Arch Gynecol Obstet (2017) 295:1105–1118
DOI 10.1007/s00404-017-4322-z

MATERNAL-FETAL MEDICINE

Genetic polymorphism of *MTHFR* C677T with preterm birth and low birth weight susceptibility: a meta-analysis

Han Wu[1] · Ping Zhu[2] · Xingyi Geng[3] · Zhong Liu[3] · Liangliang Cui[3] · Zhongchun Gao[4] · Baofa Jiang[1] · Liping Yang[1]

Received: 29 November 2016 / Accepted: 10 January 2017 / Published online: 10 March 2017

Abstract
Purpose This study aimed at clarifying the association of maternal and neonatal methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphisms with preterm birth (PTB) and low birth weight (LBW) susceptibility, respectively.
Materials and methods A systematic search of Embase, Medline, China Biological Medicine Database (CBM), Chinese National Knowledge Infrastructure (CNKI), and Wanfang Database was performed before June, 2016. The frequencies of maternal and neonatal *MTHFR* C677T genotypes in the cases and controls and other information were extracted by two independent investigators. Odds ratios (ORs) with 95% confidence intervals (CIs) were adopted to estimate the relationships between *MTHFR* C677T polymorphisms and PTB as well as LBW by random or fixed effect models.
Results Twenty-five studies from 20 articles concerning maternal and neonatal *MTHFR* C677T gene polymorphism with PTB and LBW were included in this study. Maternal *MTHFR* C677T polymorphism was associated with PTB risk under allele contrast (T vs. C, OR = 1.36, 95% CI 1.02–1.81), homozygote (TT vs. CC, OR = 1.70, 95% CI 1.07–2.68), and recessive (TT vs. CT + CC, OR = 1.49, 95% CI 1.00–2.22) model, but not dominant or heterozygote model. Maternal *MTHFR* C677T polymorphism was also associated with LBW risk under allele contrast (OR = 1.69, 95% CI 1.25–2.28), homozygote (OR = 2.26, 95% CI 1.44–3.54), dominant (OR = 1.71, 95% CI 1.19–2.47), recessive (OR = 1.79, 95% CI 1.42–2.26) model, but not heterozygote model. No associations between neonatal *MTHFR* C677T polymorphism and PTB or LBW were found under all genetic models.
Conclusions Identification of maternal *MTHFR* C677T mutation may play a key role for primary prevention of PTB as well as LBW and screening pregnant women of high risk in developing countries.

Keywords Methylenetetrahydrofolate reductase · Preterm birth · Low birth weight · Polymorphism · Meta-analysis

Introduction

Preterm birth (childbirth before 37 weeks of pregnancy, PTB) and low birth weight (birth weight less than 2500 g, LBW) are the two most common adverse birth outcomes [1, 2]. Beck et al. estimated that in 2005, approximately 13.0 million infants were preterm all over the world, representing 9.6% of all births [3]. A report of worldwide child deaths showed that from 2000 to 2013, a global total of 965,000 deaths during the neonatal period could be attributed to direct complications from preterm birth [4, 5]. According to the World Health Organization (WHO) report on LBW, there were over 20 million infants born with LBW, accounting for about 15.5% of total births globally [6]. A prospective community-based

✉ Baofa Jiang
bjiang@sdu.edu.cn

✉ Liping Yang
yliping@sdu.edu.cn

1 Department of Epidemiology, School of Public Health, Shandong University, No. 44 Wenhuaxi Road, 250012 Jinan, China

2 Jinan Maternity and Child Care Hospital, Jinan, China

3 Jinan Centers for Disease Control and Prevention, Jinan, China

4 Centre for Health Management and Policy, Shandong University, Jinan, China

study conducted in Nigeria revealed that 55.9% neonatal deaths occurred among LBW infants [7]. Besides the elevated neonatal mortality, PTB and LBW can also result in delayed effects into adolescence and adulthood such as metabolic and chronic diseases (e.g. the metabolic syndrome, diabetes, obesity, and coronary heart disease) [8].

To date, it has been identified that various factors can influence PTB or LBW, including maternal education level, gestational age, gestational hypertension, gestational diabetes mellitus, alcohol consumption, and active or passive smoking status [9–11]. In recent years, quite a number of studies have been conducted on the associations between genetic factors and PTB or LBW. Among numerous genetic variants, the single nucleotide polymorphisms (SNPs) of methylenetetrahydrofolate reductase (*MTHFR*) gene have become a research hotspot because of its implication in hyperhomocysteinemia, which may lead to an increased risk of adverse birth outcomes [12].

MTHFR, for its function of catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, plays a key role in the important enzymatic process in remethylation of homocysteine (Hcy) into methionine [12, 13]. The *MTHFR* gene is composed of 11 exons and located on the short arm of chromosome1 (1p36.3) [14]. A cytosine to thymine substitution at position 677 in exon 4 (*MTHFR* C677T) is a quite common polymorphism in this gene, leading to the substitution of valine for alanine in the corresponding protein and then followed by a reduction in enzyme activity [13]. Consequently, the conversion of Hcy into methionine may be reduced, resulting in accumulation of plasma Hcy [12].

Plenty of studies concerning a potential role of the *MTHFR* C677T in PTB and LBW susceptibility have been conducted by researchers but with inconsistent results, which might be mainly caused by insufficient population size of one single study [15]. Therefore, it is essential to perform a comprehensive meta-analysis to explore the relationship between polymorphism of *MTHFR* C677T and PTB as well as LBW susceptibility since some true associations might not be found in small-scale researches. In 2009, a meta-analysis using seven case–control studies showed a significant association of *MTHFR* mutation with intrauterine growth restriction (IUGR, one of LBW-related outcomes) and the pooled OR was 1.35 (95% CI 1.04–1.75) [16]. A meta-analysis adopting studies published before August 2014 found that there was no association of *MTHFR* C677T polymorphism with PTB [17]. However, neither of the two meta-analyses differentiated the maternal and neonatal gene sources, nor did they conduct subgroup analysis by gene sources, which might obscure the inherent association. Additionally, several new eligible studies have been published following these two meta-analyses.

With a more rigorous and precise inclusion criteria, we designed a meta-analysis on the association of maternal and neonatal *MTHFR* C677T with risk of PTB and LBW, respectively. It is worth noting that, in the view of most obstetric and neonatal physicians, small for gestational age (SGA), fetal growth retardation (FGR), and intrauterine growth restriction (IUGR) are generally deemed to manifest as LBW clinically [18]. SGA is defined as a specified birth weight less than the 10th percentile of infants born at a given gestational age. The definitions of FGR and IUGR are similar, usually defined as estimated fetal weight less than the 10th percentile, according to gestational age [19]. Thus, considering the highly similar diagnostic criteria and clinical feature, SGA, FGR, and IUGR were all included as LBW uniformly in this research.

Materials and methods

Search strategy

This meta-analysis was performed following the MOOSE guidelines [20]. A comprehensive review of studies from Embase, Medline, China Biological Medicine Database (CBM), Chinese National Knowledge Infrastructure (CNKI) and Wanfang Database was performed to identify the relevant studies before June 2016. The search was limited to English and Chinese, using the following key words: ("Methylenetetrahydrofolate reductase" OR "MTHFR" OR "C677T" OR "rs1801133") AND ("variation" OR "polymorphism" OR "mutations") AND ("Preterm Birth" OR "Low Birth Weight" OR "Intrauterine Growth Retardation" OR "Fetal Growth Retardation" OR "Small for Gestational Age"). References listed in the retrieved articles were also checked and included as additional studies if deemed eligible. For studies using overlapping data or on the same study group, only the most complete or recent studies were included.

Eligibility criteria

Studies included in this meta-analysis should be consistent with the following criteria: (1) studies with case–control design; (2) focusing on the association between *MTHFR* C677T polymorphism and PTB, LBW, SGA, IUGR, or FGR; (3) providing sufficient information on the numbers or genotype frequencies in cases and controls in each original study; (4) using healthy individuals as controls.

The exclusion criteria were as follows: (1) abstracts, reviews, comments, or case reports; (2) duplicate publications of data from the same study. Studies with the definition or raw data of interest could not be obtained after attempting to contact the corresponding authors via e-mail.

Data extraction and quality assessment

Two investigators extracted the relevant data independently and disagreements were resolved by the third investigator. The following information was extracted: the author's name, year of publication, country of origin, ethnicity, source of controls, subjects of study, gene sources, obstetric outcomes, diagnostic criteria of cases, genotyping method, and the number of cases and controls for each genotype. In addition, deviation from Hardy–Weinberg equilibrium (HWE) for distribution of the allele frequencies was analyzed by a Chi square test among the controls [21].

The quality of eligible studies was independently assessed by two investigators according to the 9-star Newcastle–Ottawa Scale [22] and the overall scores were obtained for each study. The study with best quality can be assigned nine stars and a study is considered high quality if it scored six stars or more [23].

Statistical analysis

The strength of association between the *MTHFR* C677T polymorphisms and PTB and LBW risk was estimated by ORs with 95% CIs according to allele contrast (T vs. C), homozygote (TT vs. CC), heterozygote (CT vs. CC), recessive (TT vs. CT + CC), and dominant (TT + CT vs. CC) models. Higgins I^2 statistics were performed to describe the percentage of total variation because of between-study heterogeneity rather than chance [24]. Heterogeneity between studies was deemed statistically significant if $I^2 > 50\%$ and then the random effects model (DerSimonian–Laird approach) [25] was performed to pool the effect estimates. Otherwise, a fixed effects model (Mantel–Haenszel approach) [26] was adopted. To detect the sources of heterogeneity, subgroup analysis were conducted by source of controls (hospital- or population-based controls), ethnicity (Asian, Caucasian, or others), country status (developing or developed countries), singleton pregnancy (yes or not), and case definition. To assess the impact of studies with samples not in HWE ($P < 0.05$), sensitivity analysis was performed by removing these studies and then recalculating ORs with CIs [27]. Publication bias was evaluated with the Egger's regression asymmetry test, AS-Thompson test, and trim-and-fill method. AS-Thompson test, using an arcsine transformation, has been shown to work reasonably well in studies with substantial heterogeneity [28]. The pooled effect estimates might be inflated if publication bias exists, and trim-and-fill method is usually used to adjust for publication bias [29]. This method incorporates the hypothetical "missing" studies, as though they actually existed, to recalculate a pooled effect estimate [30]. All statistical analyses were carried out using STATA (version 13.0, Stata Corporation, College Station, Texas, USA) and R (version 3.3.0, The R Project for Statistical Computing, Vienna, Austria), by two-sided P values. A P value of <0.05 was considered statistically significant.

Results

Study characteristics

The initial literature search obtained 405 records from Embase, Medline, CBM, CNKI, and Wanfang Databases and eight additional studies identified through other sources. 393 articles were excluded, and a total of 25 studies from 20 articles were included in this meta-analysis (Fig. 1). Moreover, all of the included articles were assigned 6 stars or more. Characteristics of these studies were summarized in Table 1.

Maternal MTHFR C677T polymorphism and PTB

There were eight studies [31–38] concerning maternal *MTHFR* C677T gene polymorphism and PTB in this meta-analysis (main results are presented in Table 2). Due to the existence of relatively large heterogeneity between studies (ranging from 65.9 to 81.4%), a random effects model was applied under all the five genetic inheritance models. An increased risk of PTB was indicated under allele contrast model (T vs. C, OR = 1.36, 95% CI 1.02–1.81), homozygote model (TT vs. CC, OR = 1.70, 95% CI 1.07–2.68, Fig. 2), and recessive model (TT vs. CT + CC, OR = 1.49, 95% CI 1.00–2.22), but not dominant model (TT + CT vs. CC) or heterozygote model (CT vs. CC).

In hospital-based (HB) subgroup, an increased risk of PTB was observed under four genetic inheritance models (for allele contrast model: OR = 1.46, 95% CI 1.08–1.98; for homozygote model: OR = 1.90, 95% CI 1.17–3.08; for recessive model: OR = 1.62, 95% CI 1.05–2.48; for dominant model: OR = 1.55, 95% CI 1.07–2.25; for heterozygote model: OR = 1.40, 95% CI 0.98–2.01). No significant association was found under all the models among population-based (PB) subgroup.

In the subgroup analysis by ethnicity, an increased risk of PTB was observed among Asians under the allele contrast model (OR = 1.48, 95% CI 1.02–2.15) and homozygote model (OR = 1.81, 95% CI 1.03–3.19). No significant association was found under other models of Asian and other ethnicity subgroups.

Pooled results of studies from developing countries indicated that except the heterozygote model, there were significant associations between maternal *MTHFR* C677T polymorphism and PTB risk under the other genetic inheritance models of allele contrast model (OR = 1.50, 95% CI 1.09–2.07), homozygote model (OR = 1.94, 95% CI

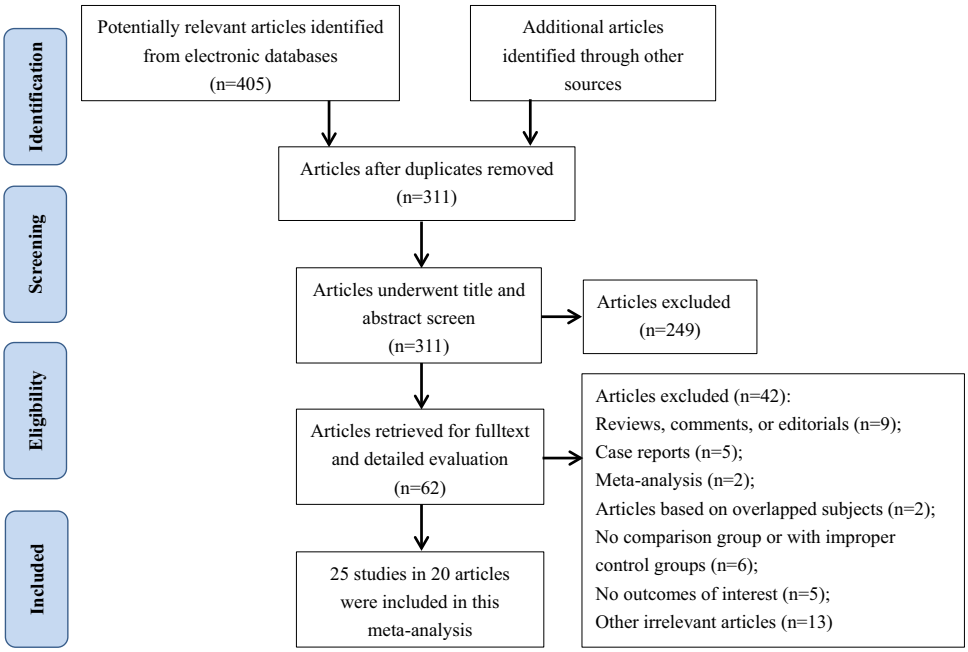

Fig. 1 Flow diagram of study selection

1.17–3.22, Fig. 3), recessive model (OR = 1.64, 95% CI 1.05–2.57), and dominant model (OR = 1.60, 95% CI 1.07–2.39). No significant association was detected in studies from developed countries (Table 3).

Maternal MTHFR C677T polymorphism and LBW risk

There were 11 studies [31, 34, 39–47] concerning maternal *MTHFR* C677T gene polymorphism and LBW in this meta-analysis (Table 2). Due to the existence of relatively large heterogeneity between studies (ranging from 54.6 to 80.0%), a random effects model was applied under all the genetic inheritance models except for the recessive model ($I^2 = 35.0\%$). An increased risk of LBW was indicated under allele contrast model (OR = 1.69, 95% CI 1.25–2.28), homozygote model (OR = 2.26, 95% CI 1.44–3.54, Fig. 4), dominant model (OR = 1.71, 95% CI 1.19–2.47), and recessive model (OR = 1.79, 95% CI 1.42–2.26), but not heterozygote model.

In HB subgroup, an increased risk of LBW was observed under all the five genetic inheritance models (for allele contrast model: OR = 1.83, 95% CI 1.31–2.55; for homozygote model: OR = 2.58, 95% CI 1.62–4.09; for recessive model: OR = 1.94, 95% CI 1.51–2.49; for dominant model: OR = 1.89, 95% CI 1.25–2.87; for heterozygote model: OR = 1.52, 95% CI 1.01–2.30), but not among PB subgroup.

In the subgroup analysis by ethnicity, an increased risk of LBW was observed among Asians and Caucasians under all the genetic inheritance models (In Asians, for allele contrast model: OR = 1.93, 95% CI 1.42–2.62; for homozygote model: OR = 3.00, 95% CI 2.02–4.46; for recessive model: OR = 2.18, 95% CI 1.58–3.02; for dominant model: OR = 2.10, 95% CI 1.36–3.25; for heterozygote model: OR = 1.77, 95% CI 1.10–2.85. In Caucasians, for allele contrast model: OR = 2.64, 95% CI 1.82–3.83; for homozygote model: OR = 3.76, 95% CI 1.77–8.01; for recessive model: OR = 3.72, 95% CI 1.77–7.82; for dominant model: OR = 2.83, 95% CI 1.75–4.60; for heterozygote model: OR = 2.45, 95% CI 1.31–4.55). No significant association was found under all the models of other ethnicity subgroups.

Pooled results from developing countries also indicated that there were significant associations between maternal *MTHFR* C677T polymorphism and LBW risk under all genetic inheritance models (for allele contrast model: OR = 1.92, 95% CI 1.53–2.42; for homozygote model: OR = 3.09, 95% CI 2.09–4.58, Fig. 5; for recessive model: OR = 2.23, 95% CI 1.62–3.08; for dominant model: OR = 2.16, 95% CI 1.55–3.01; for heterozygote model: OR = 1.88, 95% CI 1.30–2.73). However, no such association was detected in studies from developed countries under all these models except for the recessive model (OR = 1.40, 95% CI 1.00–1.96).

We also conducted subgroup analysis by case definition, and we found an increased risk of LBW under allele contrast model (OR = 4.21, 95% CI 2.15–8.25), homozygote model (OR = 4.21, 95% CI 1.63–10.90), recessive model (OR = 4.21, 95% CI 1.63–10.90), and dominant model (OR = 4.21, 95% CI 1.63–10.90) in subgroup of birthweight less than 5 percentile. Additionally, we found an increased risk of LBW under allele contrast model (OR = 1.51, 95% CI 1.02–2.24) and recessive model (OR = 1.04, 95% CI 1.04–1.99) in subgroup of birthweight less than

Table 1 Main characteristics of studies included in this meta-analysis

Authors	Year	Country	Ethnicity	Sources of controls	Gene sources	Outcomes	Genotyping method	Cases			Controls			P_{HWE}	NOS score
								CC	CT	TT	CC	CT	TT		
Kupferminc et al.	1999	Israel	Caucasian	HB	M	FGR[a]	PCR	32	0	12	101	0	9	<0.001	8
Gebhardt et al.	2001	South Africa	Mixed	HB	M	IUGR[b]	PCR	28	8	0	82	30	2	0.693	6
Infante-Rivard et al.	2002	Canada	Mixed	HB	M	IUGR[b]	PCR	260	185	45	233	199	35	0.399	7
Infante-Rivard et al.	2002	Canada	Mixed	HB	N	IUGR[b]	PCR	255	172	40	231	185	45	0.375	7
Kupferminc et al.	2002	Israel	Caucasian	HB	M	IUGR[c]	PCR	21	0	5	47	0	5	<0.001	8
Resch et al.	2004	Austria	Caucasian	HB	M	PTB	PCR	12	8	1	26	16	2	0.815	7
Chen et al.	2004	China	Asian	HB	M	PTB	PCR	48	119	76	60	97	90	<0.001	6
Chen et al.	2004	China	Asian	HB	M	LBW[d]	PCR	39	103	100	69	113	66	0.163	6
Chen et al.	2004	China	Asian	HB	N	PTB	PCR	44	115	84	32	121	94	0.473	6
Chen et al.	2004	China	Asian	HB	N	LBW[d]	PCR	32	102	108	44	134	70	0.145	6
Valdez et al.	2005	Mexico	Mixed	HB	M	PTB	PCR	14	44	28	45	86	31	0.375	6
Engel et al.	2006	USA	Mixed	PB	M	PTB	Taqman	90	38	8	357	175	41	0.003	7
Engel et al.	2006	USA	Mixed	PB	M	SGA[b]	Taqman	115	53	12	334	169	36	0.875	7
Ulukus et al.	2006	Turkey	Caucasian	HB	M	IUGR[b]	PCR	2	8	2	11	13	0	0.069	7
Glanville et al.	2006	UK	Caucasian	HB	N	LBW	PCR	126	88	29	56	59	17	0.813	6
He et al.	2007	China	Asian	HB	M	FGR[b]	PCR	29	25	8	48	12	5	0.006	6
Ozbek et al.	2008	Turkey	Caucasian	HB	M	SGA	PCR	36	28	0	78	26	0	0.145	6
Leng et al.	2009	China	Asian	HB	M	FGR[b]	PCR-RFLP	30	22	19	42	13	5	0.461	8
Zhong et al.	2009	China	Asian	HB	M	LBW[d]	PCR-RFLP	60	29	9	129	60	6	0.759	6
Yang et al.	2010	China	Asian	HB	N	PTB	PCR	27	42	32	69	72	30	0.144	6
Du et al.	2013	China	Asian	HB	M	PTB	PCR	59	91	70	89	93	38	0.114	8
Nan et al.	2015	China	Asian	HB	M	PTB	PCR-RFLP	26	44	38	40	49	19	0.554	7
Tiwari et al.	2015	India	Asian	HB	M	PTB	PCR-RFLP	148	49	12	170	20	4	0.001	6
Wang et al.	2015	China	Asian	HB	M	PTB	SNaPshot	108	142	65	53	100	35	0.312	6
Mei et al.	2015	China	Asian	HB	N	PTB	PCR	22	34	25	41	43	18	0.259	7

HB hospital-based, *PB* population-based, *M* maternal source, *N* neonatal source, *FGR* fetal growth retardation, *IUGR* intrauterine growth retardation, *PTB* preterm birth, *LBW* low birth weight, *SGA* small for gestational age, P_{HWE} *P* value for Hardy–Weinberg Equilibrium in control group

[a]Birth weight less than 5th percentile for gestational age

[b]Birth weight less than 10th percentile for gestational age

[c]Birth weight less than 3rd percentile for gestational age

[d]Birth weight less than 2500 g

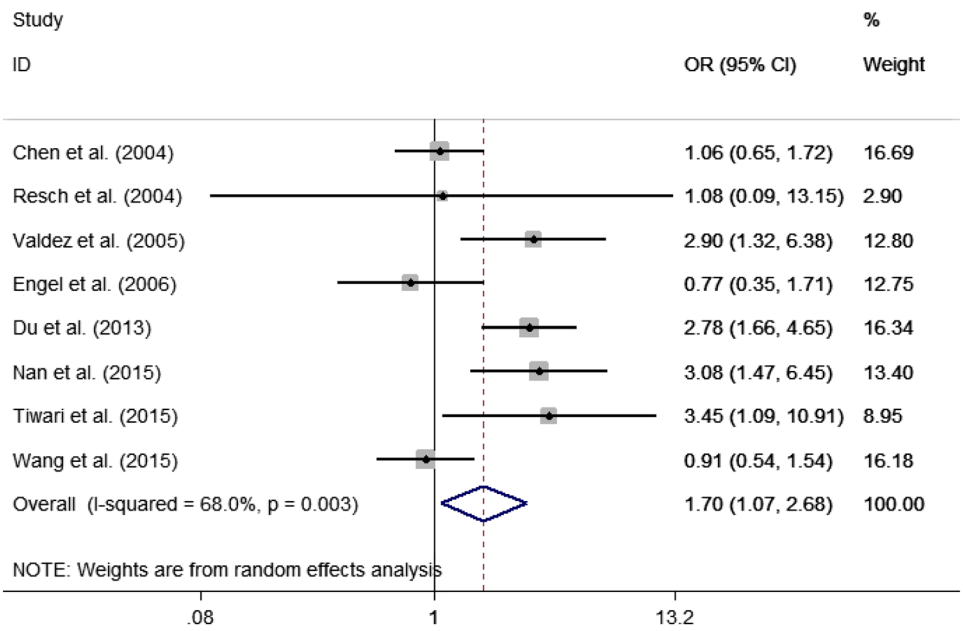

Fig. 2 Results of the random effect meta-analysis of maternal *MTHFR* C677T polymorphism and PTB under homozygote model (TT vs. CC)

10 percentile. Besides, we found significant association between *MTHFR* C677T polymorphism and LBW risk under allele contrast model (OR = 1.62, 95% CI 1.30–2.01), homozygote model (OR = 2.77, 95% CI 1.76–4.36), recessive model (OR = 2.05, 95% CI 1.43–2.93), and borderline significant association under dominant model (OR = 1.60, 95% CI 1.00–2.57) when birthweight was less than 2500 g (results of subgroup analysis are presented in Table 4).

Neonatal *MTHFR* C677T polymorphism and PTB or LBW

There were three studies concerning maternal *MTHFR* C677T gene polymorphism with PTB [31, 48, 49] and LBW [31, 41, 50], respectively, in this meta-analysis. Large heterogeneity between studies (ranging from 58.0 to 86.7%) was detected and a random effects model was applied under all the genetic inheritance models except for the heterozygote model of LBW ($I^2 = 0.0\%$). There was null association between neonatal *MTHFR* C677T polymorphism and PTB or LBW risk (main results are presented in Table 5). Due to that the total numbers of studies on PTB and LBW were only three, subgroup analysis was not conducted.

Sensitivity analysis

Three out of 8 studies of *MTHFR* polymorphism with PTB risk [31, 34, 35] and 4 out of 11 studies of *MTHFR* polymorphism with LBW risk [34, 39, 42, 45] were not consistent with HWE. However, the recalculated results were unchanged after excluding these studies (Table 2). The sensitivity analysis showed that the pooled results from this meta-analysis were statistically robust.

All of the six studies on neonatal *MTHFR* C677T polymorphism and PTB and LBW risk were consistent with HWE, so we did not perform sensitivity analysis.

Publication bias

The *P* value of Egger's regression asymmetry test and AS-Thompson test indicated that there was evidence of publication bias in the allele contrast model (Egger's test, $P = 0.048$), dominant model (Egger's test, $P = 0.013$ and AS-Thompson test, $P = 0.018$), and heterozygote model (for Egger's test, $P = 0.026$ and for AS-Thompson test, $P = 0.020$) of maternal *MTHFR* C677T gene polymorphism and LBW. By using trim-and-fill method, the adjusted effect estimates were attenuated, but their statistical significance was consistent with the unadjusted result (for allele contrast model, OR = 1.38, 95% CI 1.03–1.85; for dominant model, OR = 1.54, 95% CI 1.09–2.18; for heterozygote model, OR = 1.22, 95% CI 0.87–1.72). In addition, the results also suggested that in the three models, there were three, two, and two more hypothetical missing studies, respectively. No evidence of publication bias was found in other genetic inheritance models.

Discussion

In the present meta-analysis, 25 studies were included to explore the association between *MTHFR* gene polymorphisms and the risk of PTB (8 studies for maternal and 3

Table 2 Main results of the pooled ORs in meta-analysis of the maternal *MTHFR* C667T polymorphism with PTB and LBW outcomes

Outcomes	Comparison model	Studies (cases/controls)	Test of association		M*	I^2 (%)	Publication bias		Sensitivity analysis	
			OR (95% CI)	P_{OR}			*P* of Egger's test	*P* of AS-Thompson test	OR (95% CI)	P_{OR}
PTB	T vs. C	8 (1338/1736)	1.36 (1.02, 1.81)	0.034	R	81.4	0.385	0.546	1.43 (1.03, 1.99)	0.034
	TT vs. CC	8 (1338/1736)	1.70 (1.07, 2.68)	0.024	R	68.0	0.627	0.709	2.05 (1.13, 3.70)	0.018
	TT + CT vs. CC	8 (1338/1736)	1.42 (1.00, 2.02)	0.051	R	74.3	0.393	0.569	1.41 (0.89, 2.23)	0.143
	TT vs. CT + CC	8 (1338/1736)	1.49 (1.00, 2.22)	0.049	R	68.3	0.502	0.667	1.83 (1.30, 2.58)	0.001
	CT vs. CC	8 (1338/1736)	1.30 (0.94, 1.81)	0.113	R	65.9	0.387	0.537	1.17 (0.80, 1.71)	0.412
LBW	T vs. C	11 (1325/1978)	1.69 (1.25, 2.28)	0.001	R	80.0	0.048	0.060	1.55 (1.09, 2.20)	0.015
	TT vs. CC	10 (1261/1874)	2.26 (1.44, 3.54)	<0.001	R	54.6	0.235	0.395	2.47 (1.30, 4.70)	0.006
	TT + CT vs. CC	11 (1325/1978)	1.71 (1.19, 2.47)	0.004	R	75.5	0.013	0.018	1.58 (1.01, 2.46)	0.045
	TT vs. CT + CC	10 (1261/1874)	1.79 (1.42, 2.26)	<0.001	F	35.0	0.276	0.404	1.83 (1.40, 2.40)	<0.001
	CT vs. CC	9 (1255/1816)	1.39 (0.98, 1.96)	0.062	R	68.4	0.026	0.020	1.35 (0.92, 2.00)	0.127

*M** model of meta-analysis, *R* random effect model, *F* fixed effect model

studies for neonatal C677T mutation) and LBW (11 studies for maternal and 3 studies for neonatal C677T mutation). According to the pooled results, our study showed associations between maternal *MTHFR* C677T polymorphism with PTB and LBW under most of the genetic models, respectively. Specifically, the carriers of the TT genotype increased the risk of PTB and LBW. In addition, no association between neonatal *MTHFR* C677T polymorphism with PTB or LBW was detected under all genetic models.

Healthy pregnancy outcome depends not only on trophoblast invasion into the uterine vasculature, but also on the development and maintenance of an adequate uteroplacental circulation in the mother [43]. The placenta, transferring oxygen and nutrients required for fetal development and energy production from maternal blood to the fetus, also has the coagulation/anticoagulation system in balance to fulfill its regular functions [51]. *MTHFR* plays a key role in folate metabolism, converting 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the main form of circulatory folate and the methyl donor for the conversion of homocysteine to methionine [52]. That is to say, *MTHFR* uses folate to metabolise and thereby remove homocysteine [53]. An increase of plasma Hcy concentration happens when enzyme activity is reduced due to *MTHFR* C677T, which leads to oxidative stress, arteriolar constriction, endothelial damage, and placental thrombosis [12, 39]. All these conditions might be associated with impaired flow and prothrombotic changes in the vessel wall, inadequate trophoblast invasion into the uterine vasculature, and placental hypoperfusion that subsequently triggers poor pregnancy outcomes including PTB and LBW [33, 43].

Other than PB subgroup, a significant increased risk of PTB and LBW was observed under almost all of the genetic inheritance models in HB subgroup. Although the population-based controls could represent general population well, these results should be interpreted with caution. For the reason that there was only one study included in PB subgroup, the results may be unstable and lack of representativeness. In addition, compared to the corresponding results of HB subgroup, effect sizes of this population-based study were relatively smaller under all of the genetic inheritance models, which indicated an attenuated effect. It is worth noting that this study enrolled both black and Caucasian populations [34]. In another study enrolling black and Caucasian populations as the majority, a quite large increment of effect size was found after including ethnicity as an adjusted factor [41]. For instance, ORs were 0.93 (95% CI 0.64–1.09) and 1.15 (95% CI 0.72–1.85) before being adjusted, while ORs were 0.98 (95% CI 0.69–1.40) and 1.55 (95% CI 0.83–2.90) after being adjusted under heterozygote and homozygote model, respectively [41]. Thus, the ethnicity of subjects may confound the results above and similarly, the attenuated effect of PB subgroup in

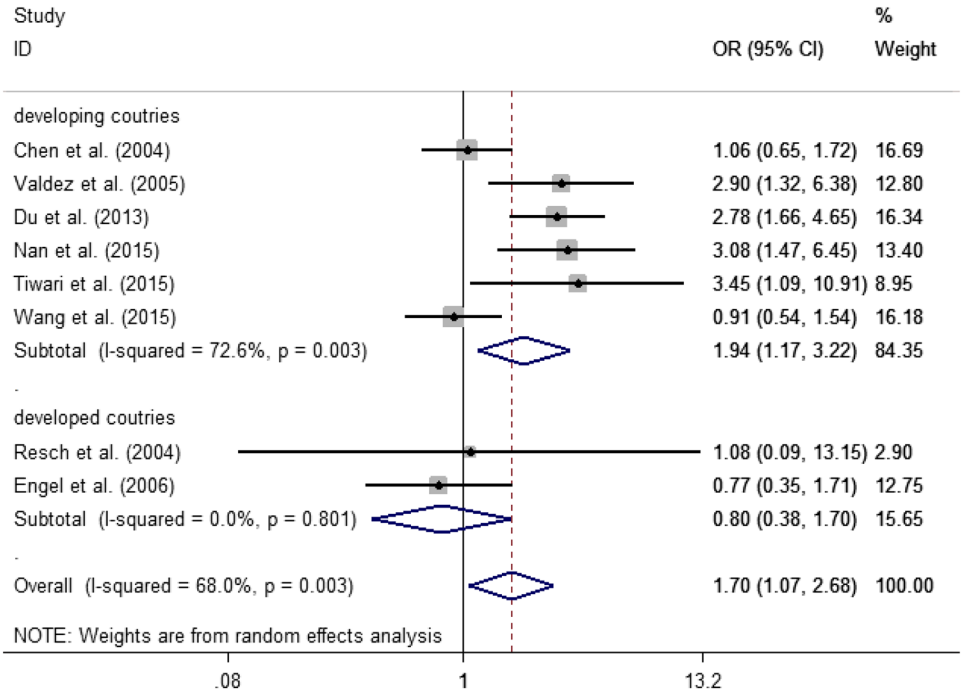

Fig. 3 Subgroup analysis of maternal *MTHFR* C677T polymorphism and PTB under homozygote model (TT vs. CC)

this meta-analysis may also be confounded by this underlying factor.

Subgroup analysis by ethnicity revealed that Asian carriers of the TT genotype of maternal *MTHFR* C677T polymorphism were associated with an increased risk of PTB. Unexpectedly, both Asian and Caucasian carriers of the TT genotype of *MTHFR* C677T polymorphism were associated with an increased risk of LBW. It is possible that diverse genetic backgrounds might interpret these differences [54]. For the reason that only one study was included in Caucasian subgroup when studying the association between PTB and maternal *MTHFR* C677T polymorphism, the nonsignificant results among Caucasians need further validation in large well-designed studies.

Based on development status, 187 countries of the world were classified into categories of 50 developed countries and 137 developing countries by the GBD 2013 group [55]. Interestingly, compared with pooled results of studies from developed countries, results of developing countries showed statistically significant association between maternal *MTHFR* C677T polymorphism and PTB as well as LBW risk. In 1996, legislation was permitted or mandated on folic acid fortification in the United States, Canada, and some other developed countries [53]. However, no such fortification has been instituted in developing countries such as China [56]. What is worse, a national survey in China revealed that only 12.1% women of childbearing age took folic acid supplements and only 8.7% of them took folic acid daily before or during early pregnancy [57]. In addition, due to the low income, inadequate prenatal care, and limited food sources, pregnant women in developing countries might not intake folate as adequately as their counterparts in developed countries do [58]. Furthermore, studies have confirmed that women with the *MTHFR* 677 TT genotype are predisposed to increased plasma homocysteine levels when folate intake is inadequate [59]. From the above, maternal carriers of the TT genotype in developing countries are more likely to deliver infants with PTB or LBW. It is also worth noting that an unexpectedly elevated risk of LBW in developed countries was observed under recessive model (TT vs. CT + CC). An interventional study confirmed that after experiencing a throughout repletion with the 1998 folate U.S. Recommended Dietary Allowance (400 μg/d as dietary folate equivalents) for 7 weeks, women with TT genotype had significant lower serum folate and significant higher plasma total homocysteine (tHcy) concentration than those with CC genotype [60]. However, CT heterozygotes did not differ in their response compared to the CC genotypes [60]. The authors of that study also concluded that the genotype response on 400 μg/d as dietary folate equivalents followed a hierarchical pattern (TT < CT < CC for serum folate and TT > CT > CC for plasma tHcy concentration) [60]. To sum up, it was the hierarchical genotype response pattern for serum folate and plasma tHcy concentration that resulted in the elevated risk of LBW in developed countries in women under recessive model.

As SGA, IUGR, and FGR were all included as LBW in this meta-analysis, subgroup analysis was used to detect the influence of different categories of cases on the final

Table 3 Results of subgroup analysis of the maternal *MTHFR* C667T polymorphism with PTB

Subgroup	No. of study	T vs. C			TT vs. CC			TT vs. CT+CC			TT+CT vs. CC			CT vs. CC		
		OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)
Source of control																
HB	7	1.46 (1.08, 1.98)	0.014	80.9	1.90 (1.17, 3.08)	0.009	67.4	1.62 (1.05, 2.48)	0.028	70.4	1.55 (1.07, 2.25)	0.021	71.5	1.40 (0.98, 2.01)	0.065	64.9
PB	1	0.86 (0.62, 1.19)	0.357	–	0.77 (0.35, 1.71)	0.526	–	0.81 (0.37, 1.77)	0.599	–	0.84 (0.57, 1.25)	0.401	–	0.86 (0.57, 1.31)	0.486	–
Ethnicity																
Asian	5	1.48 (1.02, 2.15)	0.041	86.6	1.81 (1.03, 3.19)	0.039	75.5	1.58 (0.94, 2.66)	0.081	78.5	1.55 (0.98, 2.45)	0.060	79.9	1.41 (0.90, 2.21)	0.135	75.9
Caucasian	1	1.06 (0.45, 2.53)	0.891	–	1.08 (0.09, 13.15)	0.950	–	1.05 (0.09, 12.28)	0.969	–	1.08 (0.38, 3.10)	0.882	–	1.06 (0.90, 2.21)	0.886	–
Others	2	1.18 (0.62, 2.25)	0.610	85.0	1.50 (0.41, 5.48)	0.540	81.4	1.33 (0.54, 3.29)	0.532	70.5	1.24 (0.54, 2.83)	0.616	78.4	1.12 (0.60, 2.08)	0.727	58.4
Country status																
Developing countries	6	1.50 (1.09, 2.07)	0.013	83.9	1.94 (1.17, 3.22)	0.010	72.6	1.64 (1.05, 2.57)	0.029	75.2	1.60 (1.07, 2.39)	0.022	75.8	1.44 (0.97, 2.13)	0.070	70.5
Developed countries	2	0.88 (0.65, 1.20)	0.416	0.0	0.80 (0.38, 1.70)	0.558	0.0	0.83 (0.39, 1.75)	0.625	0.0	0.87 (0.60, 1.26)	0.462	0.0	0.89 (0.60, 1.31)	0.550	0.0

pooled results. Considering that the definitions of cases vary among different studies, we conducted subgroup analysis by the concrete case definition rather than case categories. The results also indicated that maternal carriers of the TT genotype significantly increased the risk of LBW when case definitions were birthweight of less than 5th or 10th percentile, or 2500 g. In the subgroup of birthweight less than 3rd percentile, no significant association was observed. There was only one study included in these subgroups, so the results may be unstable and may lack representativeness.

In the present meta-analysis, all of the sensitivity analysis yielded similar results after excluding studies deviated from HWE, indicating that our results were statistically robust and reliable. Although publication biases were found in several genetic inheritance models, the results of the trim-and-fill method revealed that the publication biases may not affect the stability of our pooled results, strengthening this conclusion.

This meta-analysis had several strengths. Compared with the previous two meta-analyses, more newly published studies were included with a more rigorous and precise inclusion criteria. This is the first meta-analysis, to the best of our knowledge, evaluating the association of maternal and neonatal *MTHFR* C677T and PTB as well as LBW risk, respectively. By differentiating the maternal and neonatal gene sources in this meta-analysis, we have detected the inherent association between *MTHFR* C677T and outcomes of interest. We are also the first one, basing on a relatively large sample size, to report that the maternal carriers of the TT genotype in developing countries are susceptible to delivering infants with PTB or LBW.

Some potential limitations of our meta-analysis should be addressed: first, pooled results were based on unadjusted data from the original studies, and a more precise evaluation should be performed by adjusting some potential confoundings such as social-demographic, gestational, and other factors. Second, this meta-analysis was based on single-factor estimates, and was unable to analyze the potential interactions of gene–gene and gene-environment. Last, but not the least, this meta-analysis on neonatal *MTHFR* C677T polymorphism and PTB and LBW risk was based on a small sample size, indicating that power to detect the reliable association is limited and a possibility of type II error cannot be overlooked. Another consequence of the small sample size mentioned above was that only one study was included in several subgroups, and thus the significance or non-significance of association may be unstable and needs further validation in large well-designed studies.

In summary, the present meta-analysis confirms that there is a conclusive association between maternal *MTHFR* C677T polymorphism and PTB as well as LBW risk and indicates null significant association between neonatal

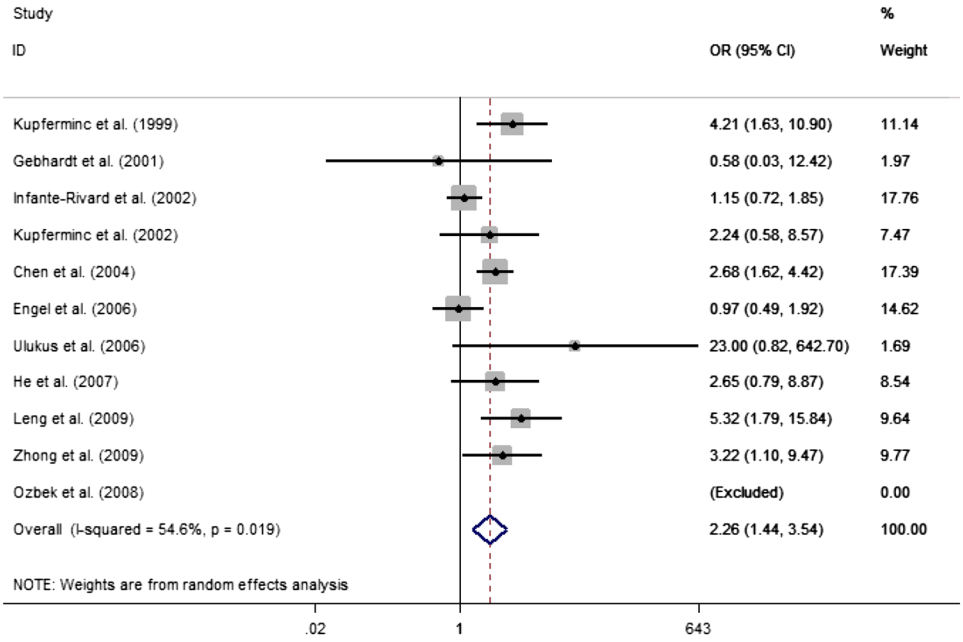

Fig. 4 Results of the random effect meta-analysis of maternal *MTHFR* C677T polymorphism and LBW under homozygote model (TT vs. CC)

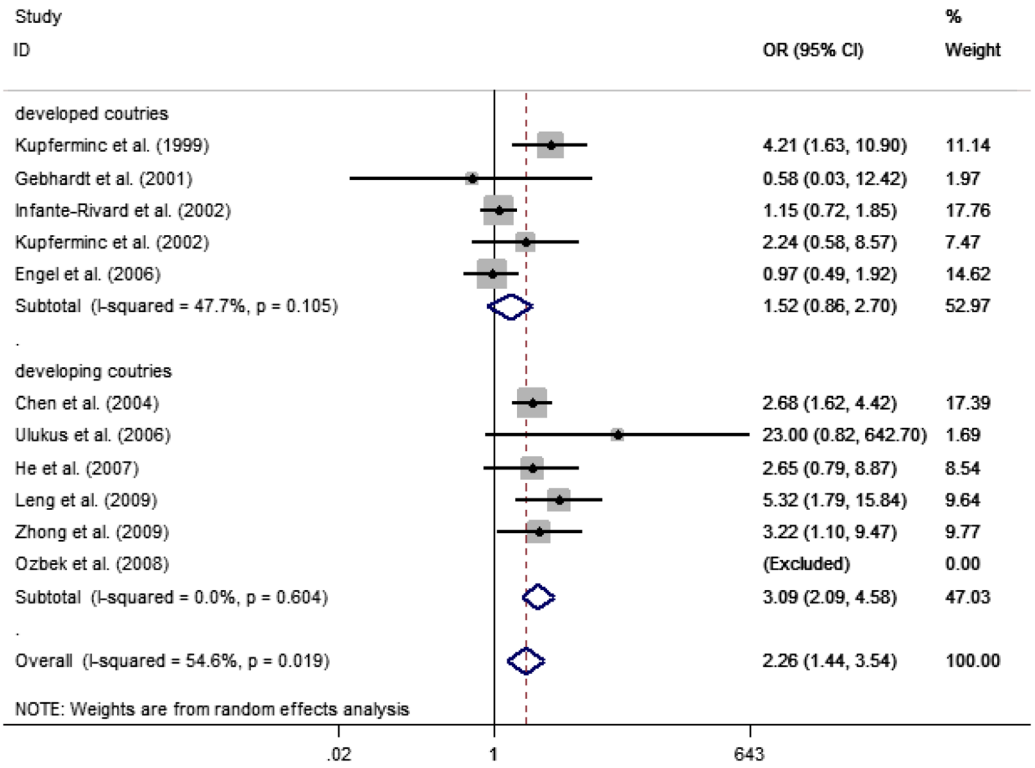

Fig. 5 Subgroup analysis of maternal *MTHFR* C677T polymorphism and LBW under homozygote model (TT vs. CC)

MTHFR C677T polymorphism with PTB or LBW. This result, however, made an implication that identification of maternal *MTHFR* C677T mutation in developing countries may play a key role for primary prevention of PTB and LBW and screening pregnant women of high-risk. Further large, well-designed studies are warranted to fully validate association.

Table 4 Results of subgroup analysis of the maternal *MTHFR* C667T polymorphism with LBW

Subgroup	No. of study	T vs. C			TT vs. CC			TT vs. CT+CC			TT+CT vs. CC			CT vs. CC		
		OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)	OR (95% CI)	P_{OR}	I^2 (%)
Source of control																
HB	10	1.83 (1.31, 2.55)	<0.001	79.3	2.58 (1.62, 4.09)	<0.001	75.6	1.94 (1.51, 2.49)	<0.001	24.5	1.89 (1.25, 2.87)	0.003	75.6	1.52 (1.01, 2.30)	0.044	70.6
PB	1	0.94 (0.71, 1.26)	0.702	–	0.97 (0.49, 1.92)	0.926	–	1.00 (0.51, 1.96)	0.995	–	0.92 (0.65, 1.31)	0.645	–	0.91 (0.63, 1.32)	0.625	–
Ethnicity																
Asian	4	1.93 (1.42, 2.62)	<0.001	50.6	3.00 (2.02, 4.46)	<0.001	54.8	2.18 (1.58, 3.02)	<0.001	0.0	2.10 (1.36, 3.25)	0.001	54.8	1.77 (1.10, 2.85)	0.019	54.6
Caucasian	4	2.64 (1.82, 3.83)	<0.001	75.7	3.76 (1.77, 8.01)	0.001	0.0	3.72 (1.77, 7.82)	0.001	0.0	2.83 (1.75, 4.60)	<0.001	0.0	2.45 (1.31, 4.55)	0.005	0.0
Others	3	0.95 (0.81, 1.11)	0.509	0.0	1.08 (0.73, 1.59)	0.704	0.0	1.15 (0.79, 1.67)	0.465	0.0	0.89 (0.72, 1.08)	0.233	0.0	0.85 (0.69, 1.05)	0.143	0.0
Country status																
Developing countries	6	1.92 (1.53, 2.42)	<0.001	24.8	3.09 (2.09, 4.58)	<0.001	33.5	2.23 (1.62, 3.08)	<0.001	0.0	2.16 (1.55, 3.01)	<0.001	33.5	1.88 (1.30, 2.73)	0.001	38.0
Developed countries	5	1.33 (0.84, 2.11)	0.221	80.8	1.52 (0.88, 2.70)	0.148	65.7	1.40 (1.00, 1.96)	0.047	43.6	1.16 (0.75, 1.80)	0.508	65.7	0.85 (0.69, 1.05)	0.143	0.0
Case definition																
<3rd percentile	1	2.24 (0.87, 5.78)	0.096	–	2.24 (0.58, 8.57)	0.239	–	2.24 (0.58, 8.57)	0.239	–	2.24 (0.58, 8.57)	0.239	–	–	–	–
<5th percentile	1	4.21 (2.15, 8.25)	<0.001	–	4.21 (1.63, 10.90)	0.003	–	4.21 (1.63, 10.90)	0.003	–	4.21 (1.63, 10.90)	0.003	–	–	–	–
<10th percentile	7	1.51 (1.02, 2.24)	0.039	79.9	1.82 (0.93, 3.59)	0.082	78.7	1.04 (1.04, 1.99)	0.030	31.2	1.58 (0.98, 2.53)	0.059	78.7	1.46 (0.93, 2.30)	0.101	73.6
<2500 g	2	1.62 (1.30, 2.01)	<0.001	0.0	2.77 (1.76, 4.36)	<0.001	50.1	2.05 (1.43, 2.93)	<0.001	0.0	1.60 (1.00, 2.57)	0.051	50.1	1.32 (0.86, 2.03)	0.205	30.5

Table 5 Main results of the pooled ORs in meta-analysis of the neonatal C667T polymorphism with PTB and LBW

Outcomes	Comparison model	Studies (cases/controls)	Test of association		M*	I^2 (%)	Publication bias	
			OR (95% CI)	P_{OR}			*P* of Egger's test	*P* of AS-Thompson test
PTB	T vs. C	3 (425/520)	1.34 (0.78, 2.27)	0.286	R	86.6	0.241	0.308
	TT + CT vs. CC	3 (425/520)	1.29 (0.65, 2.57)	0.467	R	78.7	0.478	0.511
	TT vs. CC	3 (425/520)	1.62 (0.59, 4.43)	0.347	R	85.5	0.321	0.393
	TT vs. CT + CC	3 (425/520)	1.51 (0.77, 2.98)	0.233	R	79.2	0.204	0.209
	CT vs. CC	3 (425/520)	1.12 (0.66, 1.90)	0.678	R	58.0	0.406	0.564
LBW	T vs. C	3 (952/841)	1.02 (0.68, 1.54)	0.908	R	86.7	0.934	0.944
	TT + CT vs. CC	3 (952/841)	0.90 (0.64, 1.29)	0.575	R	60.3	0.714	0.611
	TT vs. CC	3 (952/841)	1.10 (0.57, 2.12)	0.784	R	76.4	0.974	0.924
	TT vs. CT + CC	3 (952/841)	1.21 (0.65, 2.23)	0.549	R	79.9	0.516	0.504
	CT vs. CC	3 (952/841)	0.83 (0.67, 1.03)	0.085	F	0.0	0.966	0.865

Author contributions H. Wu: Project development, Manuscript writing; P. Zhu: Results interpretation; X. Geng: Data collection; Z. Gao: Data collection; Z. Liu: Data analysis; L. Cui: Data analysis; B. Jiang: Project development, Manuscript revision; L. Yang: Project supervision, Manuscript revision.

Compliance with Ethical Standards

Funding This study was funded by Shandong Provincial Natural Science Foundation, China (Grant Number ZR2015HM076).

Conflict of interest Author H. Wu declares that he has no conflict of interest. Author P. Zhu declares that she has no conflict of interest. Author X. Geng declares that he has no conflict of interest. Author Z. Liu declares that he has no conflict of interest. Author L. Cui declares that she has no conflict of interest. Author Z. Gao declares that he has no conflict of interest. Author B. Jiang declares that he has no conflict of interest. Author L. Yang declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Sapkota A, Chelikowsky AP, Nachman KE, Cohen AJ, Ritz B (2010) Exposure to particulate matter and adverse birth outcomes: a comprehensive review and meta-analysis. Air Qual Atmos Health (London) 5(4):1–13
2. Martínez-Martínez RE, Moreno-Castillo DF, Loyola-Rodríguez JP, Sánchez-Medrano AG, Miguel-Hernández JHS, Olvera-Delgado JH, Domínguez-Pérez RA (2016) Association between periodontitis, periodontopathogens and preterm birth: is it real? Arch Gynecol Obstet 294(1):47–54. doi:10.1007/s00404-015-3945-1
3. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, Rubens C, Menon R, Van Look PF (2010) The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ 88(1):31–38. doi:10.2471/blt.08.062554
4. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE (2015) Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet 385(9966):430–440. doi:10.1016/s0140-6736(14)61698-6
5. Lawn JE, Kinney M (2014) Preterm birth: now the leading cause of child death worldwide. Sci Transl Med 6(263):263ed221. doi:10.1126/scitranslmed.aaa2563
6. United Nations Children's Fund and World Health Organization (2004) Low birthweight: country, regional and global estimates. UNICEF, New York
7. Adetola AO, Tongo OO, Orimadegun AE, Osinusi K (2011) Neonatal mortality in an urban population in Ibadan, Nigeria. Pediatr Neonatol 52(5):243–250. doi:10.1016/j.pedneo.2011.06.001
8. Jiang M, Qiu J, Zhou M, He X, Cui H, Lerro C, Lv L, Lin X, Zhang C, Zhang H, Xu R, Zhu D, Dang Y, Han X, Zhang H, Bai H, Chen Y, Tang Z, Lin R, Yao T, Su J, Xu X, Liu X, Wang W, Wang Y, Ma B, Qiu W, Zhu C, Wang S, Huang H, Zhao N, Li X, Liu Q, Zhang Y (2015) Exposure to cooking fuels and birth weight in Lanzhou, China: a birth cohort study. BMC Public Health 15:712. doi:10.1186/s12889-015-2038-1
9. Oftedal AM, Busterud K, Irgens LM, Haug K, Rasmussen S (2016) Socio-economic risk factors for preterm birth in Norway 1999–2009. Scand J Public Health 44(6):587–592. doi:10.1177/1403494816653288
10. Tellapragada C, Eshwara VK (2016) Risk factors for preterm birth and low birth weight among pregnant indian women: a hospital-based prospective study. J Prev Med Public Health 49(3):165–175. doi:10.3961/jpmph.16.022
11. Handelzalts JE, Krissi H, Levy S, Freund Y, Carmiel N, Ashwal E, Peled Y (2016) Personality, preterm labor contractions, and psychological consequences. Arch Gynecol Obstet 293(3):575–582. doi:10.1007/s00404-015-3898-4
12. Chen H, Yang X, Lu M (2016) Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss in China: a systematic review and meta-analysis. Arch Gynecol Obstet 293(2):283–290. doi:10.1007/s00404-015-3894-8
13. Gong M, Dong W, He T, Shi Z, Huang G, Ren R, Huang S, Qiu S, Yuan R (2015) MTHFR 677C > T polymorphism increases the male infertility risk: a meta-analysis involving 26 studies. PLoS One 10(3):e0121147. doi:10.1371/journal.pone.0121147

14. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, Chan M, Rozen R (1998) Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome 9(8):652–656. doi:10.1007/s003359900838
15. Pan Y, Zhang W, Ma J, Du Y, Li D, Cai Q, Jiang H, Wang M, Zhang Z, Wang L (2012) Infants' MTHFR polymorphisms and nonsyndromic orofacial clefts susceptibility: a meta-analysis based on 17 case–control studies. Am J Med Genet A 158a(9):2162–2169. doi:10.1002/ajmg.a.35503
16. Facco F, You W, Grobman W (2009) Genetic thrombophilias and intrauterine growth restriction: a meta-analysis. Obstet Gynecol 113(6):1206–1216
17. Chen J, Chen L, Zhu LH, Zhang ST, Wu YL (2016) Association of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism with preterm delivery and placental abruption: a systematic review and meta-analysis. Acta Obstet Gynecol Scand 95(2):157–165
18. Wang H, Hu Y-F, Hao J-H, Chen Y-H, Su P-Y, Wang Y, Yu Z, Fu L, Xu Y-Y, Zhang C, Tao F-B, Xu D-X (2015) Maternal zinc deficiency during pregnancy elevates the risks of fetal growth restriction: a population-based birth cohort study. Sci Rep 5:11262. doi:10.1038/srep11262
19. Infante-Rivard C, Rivard GE, Guiguet M, Gauthier R (2005) Thrombophilic polymorphisms and intrauterine growth restriction. Epidemiology 16(3):281–287
20. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 283(15):2008–2012
21. Song Y-q, Gao Y-l, Pan Z-c, Zhang Y, Li J-h, Wang K-j, Li J-s, Tan H, Fu Q (2016) Preparation and characterization of controlled heparin release waterborne polyurethane coating systems. Chin J Polym Sci 34(6):679–687. doi:10.1007/s10118-016-1787-3
22. O'Connell D (2002) The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Appl Eng Agric 18(6):727–734
23. Gu WJ, Wang F, Tang L, Liu JC (2015) Single-dose etomidate does not increase mortality in patients with sepsis: a systematic review and meta-analysis of randomized controlled trials and observational studies. Chest 147(2):335–346. doi:10.1378/chest.14-1012
24. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327(7414):557–560. doi:10.1136/bmj.327.7414.557
25. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7(3):177–188
26. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22(4):719–748
27. Wu YL, Hu CY, Lu SS, Gong FF, Feng F, Qian ZZ, Ding XX, Yang HY, Sun YH (2014) Association between methylenetetrahydrofolate reductase (MTHFR) C677T/A1298C polymorphisms and essential hypertension: a systematic review and meta-analysis. Metabolism 63(12):1503–1511. doi:10.1016/j.metabol.2014.10.001
28. Sterne JAC, Sutton AJ, Ioannidis JPA, Terrin N, Jones DR, Lau J, Carpenter J, Rücker G, Harbord RM, Schmid CH, Tetzlaff J, Deeks JJ, Peters J, Macaskill P, Schwarzer G, Duval S, Altman DG, Moher D, Higgins JPT (2011) Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ 342:d4002. doi:10.1136/bmj.d4002
29. Jin ZC, Zhou XH, He J (2015) Statistical methods for dealing with publication bias in meta-analysis. Stat Med 34(2):343–360. doi:10.1002/sim.6342
30. Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J (2007) Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 298(22):2654–2664. doi:10.1001/jama.298.22.2654
31. Chen DF, Hu YH, Yang F, Wu BY, Chen L, Fang ZA, Wang LH (2004) Mother's and child's methylenetetrahydrofolate reductase C677T polymorphism is associated with preterm delivery and low birth weight. Beijing Da Xue Xue Bao 36(3):248–253 **(Chinese)**
32. Resch B, Gallistl S, Kutschera J, Mannhalter C, Muntean W, Mueller WD (2004) Thrombophilic polymorphisms—Factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations—and preterm birth. Wien Klin Wochenschr 116(17–18):622–626
33. Valdez LL, Quintero A, Garcia E, Olivares N, Celis A, Rivas F Jr, Rivas F (2004) Thrombophilic polymorphisms in preterm delivery. Blood Cells Mol Dis 33(1):51–56. doi:10.1016/j.bcmd.2004.04.011
34. Engel SM, Olshan AF, Siega-Riz AM, Savitz DA, Chanock SJ (2006) Polymorphisms in folate metabolizing genes and risk for spontaneous preterm and small-for-gestational age birth. Am J Obstet Gynecol 195(5):1231.e1–1231.e11. doi:10.1016/j.ajog.2006.07.024
35. Tiwari D, Bose PD, Das S, Das CR, Datta R, Bose S (2015) MTHFR (C677T) polymorphism and PR (PROGINS) mutation as genetic factors for preterm delivery, fetal death and low birth weight: a Northeast Indian population based study. Meta Gene 3:31–42
36. Wang BJ, Liu MJ, Wang Y, Dai JR, Tao JY, Wang SN, Zhong N, Chen Y (2015) Association between SNPs in genes involved in folate metabolism and preterm birth risk. Genet Mol Res 14(1):850–859
37. Du JY, Yang XY, Gong B, Guan YY, Mei LY, Li YM (2013) Relationship of plasma homocysteine levels, folate levels and MTHFR gene polymorphisms with premature delivery. J Sun Yat-sen Univ (Med Sci) 34(6):894–899 **(Chinese)**
38. Nan Y, Li H (2015) MTHFR genetic polymorphism increases the risk of preterm delivery. Int J Clin Exp Pathol 8(6):7397–7402
39. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G, Lessing JB (1999) Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 340(1):9–13
40. Gebhardt GS, Scholtz CL, Hillermann R, Odendaal HJ (2001) Combined heterozygosity for methylenetetrahydrofolate reductase (MTHFR) mutations C677T and A1298C is associated with abruptio placentae but not with intrauterine growth restriction. Eur J Obstet Gynecol Reprod Biol 97(2):174–177
41. Infante-Rivard C, Rivard GE, Yotov WV, Genin E, Guiguet M, Weinberg C, Gauthier R, Feoli-Fonseca JC (2002) Absence of association of thrombophilia polymorphisms with intrauterine growth restriction. N Engl J Med 347(1):19–25. doi:10.1056/nejm200207043470105
42. Kupferminc MJ, Many A, Bar-Am A, Lessing JB, Ascher-Landsberg J (2002) Mid-trimester severe intrauterine growth restriction is associated with a high prevalence of thrombophilia. BJOG 109(12):1373–1376
43. Ulukuş M, Eroğlu Z, Yeniel AÖ, Toprak E, Kosova B, Turan ÖD, Ulukuş M (2006) Frequency of factor V Leiden (G1691A), prothrombin (G20210A) and methylenetetrahydrofolate reductase (C677T) genes mutations in woman with adverse pregnancy outcome. J Turk Ger Gynecol Assoc 7(3):195–201

44. Ozbek N, Atac FB, Verdi H, Cetintas S, Gurakan B, Haberal A (2008) Relationship between small-for-gestational age births and maternal thrombophilic mutations. Thromb Res 122(2):175–178. doi:10.1016/j.thromres.2007.10.004
45. He P, Meng H, Zhang JP (2007) Relationship of MTHFR C677T polymorphisms and fetal growth restriction. Maternal Child Health Care China 22 (35):5032–5034. doi:10.3969/j.issn.1001-4411.2007.35.047 **(in Chinese)**
46. Leng JJ (2009) The relationship of MTHFR C677T polymorphism and plasma homocysteine level with fetal growth restriction. Guangzhou Medical College, China
47. Zhong QA, Qiu XQ, Zeng XY, Deng YY, Wu TY (2009) Association of low birth weight with genetic polymorphism of MTHFR and CBS in mother. Chin J Birth Health Hered 17 (3):22–24. doi:10.13404/j.cnki.cjbhh.2009.03.055 **(in Chinese)**
48. Yang Y, Jin L, Yuan JJ, Wu D, Shang Q, Wu L, Han X, Liang B, Xu L, Tang JL (2010) Association of genetic polymorphisms in methylenetetrahydrofolate reductase gene, plasminogen activator inhibitor-1 gene with preterm birth and spastic cerebral palsy. J Appl Clin Pediatr 25(20):1580–1582 **(Chinese)**
49. Mei M, Xi HL, Ren HY, Guo HY, Wang YT, Li J (2015) The relationship between MTHFR Gene, PAI gene polymorphism and preterm birth. Prog Modern Biomed 15(33):6435–6438. doi:10.13241/j.cnki.pmb.2015.33.009 **(in Chinese)**
50. Glanville T, Yates Z, Ovadia L, Walker JJ, Lucock M, Simpson NA (2006) Fetal folate C677T methylenetetrahydrofolate reductase gene polymorphism and low birth weight. J Obstet Gynaecol 26(1):11–14. doi:10.1080/01443610500363865
51. Uvuz F, Kilic S, Yilmaz N, Tuncay G, Cakar E, Yuksel B, Bilge U (2009) Relationship between preterm labor and thrombophilic gene polymorphism: a prospective sequential cohort study. Gynecol Obstet Invest 68(4):234–238
52. Colson NJ, Naug HL, Nikbakht E, Zhang P, McCormack J (2015) The impact of MTHFR 677 C/T genotypes on folate status markers: a meta-analysis of folic acid intervention studies. Eur J Nutr. doi:10.1007/s00394-015-1076-x
53. Clarke R, Bennett DA, Parish S, Verhoef P, Dotsch-Klerk M, Lathrop M, Xu P, Nordestgaard BG, Holm H, Hopewell JC, Saleheen D, Tanaka T, Anand SS, Chambers JC, Kleber ME, Ouwehand WH, Yamada Y, Elbers C, Peters B, Stewart AF, Reilly MM, Thorand B, Yusuf S, Engert JC, Assimes TL, Kooner J, Danesh J, Watkins H, Samani NJ, Collins R, Peto R (2012) Homocysteine and coronary heart disease: meta-analysis of MTHFR case–control studies, avoiding publication bias. PLoS Med 9(2):e1001177. doi:10.1371/journal.pmed.1001177
54. Alizadeh S, Djafarian K, Moradi S, Shab-Bidar S (2016) C667T and A1298C polymorphisms of methylenetetrahydrofolate reductase gene and susceptibility to myocardial infarction: a systematic review and meta-analysis. Int J Cardiol 217:99–108. doi:10.1016/j.ijcard.2016.04.181
55. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, Wolfe C, Hamadeh RR, Moore A, Werdecker A, Gessner BD, Te Ao B, McMahon B, Karimkhani C, Yu C, Cooke GS, Schwebel DC, Carpenter DO, Pereira DM, Nash D, Kazi DS, De Leo D, Plass D, Ukwaja KN, Thurston GD, Yun Jin K, Simard EP, Mills E, Park EK, Catala-Lopez F, deVeber G, Gotay C, Khan G, Hosgood HD 3rd, Santos IS, Leasher JL, Singh J, Leigh J, Jonas JB, Sanabria J, Beardsley J, Jacobsen KH, Takahashi K, Franklin RC, Ronfani L, Montico M, Naldi L, Tonelli M, Geleijnse J, Petzold M, Shrime MG, Younis M, Yonemoto N, Breitborde N, Yip P, Pourmalek F, Lotufo PA, Esteghamati A, Hankey GJ, Ali R, Lunevicius R, Malekzadeh R, Dellavalle R, Weintraub R, Lucas R, Hay R, Rojas-Rueda D, Westerman R, Sepanlou SG, Nolte S, Patten S, Weichenthal S, Abera SF, Fereshtehnejad SM, Shiue I, Driscoll T, Vasankari T, Alsharif U, Rahimi-Movaghar V, Vlassov VV, Marcenes WS, Mekonnen W, Melaku YA, Yano Y, Artaman A, Campos I, MacLachlan J, Mueller U, Kim D, Trillini M, Eshrati B, Williams HC, Shibuya K, Dandona R, Murthy K, Cowie B, Amare AT, Antonio CA, Castaneda-Orjuela C, van Gool CH, Violante F, Oh IH, Deribe K, Soreide K, Knibbs L, Kereselidze M, Green M, Cardenas R, Roy N, Tillmann T, Li Y, Krueger H, Monasta L, Dey S, Sheikhbahaei S, Hafezi-Nejad N, Kumar GA, Sreeramareddy CT, Dandona L, Wang H, Vollset SE, Mokdad A, Salomon JA, Lozano R, Vos T, Forouzanfar M, Lopez A, Murray C, Naghavi M (2015) The global burden of cancer 2013. JAMA Oncol 1(4):505–527. doi:10.1001/jamaoncol.2015.0735
56. Liu X, Lv L, Zhang H, Zhao N, Qiu J, He X, Zhou M, Xu X, Cui H, Liu S, Lerro C, Lin X, Zhang C, Zhang H, Xu R, Zhu D, Dang Y, Han X, Bai H, Chen Y, Tang Z, Lin R, Yao T, Su J, Wang W, Wang Y, Ma B, Huang H, Liang J, Qiu W, Liu Q, Zhang Y (2016) Folic acid supplementation, dietary folate intake and risk of preterm birth in China. Eur J Nutr 55(4):1411–1422. doi:10.1007/s00394-015-0959-1
57. Zeng Z, Zhu J (2010) Low folic acid supplement intake rate among women in northern China with a high-prevalence of neural tube defects, 2008. Prev Med 51(3–4):338–339. doi:10.1016/j.ypmed.2010.07.015
58. Blancquaert D, Storozhenko S, Loizeau K, De Steur H, De Brouwer V, Viaene J, Ravanel S, Rébeillé F, Lambert W, Van Der Straeten D (2010) Folates and folic acid: from fundamental research toward sustainable health. Crit Rev Plant Sci 29(1):14–35. doi:10.1080/07352680903436283
59. Shelnutt KP, Kauwell GP, Gregory JF 3rd, Maneval DR, Quinlivan EP, Theriaque DW, Henderson GN, Bailey LB (2004) Methylenetetrahydrofolate reductase 677 C→T polymorphism affects DNA methylation in response to controlled folate intake in young women. J Nutr Biochem 15(9):554–560. doi:10.1016/j.jnutbio.2004.04.003
60. Guinotte CL, Burns MG, Axume JA, Hata H, Urrutia TF, Alamilla A, McCabe D, Singgih A, Cogger EA, Caudill MA (2003) Methylenetetrahydrofolate reductase 677 C→T variant modulates folate status response to controlled folate intakes in young women. J Nutr 133(5):1272–1280