lee et al.	2005	Asian	3/195	Population-based	DHPLC	Group 1
Lee et al.	2005	Asian	10/195	Population-based	DHPLC	Group 1
Lee et al.	2005	Asian	29/195	Population-based	DHPLC	Group 1
Lee et al.	2005	Asian	25/195	Population-based	DHPLC	Group 1
Lee et al.	2005	Asian	48/195	Population-based	DHPLC	Group 1
Lee et al.	2005	Asian	37/195	Population-based	DHPLC	Group 1
Lee et al.	2005	Asian	72/195	Population-based	DHPLC	Group 1
Shaw et al.	2005	Caucasus	238/652	Population-based	Multilocus allele-specific hybridization assay	Group 1
Li et al.	2005	Asian	192/124	Population-based	RFLP-PCR	Group 1
Storti et al.	2003	Caucasus	103/200	Hospital-based	RFLP-PCR	Group 1
Group 2: Mothers with CHD offspring vs. mother controls with healthy offspring						
Jiang et al.	2015	Asian	100/100	Hospital-based	RFLP-PCR	Group 2
Shi et al.	2015	Asian	153/216	Hospital-based	Taq-Man allelic discrimination assay	Group 2
Elsayed et al.	2014	Caucasus	61/61	Hospital-based	RFLP-PCR	Group 2
Kotby et al.	2012	Caucasus	30/30	Hospital-based	RFLP-PCR	Group 2
Sánchez-Urbina et al.	2012	Caucasus	60/62	Hospital-based	RFLP-PCR	Group 2
Van et al.	2008	Caucasus	230/251	Hospital-based	RFLP-PCR	Group 2
Wintner et al.	2007	Caucasus	31/31	Hospital-based	ASO microarrays	Group 2
Zhu et al.	2006	Asian	57/104	Population-based	Taq-Man	Group 2
Li et al.	2005	Asian	192/124	Population-based	RFLP-PCR	Group 2
Storti et al.	2003	Caucasus	103/200	Hospital-based	RFLP-PCR	Group 2
Group C: Father with CHD offspring vs. father controls with healthy offspring						
Wintner et al.	2007	Caucasus	31/31	Hospital-based	ASO microarrays	Group 3
Zhu et al.	2006	Asian	57/104	Population-based	Taq-Man	Group 3
Li et al.	2005	Asian	192/124	Population-based	RFLP-PCR	Group 3
Storti et al.	2003	Caucasus	103/200	Hospital-based	RFLP-PCR	Group 3

applied [29]. In order to identify the source of heterogeneity, subgroup analyses were performed by types of CHD, ethnicity (Caucasians and Asians), source of controls (hospital-based and population-based) and number of cases (>300 vs. ≤300). Begg's funnel plot and the Egger's quantitative tests were used to evaluate and describe the possible publication bias [30]. $P<0.05$ was considered statistically significant. The statistical analyses were operated

Table 2. Distribution of *MTHFR* rs1801133 C>T polymorphism genotype and allele in each group

Study	Year	Case			Control			Case		Control		HWE
		CC	CT	TT	CC	CT	TT	C	T	C	T	
Group 1: CHD patients vs. healthy controls												
Wang et al.	2016	14	73	66	49	84	35	101	205	182	154	Yes
Li et al.	2015	31	78	41	59	66	25	140	160	184	116	Yes
Koshy et al.	2015	95	1	0	83	7	0	191	1	173	7	Yes
Sayin et al.	2015	40	33	2	43	44	8	113	37	130	60	Yes
Chao et al.	2014	10	5	2	19	12	3	25	9	50	18	Yes
Sahiner et al.	2014	50	34	13	47	39	7	134	60	133	53	Yes
Sahiner et al.	2014	19	19	1	47	39	7	57	21	133	53	Yes
Wang et al.	2013	59	76	25	53	100	35	194	126	206	170	Yes
Kotby et al.	2012	12	14	4	20	8	2	38	22	48	12	Yes
Gong et al.	2012	21	59	40	43	72	21	101	139	158	114	Yes
Gong et al.	2012	24	64	36	43	72	21	112	136	158	114	Yes
Xu et al.	2010	79	114	52	151	261	115	272	218	563	491	Yes
Xu et al.	2010	83	130	44	151	261	115	296	218	563	491	Yes
Xu et al.	2010	12	17	12	151	261	115	41	41	563	491	Yes
Kuehl et al.	2010	12	33	10	134	134	32	57	53	402	198	Yes
Li et al.	2009	16	42	46	55	114	39	74	134	224	192	Yes
Van et al.	2008	99	103	27	119	107	25	301	157	345	157	Yes
Galdieri et al.	2007	30	21	7	18	14	6	81	35	50	26	Yes
Zhu et al.	2006	3	7	12	22	57	24	13	31	101	105	Yes
Zhu et al.	2006	4	15	15	22	57	24	23	45	101	105	Yes
Lee et al.	2005	1	2	0	114	68	13	4	2	296	94	Yes
Lee et al.	2005	5	5	0	114	68	13	15	5	296	94	Yes
Lee et al.	2005	23	5	1	114	68	13	51	7	296	94	Yes
Lee et al.	2005	11	13	1	114	68	13	35	15	296	94	Yes
Lee et al.	2005	25	22	1	114	68	13	72	24	296	94	Yes
Lee et al.	2005	22	13	2	114	68	13	57	17	296	94	Yes
Lee et al.	2005	24	34	14	114	68	13	82	62	296	94	Yes
Shaw et al.	2005	69	68	16	180	202	52	206	100	562	306	Yes
Li et al.	2005	30	95	58	22	57	24	155	211	101	105	Yes
Storti et al.	2003	27	53	20	26	54	20	107	93	106	94	Yes
Group 2: mothers with CHD offspring vs. mother controls with healthy offspring												
Jiang et al.	2015	38	46	16	41	48	11	122	78	130	70	Yes
Shi et al.	2015	55	68	30	70	101	45	178	128	241	191	Yes
Elsayed et al.	2014	30	28	3	30	24	7	88	34	84	38	Yes
Kotby et al.	2012	12	16	2	20	10	1	40	20	50	12	Yes
Sánchez-Urbina et al.	2012	8	38	14	13	37	12	54	66	63	61	Yes
Van et al.	2008	91	117	22	111	104	36	299	161	326	176	Yes
Wintner et al.	2007	17	11	3	10	17	4	45	17	37	25	Yes
Zhu et al.	2006	6	27	23	20	57	25	39	73	97	107	Yes
Li et al.	2005	32	90	60	20	57	25	154	210	97	107	Yes
Storti et al.	2003	26	52	22	26	54	20	104	96	106	94	Yes
Group 3: father with CHD offspring vs. father controls with healthy offspring												
Wintner et al.	2007	17	11	3	14	14	3	45	17	42	20	Yes
Zhu et al.	2006	6	34	18	21	57	22	46	70	99	101	Yes
Li et al.	2005	25	102	52	21	57	22	152	206	99	101	Yes
Storti et al.	2003	22	60	18	26	54	20	104	96	106	94	Yes

Table 3. Quality score of each article

Study	Year	Selection				Comparability of the cases and controls	Exposure			Total Stars
		Adequate case definition	Representativeness of the cases	Selection of the controls	Definition of controls		Ascertainment of exposure	Same ascertainment method for cases and controls	Non-response rate	
Wang et al.	2016	*	*	-	*	*	*	*	-	6
Jiang et al.	2015	*	*	-	*	**	*	*	-	7
Li et al.	2015	*	*	-	*	*	*	-	-	5
Shi et al.	2015	*	*	-	*	**	*	-	-	6
Koshy et al.	2015	*	*	*	*	**	*	-	-	7
Elsayed et al.	2014	*	*	-	*	*	*	-	-	5
Sayin et al.	2014	*	*	-	*	*	*	*	-	6
Chao et al.	2014	*	*	-	*	**	*	*	-	7
Sahiner et al.	2014	*	*	-	*	**	*	*	-	7
Wang et al.	2013	*	*	-	*	*	*	-	-	5
Kotby et al.	2012	*	*	-	*	*	*	*	-	6
Gong et al.	2012	*	*	-	*	**	*	-	-	6
Sánchez-Urbina et al.	2012	*	*	*	*	**	*	*	-	8
Xu et al.	2010	*	*	-	*	**	*	*	-	7
Kuehl et al.	2010	*	*	-	-	*	*	*	-	5
Li et al.	2009	*	*	-	*	**	*	*	-	7
Van et al.	2008	*	*	-	*	**	*	-	-	6
Galdieri et al.	2007	*	*	-	*	**	*	-	-	6
Wintner et al.	2007	*	*	-	*	**	*	*	-	7
Zhu et al.	2006	*	*	*	*	**	*	*	-	8
Lee et al.	2005	*	*	-	*	**	*	*	-	7
Shaw et al.	2005	*	*	*	*	**	*	*	-	8
Li et al.	2005	*	*	*	*	**	*	*	-	8
Storti et al.	2003	*	*	-	*	**	*	*	-	7

Table 4. Results on the relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk in Group 1 (CHD patients vs. healthy controls)

	No. of study	T vs. C			TT vs. CC			TT+CT vs. CC			TT vs. CT+CC		
		OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)
Total	30	1.25 (1.06, 1.46)	0.006	<0.001	1.57 (1.14, 2.18)	0.006	<0.001	1.28 (1.05, 1.57)	0.015	<0.001	1.06 (0.81, 1.39)	0.663	<0.001
Ethnicity													
Caucasians	9	0.93 (0.75, 1.14)	0.461	0.035	1.15 (0.73, 1.80)	0.546	0.080	1.12 (0.85, 1.48)	0.418	0.361	0.69 (0.52, 0.91)	0.009	0.415
Asians	21	1.32 (1.07, 1.63)	0.008	<0.001	1.82 (1.19, 2.77)	0.005	<0.001	1.36 (1.04, 1.79)	0.027	<0.001	1.29 (0.94, 1.78)	0.117	<0.001
Number of cases													
>300	11	1.08 (0.88, 1.33)	0.107	<0.001	1.45 (0.93, 2.26)	0.105	<0.001	0.75 (0.54, 1.04)	0.192	<0.001	1.11 (0.78, 1.57)	0.562	<0.001
≤300	19	1.29 (1.04, 1.60)	0.022	<0.001	1.79 (1.17, 2.75)	0.007	0.019	1.35 (1.02, 1.78)	0.035	0.002	0.95 (0.62, 1.47)	0.828	0.001
Source of Control													
Hospital-based	18	1.26 (1.04, 1.53)	0.019	<0.001	1.59 (1.06, 2.38)	0.024	<0.001	1.32 (1.02, 1.69)	0.032	<0.001	1.09 (0.80, 1.47)	0.584	<0.001
Population-based	12	1.21 (0.90, 1.63)	0.199	0.001	1.53 (0.86, 2.72)	0.144	0.031	1.23 (0.85, 1.77)	0.278	0.008	0.93 (0.51, 1.70)	0.807	0.008
Type of CHD													
Conotruncal heart disease	5	0.85 (0.66, 1.08)	0.179	0.365	0.67 (0.36, 1.24)	0.201	0.498	0.83 (0.60, 1.15)	0.261	0.396	0.54 (0.31, 0.92)	0.023	0.135
Patent ductusarteriosus	3	1.50 (1.02, 2.21)	0.039	0.489	2.07 (0.86, 4.96)	0.102	0.437	1.57 (0.88, 2.80)	0.127	0.384	1.43 (0.74, 2.75)	0.284	0.550
Transposition of great artery	2	1.61 (1.16, 2.23)	0.005	0.398	2.80 (1.40, 5.60)	0.004	0.372	1.83 (1.09, 3.08)	0.023	0.407	1.59 (0.89, 2.83)	0.117	0.659
Venricularseptal defect	2	0.80 (0.65, 0.98)	0.034	0.122	0.67 (0.44, 1.03)	0.069	0.578	0.76 (0.56, 1.03)	0.074	0.715	0.64 (0.44, 0.93)	0.019	0.102
Atrial septal defect	3	1.28 (0.95, 1.73)	0.109	0.179	1.40 (0.75, 2.63)	0.290	0.168	1.20 (0.77, 1.87)	0.422	0.051	1.34 (0.81, 2.22)	0.254	0.704
Coarctation of the aorta	2	1.87 (1.25, 2.79)	0.002	0.840	3.44 (1.42, 8.37)	0.006	0.903	2.89 (1.50, 5.55)	0.001	0.981	1.13 (0.53, 2.41)	0.751	0.983
Others	13	1.39 (1.10, 1.75)	0.006	<0.001	1.93 (1.22, 3.06)	0.005	<0.001	1.46 (1.07, 1.99)	0.015	<0.001	1.21 (0.86, 1.70)	0.275	<0.001

Table 5. Relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk in Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring)

	No. of study	T vs. C			TT vs. CC			TT+CT vs. CC			TT vs. CT+CC		
		OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)
Total	10	1.08 (0.95, 1.23)	0.232	0.193	1.09 (0.83, 1.43)	0.521	0.240	1.70 (0.93, 3.12)	0.087	<0.001	1.11 (0.88, 1.39)	0.384	0.198
Ethnicity													
Caucasians	6	1.14 (0.95, 1.37)	0.809	0.267	0.89 (0.60, 1.33)	0.561	0.426	3.20 (1.36, 7.52)	0.008	<0.001	0.84 (0.59, 1.19)	0.322	0.515
Asians	4	1.02 (0.86, 1.22)	0.145	0.157	1.31 (0.90, 1.90)	0.156	0.183	0.82 (0.50, 1.34)	0.421	0.030	1.36 (1.01, 1.85)	0.046	0.262
Number of cases													
>300	4	1.02 (0.87, 1.20)	0.783	0.608	0.96 (0.69, 1.33)	0.125	0.492	1.55 (0.64, 3.77)	0.329	<0.001	0.99 (0.75, 1.32)	0.965	0.174
≤300	6	1.20 (0.97, 1.49)	0.098	0.102	1.47 (0.90, 2.39)	0.787	0.209	1.92 (0.76, 4.84)	0.169	<0.001	1.39 (0.93, 2.08)	0.112	0.368
Source of Control													
Hospital-based	8	1.01 (0.88, 1.17)	0.862	0.374	0.94 (0.69, 1.28)	0.697	0.494	2.30 (1.19, 4.47)	0.014	<0.001	0.92 (0.70, 1.21)	0.567	0.541
Population-based	2	1.38 (1.04, 1.82)	0.023	0.291	1.90 (1.05, 3.45)	0.034	0.280	0.58 (0.37, 0.90)	0.015	0.761	1.72 (1.12, 2.62)	0.014	0.441

Table 6. Results on the relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk in Group 3 (father with CHD offspring vs. father controls with healthy offspring)

Genetic model	OR (95% CI)	*P*	*P* (Q-test)
T vs. C	1.21 (0.98, 1.51)	0.081	0.428
TT vs. CC	3.92 (2.50, 6.15)	<0.001	0.908
TT+CT vs. CC	1.39 (0.95, 2.03)	0.087	0.337
TT vs. CT+CC	1.27 (0.88, 1.84)	0.201	0.635

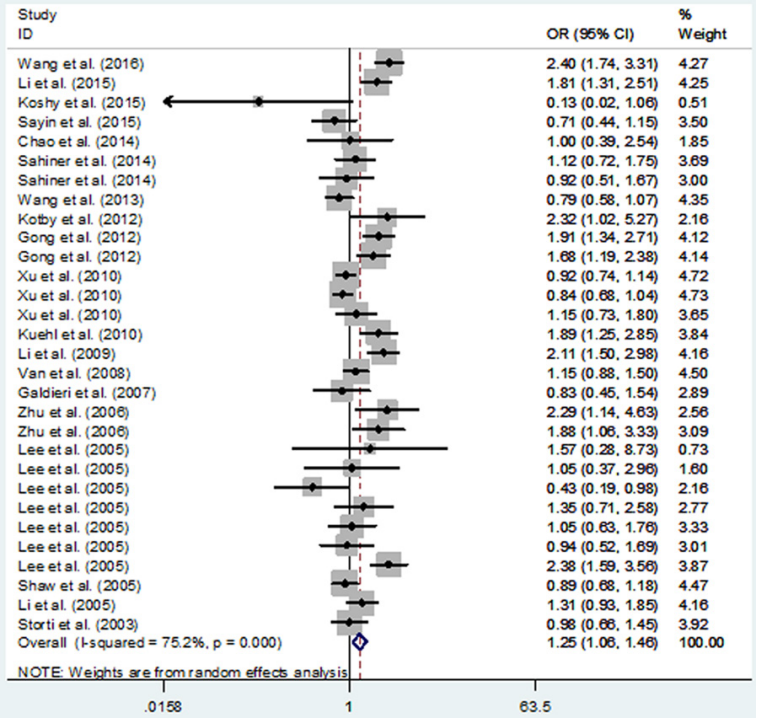

Figure 2. Random effect forest plot of allele model (T vs. C) for the relationship between the *MTHFR* rs1801133 C>T polymorphism and CHD risk: Group 1 (CHD patients vs. healthy controls).

by the STATA version 12.0 software (Stata Corp., College Station, TX, USA).

Results

Characteristics of studies

A total of 836 articles were initially identified based on the search strategy. Based on the inclusion and exclusion criteria, 44 studies case-control studies in 24 articles [4, 31-52] were included in our meta-analysis. Of the 812 excluded studies, 155 were duplicate publications; 543 were not relevant to *MTHFR* rs1801133 C>T polymorphism and CHD risk; 57 were reviews and meta-analyses; 18 were comments; 15 were not case-control studies; data was unavailable in 11 articles; seven were case reports; and one was letter. The flow chart is outlined in **Figure 1**.

In 44 studies, Group 1 included 30 studies in 19 articles [4, 31-48], Group 2 included 10 studies in 10 articles [4, 38, 43, 47-53], and Group 3 included 4 studies in 4 articles [4, 47, 48, 53]. **Table 1** showed the characteristics of all included studies. **Table 2** listed the genotype distributions among cases and controls in all eligible studies. The results of quality score for each article are shown in **Table 3**.

Main results of the overall analyses

In **Table 4**, **Table 5** and **Table 6**, the association between *MTHFR* rs1801133 C>T polymorphism and CHD risk is listed. **Table 4** is about Group 1, **Table 5** about Group 2, and **Table 6** about Group 3.

Overall, Group 1 (CHD patients vs. healthy controls): rs1801133 TT genotype and T allele increased CHD risk significantly in three genetic models [T vs. C (OR, 1.25; 95% CI, 1.06-1.46; *P* = 0.006), TT vs. CC (OR, 1.57; 95% CI, 1.14-2.18; *P* = 0.006), TT+CT vs. CC (OR, 1.28; 95% CI, 1.05-1.57; *P* = 0.015), **Table 4**, **Figure 2**]. Group 3 (father with CHD offspring vs. father controls with healthy offspring): the *MTHFR* rs1801133 TT genotype augmented CHD risk significantly in the homozygote model (TT vs. CC: OR, 3.92; 95% CI, 2.50-6.15; *P*<0.001; **Table 6** and **Figure 3**). Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring): No relationship was identified between MTHFR rs1801133 C>T polymorphism and CHD risk in Group 2 (**Table 5** and **Figure 4**).

Subgroup analyses by ethnicity

Group 1 (CHD patients vs. healthy controls): we drew a conclusion that rs1801133 TT genotype and T allele caused raised CHD incidence

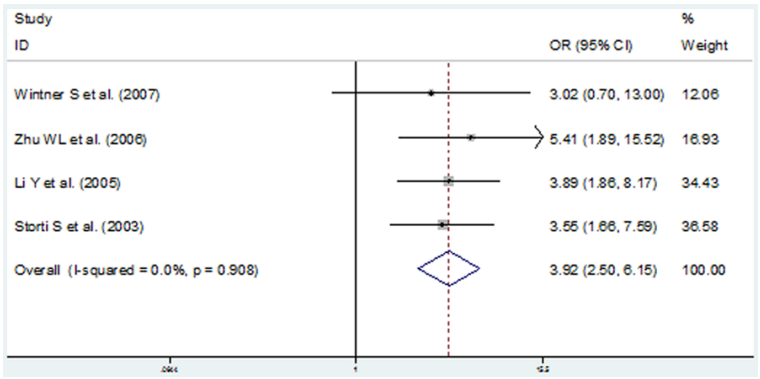

Figure 3. Fixed effect forest plot of homozygote model (TT vs. CC) for the relationship between the *MTHFR* rs1801133 C>T polymorphism and CHD risk: Group 3 (father with CHD offspring vs. father controls with healthy offspring).

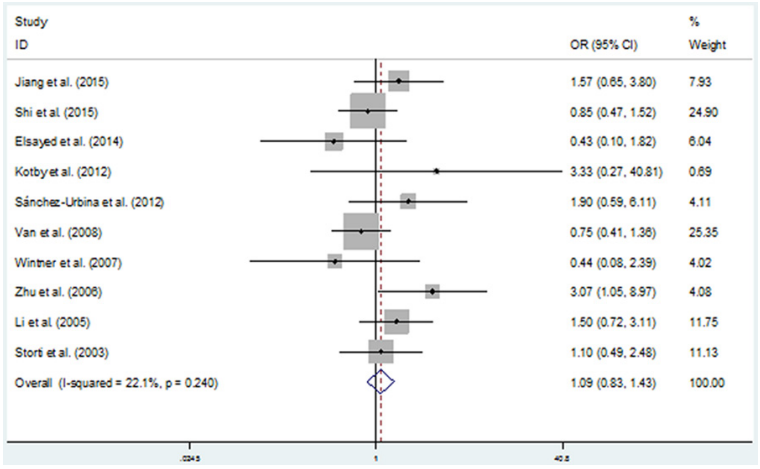

Figure 4. Fixed effect forest plot of homozygote model (TT vs. CC) for the relationship between the *MTHFR* rs1801133 C>T polymorphism and CHD risk: Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring).

in Asians in three genetic models [T vs. C (OR, 1.32; 95% CI, 1.07-1.63; *P* = 0.008), TT vs. CC (OR, 1.82; 95% CI, 1.19-2.77; *P* = 0.005), TT+CT vs. CC (OR, 1.36; 95% CI, 1.04-1.79; *P* = 0.027), **Table 4**, **Figure 5**. However, the rs1801133 TT genotype reduced CHD risk in Caucasus [TT vs. CT+CC: OR, 0.69; 95% CI, 0.52-0.91, *P* = 0.009, **Table 4** and **Figure 6**).

Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring): the mothers' *MTHFR* rs1801133 TT genotype of Caucasus might increase offspring CHD morbidity (TT+CT vs. CC: OR, 3.20; 95% CI, 1.36-7.52; *P* = 0.008, **Table 5** and **Figure 7**). And the mother's *MTHFR* rs1801133 TT genotype in Asians might increase offspring CHD morbidity (TT vs. CT+ CC: OR, 1.36; 95% CI, 1.01-1.85; *P* = 0.046, **Table 5** and **Figure 8**).

Subgroup analyses by the type of CHD

Group 1 (CHD patients vs. healthy controls): In allele model, *MTHFR* rs1801133 T allele was related to increased CHD risk in the following types of CHD: PDA (OR: 1.50, 95% CI: 1.02-2.21, *P* = 0.039); TGA (OR: 1.61; 95% CI: 1.16-2.23, P = 0.005); CoA (OR: 1.87; 95% CI: 1.25-2.79, *P* = 0.002) and other CHD type (OR: 1.39; 95% CI: 1.10-1.75). But it related to decreased VSA risk (OR, 0.80; 95% CI: 0.65-0.98, *P* = 0.034). In homozygote model, *MTHFR* rs1801133 TT polymorphism was related to increased CHD risk in the following types of CHD: TGA (OR: 2.80; 95% CI: 1.40-5.60, *P* = 0.004); CoA (OR: 3.44; 95% CI: 1.42-8.37, *P* = 0.006); and other CHD type (OR: 1.93; 95% CI: 1.22-3.06, *P* = 0.005). Similarly in dominant model, *MTHFR* rs1801133 TT polymorphism was related to increased CHD risk in the following types of CHD: TGA (OR: 1.83; 95% CI: 1.09-3.08, *P* = 0.023); CoA (OR: 2.89; 95% CI: 1.50-5.55, *P* = 0.001) and other CHD type (OR: 1.46; 95% CI: 1.07-1.99, *P* = 0.015). However, in recessive model, *MTHFR* rs1801133 TT polymorphism was related to decreased CHD risk in the following types of CHD: conotruncal heart disease (OR: 0.54; 95% CI: 0.31-0.92, *P* = 0.023) and VSD (OR: 0.64; 95% CI: 0.44-0.93, *P* = 0.019). The results are shown in **Table 4**.

Publication bias

Begg's Funnel plot and Egger's test were performed to detect the publication bias of the studies. The funnel plot shape and Egger's test showed no proofs of publication bias (data not shown).

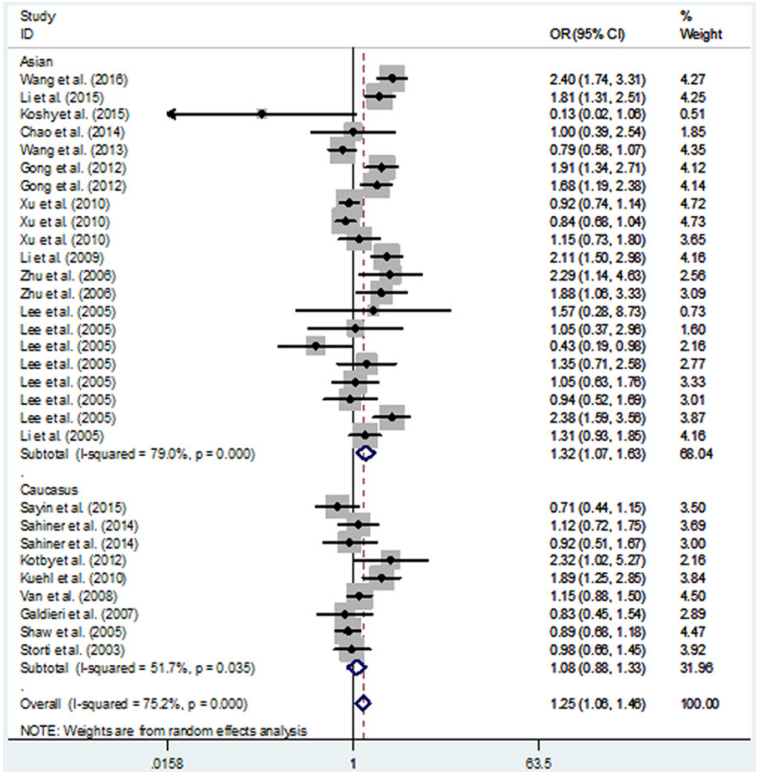

Figure 5. The relationship between the *MTHFR* rs1801133 C>T polymorphism and CHD risk in Asians: Group 1 (CHD patients vs. healthy controls), random effect model for T vs. C.

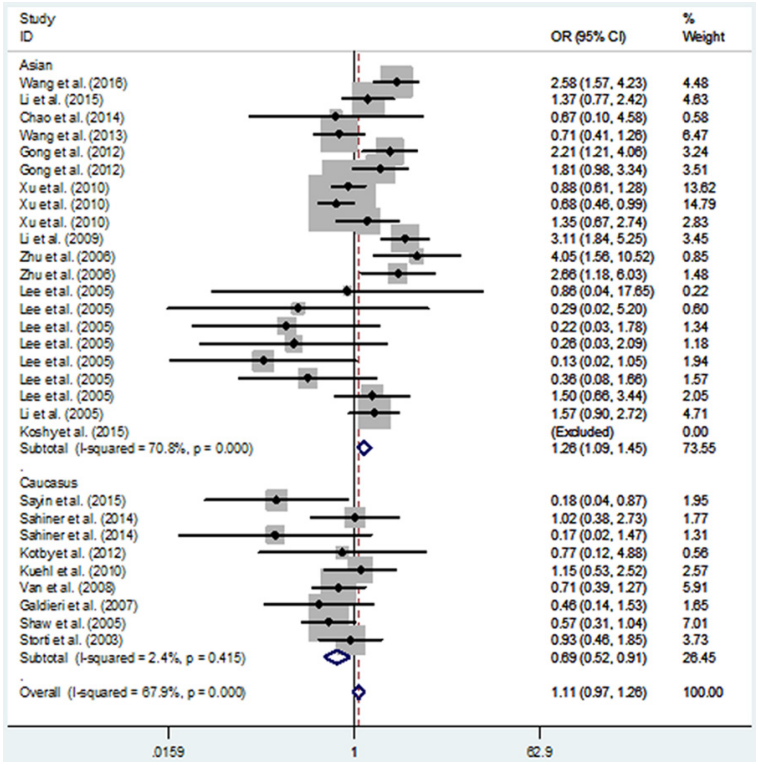

Figure 6. The relationship between the *MTHFR* rs1801133 C>T polymorphism and CHD risk in Caucasians: Group 1 (CHD patients vs. healthy controls), fixed effect model for TT vs. CC+CT.

Sensitivity analyses

Sensitivity analyses were conducted to evaluate theprimary origin of the heterogeneity. No independent study included affected the heterogeneity in Group 1 (**Figure 9**) and Group 2 (**Figure 10**). The data are not shown.

Heterogeneity test

Group 1 (CHD patients vs. healthy controls): the results indicated that Asian population, number of cases (>300 and ≤300), source of control (hospital and population based), and type of CHD (others subgroup) may contribute to the prime heterogeneities (**Table 4**).

Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring): the prime heterogeneities were derived from ethnicity (Asians and Caucasus), number of cases (>300 and ≤300), and hospital-based subgroup (**Table 5**).

Discussion

MTHFR is a critical enzyme in folic acid transformation process, and its activity may be associated with some diseases including CHD [54, 55]. In 1999, Kapusta *et al*. first reported that maternal hyperhomocysteinaemia was related to an increased risk of CHD [56]. More recently, Hobbs *et al*. identified that homocys teinamia, S-adenosylhomocysteine, and methionine were the most important predictive bio-

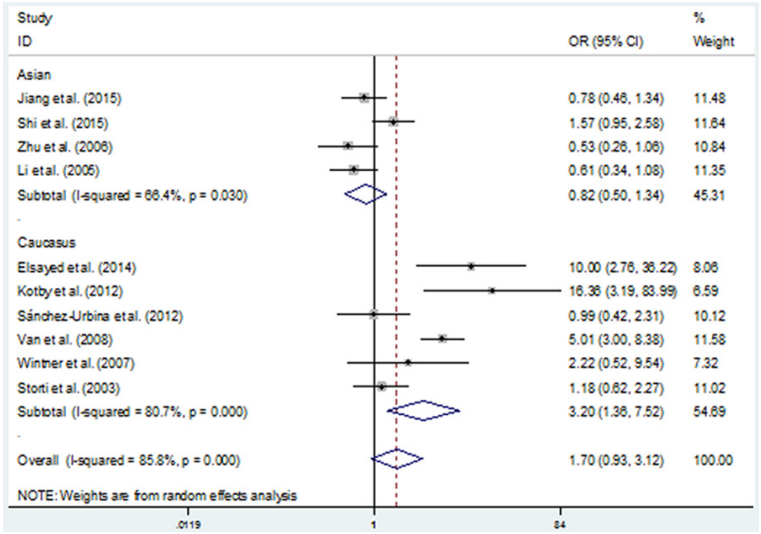

Figure 7. The relationship between the mothers' *MTHFR* rs1801133 C>T polymorphism and CHD risk in Caucasians: Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring), random effect model for TT+CT vs. CC.

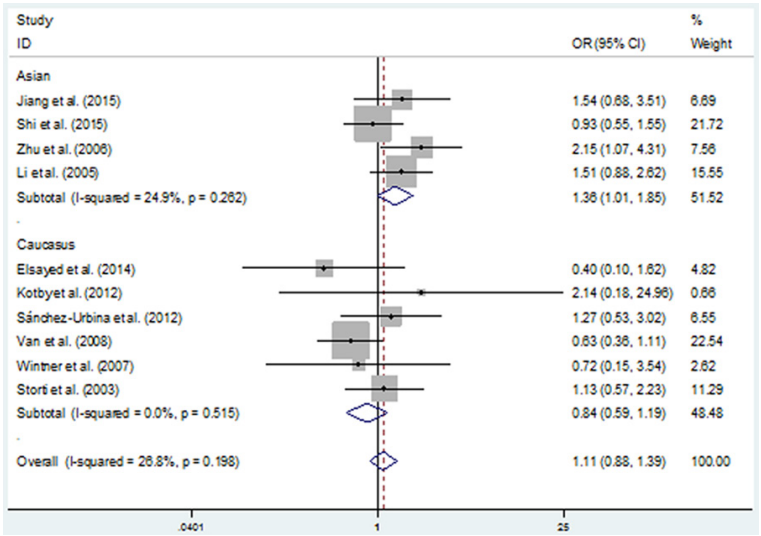

Figure 8. The relationship between the mothers' *MTHFR* rs1801133 C>T polymorphism and CHD risk in Asians: Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring), fixed effect model for TT vs. CT+CC.

markers in mothers with pregnancies affected by CHD [57]. And specifically, MTHFR protein is an essential enzyme in homocysteinemia metabolism. Therefore, the polymorphisms of *MTHFR* gene may regulate the activity of MTHFR and then may be an important decisive factor of CHD genesis and development. A number of studies have reported the possible correlations between *MTHFR* rs1801133 C>T polymorphism and CHD; nevertheless, the results were not consistent [44, 58]. Our current meta-analysis could more comprehensively evaluate the relationship between *MTHFR* rs1801133 C>T polymorphism and susceptibility of CHD from three respects. As far as we know, this is the first meta-analysis on the relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk including father's factor in CHD pathogeny. Our findings indicated that rs1801133 TT genotype and T allele increased CHD risk significantly in Group 1 and Group 3.

The homozygous TT and heterozygous CT genotypes were associated with increased Hcy concentration and decreased MTHFR enzyme concentration. Frosst *et al*. have reported that a C→T transition at nucleotide 677 on *MTHFR* gene led to the enzyme thermolabile, lowered its activity, and raised Hcy concentration [59]. Hcy has been identified to embryotoxic effects on myocardial cells in animal models [60, 61]. Studies have indicated that abnormal folic acid and Hcy metabolism influenced neural crest cells development and migration, which caused malalignment of outflow tract and defect in trunco-conal septum and resulted in CHD [62]. Results of our meta-analysis indicated that *MTHFR* rs1801133 C>T polymorphism of fetus or children was obviously related to CHD in all genetic models. The homozygosity prevalence of the polymorphism is reported to be from 5% to 16% in different ethnicities, which may explain the different incidence of CHD in different ethnicity. In our meta-analysis, further stratified analysis by ethnicity showed that *MTHFR* rs1801133 C>T polymorphism of fetus or children was intimately correlated to

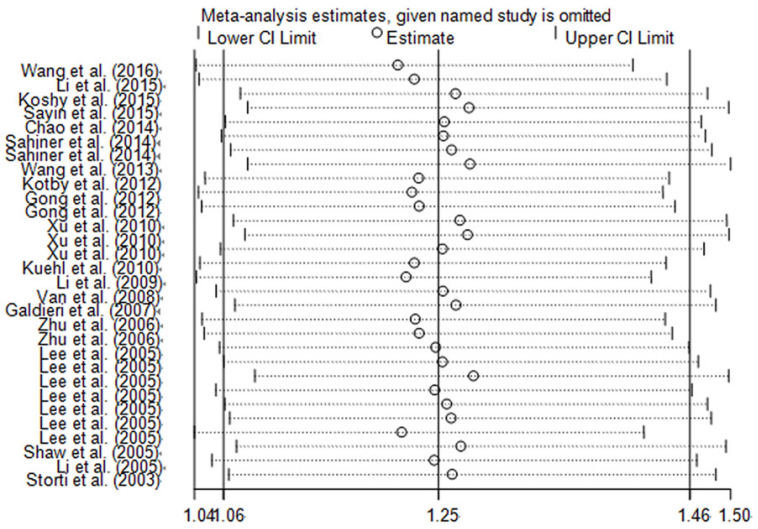

Figure 9. Sensitivity analysis for the relationship between the *MTHFR* rs1801133 C>T polymorphism and CHD risk: Group 1 (CHD patients vs. healthy controls) (C vs. T).

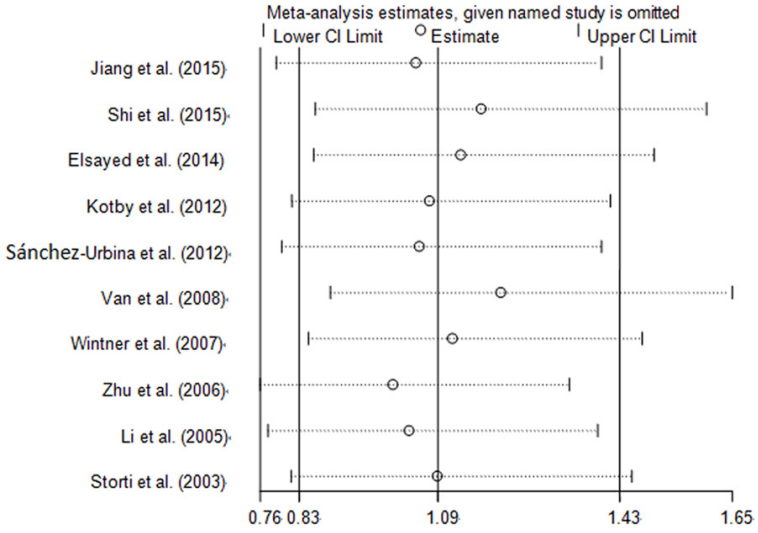

Figure 10. Sensitivity analysis for the relationship between the *MTHFR* rs1801133 C>T polymorphism and CHD risk: Group 2 (mothers with CHD offspring vs. mother controls with healthy offspring) (CC vs. TT).

increased CHD risk in Asian population, but decreased CHD risk in Caucasian population. The opposite result in Caucasian population may be due to relatively small number of including studies. What's more, the differences of environment in different regions might contribute to the distinction of *MTHFR* rs1801133 C>T polymorphism impact.

Several studies had indicated that mothers who got mutations of *MTHFR* rs1801133 C>T polymorphism had increased risk of CHD children [50, 51, 63]. So we also conducted meta-analysis on this subject. Our results indicated that mothers' *MTHFR* rs1801133 TT genotype might increase offspring CHD morbidity, both in Asians and Caucasus. Kapusta et al. first described a significant relationship between higher maternal median fasting Hcy levels and the incidence of CHD in their progeny compared to the control subjects [56].

Li Y *et al*. suggested that paternal combinative gene of *MTHFR* and cystathionine β-synthase (CBS) could raise CHD risk [47]. There were only four studies about the relationship between paternal *MTHFR* rs1801133 C>T polymorphism and CHD risk. This is first meta-analysis to simultaneously focus on this subject. We found that the *MTHFR* rs1801133 TT genotype augmented CHD risk significantly.

Although we drew these conclusions, there were several limitations in this meta-analysis. First, we only discussed the relationship between *MTHFR* rs1801133 C>T polymorphism and CHD risk. But we did not consider other polymorphisms of *MTHFR* gene, other genes, and environmental factors, such as folic acid, smoking, and drinking. Second, there was relatively small sample in several subgroup stratified by the types of CHD. This might not provide enough power to assess association between *MTHFR* rs1801133 C>T polymorphism and CHD risk. Third, we only included the published articles. Thus, publication bias might be not avoided. Forth, significant heterogeneities in our meta-analysis were found in Group 1 and Group 2. Given these results, further investigations in these areas are needed, so our conclusions of meta-analysis should be interpreted cautiously.

In conclusion, *MTHFR* rs1801133 C>T polymorphism may be related to CHD risk from three respects of children, mother and father. In order to achieve more convincible conclusion, further analyses including larger sample size and adjusted individual data were required, and further investigation of mechanism should also be performed.

Acknowledgements

A grant number of Natural Science Foundation of Fujian Province of China (2014J06018).

Disclosure of conflict of interest

None.

Abbreviations

CHD, congenital heart disease; CI, confidence interval; OR, odds ratio; MTHFR, methylenetetrahydrofolatereductase; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism; PDA, patent ductusarteriosus; TGA, transposition of great artery; VSD, ventricular septal defect; ASD, atrial septal defect; CoA, coarctation of the aorta.

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References

[1] Christensen KE, Zada YF, Rohlicek CV, Andelfinger GU, Michaud JL, Bigras JL, Richter A, Dube MP and Rozen R. Risk of congenital heart defects is influenced by genetic variation in folate metabolism. Cardiol Young 2013; 23: 89-98.

[2] Lage K, Greenway SC, Rosenfeld JA, Wakimoto H, Gorham JM, Segre AV, Roberts AE, Smoot LB, Pu WT, Pereira AC, Mesquita SM, Tommerup N, Brunak S, Ballif BC, Shaffer LG, Donahoe PK, Daly MJ, Seidman JG, Seidman CE and Larsen LA. Genetic and environmental risk factors in congenital heart disease functionally converge in protein networks driving heart development. Proc Natl Acad Sci U S A 2012; 109: 14035-14040.

[3] Kappagoda CT and Macartney FJ. Effect of environmental temperatures on oxygen consumption in infants with congenital disease of the heart. Br Heart J 1976; 38: 1-4.

[4] Zhu WL, Li Y, Yan L, Dao J and Li S. Maternal and offspring MTHFR gene C677T polymorphism as predictors of congenital atrial septal defect and patent ductus arteriosus. Mol Hum Reprod 2006; 12: 51-54.

[5] Bosi G, Scorrano M, Tosato G, Forini E and Chakrokh R. The Italian multicentric study on epidemiology of congenital heart disease: first step of the analysis. Working party of the Italian society of pediatric cardiology. Cardiol Young 1999; 9: 291-299.

[6] Caputo S, Russo MG, Capozzi G, Morelli C, Argiento P, Di Salvo G, Sarubbi B, Santoro G, Pacileo G and Calabro R. Congenital heart disease in a population of dizygotic twins: an echocardiographic study. Int J Cardiol 2005; 102: 293-296.

[7] Seliem MA, Bou-Holaigah IH and Al-Sannaa N. Influence of consanguinity on the pattern of familial aggregation of congenital cardiovascular anomalies in an outpatient population: studies from the eastern province of Saudi Arabia. Community Genet 2007; 10: 27-31.

[8] Xuan C, Li H, Zhao JX, Wang HW, Wang Y, Ning CP, Liu Z, Zhang BB, He GW and Lun LM. Association between MTHFR polymorphisms and congenital heart disease: a meta-analysis based on 9,329 cases and 15,076 controls. Sci Rep 2014; 4: 7311.

[9] WJ L. Human embryology. New York: Churchill Livingstone, Inc., 1993.

[10] Fenech M. Folate (vitamin B9) and vitamin B12 and their function in the maintenance of nuclear and mitochondrial genome integrity. Mutat Res 2012; 733: 21-33.

[11] Barua S, Kuizon S and Junaid MA. Folic acid supplementation in pregnancy and implications in health and disease. J Biomed Sci 2014; 21: 77.

[12] Hou Z and Matherly LH. Biology of the major facilitative folate transporters SLC19A1 and SLC46A1. Curr Top Membr 2014; 73: 175-204.

[13] Martinez-Frias ML. The biochemical structure and function of methylenetetrahydrofolate reductase provide the rationale to interpret the epidemiological results on the risk for infants with down syndrome. Am J Med Genet A 2008; 146A: 1477-1482.

[14] Botto LD, Mulinare J and Erickson JD. Occurrence of congenital heart defects in relation to maternal mulitivitamin use. Am J Epidemiol 2000; 151: 878-884.

[15] Botto LD, Mulinare J and Erickson JD. Do multivitamin or folic acid supplements reduce the risk for congenital heart defects? Evidence and gaps. Am J Med Genet A 2003; 121A: 95-101.

[16] van Beynum IM, Kapusta L, Bakker MK, den Heijer M, Blom HJ and de Walle HE. Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case-control study in the Northern Netherlands. Eur Heart J 2010; 31: 464-471.

[17] Czeizel AE and Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992; 327: 1832-1835.

[18] Czeizel AE. Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. Am J Med Genet 1996; 62: 179-183.

[19] Czeizel AE, Dobo M and Vargha P. Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. Birth Defects Res A Clin Mol Teratol 2004; 70: 853-861.

[20] Czeizel AE, Toth M and Rockenbauer M. Population-based case control study of folic acid supplementation during pregnancy. Teratology 1996; 53: 345-351.

[21] Czeizel AE, Rockenbauer M, Siffel C and Varga E. Description and mission evaluation of the Hungarian case-control surveillance of congenital abnormalities, 1980-1996. Teratology 2001; 63: 176-185.

[22] Warkany J, Beaudry PH and Hornstein S. Attempted abortion with aminopterin (4-aminopteroylglutamic acid); malformations of the child. AMA J Dis Child 1959; 97: 274-281.

[23] Wenstrom KD, Johanning GL, Johnston KE and DuBard M. Association of the C677T methylenetetrahydrofolate reductase mutation and elevated homocysteine levels with congenital cardiac malformations. Am J Obstet Gynecol 2001; 184: 806-812; discussion 812-807.

[24] Li Z, Jun Y, Zhong-Bao R, Jie L and Jian-Ming L. Association between MTHFR C677T polymorphism and congenital heart disease. A family-based meta-analysis. Herz 2015; 40 Suppl 2: 160-167.

[25] Wang W, Hou Z, Wang C, Wei C, Li Y and Jiang L. Association between 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms and congenital heart disease: a meta-analysis. Meta Gene 2013; 1: 109-125.

[26] Tang W, Yu P, Wang Y, Kang M, Sun B, Yin J and Gu H. Lack of association between cyclin D1 A870G (rs9344) polymorphism and esophageal squamous cell carcinoma risk: case-control study and meta-analysis. Int J Clin Exp Med 2015; 8: 12685-12695.

[27] Tang W, Qiu H, Ding H, Sun B, Wang L, Yin J and Gu H. Association between the STK15 F31I polymorphism and cancer susceptibility: a meta-analysis involving 43,626 subjects. PLoS One 2013; 8: e82790.

[28] Tang W, Wang Y, Jiang H, Liu P, Liu C, Gu H, Chen S and Kang M. Programmed death-1 (PD-1) rs2227981 C>T polymorphism is associated with cancer susceptibility: a meta-analysis. Int J Clin Exp Med 2015; 8: 22278-22285.

[29] Wang Y, Jiang H, Liu T, Tang W and Ma Z. Cyclooxygenase-2 -1195G>A (rs689466) polymorphism and cancer susceptibility: an updated meta-analysis involving 50,672 subjects. Int J Clin Exp Med 2015; 8: 12448-12462.

[30] Tang W, Wang Y, Jiang H, Liu C, Dong C, Chen S, Kang M and Gu H. Insulin receptor substrate-1 (IRS-1) rs1801278G>A polymorphism is associated with polycystic ovary syndrome susceptibility: a meta-analysis. Int J Clin Exp Med 2015; 8: 17451-17460.

[31] Wang Y, Zhang H, Yue S, Zhang K, Wang H, Dong R, Yang X, Liu Y and Ma Y. Evaluation of high resolution melting for MTHFR C677T genotyping in congenital heart disease. PLoS One 2016; 11: e0151140.

[32] Li D, Yu K, Ma Y, Liu Y and Ji L. [Correlationship between congenital heart disease and polymorphism of MTHFR gene]. Wei Sheng Yan Jiu 2015; 44: 933-938.

[33] Koshy T, Venkatesan V, Perumal V, Hegde S and Paul SF. The A1298C methylenetetrahydrofolate reductase gene variant as a susceptibility gene for non-syndromic conotruncal heart defects in an indian population. Pediatr Cardiol 2015; 36: 1470-1475.

[34] Sayin Kocakap BD, Sanli C, Cabuk F, Koc M and Kutsal A. Association of MTHFR A1298C polymorphism with conotruncal heart disease. Cardiol Young 2015; 25: 1326-1331.

[35] Chao CS, Wei J, Huang HW and Yang SC. Correlation between methyltetrahydrofolate reductase (MTHFR) polymorphisms and isolated patent ductus arteriosus in Taiwan. Heart Lung Circ 2014; 23: 655-660.

[36] Sahiner UM, Alanay Y, Alehan D, Tuncbilek E and Alikasifoglu M. Methylene tetrahydrofolate reductase polymorphisms and homocysteine level in heart defects. Pediatr Int 2014; 56: 167-172.

[37] Wang B, Liu M, Yan W, Mao J, Jiang D, Li H and Chen Y. Association of SNPs in genes involved in folate metabolism with the risk of congenital heart disease. J Matern Fetal Neonatal Med 2013; 26: 1768-1777.

[38] Kotby A, El-Masry, Awady M, El-Nashar, Meguid NA. Genetic variants in the methylenetetrahydrofolate reductase gene in Egyptian children with conotruncal heart defects and their mothers. Macedonian Journal of Medical Sciences 2012; 5: 78-84.

[39] Gong D, Gu H, Zhang Y, Gong J, Nie Y, Wang J, Zhang H, Liu R, Hu S and Zhang H. 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 2012; 50: 1455-1461.

[40] Xu J, Xu X, Xue L, Liu X, Gu H, Cao H, Qiu W, Hu Z, Shen H and Chen Y. MTHFR c.1793G>A polymorphism is associated with congenital cardiac disease in a Chinese population. Cardiol Young 2010; 20: 318-326.

[41] Kuehl K, Loffredo C, Lammer EJ, Iovannisci DM and Shaw GM. Association of congenital cardiovascular malformations with 33 single nucleotide polymorphisms of selected cardiovascular disease-related genes. Birth Defects Res A Clin Mol Teratol 2010; 88: 101-110.

[42] Li D, Jing XA, Wang HY, Ye WJ and Fan H. [Study of correlationship between congenital heart disease and 5,10-methylenetetra hydrofolate reductase gene's polymorphism or folacin intakes]. Zhonghua Yu Fang Yi Xue Za Zhi 2009; 43: 700-704.

[43] van Driel LM, Verkleij-Hagoort AC, de Jonge R, Uitterlinden AG, Steegers EA, van Duijn CM, Steegers-Theunissen RP. Two MTHFR polymorphisms, maternal B-vitamin intake, and CHDs. Birth Defects Res A Clin Mol Teratol 2008; 82:6: 474-481.

[44] Galdieri LC, Arrieta SR, Silva CM, Pedra CA and D'Almeida V. Homocysteine concentrations and molecular analysis in patients with congenital heart defects. Arch Med Res 2007; 38: 212-218.

[45] Lee CN, Su YN, Cheng WF, Lin MT, Wang JK, Wu MH and Hsieh FJ. Association of the C677T methylenetetrahydrofolate reductase mutation with congenital heart diseases. Acta Obstet Gynecol Scand 2005; 84: 1134-1140.

[46] Shaw GM, Iovannisci DM, Yang W, Finnell RH, Carmichael SL, Cheng S and Lammer EJ. Risks of human conotruncal heart defects associated with 32 single nucleotide polymorphisms of selected cardiovascular disease-relatedgenes. Am J Med Genet A 2005; 138: 21-26.

[47] Li Y, Cheng J, Zhu WL, Dao JJ, Yan LY, Li MY and Li SQ. [Study of serum Hcy and polymorphisms of Hcy metabolic enzymes in 192 families affected by congenital heart disease]. Beijing Da Xue Xue Bao 2005; 37: 75-80.

[48] Storti S, Vittorini S, Iascone MR, Sacchelli M, Collavoli A, Ripoli A, Cocchi G, Biagini A and Clerico A. Association between 5,10-methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and conotruncal heart defects. Clin Chem Lab Med 2003; 41: 276-280.

[49] Jiang Y, Mei J, Zhang W, Qian X, Zhang S, Liu C and Yang H. [Correlation between offspring congenital heart disease and MTHFR 677C/T polymorphism and general status of pregnant women]. Zhonghua Liu Xing Bing Xue Za Zhi 2015; 36: 1072-1076.

[50] Shi H, Yang S, Liu Y, Huang P, Lin N, Sun X, Yu R, Zhang Y, Qin Y and Wang L. Study on environmental causes and SNPs of MTHFR, MS and CBS genes related to congenital heart disease. PLoS One 2015; 10: e0128646.

[51] Elsayed GM, Elsayed SM, Ezz-Elarab SS. Maternal MTHFR C677T genotype and septal defects in offspring with Down syndrome: a pilot study. Egyptian Journal of Medical Human Genetics 2014; 15: 39-44.

[52] Sanchez-Urbina R, Galaviz-Hernandez C, Sierra-Ramirez JA, Rangel-Villalobos H, Torres-Saldua R, Alva-Espinoza C, Ramirez-Duenas Mde L, Garcia-Cavazos R and Arambula-Meraz E. Methylenetetrahydrofolate reductase gene 677CT polymorphism and isolated congenital heart disease in a Mexican population. Rev Esp Cardiol (Engl Ed) 2012; 65: 158-163.

[53] Wintner S, Hafner E, Stonek F, Stuempflen I, Metzenbauer M and Philipp K. Association of congenital cardiac defects and the C677T methylenetetrahydrofolate reductase polymorphism. Prenat Diagn 2007; 27: 704-708.

[54] Li R, Wang R, Li Y, Li X, Feng Y, Li Y and Jiang C. Association study on MTHFR polymorphisms and meningioma in northern China. Gene 2013; 516: 291-293.

[55] Long S, Yang X, Liu X and Yang P. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and susceptibility for cervical lesions: a meta-analysis. PLoS One 2012; 7: e52381.

[56] Kapusta L, Haagmans ML, Steegers EA, Cuypers MH, Blom HJ and Eskes TK. Congenital heart defects and maternal derangement of homocysteine metabolism. J Pediatr 1999; 135: 773-774.

[57] Hobbs CA, Cleves MA, Melnyk S, Zhao W and James SJ. Congenital heart defects and abnormal maternal biomarkers of methionine and homocysteine metabolism. Am J Clin Nutr 2005; 81: 147-153.

[58] Balderrabano-Saucedo NA, Sanchez-Urbina R, Sierra-Ramirez JA, Garcia-Hernandez N, Sanchez-Boiso A, Klunder-Klunder M, Arenas-Aranda D, Bravo-Hernandez G, Noriega-Zapata P and Vizcaino-Alarcon A. Polymorphism 677C → T MTHFR gene in Mexican mothers of children with complex congenital heart disease. Pediatr Cardiol 2013; 34: 46-51.

[59] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M,

Kluijtmans LA, van den Heuvel LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-113.

[60] Burgoon JM, Selhub J, Nadeau M and Sadler TW. Investigation of the effects of folate deficiency on embryonic development through the establishment of a folate deficient mouse model. Teratology 2002; 65: 219-227.

[61] Miller PN, Pratten MK and Beck F. Growth of 9.5-day rat embryos in folic-acid-deficient serum. Teratology 1989; 39: 375-385.

[62] Yelbuz TM, Waldo KL, Kumiski DH, Stadt HA, Wolfe RR, Leatherbury L and Kirby ML. Shortened outflow tract leads to altered cardiac looping after neural crest ablation. Circulation 2002; 106: 504-510.

[63] van Driel LM, Verkleij-Hagoort AC, de Jonge R, Uitterlinden AG, Steegers EA, van Duijn CM, Steegers-Theunissen RP. Two MTHFR polymorphisms, maternal B-vitamin intake, and CHDs. Birth Defects Res A Clin Mol Teratol 2008; 82: 474-481.