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The association between 5, 10 – methylenetetrahydrofolate reductase and the risk of unexplained recurrent pregnancy loss in China

A Meta-analysis

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Abstract

Backgroud: To analyze the correlation between gene polymorphisms of 5,10- methylenetetrahydrofolate reductase (MTHFR) and risk of unexplained recurrent pregnancy loss (URPL) in Chinese women.

Methods: Eligible studies were searched in Pubmed, Embase, Web of Science, Wanfang, and China National Knowledge Infrastructure (CNKI) databases. Established inclusion criteria were used to screening articles, subsequently evaluate the quality of the included studies, Stata 16.0 PM and RevMan 5.3 software were conducted for meta-analysis. The pooled odds ratio (OR) with 95% confidence interval (CI) was determined to assess the relationship between MTHFR and risk of URPL in Chinese women.

Results: For MTHFR C677T, fifty studies were included, involving 6677 URPL cases and 8111 controls. The overall results showed that MTHFR C677T was significantly correlated with URPL risk, especially in the homozygous model (TT vs CC; OR 3.06; 95% CI 2.56–3.66). For MTHFR A1298C, twenty-first studies were included, involving 3439 URPL cases and 3155 controls. The results showed that MTHFR A1298C was also significantly correlated with URPL risk in recessive (CC vs AC + AA; OR 1.55; 95% CI 1.25–1.93) and homozygous (CC vs AA; OR 1.53; 95% CI 1.22–1.91) models. In addition, sub-group results showed that no significant difference between north and south China populations in the MTHFR gene polymorphisms and URPL risk. Of note, the patients carrying MTHFR C677T and MTHFR A1298C joint mutants had no synergistic effect (OR 2.71; 95% CI 0.84–8.70) on the occurrence of URPL compared with the wild-type homozygous genotype (MTHFR 677CC/ MTHFR 1298AA).

Conclusion: Studies included in this meta-analysis suggested that MTHFR 677T allele and 677TT genotype and MTHFR 1298CC genotype were both associated with URPL; testing MTHFR C677T gene polymorphism was a more appropriate target compared with other mutations in the prediction of URPL.

Abbreviations: CI = confidence interval, CNKI = China National Knowledge Infrastructure, FEM = fixed effects model, HWE = Hardy–Weinberg equilibrium, MTHFR = 5,10-methylenetetrahydrofolate reductase, 5,10-MTHF = 5,10-methylenetetrahydrofolate, 5-MTHF = 5-methylenetetrahydrofolate, OR = odds ratio, PRISMA = the Preferred Reporting Items for Systematic Reviews and Meta-Analyses, RPL = recurrent pregnancy loss, NOS = Newcastle-Ottawa Scale, REM = random effects model, URPL = unexplained recurrent pregnancy loss.

Keywords: 5, 10 – methylenetetrahydrofolate reductase, unexplained recurrent pregnancy loss, meta-analysis

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Compliance with ethical standards

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Ethical approval: All data utilized in our meta-analysis are extracted from publicly available material; therefore, ethical approval is waived.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Recurrent pregnancy loss (RPL) was defined as two or more failed clinical pregnancies by American Society for Reproductive Medicine.[1] A total of 1–5% of women in reproductive age will experience RPL.[2,3] Although abnormalities of the genital tract and the uterine structure, autoimmune diseases, genetic disorders, and inherited thrombophilia had been identified as risk factors for RPL, nearly 50% of RPL cases were still classified as URPL.[4] MTHFR reduced 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methylenetetrahydrofolate (5-MTHF), and 5-MTHF participated in the conversion of homocysteine to methionine. The reduction of MTHFR resulted in the increased level of homocysteine in the blood.[5] High level blood homocysteine was a significant risk factor for URPL and detected in about 30% URPL cases.[6,7] MTHFR gene polymorphisms were widely believed to play a key role in the risk of URPL, MTHFR C677T and MTHFR A1298C of which were the two most-investigated single nucleotide polymorphisms.[8–11]

Numerous case-control studies and meta-analyses reported that MTHFR C677T was significantly associated with URPL.[10–12] The frequencies of the MTHFR 677T allele and 677TT genotype increased along the south-north direction, and showed significantly geographical variations among Chinese population.[13–15] However, to our best knowledge, no meta-analysis compared the relationship between MTHFR C677T geographical distribution and the risk of URPL by meta-analysis.

The correlations between MTHFR A1298C and the risk of URPL were also reported by lots of meta-analyses. However, these results were controversial.[8–11] Three meta-analyses indicated MTHFR A1298C polymorphisms were significantly associated with URPL,[9,11,16] whereas others showed no significant correlation between them.[8,10] Considered that a series of novel case-control studies about Chinese women's URPL had been published, an updated meta-analysis needed to further validate the association between MTHFR A1298C and the risk of URPL. The frequencies of MTHFR 1298C allele and 1298CC genotype decreased along the southern-northern direction, and also showed significantly geographical variations.[13,14] Hence, it is also important to clarify the correlation between MTHFR A1298C geographical distributions and the risk of URPL by meta-analysis.

The MTHFR 677T allele and the 677TT genotype reduced the activity of MTHFR, and the MTHFR 1298C allele and the 1298CC genotype increased folate level in the blood.[9] To our best knowledge, no meta-analysis investigates the association between MTHFR C677T and MTHFR A1298C joint mutation and the risk of URPL.

Therefore, we performed a comprehensive and updated meta-analysis to drive a precise estimation of association between MTHFR C677T or MTHFR A1298C gene polymorphisms and the risk of URPL in Chinese women, to unravel the effects of geographical variations of MTHFR C677T or MTHFR A1298C gene polymorphisms on the risk of URPL, and to analyze the association between MTHFR C677T and MTHFR A1298C joint mutation and the risk of URPL. The findings of this meta-analysis may help predict the risk of URPL in Chinese women from the angle of MTHFR C677T or / and MTHFR A1298C gene polymorphisms.

2. Materials and methods

The meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[17] This review has been registered on the PROSPERO website as No. CRD42020173815 (To enable PROSPERO to focus on COVID-19 registrations during the 2020 pandemic, this registration record was automatically published exactly as submitted. The PROSPERO team has not checked eligibility).

2.1. Search strategy

A systematic literature search for studies published up to March 25, 2020, was performed. Relevant studies were identified by searching Pubmed, Embase, Web of Science, Wanfang, and CNKI databases with the following key words: 'Chinese' or 'China', 'methylenetetrahydrofolate reductase' or 'MTHFR', 'recurrent miscarriage' or 'recurrent abortion' or 'spontaneous abortion' or 'recurrent pregnancy loss' or 'recurrent fetal loss'. Only studies that had been published in English or Chinese were included.

2.2. Inclusion and exclusion criteria

Studies were selected according to the following criteria:

1. case-control studies;
2. all the participants were Chinese women. URPL was defined as at least two miscarriages, and the controls were women with at least one live birth;
3. MTHFR C677T and/or MTHFR A1298C gene polymorphisms were detected and sufficient data regarding genotype distributions were provided; and
4. articles were published in English or Chinese.

For duplicates, only the studies with more complete data were included. Case reports, comments, review papers were excluded. The full-text screening according to the inclusion and exclusion procedures was summarized in Figure 1.

2.3. Data extraction

The following information from each eligible publication was recorded: first name of the first author, year of publication, province, sample size, number of cases and controls with genotypes, the genotype frequencies of control group were evaluated for Hardy–Weinberg equilibrium (HWE), and the quality of included studies was estimated by the 9-star Newcastle-Ottawa Scale (NOS).[18] The stars of 0–3, 4–6, and 7–9 were considered of low, moderate, and high quality, respectively.

2.4. Data analysis

All statistical analyses were performed using the STATA 16.0PM and RevMan 5.3 software. The two-tailed Student t test was employed to analyze the geographical distribution data, $P < .05$ was considered statistically significant. The strength of the association between the MTHFR gene polymorphisms (C677T and A1298C) and URPL risk in the Chinese women was assessed by the odds ratios (ORs) with 95% confidence intervals (CIs). For MTHFR C677T, the pooled ORs were calculated for the dominant model (TT + CT vs CC), recessive model (TT vs CT + CC), heterozygous model (CT vs CC), homozygous model (TT vs CC), and an allele model (T vs C); for MTHFR A1298C, the pooled ORs were also calculated for the dominant model (CC + AC vs AA), recessive model (CC vs AC + AA), heterozygous

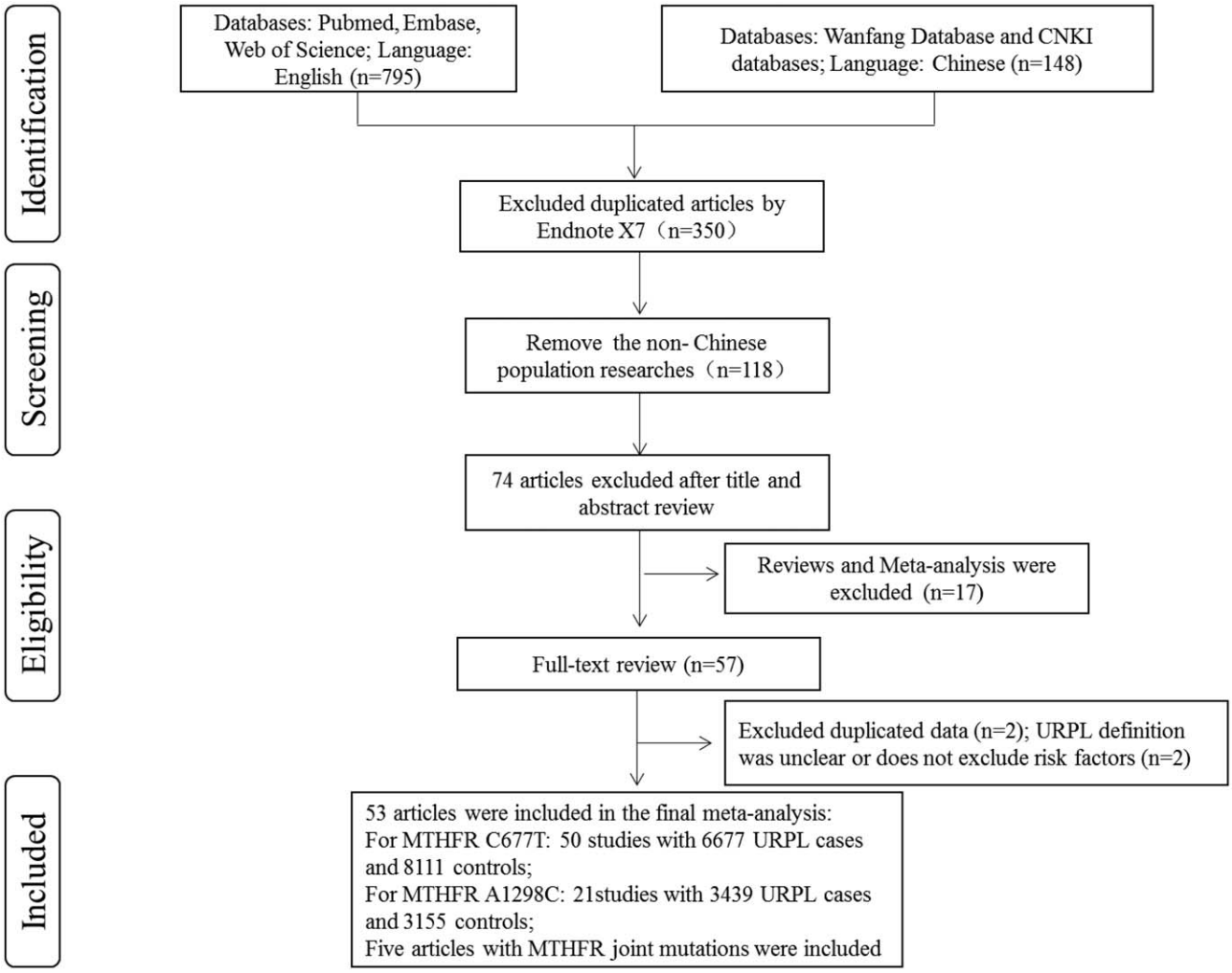

Figure 1. Flowchart of the literature search.

model (AC vs AA), homozygous model (CC vs AA), and an allele model (C vs A). χ^2 and Higgins I^2 statistics were performed to assess heterogeneity between studies. If $I^2 > 50\%$ and $P < .01$, the pooled ORs were analyzed using the random effects model (REM), or else, the fixed effects model (FEM) was used. Publication bias was estimated by Egger's linear regression test. If $P < .05$, a significant publication bias was considered.

3. Results

3.1. Main characteristics and quality assessment of the included studies

Overall, fifty-three articles were included in the final meta-analysis. For MTHFR C677T, fifty studies with 6677 URPL cases and 8111 controls were included.[19–68] The stars of NOS ranged from 5-9, and only two studies were classed as moderate quality.[24,51] The main characteristics of all included studies were shown in Table 1. Twenty-one articles demonstrated the association of MTHFR A1298C polymorphism with risk of URPL involving 3439 URPL cases and 3155 controls.[23,24,33–35,37,39,42,43,46,47,56,59,63,64,66–71] All studies were classed as high quality according to the NOS score. The main characteristics were shown in Table 2. Five studies investigated the relationship between the MTHFR joint mutations and the risk of URPL,[23,56,59,66,68] all studies were classed as high quality, and the main characteristics were listed in Table 3.

3.2. Geographical distributions of MTHFR gene polymorphisms

The prevalence of the two gene polymorphisms varied significantly among different populations and showed apparent geographical gradients. For MTHFR C677T, the MTHFR 677TT genotype frequency was significantly higher in URPL group compared with control group ($P < .001$), and the frequency increased along the south-north direction. For MTHFR A1298C, the MTHFR 1298CC genotype frequency was slightly higher in URPL group compared with control (not statistically significant), and the frequency decreased along the south-north direction (Table 4).

Table 1

Characteristics of all selected studies included on MTHFR C677T.

Author	Provence *	Publication year	Pregnancy loss(times)	Cases No.				Control No.				HWE †	*P* values ‡	NOS score §
				Total	CC	CT	TT	Total	CC	CT	TT			
Wang et al., [19]	**Shanxi**	2002	≥2	62	13	33	16	119	43	53	23	Y	.36	7
Song et al., [20]	Guangdong	2003	≥2	50	36	2	12	56	40	12	4	N	.04	8
Li et al, [21]	**Shandong**	2004	≥2	57	16	32	9	50	25	20	5	Y	.74	7
Guan et al, [22]	**Shandong**	2005	≥3	127	13	59	55	117	19	73	25	N	.01	8
Wang et al, [23]	Shanghai	2006	≥2	147	49	78	20	82	43	34	5	Y	.61	8
Ren et al, [24]	**Shanxi**	2007	≥2	71	9	40	22	93	29	38	26	Y	.08	5
Wan et al, [25]	Shandong	2007	≥2	80	6	46	28	60	19	33	8	Y	.28	9
Xu et al, [26]	Shandong	2007	≥2	112	21	48	43	100	32	50	18	Y	.84	8
Ma et al, [27]	**Shanxi**	2008	≥2	60	12	32	16	60	19	34	7	Y	.16	8
Zhang et al, [29]	**Jilin**	2009	≥2	56	12	25	19	50	20	22	8	Y	.64	7
Wang et al, [28]	Jiangsu	2009	≥2	50	36	2	12	125	89	27	9	N	.00	7
Zhong et al, [30]	**Ningxia**	2010	≥3	141	72	53	16	160	114	43	3	Y	.65	9
Wang et al, [31]	**Shandong**	2011	≥2	159	18	82	59	127	28	78	21	N	.01	8
Han et al, [32]	**Beijing**	2012	≥2	71	10	35	26	58	25	15	18	N	.00	8
Hu et al, [34]	Guangdong	2014	≥3	52	29	14	9	16	11	4	1	Y	.47	8
Cao et al, [33]	Shanghai	2014	≥2	166	53	83	30	82	29	43	10	Y	.33	9
Luo et al, [37]	Zhejing	2015	≥2	125	40	70	15	135	60	65	10	Y	.18	9
Zhu et al, [39]	**Henan**	2015	≥2	118	60	40	18	174	100	72	2	N	.01	9
Wang et al, [38]	Zhejiang	2015	≥2	125	40	70	15	905	374	471	60	N	.00	9
Guo et al, [36]	Guangdong	2015	≥2	62	15	29	18	59	31	16	11	N	.00	9
Gao et al, [35]	**Henan**	2015	≥2	378	130	185	63	423	224	160	39	Y	.18	7
Tang et al, [41]	Guizhou	2016	≥2	100	38	37	25	50	25	16	9	N	.04	8
Wang et al, [42]	Jiangsu	2016	≥2	190	97	64	29	180	103	75	2	N	.00	9
Yue et al, [44]	**Shanxi**	2016	≥2	130	30	68	32	130	32	70	28	Y	.37	8
Shang et al, [40]	**Henan**	2016	≥2	349	79	150	120	421	220	175	26	Y	.25	9
Xie et al, [43]	**Tianjin**	2016	≥2	244	31	94	119	116	23	62	31	Y	.42	8
Huang et al, [47]	Guangdong	2017	≥2	83	19	39	25	90	30	48	12	Y	.29	9
Shen et al, [50]	**Henan**	2017	≥2	100	10	40	50	100	14	54	32	Y	.25	8
Hua et al, [46]	Shanghai	2017	≥2	140	32	72	36	143	51	71	21	Y	.64	8
Wang et al, [54]	Zhejiang	2017	≥3	79	20	43	16	280	116	122	42	Y	.29	8
Zhan et al, [55]	Anhui	2017	≥2	120	31	48	41	98	55	32	11	Y	.07	9
Wang et al, [52]	Zhejiang	2017	≥2	100	15	45	40	50	19	21	10	Y	.35	7
Wang et al, [53]	Zhejiang	2017	≥2	50	11	21	18	50	20	24	8	Y	.86	7
Jiang et al, [48]	Guangxi	2017	≥2	152	76	60	16	313	197	96	20	Y	.08	9
Ding et al, [45]	Hubei	2017	≥2	100	20	48	32	100	34	48	18	Y	.88	7
Li et al, [49]	**Shanxi**	2017	≥2	50	7	25	18	50	22	13	15	N	.00	7
Shi et al, [51]	**Shandong**	2017	≥2	69	8	35	26	169	46	92	31	Y	.21	6
Zhang et al, [56]	Zhejiang	2017	≥2	50	14	24	12	10	4	6	0	Y	.18	7
Zhu et al, [59]	**Beijing**	2018	≥2	370	166	157	47	144	66	59	19	Y	.32	9
Sun et al, [58]	Anhui	2018	≥2	108	26	60	22	181	53	91	37	Y	.86	9
Li et al, [57]	**Beijing**	2018	≥2	100	18	44	38	100	35	41	24	Y	.09	9
Lin et al, [63]	Guangdong	2019	≥2	403	213	153	37	342	253	78	11	Y	.11	9
Xu et al, [67]	**Henan**	2019	≥2	218	26	87	105	264	40	122	102	Y	0.72	9
Li et al, [62]	Gansu	2019	≥2	264	64	124	76	381	118	209	54	N	0.01	8
Xu et al, [66]	Zhejiang	2019	≥2	108	38	41	29	140	69	53	18	Y	0.13	8
Bai et al, [60]	Zhejiang	2019	≥2	72	27	28	17	116	48	54	14	Y	0.84	9
Wu et al, [65]	**Henan**	2019	≥2	109	14	49	46	487	91	242	154	Y	0.81	8
Cai et al, [61]	Fujian	2019	≥2	150	32	69	49	120	65	47	8	Y	0.90	8
Liu et al, [64]	Jiangsu	2019	≥2	170	38	84	48	170	55	86	29	Y	0.64	8
Xu et al, [68]	**Henan**	2020	≥2	230	29	90	111	264	40	122	102	Y	0.72	9

MTHFR = 5,10-methylenetetrahydrofolate reductase, HWE = Hardy–Weinberg equilibrium.

* Bold for north China, regular for south China.

† Y: genotype frequencies of control group were meet HWE criteria; N: genotype frequencies of control group were not meet HWE criteria.

‡ *P* value for HWE in control group.

§ Scores estimated by the 9-star Newcastle–Ottawa Scale.

3.3. MTHFR C677T and/or MTHFR A1298C gene polymorphisms and URPL risk

The ORs and 95% CIs of the association between MTHFR gene polymorphisms and URPL risk were considered under different models (Figure S1 and S2 http://links.lww.com/MD/GB, http://links.lww.com/MD/G9, http://links.lww.com/MD/G10, http://links.lww.com/MD/G11, http://links.lww.com/MD/G12, http://links.lww.com/MD/G13). Main meta-analyzed results of the association of MTHFR C677T and URPL risk were summarized in Table 5. The overall pooled results indicated significant

Table 2

Characteristics of all selected studies included on MTHFR A1298C.

Author	Province *	Publication year	Pregnancy loss(times)	Cases No.				Control No.				HWE †	*P* values ‡	NOS score §
				Total	AA	AC	CC	Total	AA	AC	CC			
Li et al, [69]	**Shandong**	2003	≥2	57	33	21	3	50	29	18	3	Y	.93	8
Wang et al, [23]	Shanghai	2006	≥2	148	103	35	10	82	60	20	2	Y	.83	7
Ren et al, [24]	**Shanxi**	2007	≥2	71	49	20	2	93	69	23	1	Y	.54	7
Chen et al, [70]	Hainan	2013	≥2	59	24	29	6	87	38	44	5	Y	.09	8
Hu et al, [34]	Guangdong	2014	≥3	52	33	12	7	16	12	3	1	Y	.25	8
Cao et al, [33]	Shanghai	2014	≥2	166	132	31	3	82	49	31	2	Y	.25	8
Luo et al, [37]	Zhejiang	2015	≥2	125	82	40	3	135	78	54	3	Y	.07	9
Zhu et al, [39]	**Henan**	2015	≥2	118	48	58	12	174	76	88	10	N	.02	8
Gao et al, [35]	**Henan**	2015	≥2	378	180	118	80	423	210	165	48	Y	.08	7
Li et al, [71]	Jiangsu	2015	≥2	60	31	21	8	150	84	61	5	Y	.12	7
Wang et al, [42]	Jiangsu	2016	≥2	190	77	93	19	180	79	91	10	N	.01	9
Xie et al, [43]	**Tianjin**	2016	≥2	244	165	74	5	116	82	29	5	Y	.25	8
Huang et al, [47]	Guangdong	2017	≥2	83	52	28	3	90	64	23	3	Y	.60	9
Hua et al, [46]	Shanghai	2017	≥2	140	100	36	4	143	86	53	4	Y	.21	9
Zhang et al, [56]	Zhejiang	2017	≥2	50	30	19	1	10	5	4	1	Y	.88	7
Zhu et al, [59]	Guangdong	2018	≥2	370	243	114	13	144	83	56	5	Y	.23	8
Lin et al, [63]	Guangxi	2019	≥2	403	231	144	28	342	221	102	19	Y	.12	9
Xu et al, [67]	**Henan**	2019	≥2	218	155	58	5	264	214	44	6	N	.05	9
Xu et al, [66]	Zhejiang	2019	≥2	108	61	37	10	140	83	46	11	Y	.21	8
Liu et al, [64]	Jiangsu	2019	≥2	170	119	49	2	170	114	53	3	Y	.26	8
Xu et al, [68]	**Henan**	2020	≥2	230	156	67	7	264	214	44	6	N	.05	9

MTHFR = 5,10-methylenetetrahydrofolate reductase, HWE = Hardy–Weinberg equilibrium.

* Bold for north China, regular for south China.

† Y: genotype frequencies of control group were meet HWE criteria; N: genotype frequencies of control group were not meet HWE criteria.

‡ *P* value for HWE in control group.

§ Scores estimated by the 9-star Newcastle–Ottawa Scale.

Table 3

Characteristics of all selected studies included on MTHFR joint mutations.

Author	Provence	Publication year	Pregnancy loss(times)	Case No.		Control No.		HWE *	*P* value †	NOS score ‡
				Joint mutation	No mutation	Joint mutation	No mutation			
Wang et al, [23]	Shanghai	2006	≥2	23	27	7	28	Y	.61	8
Zhang et al, [56]	Zhejiang	2017	≥2	12	6	2	1	Y	.18	7
Zhu et al, [59]	Beijing	2018	≥2	48	88	23	28	Y	.32	9
Xu et al, [66]	Zhejiang	2019	≥2	41	4	28	18	Y	.72	9
Xu et al, [68]	Henan	2020	≥2	49	4	28	18	Y	.72	9

MTHFR = 5,10-methylenetetrahydrofolate reductase, HWE = Hardy–Weinberg equilibrium, No. = number.

* Y: genotype frequencies of control group were meet HWE criteria; N: genotype frequencies of control group were not meet HWE criteria.

† *P* value for HWE in control group.

‡ Scores estimated by the 9-star Newcastle–Ottawa Scale.

Table 4

Distribution frequencies under different MTHFR gene polymorphisms.

Groups			No.	Genotype frequencies [n (%)]		
				Wild type	Heterozygous type	Homozygous type
C677T	Total	Cases	6677	1919 (28.84)	2957 (44.48)	1801 (26.69) *
		Control	8111	3318 (41.03)	3592 (44.26)	1201 (14.72)
	North	Cases	3725	874 (23.46)	1673 (44.91)	1178 (31.26)
		Control	4217	1444 (34.24)	1952 (46.29)	821 (19.47)
	South	Cases	2952	1045 (35.40)	1284 (43.50)	623 (21.10)
		Control	3894	1874 (48.13)	1640 (42.12)	380 (9.76)
A1298C	Total	Cases	3439	2206 (61.18)	1139 (32.10)	241 (6.72)
		Control	3155	2010 (61.81)	1072 (33.34)	155 (4.85)
	North	Cases	1686	1029 (61.03)	530 (31.44)	127 (7.53)
		Control	1528	977 (63.94)	467 (30.56)	84 (5.50)
	South	Cases	1753	1075 (61.32)	574 (32.74)	104 (5.93)
		Control	1627	973 (59.80)	585 (35.96)	69 (4.24)

* $P < .001$ *vs* control, MTHFR = 5,10-methylenetetrahydrofolate reductase, No. = number.

Table 5

ORs and 95% Cis for the MTHFR C677T and URPL under different models.

Contrast	Group	No.	I^2(%)	Model *	Pooled OR(95% CI)	Egger (*P* value)	*P* value †
TT + CT vs CC	Overall	50	51.9	REM	1.91 (1.70–2.15)	.61	
	HWE: yes	38	49.7	FEM	2.01 (1.84–2.19)	.75	
	Distribution: North	25	59.6	REM	1.99 (1.65–2.39)		.57
	Distribution: South	25	43.0	FEM	1.86 (1.67–2.07)		
TT vs CT + CC	Overall	50	57.0	REM	2.24 (1.93–2.60)	.03	
	HWE: yes	38	58.5	REM	2.16 (1.83–2.56)	.11	
	Distribution: North	25	69.1	REM	2.16 (1.74–2.68)		.47
	Distribution: South	25	31.2	FEM	2.24 (1.92–2.61)		
CT vs CC	Overall	50	50.7	REM	1.59 (1.40–1.80)	.84	
	HWE: yes	38	25.5	FEM	1.70 (1.55–1.87)	.44	
	Distribution: North	25	53.9	REM	1.63 (1.36–1.96)		.98
	Distribution: South	25	49.4	FEM	1.60 (1.42–1.80)		
TT vs CC	Overall	50	57.8	REM	3.06 (2.56–3.66)	.06	
	HWE: yes	38	63.9	REM	2.95 (2.38–3.67)	.28	
	Distribution: North	25	70.3	REM	3.10 (2.33–4.11)		.91
	Distribution: South	25	31.9	FEM	2.95 (2.48–3.49)		
T vs C	Overall	50	61.3	REM	1.74 (1.60–1.90)	.70	
	HWE: yes	38	69.2	REM	1.75 (1.57–1.95)	.99	
	Distribution: North	25	69.8	REM	1.74 (1.53–1.97)		.92
	Distribution: South	25	49.3	FEM	1.73 (1.60–1.87)		

OR = odds ratio, CI = confidence interval, MTHFR = 5,10-methylenetetrahydrofolate reductase, URPL = unexplained recurrent pregnancy loss, No. = number, HWE = Hardy–Weinberg equilibrium, NOS = Newcastle–Ottawa Scale.

* REM: random effects model; FEM: fixed effects model.

† *P* value for north *vs* south.

associations between all the MTHFR C677T gene polymorphisms and the risk of URPL, especially homozygous model (TT vs CC; OR 3.06; 95% CI 2.56–3.66). When excluding studies that deviated from HWE, significant associations were also found in all these models (Table 5). The overall results of MTHFR A1298C polymorphism also showed significant association in the recessive (CC vs AC + AA; OR 1.55; 95% CI 1.25–1.93) and homozygous (CC vs AA; OR 1.53; 95% CI 1.22–1.91) models. When excluding studies that deviated from HWE, significant associations were still found in these two models (Table 6). Through linkage analysis of the MTHFR C677T and A1298C loci, our results found that the patients carrying these two MTHFR mutants had no synergistic effect (OR 2.71; 95% CI 0.84–8.70) on the occurrence of URPL compared with the

Table 6

ORs and 95% CIs for the MTHFR A1298C and URPL under different models.

Contrast	Group	No.	I^2(%)	Model *	Pooled OR(95% CI)	Egger (*P* value)	*P* value †
CC + AC vs AA	Overall	21	54.5	REM	1.07 (0.90–1.26)	.06	
	HWE: yes	17	43.1	FEM	0.99 (0.88–1.12)	.64	
	Distribution: North	8	58.2	REM	1.24 (0.95–1.56)		.10
	Distribution: South	13	50.9	REM	0.97 (0.78–1.24)		
CC vs AA + AC	Overall	21	0.0	FEM	1.55 (1.25–1.93)	.04	
	HWE: yes	17	1.6	FEM	1.54 (1.21–1.97)	.08	
	Distribution: North	8	9.6	FEM	1.63 (1.21–2.19)		.64
	Distribution: South	13	0.0	FEM	1.47 (1.06–1.93)		
AC vs AA	Overall	21	56.5	REM	1.02 (0.85–1.21)	.75	
	HWE: yes	17	39.3	FEM	0.92 (0.81–1.05)	.97	
	Distribution: North	8	67.9	REM	1.17 (0.87–1.58)		.15
	Distribution: South	13	45.6	FEM	0.95 (0.82–1.11)		
CC vs AA	Overall	21	0.0	FEM	1.53 (1.22–1.91)	.06	
	HWE: yes	17	0.0	FEM	1.49 (1.16–1.91)	.10	
	Distribution: North	8	0.0	FEM	1.57 (1.15–2.13)		.82
	Distribution: South	13	0.0	FEM	1.49 (1.07–2.07)		
C vs A	Overall	21	52.7	REM	1.10 (0.97–1.26)	.29	
	HWE: yes	17	49.9	FEM	1.07 (0.97–1.18)	.29	
	Distribution: North	8	51.1	REM	1.22 (1.01–1.47)		.08
	Distribution: South	13	51.9	REM	1.02 (0.85–1.23)		

OR = odds ratio, CI = confidence interval, MTHFR = 5,10-methylenetetrahydrofolate reductase URPL = unexplained recurrent pregnancy loss, No. = number, HWE = Hardy–Weinberg equilibrium.

* REM: random effects model; FEM: fixed effects model.

† *P* value for north *vs* south.

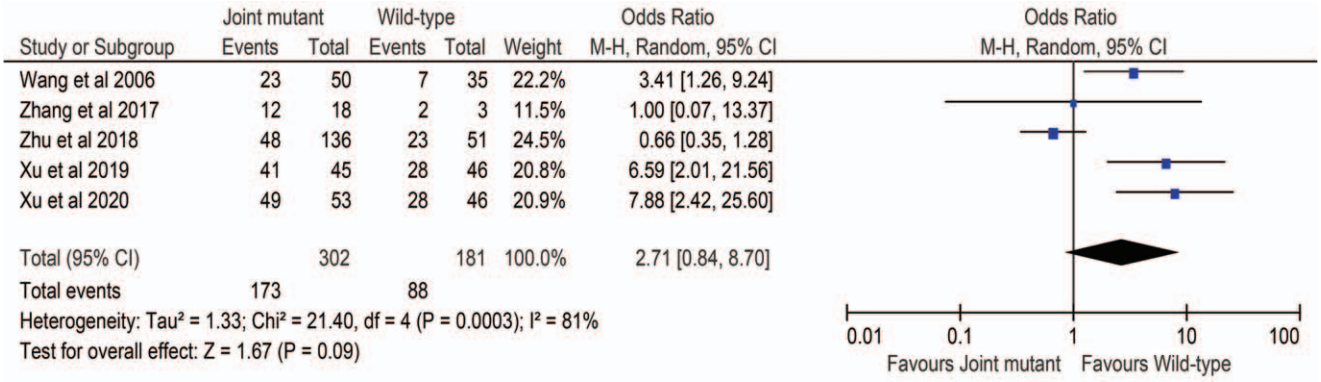

Figure 2. Random effect forest plot of MTHFR joint mutant *vs* the wild-type homozygous genotype.

individuals carrying the wild-type homozygous genotype (677CC/1298AA) (Figure 2).

3.4. Relationship between MTHFR C677T or MTHFR A1298C geographical distribution and the risk of URPL

We also performed a sub-group analysis stratified by north and south China (Table 5 and 6). For MTHFR C677T, twenty-five studies with 3725 URPL cases and 4217 controls were included in the north China and twenty-five studies with 2952 URPL cases and 3894 controls were included in the south China. Significant associations between MTHFR C677T and URPL risk were found in both north and south China in all the models. However, no significant difference was observed in all the genotype groups between north and south China in the risk of UPRL (Table 5). For MTHFR A1298C, eight studies with 1686 URPL cases and 1528 controls were included in the north China and thirteen studies with 1753 URPL cases and 1627 controls were included in the south China. Sub-group analyzed results still showed significant association in the recessive and homozygous models of MTHFR A1298C polymorphism in north and south China. In addition, we also found a significant association in the allele model (C vs A; OR 1.22; 95% CI 1.01–1.47) in north China. There was also no significant difference in all the genotype groups between north and south of China in the risk of UPRL (Table 6).

3.5. Sensitivity analysis

We conducted sensitivity analysis to ascertain the primary origin of the heterogeneity. Through sensitivity analysis, the present study showed that no individual study had affected the pooled ORs (see Figure S1 and S2 http://links.lww.com/MD/GB, http://links.lww.com/MD/G9, http://links.lww.com/MD/G10, http://links.lww.com/MD/G11, http://links.lww.com/MD/G12, http://links.lww.com/MD/G13, Supplemental Content, which illustrates the sensitivity analysis results).

3.6. Publication bias

Egger's test was performed to evaluate funnel plot symmetry statistically (see Figure S3 and S4 http://links.lww.com/MD/G14, http://links.lww.com/MD/G15, http://links.lww.com/MD/G16, http://links.lww.com/MD/G17, http://links.lww.com/MD/G18, http://links.lww.com/MD/G19, Supplemental Content, which illustrates the publication bias results). For MTHFR C677T, the egger's regression asymmetry test showed significant publication bias ($P = .03$) in the recessive model (Table 5). For MTHFR A1298C, significant publication bias ($P = .04$) was also found in the recessive model (Table 6). However, after excluding non-HWE studies, no publication bias was found on the recessive model of both MTHFR C677T and MTHFR A1298C mutations (Table 5 and 6).

4. Discussion

In our meta-analysis, we collected 50 studies with 6677 URPL cases and 8111 controls to investigate the association between MTHFR C677T gene polymorphism and the risk of URPL. The results revealed that all the C677T mutations of MTHFR were significantly associated with the risk of URPL in the Chinese population. The MTHFR 677 T allele and TT genotype may increase the risk of URPL. These results were expected because all the individual studies included in our meta-analysis presented these trends in their populations. The results were also consistent with previous meta-analysis results among Chinese population reported by Chen et al and Ren et alet alet al,[8,72] and among Asian population reported by Parveen et al, Cao et al, and Wu et al,[73–75]. For MTHFR A1298C, a total of 21 studies involving 3439 URPL cases and 3155 controls were included. A significant association between MTHFR A1298C mutations (recessive [CC vs AC + AA; OR 1.55; 95% CI 1.25–1.93] and homozygous [CC vs AA; OR 1.53; 95% CI 1.22–1.91] models) and URPL was observed. Among the related 21 studies, two studies showed that the MTHFR 1298CC genotype occurred more significantly frequent in the URPL population than in the control group,[35,71] and eleven studies described the same trend,[23,24,34,39,42,63,66–68,70] while eight studies showed the opposite trend.[33,37,43,46,56,59,64,69] Our present meta-analysis showed significantly positive results, which were similar with the meta-analyzed ones reported by Yang et al and Zhang et al,[9,11], but were inconsistent with previous meta-analyzed results reported by Chen et al, Rai et al, and Cao et al, [8,74,76]. We hypothesize that part of the reasons for these controversial results might be related to negative results occupied high weights and different ethnic background in their meta-analyses.[9] The relationship between MTHFR C677T and MTHFR A1298C joint mutation and the risk of URPL were investigated by five studies and our result showed

no significant association among Chinese women (OR 2.71; 95% CI 0.84–8.70). Further analysis revealed that among the five studies, one study presented MTHFR C677T and MTHFR A1298C joint mutation more frequent in the control than in the URPL group and has the highest weight (24.52%).[59] And this might be the reason why we did not get statistically significant result.

The association of geographical distributions of the MTHFR C677T and MTHFR A1298C gene polymorphisms with URPL was not clear among Chinese population. Our results showed that geographical distributions of the MTHFR C677T or MTHFR A1298C gene polymorphisms were not associated with URPL.

Some potential limitations of this meta-analysis should be addressed. One of the limitations was high heterogeneity. It influenced the interpretation of the meta-analysis result. Sample size, racial difference, and deviations of allele distributions from the HWE law played important roles.[77] After excluded non HWE studies, the results still played high heterogeneity in the recessive, homozygous, and additive comparisons in MTHFR C677T group. Our further analysis showed the high heterogeneity was caused by Shang et al, whose pooled OR value and sample size were high.[40] After we removed this study, all the I^2 values decreased to less than 50%. While for MTHFR A1298C, no significant heterogeneity was found in all the comparisons after remove non HWE articles. The other limitation was publication bias. Positive results interested only by researchers and journals might be the possible reason for publication bias.[78,79] However, in our present study, deviation from HWE law was an indication of potential publication bias. After excluding the non HWE studies, all the *P* values increased to over.05 in both MTHFR C677T and MTHFR A1298C groups. In addition, for the association between MTHFR C677T and MTHFRA1298C joint mutation and the risk of URPL, only five studies were involved, so heterogeneity and publication bias were not investigated. Therefore, additional studies were needed to reevaluate the risk of MTHFR C677T and MTHFR A1298C joint mutation in URPL. In addition, although we performed a broad search in five different databases to find studies for inclusion criteria, it is impossible to confirm that all available studies were included, which may exhibit another limitation of our meta-analysis.

In conclusion, this meta-analysis suggested that MTHFR 677T allele and 677TT genotype and MTHFR 1298CC genotype may be risk factors for the development of URPL. Moreover, geographical distribution of the MTHFR C677T or MTHFR A1298C gene polymorphisms was not associated with the risk of URPL for Chinese women. We suggested that MTHFR C677T and MTHFR A1298C mutants should be tested in Chinese pregnant women.

Author contributions

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