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Association between 5, 10-methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms and congenital heart disease: A meta-analysis ☆

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ABSTRACT

Background: Inconsistent results were reported in recent literature regarding the association between methylenetetrahydrofolate reductase (MTHFR) C677T/A1298C polymorphisms and the susceptibility of congenital heart disease (CHD). In this study, we performed a meta-analysis to investigate the associations by employing multiple analytical methods.
Methods: Literature search was performed and published articles were obtained from PubMed, Embase and CNKI databases based on the exclusion and inclusion criteria. Data were extracted from eligible studies and the crude odds ratios and their corresponding 95% confidence intervals (CIs) were calculated using random or fix effects model to evaluate the associations between the MTHFR C677T/A1298C polymorphisms and CHD development. Subgroup based analysis was performed by Hardy–Weinberg equilibrium, ethnicity, types of CHD, source of control and sample size.
Results: Twenty-four eligible studies were included in this meta-analysis. Significant association was found between fetal MTHFR C677T polymorphism and CHD development in all genetic models. The pooled ORs and 95% CIs in all genetic models indicated that MTHFR C677T polymorphism was significantly associated with CHD

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in Asian, but not Caucasian in subgroup analysis. The maternal MTHFR C677T polymorphism was not associated with CHD except for recessive model. Moreover, neither maternal nor fetal MTHFR A1298C polymorphism was associated with CHD.
Conclusion: The fetal MTHFR C677T polymorphism may increase the susceptibility to CHD. Fetal MTHFR C677T polymorphism was more likely to affect Asian fetus than Caucasian. The MTHFR A1298C polymorphism may not be a risk of congenital heart disease.

1. Introduction

Congenital heart disease (CHD) is one of most common congenital anomalies. CHD is a major cause of fetal loss and death in newborns less than one year of age all over the world. Approximately, CHD accounts for 28% of the major congenital anomalies (van der Linde et al., 2011). The generally accepted prevalence of CHD was about 8 per 1000 live births, which poses a serious challenge to healthcare (Bernier et al., 2010). Remarkable progresses have been achieved in CHD diagnosis and cardiac surgery during the past decades, resulting in an increased survival rate of neonates with CHD (Greutmann and Tobler, 2012). However, more patients with CHD have grown up who comprised of a special population: patients with grown-up congenital heart disease (GUCH) (Khairy et al., 2010; van der Linde et al., 2011). It was reported that the prevalence of patients with GUCH was estimated to be 4 per 1000 adults. Long-term medical care and related resource cost are needed for patients with GUCH, and rapidly increase healthcare burden.

Since more GUCH patients survive, more are now in childbearing age. Thus, it is very important to characterize the etiology of congenital heart disease, which has not been well understood yet. Several classic studies including the Baltimore–Washington Infant Study have indicated that the cause of CHD was multifactorial, and both genetic background and environmental factors may play important roles in the development of CHD (Richards and Garg, 2010; Shieh et al., 2012). Importantly, due to the advances in molecular techniques, accumulating evidences have suggested that genetic factors were dominant (Bruneau, 2008). It was known that a large proportion of CHDs were characterized with aneuploidy or abnormal chromosomal number (Blue et al., 2012; Pierpont et al., 2007). About 50% of children who were born with Trisomy 21 have atrial and ventricular septal defects or atrioventricular canal lesion. With completion of the Human Genome Project, associations between single gene mutations and CHD have also been extensively studied. It has been reported that the mutations in single genes including *TBX5*, *JAG1*, *NKX2.5* and *GATA4* have been associated with the development of CHD (Basson et al., 1997; Oda et al., 1997; Schott et al., 1998; Zhang et al., 2008).

The association between folic acid metabolism and the development of CHD has been explored recently. Maternal supplement of folic acid has been proved to reduce the incidence of CHD as well as other congenital heart disease (van Beynum et al., 2010). Single nucleotide polymorphisms of many genes involved in the folate pathway have been identified to affect the function of the genes or folic acid metabolism and thus increase the risk of CHD (Locke et al., 2010; Shaw et al., 2009). The flavin adenine dinucleotide-dependent enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is required for the remethylation of homocysteine to methionine (Ueland et al., 2001). Hyperhomocysteinemia was believed to be a high risk for the development of heart defects (Verkleij-Hagoort et al., 2006, 2007). Elevating the level of 5-methyltetrahydrofolate, a major circulating folic acid, prevented CHD by reducing maternal homocysteine plasma level (Lamers et al., 2004). Therefore, the polymorphisms of *MTHFR* may be closely related to the risk of CHD. It was reported that two *MTHFR* SNPs including *MTHFR* C677T (p.Ala222Val, ID: rs1801133) and *MTHFR* A1298C (p.Glu429Ala, rs1801131) were potentially associated with CHD (van Driel et al., 2008). The amino acid transition in *MTHFR* C677T (Ala-Val) has resulted in a thermolabile protein associated with reduced enzyme activity in vivo, which may increase plasma homocysteine level (Huhta and Hernandez-Robles, 2005). The *MTHFR* A1298 C has also been reported to moderately reduce MTHFR activity in vivo (Weisberg et al., 1998).

To date, a large number of studies regarding the associations between *MTHFR* gene polymorphisms and risk of CHD have been published. However, the results of these studies were confounding and

inconsistent. Herein, we performed a meta-analysis of all published studies until January 2013 to investigate the association between the two SNPs (*MTHFR* 677CT and *MTHFR* 1298AC) and CHD patients and their mothers.

2. Materials and methods

2.1. Literature and search strategy

The PubMed, Embase, Web of knowledge and CNKI (China National Knowledge Infrastructure) database searches were performed to identify all the eligible papers. The search terms were used as the following: (MTHFR or methylenetetrahydrofolate reductase or folic acid) and (variant or polymorphism or SNP) and (congenital heart disease or heart defect or CHD or congenital anomalies). The publication languages were restricted to English and Chinese. Moreover, potentially relevant studies were evaluated by reviewing the titles and abstracts, and studies matching the criteria were carefully retrieved. If more than one study was published using the same data, only the study with a larger population was included. The literature search was updated on January, 31, 2013.

2.2. Inclusion criteria and data extraction

The eligible studies should meet the following inclusion criteria: (1) Investigation of association between the MTHFR polymorphisms (including C677T or A1298C or both) and congenital heart disease; (2) a case–control study; (3) providing sufficient data on genotype frequencies of the MTHFR C677T and/or A1298C polymorphisms and sufficient data for calculation of an odd ratio (OR) with 95% confidence interval (CI). The exclusion criteria were as follows: (1) reviews, case report, editorial or comment; (2) a duplicated study; (3) studies providing insufficient data or data in poor quality; and (4) studies without control. Based on the inclusion and exclusion criteria, data extraction from each study was performed by two authors (Wang, Hou) independently to ensure that the data extraction were accurate. The following information was extracted from each study: (1) name of the first author; (2) year of publication;

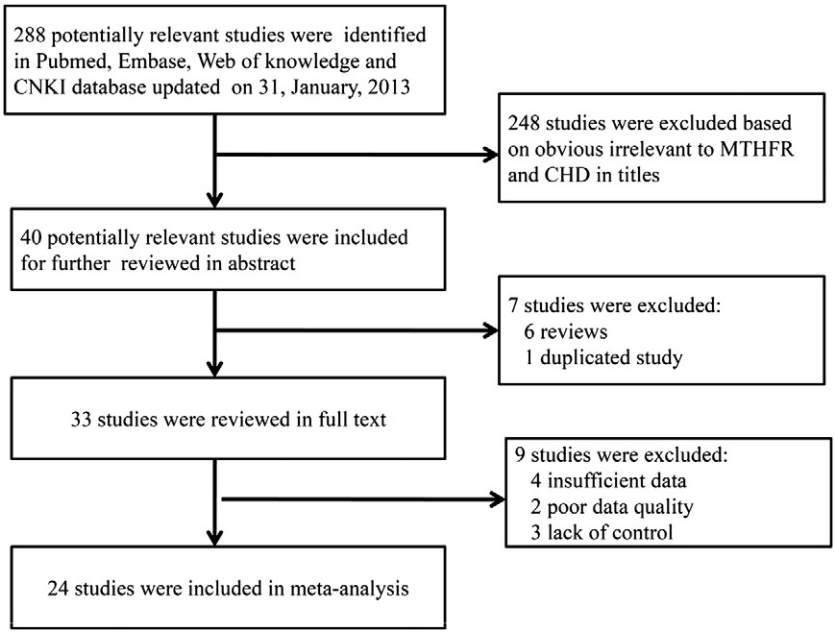

Fig. 1. Flow diagram of the study selection process.

Table 1
Characteristics of the studies included on associations between MTHFR C677T/A1298C polymorphisms and congenital heart disease.

First author	Year	Country	Ethnicity	Source of controls	Genotyping method	Types of CHD	Maternal or fetal	SNP sites		HWE
								MTHFR 677CT	*MTHFR* 1298AC	
Balderrabano-Saucedo (Balderrabano-Saucedo et al., 2013)	2013	Mexico	Caucasian	HB	RFLP	All types	Maternal	Yes		Yes
Božovic (Bozovic et al., 2011)	2011	Croatia	Caucasian	PB	RFLP	All types	Both	Yes	Yes	Yes
Hobbs (Hobbs et al., 2010)	2010	United States	Caucasian	PB	TaqMan	All types	Maternal	Yes		Yes
García-Fragoso (Garcia-Fragoso et al., 2010)	2010	Puerto Rico	Caucasian	HB	RFLP	All types	Both	Yes		No
Xu (Xu et al., 2010)	2010	Chinese	Asian	HB	RFLP	All types	Fetal	Yes	Yes	Yes
Li (Li et al., 2009b)	2009	Chinese	Asian	HB	RFLP	All types	Fetal	Yes		Yes
van Driel (van Driel et al., 2008)	2008	Netherlands	Caucasian	PB	RFLP	All types	Both	Yes	Yes	Yes
Wintner (Wintner et al., 2007)	2007	Austria	Caucasian	HB	Microarray	All types	Maternal	Yes		Yes
van Beynum (van Beynum et al., 2006)	2006	Netherlands	Caucasian	PB	RFLP	All types	Maternal	Yes		Yes
Galdieri (Galdieri et al., 2007)	2007	Brazil	Caucasian	HB	RFLP	All types	Both	Yes	Yes	Yes
Zhu (Zhu et al., 2006)	2006	Chinese	Asian	NA	RFLP	ASD/PDA	Both	Yes		Yes
Lee (Lee et al., 2005)	2005	Chinese	Asian	HB	DHPLC	All types	Fetal	Yes		Yes
Shaw (Shaw et al., 2005)	2005	United States	Caucasian	PB	Hybridization	All types	Fetal	Yes		Yes
Storti (Storti et al., 2003)	2003	Italy	Caucasian	HB	RFLP	CD	Both	Yes	Yes	Yes
Junker (Junker et al., 2001)	2001	Germany	Caucasian	NA	NA	All types	Fetal	Yes		Yes
Sanchez-Urbina (Sanchez-Urbina et al., 2012)	2012	Mexico	Caucasian	PB	RFLP	All types	Both	Yes		No
Yan (Yan and Li, 2003)	2003	Chinese	Asian	HB	RFLP	All types	Fetal	Yes		Yes
Gong (Gong et al., 2012)	2012	Chinese	Asian	HB	MassArray	CD	Fetal	Yes		Yes
Wang (Wang, 2006)	2004	Chinese	Asian	HB	RFLP	All types	Both	Yes		No
Peng (Peng et al., 2009)	2009	Chinese	Asian	HB	DHPLC	All types	Maternal	Yes		Yes
Li (Li et al., 2009a)	2009	Chinese	Asian	HB	RFLP	All types	Fetal	Yes		Yes
Gong (Gong et al., 2009)	2009	Chinese	Asian	HB	RFLP	All types	Fetal	Yes		Yes
Liu (Liu et al., 2005)	2005	Chinese	Asian	HB	RFLP	CD	Fetal	Yes		Yes
Li (Li et al., 2005)	2005	Chinese	Asian	PB	RFLP	All types	Both	Yes		Yes

Abbreviations: HWE, Hardy–Weinberg equilibrium; NA, not available; RFLP, restriction fragment length polymorphism; DHPLC, denaturing high performance liquid chromatography; CD, conotruncal heart defects; ASD, atrial septal defect; PDA, patent ductus arteriosus; PB, population-based; HB, hospital-based.

Table 2
Genotype and allele distributions of maternal MTHFR C677T/A1298C polymorphisms in case-control studies included.

Study	Sample size case/control	Genotype distribution								Allele distribution	
		Case				Control				A (case/control)	B (case/control)
		AA	AB	BB	AB + BB	AA	AB	BB	AB + BB		
MTHFR 677CT polymorphism		CC	CT	TT	CT + TT	CT	TT	CT + TT	C	T	CC
Balderrabano-Saucedo	31/62	7 (22.6%)	12 (38.7%)	12 (38.7%)	24 (77.4%)	24 (38.7%)	31 (50%)	7 (11.3%)	38 (61.3%)	26/79	36/45
Božovic	52/55	26 (50%)	20 (38%)	6 (12%)	26 (50%)	19 (35%)	28 (51%)	8 (14%)	36 (65%)	72/66	32/44
Hobbs	572/363	285 (51.5%)	203 (36.7%)	65 (11.8%)	268 (48.5%)	191 (53.7%)	128 (36%)	37 (10.4%)	165 (46.4%)	773/510	333/202
García-Fragoso	27/220	10 (37%)	11 (41%)	6 (22%)	17 (63%)	84 (38%)	115 (52%)	21 (10%)	136 (62%)	31/283	23/157
van Driel	230/251	91 (40%)	117 (51%)	22 (9%)	139 (60%)	111 (44%)	104 (42%)	36 (14%)	140 (56%)	299/326	161/176
Wintner	31/31	17 (54.84%)	11 (35.48%)	3 (9.68%)	14 (45.16%)	10 (32.26%)	17 (54.84%)	4 (12.9%)	21 (67.74%)	45/37	17/25
Van Beynum	158/261	72 (45.6%)	68 (43%)	18 (11.4%)	86 (54.4%)	131 (50.2%)	107 (41%)	23 (8.8%)	130 (49.8%)	212/369	104/153
Galdieri	47/26	27 (57.45%)	15 (31.91%)	5 (10.64%)	20 (42.55%)	10 (38.46%)	15 (57.70%)	1(3.84%)	16 (61.54)	69/17	25/17
Zhu	56/102	6 (10.71%)	27 (48.21%)	23 (41.08%)	50 (89.29%)	20 (19.61)	57 (55.88%)	25 (24.51%)	82 (80.39%)	39/97	73/107
Storti	103/200	27 (26%)	53 (52%)	23 (22%)	76 (74%)	52 (26%)	108 (54%)	40 (20%)	148 (74%)	107/212	99/188
Sanchez-Urbina	60/62	8 (13.3%)	38 (63.3%)	14 (23.3%)	52 (86.6%)	13 (21%)	37 (59.7%)	12 (19.3%)	49 (79%)	54/63	66/61
Wang	104/208	25 (24.04%)	60 (57.69%)	19 (18.27%)	79 (76.39%)	49 (23.56%)	120 (57.69%)	39 (18.75%)	159 (76.44%)	110/218	98/198
Li	183/102	32 (17.49%)	90 (49.18%)	61 (33.33%)	151 (82.51%)	20 (19.61%)	57 (55.88%)	25 (24.51%)	82 (80.39%)	154/97	212/107
Peng	91/101	32 (35.2%)	48 (52.7%)	11 (12.1%)	59 (64.8%)	46 (45.5%)	44 (43.6%)	11 (10.9%)	55 (54.5%)	112/136	70/66
MTHFR 1298AC polymorphism		AA	AC	CC	AC + CC	AA	AC	CC	AC + CC	A	C
Božovic	52/55	21 (40%)	27 (52%)	4 (8%)	31 (60%)	27 (49%)	27 (49%)	1 (2%)	28 (51%)	69/81	35/29
van Driel	230/251	104 (45%)	102 (45%)	24 (10%)	126 (55%)	116 (46%)	104 (42%)	31 (12%)	135 (54%)	310/336	150/166
Galdieri	47/26	26 (55.32%)	17 (36.17%)	4 (8.51%)	21 (44.68%)	15 (57.7%)	10 (38.46%)	1 (3.84%)	11 (42.3%)	69/25	25/12
Storti	103/200	49 (48%)	46 (45%)	8 (7%)	54 (52%)	101 (50%)	86 (43%)	13 (7%)	99 (50%)	144/288	62/112

Table 3
Genotype and allele distributions of fetal MTHFR C677T/A1298C polymorphisms in case–control studies included.

Study	Sample size case/control	Genotype distribution								Allele distribution	
		Case				Control				A (case/control)	B (case/control)
		AA	AB	BB	AB + BB	AA	AB	BB	AB + BB		
MTHFR 677CT polymorphism		CC	CT	TT	CT + TT	CC	CT	TT	CT + TT	C	T
Božovic	54/58	20 (37%)	28 (52%)	6 (11%)	34 (63%)	25 (43%)	26 (45%)	7 (12%)	33 (57%)	68/76	40/40
García-Fragoso	27/220	9 (33%)	14 (52%)	4 (15%)	28 (67%)	84 (38%)	115 (52%)	21 (10%)	136 (62%)	32/283	22/157
Xu	502/527	162 (32.2%)	244 (48.6%)	96 (19.1%)	340 (67.7%)	151 (28.7%)	261 (49.5%)	115 (21.8%)	376 (71.3%)	568/563	436/491
Li	104/208	16 (15.38%)	42 (40.38%)	46 (44.24%)	88 (84.62%)	55 (26.44%)	114 (54.81%)	39 (18.75%)	153 (73.56%)	74/224	134/192
van Driel	229/251	99 (43%)	103 (45%)	27 (12%)	130 (57%)	119 (47%)	107 (43%)	25 (10%)	132 (53%)	301/345	157/157
Galdieri	58/38	30 (51.72%)	21 (36.21%)	7 (12.07%)	28 (48.28%)	18 (47.37%)	14 (36.84%)	6 (15.79%)	20 (52.63%)	81/50	35/26
Zhu	56/103	7 (12.5%)	22 (39.28%)	27 (48.21%)	49 (87.49%)	22 (21.4%)	57 (55.3%)	24 (23.3%)	81 (78.6%)	36/101	76/105
Lee	213/195	110 (51.64%)	89 (41.78%)	14 (6.57%)	103 (48.35%)	114 (58.46%)	68 (34.87%)	13 (6.67%)	81 (41.54%)	309/296	117/94
Shaw	151/428	67 (44.37%)	68 (45.03%)	16 (10.6%)	84 (55.63%)	177 (41.36%)	199 (46.5%)	52 (12.14%)	251 (58.64%)	202/553	100303
Storti	103/200	28 (27%)	55 (53%)	20 (20%)	75 (73%)	52 (26%)	108 (54%)	40 (20%)	148 (74%)	111/212	95/188
Junker	114/228	51 (44.7%)	42 (36.8%)	21 (18.4%)	63 (55.2%)	129 (56.6%)	78 (34.2%)	21 (9.2%)	99 (43.4%)	144/336	84/120
Sanchez-Urbina	60/62	7 (11.7%)	41 (68.3%)	12 (20%)	53 (88.3%)	9 (14.5%)	46 (74.2%)	7 (11.3%)	53 (85.5%)	55/64	65/60
Yan	174/103	28 (16.1%)	89 (51.14%)	57 (32.76%)	146 (83.9%)	22 (21.36%)	57 (55.34%)	24 (23.3%)	81 (78.64%)	145/101	203/105
Gong	244/136	45 (18.4%)	123 (50.4%)	76 (31.1%)	199 (81.5%)	43 (31.6%)	72 (52.9%)	21 (15.4%)	93 (68.3%)	213/158	275/114
Wang	104/208	16 (15.38%)	42 (40.38%)	39 (18.75%)	81 (59.13%)	55 (26.44%)	114 (54.81%)	39 (18.75%)	153 (73.56%)	74/224	120/192
Li	144/168	26 (18.06%)	52 (36.11%)	66 (45.83%)	118 (81.94%)	49 (29.17%)	84 (50%)	35 (20.83%)	119 (70.83%)	104/182	184/154
Gong	80/80	10 (12.5%)	41 (51.3%)	29 (36.3%)	70 (87.6%)	17 (21.3%)	40 (50%)	23 (28.8%)	63 (78.8%)	61/74	99/86
Liu	97/118	19 (19.6%)	54 (55.7%)	24 (24.7%)	78 (80.4%)	33 (27.9%)	69 (58.5%)	16 (13.6%)	85 (72.1%)	92/135	102/101
Li	183/103	30 (16.4%)	95 (51.91%)	58 (31.69%)	153 (83.6%)	22 (21.36%)	57 (55.34%)	24 (23.3%)	81 (78.64%)	155/101	211/105
MTHFR 1298AC polymorphism		AA	AC	CC	AC + CC	AA	AC	CC	AC + CC	A	C
Božovic	54/58	30 (55%)	22 (41%)	2 (4%)	24 (45%)	25 (43%)	30 (52%)	3 (5%)	33 (57%)	82/80	26/36
Xu	502/527	316 (62.9%)	168 (33.5%)	18 (3.6%)	186 (37.1%)	326 (61.9%)	185 (35.1%)	16 (3%)	201 (38.1%)	800/837	204/217
van Driel	229/251	112 (49%)	90 (39%)	27 (12%)	117 (51%)	97 (39%)	129 (51%)	25 (10%)	154 (61%)	314/323	144/179
Galdieri	57/38	35 (61.40%)	21 (36.84%)	1 (1.76%)	22 (38.6%)	19 (50%)	16 (42.11%)	3 (7.89%)	19 (50%)	91/54	23/22
Storti	103/200	45 (43%)	47 (46%)	11 (11%)	58 (57%)	101 (50%)	86 (43%)	13 (7%)	99 (50%)	137/288	69/112

Table 4
Pooled ORs and 95% CIs of the association between maternal MTHFR C677T polymorphism and CHD.

Contrasts	No. of studies	Total case/control	T vs. C			TT vs. CC			TT + CT vs. CC			TT vs. TC + CC		
			OR	95% CI	P_H	OR	95% CI	P_H	OR	95% CI	P_H	OR	95% CI	P_H
All	14	1745/2044	1.103	0.999–1.218	0.018	1.254	1.012–1.553	0.167	1.105	0.958–1.275	0.220	1.235	1.024–1.489	0.098
Study in HWE	12	1523/1515	1.098	0.984–1.224	0.007	1.247	0.986–1.578	0.104	1.092	0.935–1.275	0.130	1.244	1.012–1.528	0.076
Ethnicity														
Caucasian	10	1311/1531	1.062	0.945–1.193	0.014	1.189	0.925–1.527	0.121	1.067	0.909–1.251	0.145	1.165	0.926–1.466	0.066
Asian	4	434/513	1.204	0.973–1.490	0.175	1.454	0.921–2.295	0.207	1.170	0.802–1.707	0.438	1.431	1.011–2.024	0.228
Types of CHD														
All types	11	1586/1742	1.072	0.960–1.197	0.016	1.202	0.947–1.525	0.140	1.071	0.917–1.250	0.197	1.192	0.964–1.474	0.067
CD	1	103/200	1.043	0.745–1.461	–	1.107	0.554–2.213	–	0.989	0.576–1.699	–	1.150	0.645–2.052	–
ASD/PDA	1	56/102	1.697	1.054–2.731	–	3.067	1.048–8.974	–	2.033	0.765–5.403	–	2.147	1.068–4.314	–
Source of controls														
HB	6	514/750	1.092	0.883–1.351	0.004	1.504	0.999–2.264	0.120	0.952	0.701–1.293	0.156	1.550	1.106–2.170	0.099
PB	6	1175/1192	1.060	0.936–1.201	0.573	1.078	0.823–1.412	0.500	1.090	0.914–1.300	0.135	1.028	0.803–1.318	0.500
Sample size														
Small	6	1441/1486	1.081	0.963–1.214	0.884	1.110	0.865–1.425	0.672	1.115	0.947–1.314	0.977	1.090	0.874–1.361	0.333
Large	7	304/558	1.136	0.913–1.412	0.001	1.854	1.161–2.961	0.090	0.953	0.682–1.331	0.034	1.838	1.252–2.700	0.199

Abbreviations: OR, odds ratio; CI, confidence interval; P_H, p value based on Q test for between-study heterogeneity; HWE, Hardy–Weinberg equilibrium; CD, conotruncal heart defects; ASD, atrial septal defect; PDA, patent ductus arteriosus; PB, population-based; HB, hospital-based.

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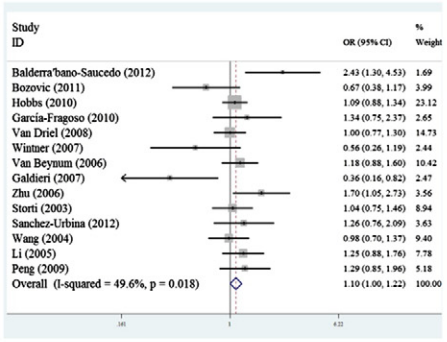
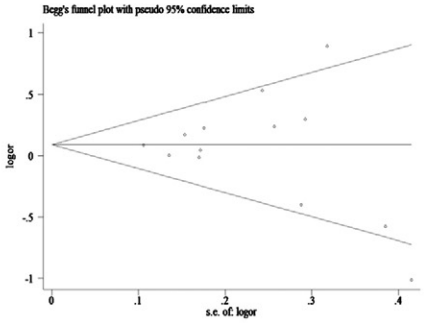

Study
ID
OR (95% CI)
%
Weight
Balderra'bano-Saucedo (2012) 2.43 (1.30, 4.53) 1.69
Bozovic (2011) 0.67 (0.38, 1.17) 3.99
Hobbs (2010) 1.09 (0.88, 1.34) 23.12
García-Fragoso (2010) 1.34 (0.75, 2.37) 2.65
Van Driel (2008) 1.00 (0.77, 1.30) 14.73
Wintner (2007) 0.56 (0.26, 1.19) 2.44
Van Beynum (2006) 1.18 (0.88, 1.60) 10.42
Galdieri (2007) 0.36 (0.16, 0.82) 2.47
Zhu (2006) 1.70 (1.05, 2.73) 3.56
Storti (2003) 1.04 (0.75, 1.46) 8.94
Sanchez-Urbina (2012) 1.26 (0.76, 2.09) 3.63
Wang (2004) 0.98 (0.70, 1.37) 9.40
Li (2005) 1.25 (0.88, 1.76) 7.78
Peng (2009) 1.29 (0.85, 1.96) 5.18
Overall (I-squared = 49.6%, p = 0.018) 1.10 (1.00, 1.22) 100.00


Begg's funnel plot with pseudo 95% confidence limits
logor
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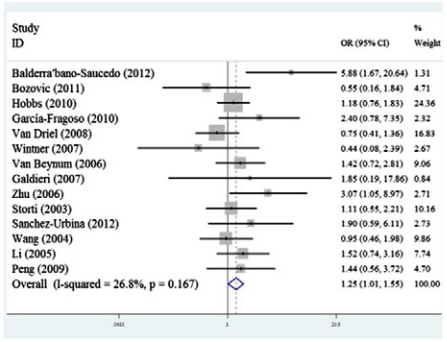
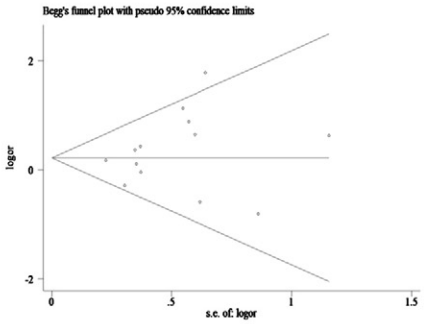
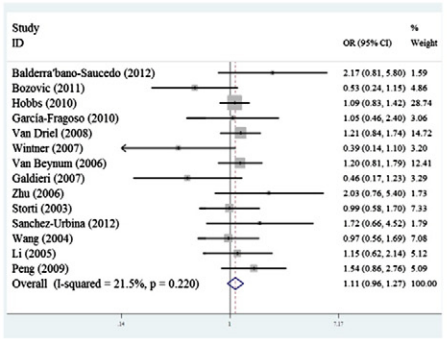
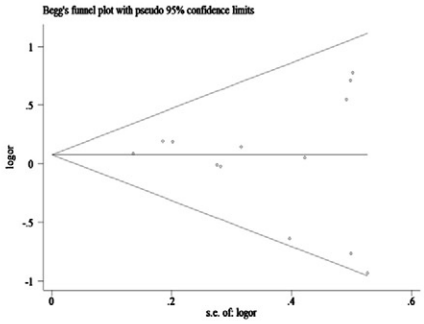
B


Study
ID
OR (95% CI)
%
Weight
Balderra'bano-Saucedo (2012) 5.88 (1.67, 20.64) 1.31
Bozovic (2011) 0.55 (0.16, 1.84) 4.71
Hobbs (2010) 1.18 (0.76, 1.83) 24.36
García-Fragoso (2010) 2.40 (0.78, 7.35) 2.32
Van Driel (2008) 0.75 (0.41, 1.36) 16.83
Wintner (2007) 0.44 (0.08, 2.39) 2.67
Van Beynum (2006) 1.42 (0.72, 2.81) 9.06
Galdieri (2007) 1.85 (0.19, 17.86) 0.84
Zhu (2006) 3.07 (1.05, 8.97) 2.71
Storti (2003) 1.11 (0.55, 2.21) 10.16
Sanchez-Urbina (2012) 1.90 (0.59, 6.11) 2.73
Wang (2004) 0.95 (0.46, 1.98) 9.86
Li (2005) 1.52 (0.74, 3.16) 7.74
Peng (2009) 1.44 (0.56, 3.72) 4.70
Overall (I-squared = 26.8%, p = 0.167) 1.25 (1.01, 1.55) 100.00

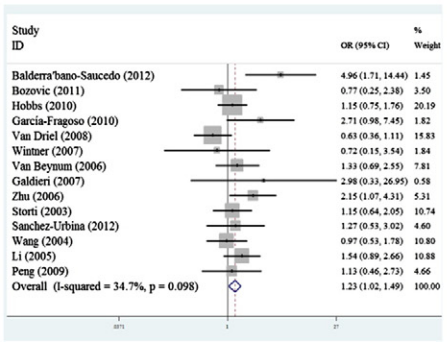

Begg's funnel plot with pseudo 95% confidence limits
logor
s.e. of: logor

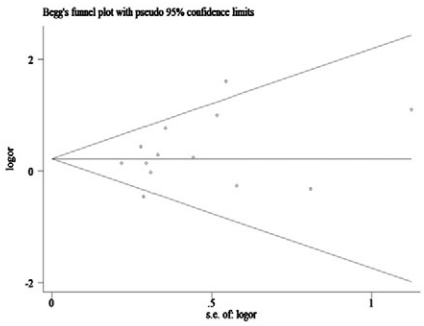
C


Study
ID
OR (95% CI)
%
Weight
Balderra'bano-Saucedo (2012) 2.17 (0.81, 5.80) 1.59
Bozovic (2011) 0.53 (0.24, 1.15) 4.86
Hobbs (2010) 1.09 (0.83, 1.42) 28.74
García-Fragoso (2010) 1.05 (0.46, 2.40) 3.06
Van Driel (2008) 1.21 (0.84, 1.74) 14.72
Wintner (2007) 0.39 (0.14, 1.10) 3.20
Van Beynum (2006) 1.20 (0.81, 1.79) 12.41
Galdieri (2007) 0.46 (0.17, 1.23) 3.29
Zhu (2006) 2.03 (0.76, 5.40) 1.73
Storti (2003) 0.99 (0.58, 1.70) 7.33
Sanchez-Urbina (2012) 1.72 (0.66, 4.52) 1.79
Wang (2004) 0.97 (0.56, 1.69) 7.08
Li (2005) 1.15 (0.62, 2.14) 5.12
Peng (2009) 1.54 (0.86, 2.76) 5.09
Overall (I-squared = 21.5%, p = 0.220) 1.11 (0.96, 1.27) 100.00


Begg's funnel plot with pseudo 95% confidence limits
logor
s.e. of: logor

D


Study
ID
OR (95% CI)
%
Weight
Balderra'bano-Saucedo (2012) 4.96 (1.71, 14.44) 1.45
Bozovic (2011) 0.77 (0.25, 2.38) 3.50
Hobbs (2010) 1.15 (0.75, 1.76) 20.19
García-Fragoso (2010) 2.71 (0.98, 7.45) 1.82
Van Driel (2008) 0.63 (0.36, 1.11) 15.83
Wintner (2007) 0.72 (0.15, 3.54) 1.84
Van Beynum (2006) 1.33 (0.69, 2.55) 7.81
Galdieri (2007) 2.98 (0.33, 26.95) 0.58
Zhu (2006) 2.15 (1.07, 4.31) 5.31
Storti (2003) 1.15 (0.64, 2.05) 10.74
Sanchez-Urbina (2012) 1.27 (0.53, 3.02) 4.60
Wang (2004) 0.97 (0.53, 1.78) 10.80
Li (2005) 1.54 (0.89, 2.66) 10.88
Peng (2009) 1.13 (0.46, 2.73) 4.66
Overall (I-squared = 34.7%, p = 0.098) 1.23 (1.02, 1.49) 100.00


Begg's funnel plot with pseudo 95% confidence limits
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(3) country of origin; (4) ethnicity of the study population; (5) source of controls (population based or hospital based); (6) sample size of case and controls; (7) types of congenital heart disease; (8) genotype distributions in cases and controls; and (9) whether population involved in the study was in Hardy–Weinberg equilibrium (HWE).

2.3. Statistical analysis

Meta-analysis was performed to evaluate the association between MTHFR polymorphisms and risk of developing CHD. Firstly, crude ORs with 95% CIs were calculated to assess the strength of the correlation between the MTHFR C677T/A1298C polymorphisms (including maternal and fetal) and risk of CHD. Pooled ORs and 95% CIs were calculated for the multiplicative, co-dominant, dominant, and recessive genetic models respectively. The significances of pooled ORs were analyzed by Z tests, and the criteria for statistically significant were $p < 0.05$. A Q test was conducted to determine the possible heterogeneity, and $p < 0.10$ or $I > 50\%$ indicated an obvious heterogeneity. Pooled ORs (95% CI) were calculated by random effects model (DerSimonian–Laird method) or fix effects model (Mantel–Haenszel method). Subgroup analysis was performed by ethnicity, types of CHD, source of controls and sample size ($n < 100$ vs. $n > 100$). Sensitivity analysis were performed to evaluate the stability of the results by removing one case–control study each time to assess the influence of the individual data on pooled ORs. Begg's funnel plot was generated to indicate the possible publication bias. Moreover, the Egger quantitative tests were also performed, and $p < 0.05$ was considered statistically significant. To obtain reliable data, two authors (Wang, Hou) have performed the statistical analysis independently by using the same data and the programs. Data analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA).

3. Results

3.1. Characteristics of the studies included

Totally, we have identified 288 potentially relevant studies by employing the search strategy described above. Based on obvious irrelevance to MTHFR and CHD in titles, 248 papers from the 288 potentially relevant papers were excluded. After reading the abstracts of the remaining 40 studies, 7 studies were further excluded, as 6 studies were reviews and one study was a duplicated study. To further polish target studies, the remaining studies were reviewed in full text. Of these, 9 studies were excluded, due to insufficient data, data with poor quality or papers without control. After careful screening, 24 eligible studies were finally included in this meta-analysis (Balderrabano-Saucedo et al., 2013; Bozovic et al., 2011; Galdieri et al., 2007; Garcia-Fragoso et al., 2010; Gong et al., 2009, 2012; Hobbs et al., 2010; Junker et al., 2001; Lee et al., 2005; Li et al., 2005, 2009a, 2009b; Liu et al., 2005; Peng et al., 2009; Sanchez-Urbina et al., 2012; Shaw et al., 2005; Storti et al., 2003; van Beynum et al., 2006; van Driel et al., 2008; Wang, 2006; Wintner et al., 2007; Xu et al., 2010; Yan and Li, 2003; Zhu et al., 2006). The search strategy and inclusion/exclusion of studies were shown in a flow chart (Fig. 1). Among these studies, fourteen studies investigated the maternal MTHFR C677T polymorphism with 1745 cases and 2044 controls and nineteen studies investigated the fetal MTHFR C677T polymorphism with 2697 cases and 3434 controls. In addition, there were 4 studies investigating maternal MTHFR A1298C polymorphism with 432 cases and 532 controls and 5 studies investigating fetal MTHFR A1298C polymorphism with 945 cases and 1074 controls. Concerning Hardy–Weinberg equilibrium, 3 studies were not conformed to HWE. In these papers, 9 studies included both maternal and fetal MTHFR

Fig. 2. Forest plot of meta-analysis of association between maternal MTHFR C677T polymorphism and CHD risk and funnel plot analysis on the detection of publication bias. (A) Meta-analysis in a random effects model for C vs. T (additive model); (B) meta-analysis in a random effects model for CC vs. TT (co-dominant model); (C) meta-analysis in a random effects model for TT + CT vs. CC (dominant model); (D) meta-analysis in a random effects model for TT vs. CC + CT (recessive model). Left panel: forest plot analysis, each study is shown by the point of estimating the OR and 95% CIs for corresponding ORs were shown by extending lines; right panel: funnel plot analysis, each point represents an individual study. LogOR, natural logarithm of OR, perpendicular line denotes the mean effect size.

polymorphisms and 5 studies included both MTHFR C677T and A1298C polymorphisms. Moreover, 11 studies were performed in Caucasian and 12 studies were performed in Asian. The general characteristics of the studies included were listed in Table 1. The genotype and allele distributions of maternal C677T and A1298C polymorphisms in all the studies included were shown in Table 2. For the fetal polymorphisms, the genotype and allele frequencies of C677T and A1298C were shown in Table 3.

3.2. Quantitative data analysis

For the maternal MTHFR C677T polymorphism, the results indicated no statistically significant association between the polymorphism and the susceptibility to CHD in all genetic models except for recessive model and co-dominant model (T vs. C: OR = 1.103, 95% CI 0.999–1.218; TT vs. CC: OR = 1.254, 95% CI 1.012–1.553; TT + CT vs. CC: 1.105, 95% CI 0.958–1.275; TT vs. TC + CC: OR = 1.235, 95% CI 1.024–1.489) (Table 4 Fig. 2). In the subgroup analysis by ethnicity, no significant association was observed in Asian population in all genetic models except for recessive model (T vs. C: OR = 1.204, 95% CI 0.973–1.490; TT vs. CC: OR = 1.454, 95% CI 0.921–2.295; TT + CT vs. CC: OR = 1.170, 95% CI 0.802–1.707; TT vs. TC + CC: OR = 1.431, 95% CI 1.011–2.024) (Table 4). No association was detected in Caucasians in all genetic models. In the stratified analysis by types of CHD, there was a significant association between C667T and ASD/PDA, however, the results were not reliable because only one study was performed in ASD/PDA patients (Table 4). In the subgroup of source of control, association was only observed in the recessive model of hospital based control subgroup (TT vs. TC + CC: OR = 1.550, 95% CI 1.106–2.170) (Table 4). In the sample size subgroup analysis, there was no significant association between CHD and maternal C677T in all genetic models of large sample studies. However, we have observed a significant association in co-dominant model and recessive model with small sample studies (Table 4).

For the fetal MTHFR C667T polymorphism, the overall results suggested a significant association of polymorphism with CHD susceptibility (T vs. C: OR = 1.271, 95% CI 1.178–1.372; TT vs. CC: OR = 1.610, 95% CI 1.374–1.885; TT + CT vs. CC: OR = 1.258, 95% CI 1.120–1.414; TT vs. TC + CC: OR = 1.565, 95% CI 1.370–1.788) (Table 5, Fig. 3). In the subgroup by ethnicity, fetal MTHFR C677T was associated with CHD in Asian populations for all genetic models, however, no significant association was found in Caucasian (Table 5). In the stratified analysis by types of CHD, significant associations were detected between fetal MTHFR C677T and all types of CHD for all genetic models (Table 5). Similar significant association was also observed in CD and ASD/PDA, however, the positive result in CD was not reliable because it was derived from one study. Interestingly, a significant association was observed in hospital based control subgroup rather than in population based control subgroup. By considering sample size, significant results were also found in all genetic models in both small and large sample subgroups (Table 5).

For MTHFR A1298C polymorphism, the results showed no significant association between this polymorphism and CHD risk in either maternal or fetal groups (maternal: T vs. A: OR = 1.043, 95% CI 0.855–1.271; CC vs. AA OR = 1.109, 95% CI 0.692–1.775; CC + AC vs. AA: OR = 1.108, 95% CI 0.856–1.435; CC vs. AC + AA: OR = 0.735, 95% CI 0.467–1.157; fetal: C vs. A OR = 0.938, 95% CI 0.812–1.083; CC vs. AA: OR = 1.058, 95% CI 0.719–1.558; CC + AC vs. AA: OR = 0.871, 95% CI 0.728–1.042; CC vs. AC + AA: OR = 1.184, 95% CI 0.815–1.721). In the subgroup analysis of either maternal or fetal polymorphisms, there was no statistically significant association in each subgroup by ethnicity, types of CHD, source of controls and sample size under all genetic models (Table 6).

3.3. Source of heterogeneity

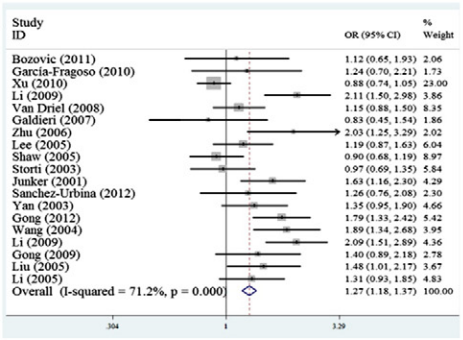
As shown in Table 4, heterogeneity between studies was significant ($p < 0.10$) under additive, and recessive genetic models for maternal MTHFR C677T. Moreover, evidence for heterogeneity between studies was also found in all genetic models for fetal MTHFR C677T. For the MTHFR A1298C polymorphism, significant heterogeneity was only found in recessive model of maternal polymorphism. No evidence for heterogeneity between studies was detected for maternal MTHFR C677T in the co-dominant and dominant models, for maternal and fetal MTHFR A1298C under all genetic models except for maternal recessive model.

Table 5
Pooled ORs and 95% CIs of the association between fetal MTHFR C677T polymorphism and CHD.

Contrasts	No. of studies	Total case/control	T vs. C			TT vs. CC			TT + CT vs. CC			TT vs. TC + CC		
			OR	95% CI	P_H	OR	95% CI	P_H	OR	95% CI	P_H	OR	95% CI	P_H
All	19	2697/3434	1.271	1.178–1.372	0.000	1.610	1.374–1.885	0.000	1.258	1.120–1.414	0.057	1.565	1.370–1.788	0.000
Study in HWE	17	2657/3006	1.247	1.155–1.348	0.000	1.537	1.308–1.807	0.000	1.238	1.101–1.392	0.070	1.492	1.302–1.711	0.000
Ethnicity														
Caucasian	8	796/1485	1.108	0.970–1.264	0.252	1.233	0.925–1.643	0.296	1.151	0.956–1.385	0.444	1.196	0.920–1.556	0.399
Asian	11	1901/1949	1.358	1.240–1.487	0.000	1.793	1.487–2.162	0.000	1.345	1.164–1.555	0.042	1.695	1.455–1.976	0.000
Types of CHD														
All types	15	2197/2877	1.233	1.136–1.339	0.000	1.496	1.259–1.777	0.000	1.230	1.087–1.391	0.099	1.474	1.274–1.707	0.000
CD	3	444/454	1.391	1.148–1.686	0.026	2.053	1.371–3.075	0.021	1.471	1.074–2.015	0.106	1.751	1.249–2.455	0.059
ASD/PDA	1	56/103	2.031	1.255–3.287	–	3.536	1.284–9.735	–	1.901	0.757–4.778	–	3.065	1.529–6.142	–
Source of controls														
HB	12	1850/2201	1.322	1.208–1.448	0.000	1.720	1.431–2.067	0.000	1.323	1.146–1.526	0.021	1.633	1.401–1.904	0.000
PB	5	677/902	1.101	0.945–1.283	0.509	1.225	0.871–1.723	0.488	1.101	0.881–1.376	0.688	1.215	0.902–1.638	0.542
Sample size														
Small	7	432/679	1.332	1.134–1.565	0.537	1.877	1.294–2.722	0.570	1.501	1.147–1.964	0.875	1.655	1.224–2.237	0.390
Large	12	2265/2755	1.254	1.154–1.363	0.000	1.551	1.306–1.843	0.000	1.221	1.077–1.385	0.013	1.531	1.323–1.772	0.000

Abbreviations: OR, odds ratio; CI, confidence interval; P_H, p value based on Q test for between-study heterogeneity; HWE, Hardy–Weinberg equilibrium; CD, conotruncal heart defects; ASD, atrial septal defect; PDA, patent ductus arteriosus; PB, population-based; HB, hospital-based

A


Study
ID
OR (95% CI)
%
Weight
Bozovic (2011) 1.12 (0.65, 1.93) 2.06
Garcia-Fragoso (2010) 1.24 (0.70, 2.21) 1.73
Xu (2010) 0.88 (0.74, 1.05) 23.00
Li (2009) 2.11 (1.50, 2.98) 3.86
Van Driel (2008) 1.15 (0.88, 1.50) 8.35
Galdieri (2007) 0.83 (0.45, 1.54) 1.86
Zhu (2006) 2.03 (1.25, 3.29) 2.02
Lee (2005) 1.19 (0.87, 1.63) 6.04
Shaw (2005) 0.90 (0.68, 1.19) 8.97
Storti (2003) 0.97 (0.69, 1.35) 5.84
Junker (2001) 1.63 (1.16, 2.30) 4.29
Sanchez-Urbina (2012) 1.26 (0.76, 2.08) 2.30
Yan (2003) 1.35 (0.95, 1.90) 4.66
Gong (2012) 1.79 (1.33, 2.42) 5.42
Wang (2004) 1.89 (1.34, 2.68) 3.95
Li (2009) 2.09 (1.51, 2.89) 4.36
Gong (2009) 1.40 (0.89, 2.18) 2.78
Liu (2005) 1.48 (1.01, 2.17) 3.67
Li (2005) 1.31 (0.93, 1.85) 4.83
Overall (I-squared = 71.2%, p = 0.000) 1.27 (1.18, 1.37) 100.00
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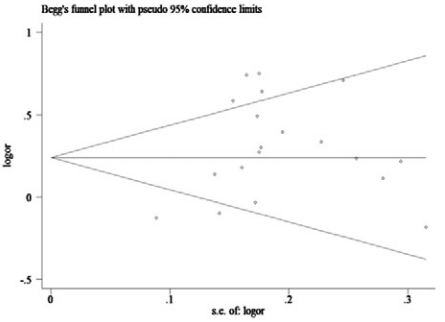
1
3.29


Begg's funnel plot with pseudo 95% confidence limits
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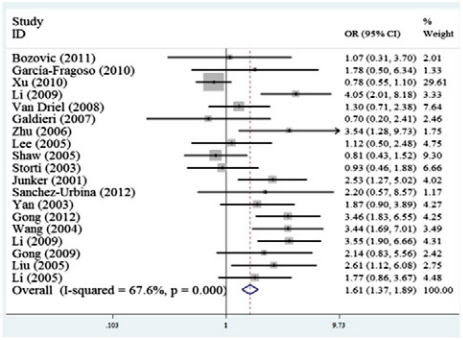
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ID
OR (95% CI)
%
Weight
Bozovic (2011) 1.07 (0.31, 3.70) 2.01
Garcia-Fragoso (2010) 1.78 (0.50, 6.34) 1.33
Xu (2010) 0.78 (0.55, 1.10) 29.61
Li (2009) 4.05 (2.01, 8.18) 3.33
Van Driel (2008) 1.30 (0.71, 2.38) 7.64
Galdieri (2007) 0.70 (0.20, 2.41) 2.46
Zhu (2006) 3.54 (1.28, 9.73) 1.75
Lee (2005) 1.12 (0.50, 2.48) 4.75
Shaw (2005) 0.81 (0.43, 1.52) 9.30
Storti (2003) 0.93 (0.46, 1.88) 6.66
Junker (2001) 2.53 (1.27, 5.02) 4.02
Sanchez-Urbina (2012) 2.20 (0.57, 8.57) 1.17
Yan (2003) 1.87 (0.90, 3.89) 4.27
Gong (2012) 3.46 (1.83, 6.55) 4.25
Wang (2004) 3.44 (1.69, 7.01) 3.49
Li (2009) 3.55 (1.90, 6.66) 4.31
Gong (2009) 2.14 (0.83, 5.56) 2.42
Liu (2005) 2.61 (1.12, 6.08) 2.75
Li (2005) 1.77 (0.86, 3.67) 4.48
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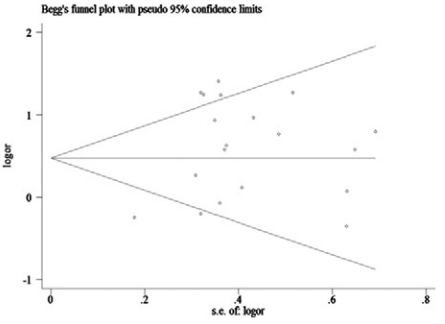
9.73


Begg's funnel plot with pseudo 95% confidence limits
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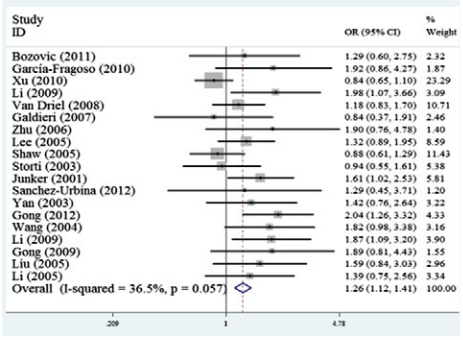
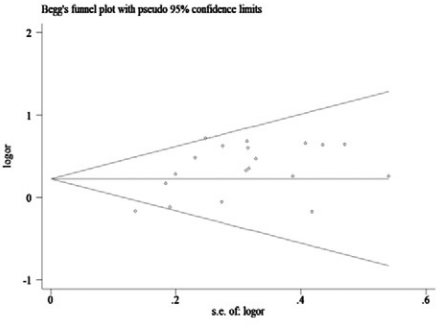

Study
ID
OR (95% CI)
%
Weight
Bozovic (2011) 1.29 (0.60, 2.75) 2.32
Garcia-Fragoso (2010) 1.92 (0.86, 4.27) 1.87
Xu (2010) 0.84 (0.65, 1.10) 23.29
Li (2009) 1.98 (1.07, 3.66) 3.09
Van Driel (2008) 1.18 (0.83, 1.70) 10.71
Galdieri (2007) 0.84 (0.37, 1.91) 2.46
Zhu (2006) 1.90 (0.76, 4.78) 1.40
Lee (2005) 1.32 (0.89, 1.95) 8.59
Shaw (2005) 0.88 (0.61, 1.29) 11.43
Storti (2003) 0.94 (0.55, 1.61) 5.38
Junker (2001) 1.61 (1.02, 2.53) 5.81
Sanchez-Urbina (2012) 1.29 (0.45, 3.71) 1.20
Yan (2003) 1.42 (0.76, 2.64) 3.22
Gong (2012) 2.04 (1.26, 3.32) 4.33
Wang (2004) 1.82 (0.98, 3.38) 3.16
Li (2009) 1.87 (1.09, 3.20) 3.90
Gong (2009) 1.89 (0.81, 4.43) 1.55
Liu (2005) 1.59 (0.84, 3.03) 2.96
Li (2005) 1.39 (0.75, 2.56) 3.34
Overall (I-squared = 36.5%, p = 0.057) 1.26 (1.12, 1.41) 100.00
.209
1
4.78


Begg's funnel plot with pseudo 95% confidence limits
logor
s.e. of: logor
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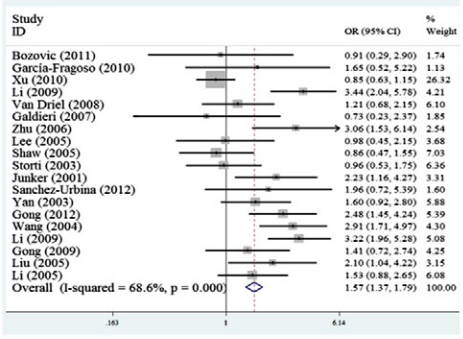
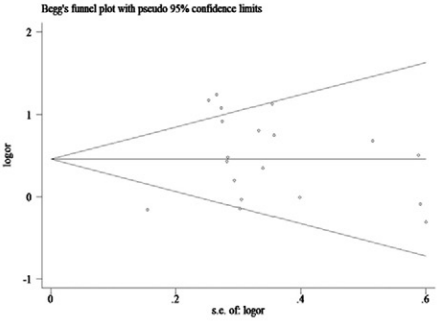

Study
ID
OR (95% CI)
%
Weight
Bozovic (2011) 0.91 (0.29, 2.90) 1.74
Garcia-Fragoso (2010) 1.65 (0.52, 5.22) 1.13
Xu (2010) 0.85 (0.63, 1.15) 26.32
Li (2009) 3.44 (2.04, 5.78) 4.21
Van Driel (2008) 1.21 (0.68, 2.15) 6.10
Galdieri (2007) 0.73 (0.23, 2.37) 1.85
Zhu (2006) 3.06 (1.53, 6.14) 2.54
Lee (2005) 0.98 (0.45, 2.15) 3.68
Shaw (2005) 0.86 (0.47, 1.55) 7.03
Storti (2003) 0.96 (0.53, 1.75) 6.36
Junker (2001) 2.23 (1.16, 4.27) 3.31
Sanchez-Urbina (2012) 1.96 (0.72, 5.39) 1.60
Yan (2003) 1.60 (0.92, 2.80) 5.88
Gong (2012) 2.48 (1.45, 4.24) 5.39
Wang (2004) 2.91 (1.71, 4.97) 4.30
Li (2009) 3.22 (1.96, 5.28) 5.08
Gong (2009) 1.41 (0.72, 2.74) 4.25
Liu (2005) 2.10 (1.04, 4.22) 3.15
Li (2005) 1.53 (0.88, 2.65) 6.08
Overall (I-squared = 68.6%, p = 0.000) 1.57 (1.37, 1.79) 100.00
.163
1
6.14


Begg's funnel plot with pseudo 95% confidence limits
logor
s.e. of: logor
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.2
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.6

3.4. Sensitivity analysis

In the sensitivity analysis, the overall association between the maternal MTHFR C677T genotype and CHD was not substantially changed by excluding one study at a time (data not shown). Similar results were also found in fetal MTHFR C677T, maternal and fetal MTHFR A1298C.

3.5. Potential publication bias

Except for dominant model of fetal MTHFR C677T and co-dominant model of maternal A1298C polymorphisms, no publication bias could be detected by employing Egger's test for studies on maternal MTHFR C677T polymorphism (T vs. C: $p = 0.647$; TT vs. CC: $p = 0.324$; TT + CT vs. CC $p = 0.533$; TT vs. TC + CC: $p = 0.269$); fetal MTHFR C677T polymorphism (T vs. C: $p = 0.077$; TT vs. CC: $p = 0.110$; TT vs. TC + CC: $p = 0.057$); maternal MTHFR A1298C polymorphism (C vs. A: $p = 0.882$; CC + AC vs. AA: $p = 0.330$; CC vs. AC + AA: $p = 0.107$); and fetal MTHFR A1298C polymorphism (C vs. A: $p = 0.493$; CC vs. AA: $p = 0.576$; CC + AC vs. AA: $p = 0.576$; CC vs. AC + AA: $p = 0.576$). The results of Egger's test suggested publication bias in dominant model of fetal MTHFR C677T polymorphism (TT + CT vs. CC: $p = 0.006$) and co-dominant model of maternal A1298C polymorphism (CC vs. AA: $p = 0.049$). The Begg's tests of corresponding genetic models in forest plots were shown in Figs. 2 and 3.

4. Discussion

To date, it is known that genetic and environmental risks may be the causes of congenital heart diseases. Importantly, numerous studies have suggested the role of folic acid metabolism in the CHD development (Ueland et al., 2001). MTHFR is a key enzyme in folic acid conversion process, and its activity may be related with a variety of diseases including CHD (Li et al., 2013; Long et al., 2012). It was reported that the C677T mutation of MTHFR could render the enzyme thermolabile with approximately 50% reduced activity and increased plasma homocysteine concentrations (Huhta and Hernandez-Robles, 2005). Therefore, the variants of the MTHFR gene may modulate the activity of MTHFR and may be an important determinant of CHD development. Several studies have reported the potential association between MTHFR polymorphisms (C677T and A1298C) and CHD, however, the results were not consistent (Balderrabano-Saucedo et al., 2013; Galdieri et al., 2007). Our current comprehensive meta-analysis could better evaluate the association between MTHFR C677T/A1298C and susceptibility of CHD. Two studies have performed meta-analysis in association between MTHFR C677T polymorphism and CHD two years ago (Nie et al., 2011; Yin et al., 2012). However, the MTHFR A1298C polymorphism was not analyzed in either of the two studies. Moreover, subgroup analysis based methods were not employed in previous studies. We have analyzed the association between MTHFR polymorphisms and CHD by multiple methods in all genetic models and included more recent studies. To our knowledge, this is the first meta-analysis on association between MTHFR polymorphisms and CHD including both C677T and A1298C.

For the MTHFR C677T polymorphism, most studies have indicated that maternal C677T was not a strong risk of CHD, however, some reports have suggested its potential role in CHD development. In our finding, no statistically significant difference was detected in genotype or allele frequencies of MTHFR C677T polymorphism in the mothers of CHD patients compared with controls. Only marginal association between maternal C677T polymorphism and CHD was found in recessive model. The finding was consistent with the previous studies involving both maternal and fetal C677T polymorphism. Particularly, we found a significant association between maternal C677T and CHD in recessive genetic models of Asian subgroup, however, similar result was not

Fig. 3. Forest plot of meta-analysis of association between fetal MTHFR C677T polymorphism and CHD risk and funnel plot analysis on the detection of publication bias. (A) Meta-analysis in a random effects model for C vs. T (additive model); (B) meta-analysis in a random effects model for CC vs. TT (co-dominant model); (C) meta-analysis in a random effects model for TT + CT vs. CC (dominant model); (D) meta-analysis in a random effects model for TT vs. CC + CT (recessive model). Left panel: forest plot analysis, each study is shown by the point of estimating the OR and 95% CIs for corresponding ORs were shown by extending lines; right panel: funnel plot analysis, each point represents an individual study. LogOR, natural logarithm of OR, perpendicular line denotes the mean effect size.

Table 6
Pooled ORs and 95% CIs of the association between maternal/fetal MTHFR A1298C polymorphism and CHD.

Contrasts	No. of studies	Total case/control	C vs. A			CC vs. AA			CC + AC vs. AA			CC vs. AC + AA		
			OR	95% CI	P_H	OR	95% CI	P_H	OR	95% CI	P_H	OR	95% CI	P_H
Maternal														
All studies	4	432/532	1.043	0.855–1.271	0.585	1.109	0.692–1.775	0.405	1.108	0.856–1.435	0.912	0.735	0.467–1.157	0.053
All types of CHD	3	329/332	1.018	0.805–1.287	0.407	1.062	0.617–1.825	0.250	1.102	0.810–1.499	0.769	0.615	0.366–1.034	0.063
CD	1	103/200	1.107	0.765–1.601	–	1.268	0.493–3.262	–	1.124	0.699–1.809	–	1.276	0.512–3.183	–
HB	2	150/226	1.040	0.741–1.458	0.407	1.405	0.594–3.323	0.634	1.120	0.731–1.716	0.970	1.412	0.613–3.253	0.627
PB	2	282/306	1.044	0.818–1.344	0.263	1.003	0.572–1.761	0.134	1.102	0.796–1.524	0.468	0.557	0.324–0.957	0.048
Small	2	99/81	1.150	0.712–1.856	0.224	3.476	0.700–17.271	0.625	1.289	0.708–2.350	0.684	3.266	0.674–15.825	0.682
Large	2	333/451	1.022	0.822–1.270	0.599	0.961	0.581–1.591	0.500	1.071	0.804–1.426	0.800	0.608	0.372–0.993	0.059
Fetal														
All studies	5	945/1074	0.938	0.812–1.083	0.171	1.058	0.719–1.558	0.331	0.871	0.728–1.042	0.128	1.184	0.815–1.721	0.516
All types of CHD	4	842/874	0.884	0.756–1.034	0.422	0.924	0.600–1.421	0.460	0.813	0.670–0.987	0.281	1.085	0.715–1.646	0.496
CD	1	103/200	1.295	0.901–1.861	–	1.899	0.791–4.562	–	1.315	0.815–2.121	–	1.720	0.742–3.987	–
HB	3	662/765	1.017	0.851–1.216	0.145	1.221	0.725–2.057	0.168	0.991	0.799–1.229	0.275	1.216	0.729–2.027	0.233
PB	2	283/309	0.805	0.630–1.028	0.627	0.889	0.499–1.582	0.603	0.648	0.467–0.898	0.846	1.149	0.664–1.989	0.582
Small	2	111/96	0.667	0.428–1.041	0.781	0.351	0.084–1.460	0.461	0.616	0.354–1.073	0.949	0.427	0.105–1.730	0.416
Large	3	834/978	0.976	0.838–1.136	0.150	1.166	0.778–1.749	0.429	0.907	0.750–1.096	0.064	1.293	0.875–1.912	0.758
Caucasian	4	443/547	0.902	0.743–1.095	0.108	1.014	0.636–1.618	0.209	0.793	0.615–1.023	0.106	1.183	0.757–1.848	0.353
Asian	1	502/527	0.984	0.794–1.219	–	1.161	0.582–2.316	–	0.955	0.742–1.229	–	1.188	0.599–2.356	–

Abbreviations: OR, odds ratio; CI, confidence interval; P_H, p value based on Q test for between-study heterogeneity; HWE, Hardy–Weinberg equilibrium; CD, conotruncal heart defects; PB, population-based; HB, hospital-based.

observed in Caucasian. This discrepancy of association between Asian and Caucasian groups may be attributed to the different genetic background and environmental factors.

Our results have indicated that fetal MTHFR C677T polymorphism was significantly associated with CHD in all genetic models. It was evident that fetal MTHFR C677T polymorphism was an important risk in the development of CHD. To explain the results, we speculated that decreased fetal MTHFR enzyme activity may result in a local hyperhomocystein environment, in which the heart could not develop normally (Lu et al., 2011). These evidences have supported the viewpoint that fetal MTHFR C677T polymorphism was more important than maternal MTHFR C677T polymorphism, and concentration of homocystein in fetus may influence heart development rather than maternal homocystein concentration. In addition, we found that fetal MTHFR C677T was significantly associated with CHD in Asian, while no statistically significant association was found in Caucasian population. Consistent with the result from recessive model of maternal analysis, the fetal MTHFR C677T was more likely to be associated with CHD in Asian than Caucasian. The results have validated the notion that MTHFR C667T may be in combination with other genetic background and environment factors to affect the fetal heart development. By considering the source of controls, the association between MTHFR C667T polymorphism and CHD was significant in hospital based control group, though, not significant in population based control. The confounding results from two subgroups categorized by source of control have indicated that hospital based and population based control was not homogenous in this study. We believe that the comparability between cases and controls contributes to the disagreement of these two subgroups.

For the MTHFR A1298C polymorphism, we found no statistically significant association between this polymorphism and CHD either in maternal or fetal analysis. Our finding has demonstrated that MTHFR A1298C may not be a risk of congenital heart disease development. However, some studies indicated that the interaction between MTHFR 1298 C allele and folic acid supplement increased the risk of having a child with CHD (van Driel et al., 2008). Considering that minimal eligible studies included in our meta-analysis, this result should be validated with more studied and large pooled samples in future. In subgroup analysis by ethnicity, only one study was performed in Asian population to investigate fetal A1298C polymorphism and four studies were performed in Caucasian population. Because of the importance of MTHFR polymorphisms in Asian CHD development, we suggest that more studies investigating association between A1298C polymorphism and CHD be performed in Asian population.

5. Potential study limitations

Although we made these findings in this meta-analysis, there were several limitations. First, our study was mainly based on unadjusted odd ratios, and the potential covariates including gender, age, vitamin supplement, smoking or other environmental factors, which might influence the final results, were unable to control. Second, significant heterogeneity in the study was presented in overall and subgroup analysis. We have investigated the study heterogeneity including geographic region, ethnicity, and source of control. However, none of them was identified as the potential source of heterogeneity between studies by meta-regression (data not shown). We estimated that other unknown confounding factors may help explain the between-study heterogeneity. Third, it was known that there were several subtypes of congenital heart diseases. However, only a few studies included in our meta-analysis have classified their cases by types of CHD. To analyze this issue, we need more studies involving CHD cases with clear subtypes.

6. Conclusion

Despite the limitations mentioned above, the present study has demonstrated that the fetal MTHFR C677T polymorphism is an important risk of developing congenital heart diseases. Our findings also suggest that MTHFR A1298C polymorphism does not increase the susceptibility to CHD. Interestingly, we found that fetal MTHFR C677T polymorphism more likely affects Asian fetus than Caucasian fetus in the development of CHD.

Conflicts of interest

There was no potential conflict of interest.

Acknowledgments

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