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REVIEW

Polymorphisms of methalenetetrahydrofolate reductase in recurrent pregnancy loss: an overview of systematic reviews and meta-analyses

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Abstract

Purpose The aim is to summarize and evaluate current systematic reviews and meta-analyses on MTHFR polymorphisms in recurrent pregnancy loss (RPL).

Methods We searched Pubmed and Embase databases and selected in form of PICOS (participants, interventions, comparisons, outcomes, and study design). Our methodology was registered on PROSPERO (CRD42017042762). Systematic reviews and meta-analyses containing primary studies were extracted for meta-analyses, along with their OR and 95%CI. We assessed the quality of the included studies using AMSTAR and OQAQ criteria.

Results Eleven systematic reviews and meta-analyses were identified. C677T was significantly related to RPL overall in Allele (OR, 95%CI 1.43, 1.29–1.60), Recessive (OR, 95%CI 1.66, 1.42–1.95), and Homozygous (OR, 95%CI 2.08, 1.66–2.61). There was no correlation observed between A1298C and RPL, except for in Heterozygous (OR, 95%CI 1.62, 1.17–2.25).

Conclusions We identified a difference in the association between MTHFR C677T polymorphism and RPL, especially in Asian population. No significant correlation was found between A1298C and RPL.

Keywords Recurrent pregnancy loss (RPL) · Methalenetetrahydrofolate reductase (MTHFR) · Polymorphism · C677T · A1298C · Systematic review and meta-analysis

Introduction

Recurrent pregnancy loss (RPL) is defined as two or more consecutive spontaneous miscarriages before 20 weeks of gestation. RPL affects at least 2% of women in reproductive age. Despite anatomical abnormalities, autoimmune diseases, and environmental factors, the causes of RPL in 50% of cases are still unknown [1]. Methylenetetrahydrofolate reductase (MTHFR) plays a critical role in the folate pathway, which is widely believed to play a key role in pregnancy outcome. MTHFR gene is located on chromosome 1p36.6 and features 11 exons. Polymorphisms of MTHFR, especially C677T (rs1801133) and A1298C (rs1801131), are believed to be associated with RPL [2]. These mutations cause a reduction in the activity of MTHFR, a vital enzyme which catalyzes 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate (5-MTHF). Then, the reduction of 5-MTHF, which participates in the conversion of homocysteine to methionine, results in elevated levels of total homocysteine (tHcy) in both blood and urine [2]. Subsequently, hyperhomocysteinemia, or homocystinuria, can induce inherited thrombophilia, a significant risk factor for RPL [3]. Accumulation of homocysteine is also associated with arteriosclerosis, pre-eclampsia, and neural tube defects [4].

A few systematic reviews and meta-analyses have investigated the correlation between MTHFR polymorphisms and RPL; however, the outcome on this work remains controversial as results have been inconsistent. The reasons for such discrepancies may be related to differences in ethnicities and the selection of primary case-control studies. The accumulation of primary case-control studies may also contribute to variation in views of different authors in term of MTHFR polymorphisms and RPL [4].

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Therefore, in the present study, we conducted an overview of systematic reviews and meta-analyses in a comprehensive manner, including different ethnicities and genotype models, in order to assess the association between MTHFR genotype variants and the risk of RPL. A citation matrix was created to analyze the influence of selection in primary studies by different authors. Cumulative meta-analyses were also performed to investigate the tendency of results in genotype models with possible risk for RPL. We also evaluated systematic reviews with Assessment of Multiple Systematic Reviews (AMSTAR) and Overview Quality Assessment Questionnaire (OQAQ) scales to assess the quality of the systematic reviews and meta-analyses which have already been published on this topic.

Materials and methods

Search strategy

We identified potential articles by searching Medline and Embase databases with the following key terms: "recurrent pregnancy loss"; "recurrent miscarriage"; "spontaneous abortion"; "methylenetetrahydrofolate reductase"; "MTHFR"; "homocysteine"; "polymorphisms"; "single nucleotide polymorphisms"; and "SNP". Only systematic reviews and meta-analyses that had been published in English were included.

Eligibility criteria and study selection

According to methodology registered on PROSPERO (CRD42017042762), two researchers independently screened and selected potential articles in accordance with the following eligibility criteria.

Inclusion criteria: (1) concerning the association between MTHFR C677T or A1298C polymorphisms and recurrent pregnancy loss; (2) human studies; (3) systematic reviews and meta-analyses
Exclusion criteria: (1) reviews, comments, editorials; (2) animal studies; (3) without the number of pregnancy losses ≥ 2; (4) controls without having at least one successful live birth

In form of PICOS (participants, interventions, comparisons, outcomes, and study design), the study was described as follows [5]:

P: women with two or more pregnancy losses;
I: N/A;
C: women with at least one successful live birth;
O: MTHFR polymorphisms including C677T or A198C;
S: systematic reviews and meta-analysis; case-control studies in the systematic reviews included

Table 1 Genotype contrast model of MTHFR polymorphisms and RPL(C677T and A1298C)

Contrast model	C677T	A1298C
Allele	T/C	C/A.
Dominant	CT+TT/CC	AC+CC/AA
Recessive	TT/CT+CC	CC/AC+AA
Homozygous	TT/CC	CC/AA
Heterozygous	CT/CC	AC/AA.

Data extraction and quality assessment

Two reviewers (Boran-Du, Xiangjun-Shi) screened titles and abstracts according to the eligibility criteria. Studies which met the inclusion criteria were evaluated with full text. For the articles finally included, two reviewers independently extracted the following information: first author, year of the publication, ethnicity, and frequencies of MTHFR in cases and controls. Any disagreement was resolved through discussion. If necessary, a third reviewer (Xin-Feng) made the final decision with regard to discrepancies.

We assessed the methodological quality of systematic reviews and meta-analyses with Assessment of Multiple Systematic Reviews (AMSTAR) [6], which consists of 11 items relating to search bias, selection, and the process of data synthesis. The quality of the systematic reviews and meta-analyses

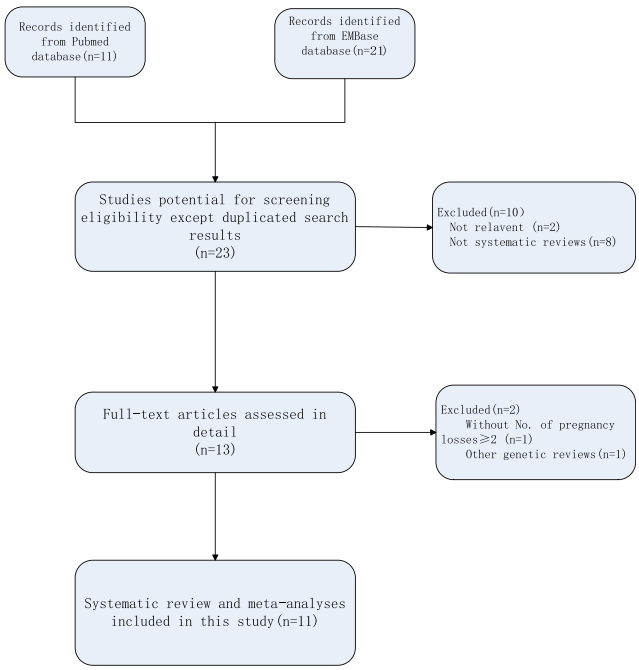

Fig. 1 Flowchart of selection of systematic reviews published

Table 2 Characteristics of systematic reviews included

Author	Year of publication	C677T				A1298C			
		Number of studies	Case number	Control number	Conclusion	Number of studies	Case number	Control number	Conclusion
Nelen [11]	2000	6			Pooled estimate OR of C677T TT genotype was 1.4 (1.0–2.0). The role of C677T TT genotype was not clear in RPL.				
Wiwanitkit [26]	2005	8			53.1% subjects with T allele have RPL; on the other hand, 55.3% subjects without T allele have RPL. C677T might not be a useful marker for RPL. There was no association between C677T and ethnicities.				
Ren [13]	2006	26	2120	2949	C667T was not a genetic risk factor for RPL, except in a Chinese population.				
Wu [14]	2012	27	2427	3118	C667T was associated with increased risk of RPL, except for Caucasians.				
Cao [8]	2012	37	3559	5097	Significant association between C677T and RPL in East Asian and mixed subgroup.	8	1163	1061	No significance between A1298C and RPL.
Nair [10]	2013					5	1080	709	A1298C was a genetic risk factor for RPL.
Parveen [27]	2013	12	1725	2392	Estimated OR of C677T was 1.383 (1.045–1.830). Moderate risk of C677T in RPL.				
Rai [12]	2014					17	2338	2588	A1298C was not associated with RPL
Chen [24]	2015	16	1420	453	Significant association between C677T and RPL in Chinese population.	5	1408	376	No association between A1298C and increased risk of RPL.
Yang [15]	2015	53	6078	9441	C677T was associated with RPL.	16	2924	4759	A1298C was associated with RPL.
Rai [28]	2016	29	3725	4105	Strong association between C677T and RPL in Asian population.				

included was also evaluated using Overview Quality Assessment Questionnaire (OQAQ) scale [7], which consists of 9 items and a final total score item. In this research study, we omitted the last item in comparison to AMSTAR scale.

Data synthesis

We described and synthesized data from all selected publications. The odds ratio (OR) with 95% confidence interval (95%CI) was used to compare differing results from systematic reviews relating to associations between MTHFR polymorphisms and RPL. Data synthesis was performed with Stata 12.0 (Stata Corporation, College Station, TX, USA). A two-sided p value < 0.5 was considered to be statistically significant. Heterogeneity was identified if $I^2 > 50\%$, and a random-effect model was used to synthesize data. In order to explore the optimal genotype model of MTHFR and its relationship with RPL, we analyzed a range of different genetic models including Allele model, Dominant model, Recessive model, Homozygous model, and Heterozygous model (Table 1).

Results

Literature searches and study selection

Our initial search generated 29 articles, of which 16 were excluded due to the duplication of results ($n = 8$) [8–15],

Table 3 Characteristics of primary studies included on C677T

	Author	Year of publication	Ethnicity	Case			Control			Nelen 2000	Wiwanitkit 2005	Ren 2006	Wu 2012	Cao 2013	Parveen 2013	Chen 2015	Yang 2015	Rai 2016
				CC	CT	TT	CC	CT	TT									
1	Nelen	1997	Caucasian	77	79	29	48	59	6	√		√	√	√				
2	Grandone	1998	Caucasian	35	42	17	45	77	28	√		√	√	√			√	
3	Quere	1998	Caucasian	28	52	20	32	54	14	√		√	√	√				
4	Brenner	1999	Asian	54	8	14	86	9	11		√	√	√	√				√
5	Holmes	1999	Caucasian	102	57	14	31	30	6	√	√	√	√				√	
6	Kutteh	1999	Caucasian	50		4	50		2	√		√		√				
7	Lissak	1999	Caucasian	17	20	4	7	7	4	√		√	√				√	√
8	Foka	2000	Caucasian	80		6	100		15		√	√						
9	Murphy	2000	Caucasian	24	3	0	527	13	0			√	√	√			√	
10	Wramsby	2000	Caucasian	17	17	2	27	35	7			√	√	√				
11	Pihusch	2001	Caucasian	41	47	14	55	61	12			√	√	√	√		√	
12	Carp	2002	Caucasian	108		14	82		7		√	√						
13	Unfried	2002	Caucasian	64	46	23	46	24	4		√						√	
14	Dilley	2002	Asian	27	26	7	39	45	8			√	√	√				
15	Wang	2002	Asian	13	33	16	43	53	23			√	√	√	√	√	√	√
16	Pauer	2003	Caucasian	28	32	9	56	51	15		√	√	√	√				
17	Kumar	2003	Asian	18	6	0	22	2	0		√	√	√	√			√	√
18	Hohlagschwandtner	2003	Caucasian	72	52	21	53	41	7			√	√	√	√		√	
19	Wang	2004	Asian	49	78	20	43	34	5		√					√	√	√
20	Dossenbach-Glaninger	2004	Caucasian	49		8	48		5			√						
21	Li	2004	Asian	16	32	9	25	20	5			√	√	√		√	√	√
22	Makino	2004	Caucasian	56	55	14	29	32	15			√	√	√			√	√
23	Wang	2004	Asian	14	17	8	43	34	5			√	√				√	
24	Couto	2005	Caucasian	29	47	12	53	26	9			√	√	√			√	
25	Gerhardt	2005	Caucasian	104		14	277		28			√						
26	Guan	2005	Asian	13	59	55	19	73	25			√	√	√	√	√	√	√
27	Kobashi	2005	Asian	34	40	9	67	82	25			√	√	√			√	√
28	Song	2005	Asian	36	2	12	40	12	4			√	√	√		√	√	√
29	Parle-McDermott	2005	Caucasian	55	55	14	271	270	73					√	√		√	
30	Mtiraoui	2006	Caucasian	92	47	61	156	30	14				√	√	√		√	√
31	Dong	2006	Asian	2	12	20	11	26	18					√			√	
32	Wang	2006	Asian	36	2	12	89	27	9					√		√	√	
33	Jivraj	2006	Caucasian	136		32	714		58					√				
34	Sotiriadis	2006	Caucasian	24	61	12	32	57	13					√			√	

Table 3 (continued)

	Author	Year of publication	Ethnicity	Case			Control			Nelen 2000	Wiwanitkit 2005	Ren 2006	Wu 2012	Cao 2013	Parveen 2013	Chen 2015	Yang 2015	Rai 2016
				CC	CT	TT	CC	CT	TT									
35	Wan	2006	Asian	6	46	28	19	33	8							√		
36	Ren	2007	Asian	9	40	22	29	38	26							√		
37	Xu	2007	Asian	21	48	43	32	50	18							√		
38	D'Uva	2007	Caucasian	0	5	15	8	9	3								√	
39	Callejon	2007	Caucasian	10	233	99	195	170	70					√			√	
40	Vettriselvi	2008	Asian	86	15	3	98	19	3				√	√	√		√	√
41	Makino	2008	Asian	33	42	10	29	32	15									√
42	Toth	2008	Caucasian	71	68	12	68	70	19					√			√	
43	Ma	2008	Asian	12	32	16	19	34	7					√		√	√	
44	Cardona	2008	Caucasian	38	43	12	93	83	30								√	
45	Zhong	2008	Asian	72	50	16	116	43	3								√	
46	Morales-Machin	2009	Caucasian	10	18	2	19	29	2				√					
47	Govindaiah	2009	Caucasian	111	25	4	112	28	0				√		√		√	√
48	Mukhopadhyay	2009	Asian	75	6	3	78	2	0				√				√	√
49	Ciacci	2009	Caucasian	47	0	31	85	0	59					√			√	
50	Bae	2009	Asian	82	104	36	45	63	14					√			√	
51	Rodriguez-Guillen	2009	Caucasian	6	7	10	16	39	19					√			√	
52	Zhang	2009	Asian	12	25	19	20	22	8					√		√	√	
53	Abu-Asab	2010	Asian	145	151	33	182	177	43						√			√
54	Kim	2010	Asian	26	19	12	63	60	32					√			√	
55	Zhong	2010	Asian	72	53	16	114	43	3					√		√	√	
56	Settin	2011	Caucasian	40	26	4	67	68	1				√	√			√	√
57	Nair	2011	Asian	75	26	5	118	21	1					√	√		√	√
58	Wang	2011	Asian	18	82	59	28	78	21							√	√	
59	Jeddi-Tehrani	2011	Asian	43	42	15	66	25	9									√
60	Park	2011	Asian	14	16	9	17	26	7								√	
61	Ozdemir	2011	Asian	231	239	73	76	30	0								√	√
62	Parveen	2012	Asian	110	70	20	196	90	14						√		√	√
63	Han	2012	Asian	10	35	26	25	15	18							√	√	
64	Dissanayke	2012	Asian	158	39	3	142	27	2									√
65	Torabi	2012	Asian	43	42	15	66	25	9									√
66	Zonouzi	2012	Asian	53	30	9	27	22	1								√	√
67	Creus	2012	Caucasian	23	26	11	13	13	4								√	
68	Puri	2012	Asian	86	16	5	263	69	11								√	

Table 3 (continued)

	Author	Year of publication	Ethnicity	Case			Control			Nelen 2000	Wiwanitkit 2005	Ren 2006	Wu 2012	Cao 2013	Parveen 2013	Chen 2015	Yang 2015	Rai 2016
				CC	CT	TT	CC	CT	TT									
69	Yin	2012	Asian	13	25	15	33	18	12								√	
70	Kaur	2013	Asian	86	16	5	463	109	21									√
71	Cao	2013	Asian	29	43	10	53	83	30								√	√
72	Chen	2013	Asian	30	20	9	50	36	1								√	
73	Hu	2014	Asian	29	14	9	11	4	1							√		
74	Luo	2014	Asian	40	70	15	60	65	10							√		
75	Yousefian	2014	Asian	96	90	18	63	43	10								√	√
76	Farahmand	2015	Asian	180	114	36	230	85	35									√
77	Vanill	2015	Asian	13	2	0	13	2	0									√
78	Hubacek	2015	Caucasian	208	214	42	1068	1116	302								√	

non-relevance ($n = 2$) [16, 17], or the fact that they were not systematic reviews and meta-analyses ($n = 6$) [18–23] (Fig. 1). After full-text screening, 2 studies were removed according to the eligibility criteria (without the number of pregnancy losses ≥ 2 [24], which did not concern MTHFR polymorphisms [25]). Finally, 11 reviews and meta-analyses remained; all of these were published in English between 2000 and 2016.

Characteristics of the systematic reviews and meta-analyses included in this study

The characteristics of the 11 systematic reviews and meta-analyses included in our final analysis are summarized in Table 2. These publications all focused on the correlation between RPL and MTHFR polymorphisms, of which there were 9 reviews related to C677T and 5 reviews related to A1298C (Table 2). The number of primary studies included in each systematic review ranged from 6 to 53. There were ten systematic reviews which involved the analysis of ethnicity and MTHFR polymorphisms; one of these was especially focused on Asian population while another focused on Chinese population. Two reviews were partial systematic reviews and meta-analyses. The researchers (Nair, 2013 [10]; Parveen, 2013 [27]) of these two reviews conducted a case-control study in their own country and compared with results of the systematic reviews and meta-analyses they executed.

The conclusions from 11 of these reviews clearly differed. Seven reviews reached the conclusion that C677T was associated with RPL in Asian population, while two reviews held the opinion that C677T has no association with RPL. In the case of A1298C, two studies claimed this mutation as a genetic factor for RPL, while three other systematic reviews reached the opposite conclusion.

Primary study characteristics

The primary studies included in each systematic review were extracted and compared, including citation matrixes (Tables 3 and 4). With regard to C677T, six studies presented only the number of Homozygous type (TT) and total numbers of patients in RPL and control group; the other 72 provided a complete set of data for case-control studies. Of these 78 studies, 45 were conducted in Asian populations and 33 were conducted in Caucasian populations (Table 3). In term of A1298C, 31 case-control studies provided a complete data set; of these, 15 were based on Asian populations and 16 on Caucasian populations (Table 4).

The citation matrix showed the differences of selection for each systematic review, along with a meta-analysis which identified similarities and controversies in the published conclusions (Tables 3 and 4).

Table 4 Characteristics of primary studies included on A1298C

	Author	Year of publication	Ethnicity	Case			Control			Nair 2012	Cao 2013	Rai 2014	Chen 2015	Yang 2016
				AA	AC	CC	AA	AC	CC					
1	Hohlagschwandtner	2003	Caucasian	63	67	15	35	50	16	√				√
2	Li	2004	Asian	33	21	3	29	18	3				√	
4	Mtiraoui	2006	Caucasian	108	65	27	130	62	8	√	√	√		√
5	Sotiriadis	2006	Caucasian	44	37	7	45	39	6		√	√		
6	Wang	2006	Asian	103	35	10	60	20	2		√	√	√	√
8	Callejon	2007	Caucasian	209	89	44	248	149	37		√	√		√
9	Ren	2007	Asian	0	17	54	1	6	86				√	
10	Bae	2009	Asian	144	68	9	75	43	4		√			√
11	Ciacci	2009	Caucasian	22	0	56	52	0	92		√	√		√
12	Rodriguez-Guillen	2009	Caucasian	18	5	0	60	14	0					√
13	Kim	2011	Asian	34	21	2	113	38	4		√	√		√
14	Jeddi-Tehrani	2011	Asian	69	27	4	94	6	0			√		
15	Klai	2011	Asian	47	14	2	93	7	0	√				
16	Ozdemir	2011	Caucasian	201	257	85	71	35	0	√		√		√
17	Settin	2011	Caucasian	15	49	6	36	97	3		√	√		√
18	Dissanayke	2012	Asian	74	78	43	72	79	46			√		
19	Nair	2012	Asian	48	68	13	116	80	6	√		√		
20	Torabi	2012	Asian	69	27	4	94	6	66			√		
21	Zonouzi	2012	Caucasian	35	46	8	13	34	3			√		√
22	Chen	2013	Asian	24	29	6	38	44	5					√
23	Herodez	2013	Caucasian	36	48	16	43	47	18			√		
24	Parveen	2013	Caucasian	88	92	20	157	127	16			√		√
25	Cao	2014	Asian	49	31	2	132	31	3					√
26	Hefler	2014	Caucasian	49	38	7	43	40	11			√		
27	Hu	2014	Asian	33	12	7	12	3	1				√	
28	Lino	2014	Caucasian	71	32	9	52	43	3			√		
29	Luo	2014	Asian	82	40	3	78	54	3				√	
30	Yousefian	2014	Asian	98	81	25	68	39	9					√
31	Hubacek	2015	Caucasian	209	212	43	1145	1066	275					√

A summarized meta-analysis combining primary studies reported in 11 systematic reviews and meta-analyses (overall analysis and sub-group analyses by ethnicity)

According to the citation matrixes and the primary studies included in 11 systematic reviews and meta-analyses, we re-calculated the OR and 95%CI of different genotype models (Table 5). Due to high heterogeneity, the OR and 95%CI were removed in a random-effect model. The OR and 95%CI of C677T in Recessive model were not heavily influenced by the 6 primary studies in which only the number of TT genotypes and the total number of cases and controls were provided.

In term of the MTHFR C677T polymorphism and RPL, there were 78 primary studies, including 8907 cases and 13,636 controls. The OR and 95%CI of C677T in Allele model were 1.54 (1.38, 1.72) in Asian population, 1.28 (1.03, 1.58) in Caucasian population, and 1.43 (1.29, 1.60) overall. The most significant genotype model for C677T was Homozygous model, in which OR and 95%CI were 2.32 (1.84, 2.92) in Asian population compared with 1.66 (1.07, 2.58) in Caucasian population and 2.08 (1.66, 2.61) overall.

In term of A1298C polymorphism and RPL, 31 primary studies were analyzed, including 4211 cases and 6208 controls. The OR and 95%CI of A1298C in Allele model were 1.25 (0.90, 1.75) in Asian group, 1.16 (0.95, 1.41) in Caucasian group, and 1.19 (1.00, 1.42) overall. Analysis showed no association between A1298C polymorphism and RPL, except that the OR and 95%CI of A1298C in Heterozygous model were 1.62 (1.17, 2.25) in Asians, 1.02 (0.81, 1.28) in Caucasians, and 1.25 (1.03, 1.53) overall,

Table 5 Re-meta-analysis of primary studies on MTHFR polymorphisms

	Allele		Dominant		Recessive		Recessive (6 included)		Homozygous		Heterozygous	
	OR, 95%CI	I^2	OR, 95%CI	I^2	OR, 95%CI	I^2	OR, 95%CI	I^2	OR, 95%CI	I^2	OR, 95%CI	I^2
C677T												
Asian	1.54 (1.38, 1.72)	62.30%	1.61 (1.39, 1.86)	57.90%	1.85 (1.53, 2.25)	46.70%	1.85 (1.53, 2.25)	46.70%	2.32 (1.84, 2.92)	53.20%	1.44 (1.24, 1.67)	53.80%
Caucasian	1.28 (1.03, 1.58)	87.20%	1.33 (1.01, 1.76)	84.70%	1.42 (1.05, 1.91)	70.60%	1.43 (1.10, 1.86)	68.80%	1.66 (1.07, 2.58)	83.60%	1.27 (0.96, 1.67)	81.90%
Total	1.43 (1.29, 1.60)	78.50%	1.50 (1.30, 1.73)	75.20%	1.68 (1.42, 1.99)	60.30%	1.66 (1.42, 1.95)	59.60%	2.08 (1.66, 2.61)	72.90%	1.37 (1.18, 1.57)	70.80%
A1298C												
Asian	1.25 (0.90, 1.75)	85.50%	1.44 (1.05, 1.98)	73.70%	1.15 (0.62, 2.14)	73%			1.47 (0.79, 2.75)	67.40%	1.62 (1.17, 2.25)	71.50%
Caucasian	1.16 (0.95, 1.41)	76.60%	1.11 (0.88, 1.41)	72%	1.44 (1.00, 2.08)	63.50%			1.43 (0.97, 2.12)	65.70%	1.02 (0.81, 1.28)	65.30%
Total	1.19 (1.00, 1.42)	81.60%	1.25 (1.03, 1.50)	72.60%	1.29 (0.93, 2.12)	68%			1.43 (1.03, 1.98)	64.20%	1.25 (1.03, 1.53)	70.60%

which might indicate moderate RPL risk for the heterozygous genotype AC of MTHFR.

Cumulative meta-analyses of MTHFR polymorphisms and RPL (overall analysis and sub-group analyses by ethnicity)

Cumulative studies were performed on MTHFR polymorphisms and RPL risk sorted by publication year. Differences of tendency across ethnicities were also evident in subgroups.

Trends of C677T and RPL risk in Allele model are shown in Fig. 2 a and b. The first statistical change appeared in the 16th study in 2003, which remained stable in the next 56 primary studies over 12 years (Fig. 2a). Compared with wagging trends for Caucasians in Allele model, C677T mutation of Asians in the Allele model remained unchanged except for the first 3 studies (Fig. 2b).

With 6 primary studies only providing TT and total number, the first statistical association of C677T in Recessive model was achieved in the 14th study in 2002 (Fig. 2c). In the recessive model, C677T showed a similar tendency to Allele model for Asian population (Fig. 2d). Following the publication of 4 studies showing uncertainty, the association did not change in Asian population but remained uncertain in 33 primary studies involving Caucasians.

In Homozygous model, C677T mutation in Asian group did not change after the 3rd study and the width of the 95%CI became more narrow and more stable (Fig. 2e, f).

In all three models, the OR and 95%CI changed in a swinging trend across 31 primary studies (Fig. 3a, b). Heterozygous genotype showed only a moderate risk between A1298C and RPL (Fig. 3c, d). However, a statistical change appeared only in the last 6 studies, published between 2012 and 2014.

Publication bias and sensitivity analysis

Begg's test and Egger's test were performed for checking the publication bias of the primary case-control studies (Table 6). On C677T of MTHFR polymorphism, publication bias appeared in Dominant model of overall analysis and Caucasian subgroup, Homogeneous model of overall analysis and Asian subgroup, and Recessive model of overall analysis and Asian subgroup. Other primary studies on C677T did not have publication bias. On A1298C of MTHFR polymorphism, publication bias did not exist.

We conducted sensitivity study to test the origin of heterogeneity. The results showed no individual case-control study had marked effect on the meta-analysis of primary studies of C677T or A1298C.

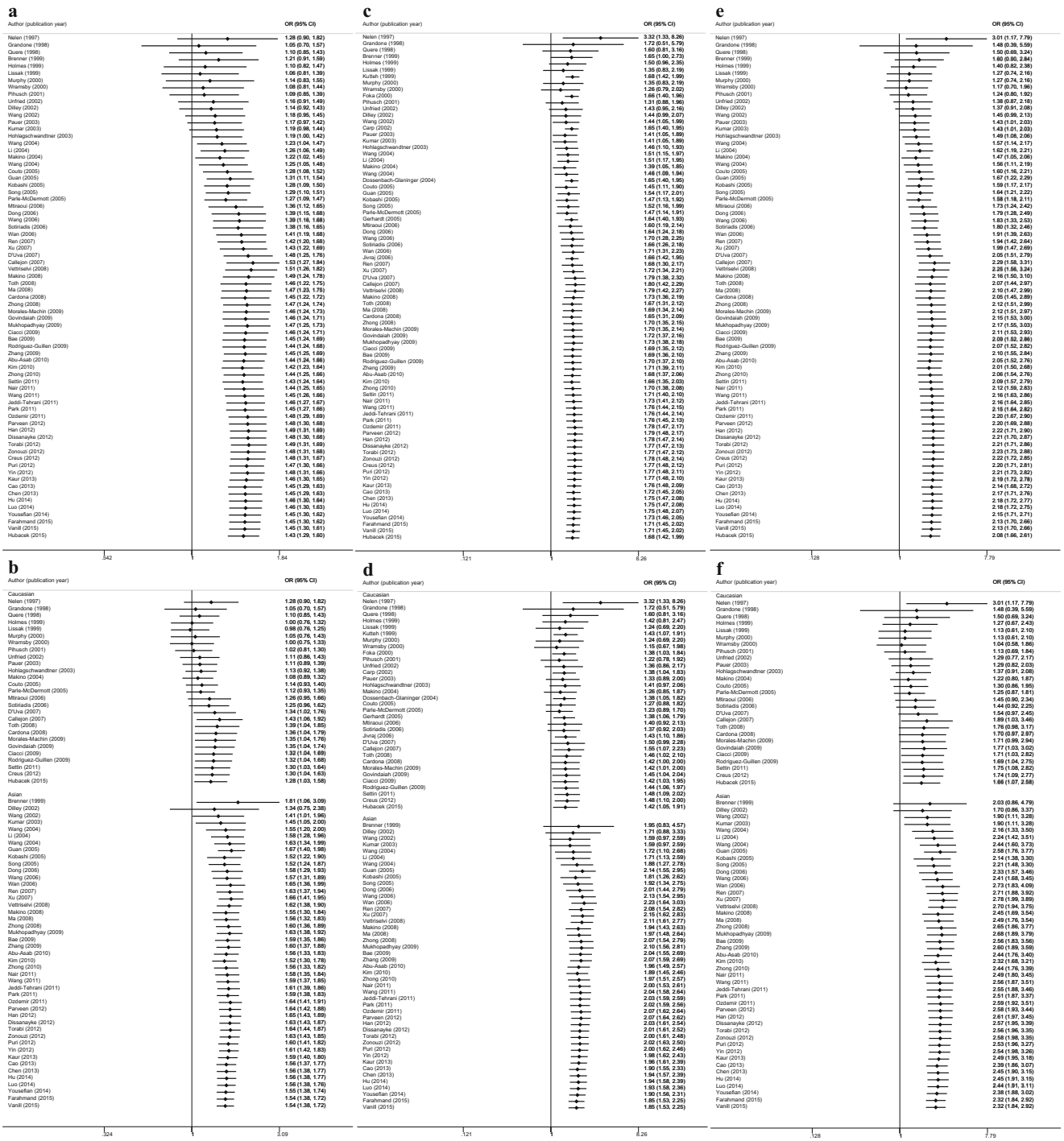

Fig. 2 Cumulative meta-analysis of **a** C677T in Allele model, **b** C677T in Allele model of different ethnicities, **c** C677T in Recessive model, **d** C677T in Recessive model of different ethnicities, **e** C677T in Homogeneous model, **f** C677T in Homogeneous model of different ethnicities

Quality of systematic reviews and meta-analyses

Table 6 and Table 7 show the quality assessments of 11 systematic reviews and meta-analyses executed with AMSTAR and OQAQ scales. The AMSTAR assessment showed one review of high methodology quality (≥ 8), 2 of low quality (≤ 3), and 8 of moderate quality with scores ranging from 5 to7 (Table 7). The OQAQ scale showed similar results for the quality of 11 systematic reviews, of which there were 2 of low quality (≤ 3) and 9 of moderate quality with scores between 5 and 7 (Table 8).

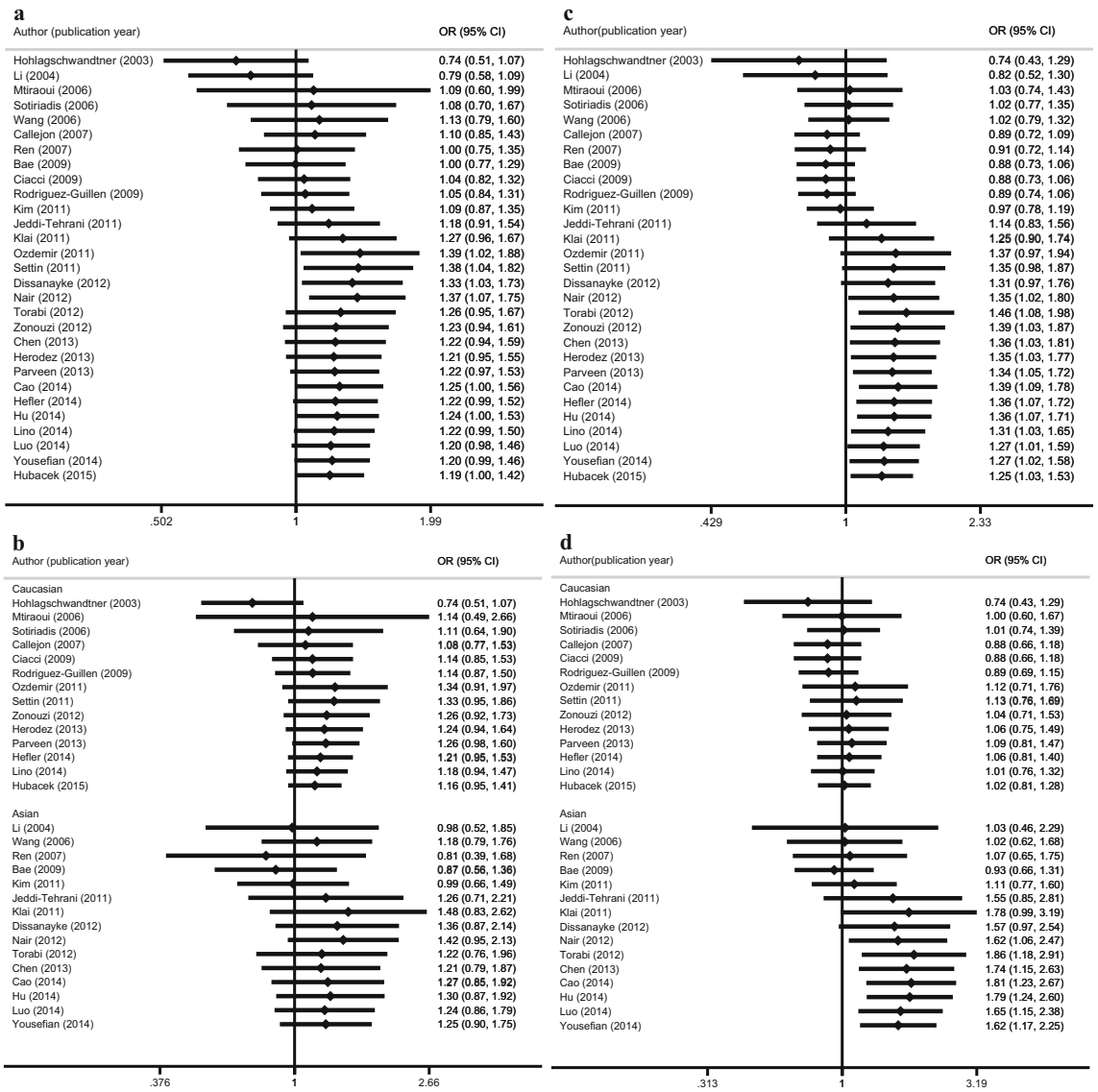

Fig. 3 Cumulative meta-analysis of **a** A1298C in Allele model, **b** A1298C in Allele model of different ethnicities, **c** A1298C in Heterogeneous model, **d** A1298C in Heterogeneous model of different ethnicities

Discussion

MTHFR plays a key role in catalyzing synthetic folic acid to 5-MTHF, also absorbed as natural folate from food [29], which participates in the conversion of homocysteine to methionine (Fig. 4). The transformation of homocysteine causes a reduction in the level of homocysteine in the plasma and promotes the methylation of substances such as DNA, protein, and lipids involving S-adenosylmethionine to S-adenosylhomocysteine [30].

DNA methylation plays a crucial role in trophoblast development, affecting imprinted or non-imprinted genes after global demethylation on morula stage (third day postconception) [31]. Epigenetic modification in the placenta leads to intrauterine growth restriction, through methylation on gene promotors [32]. Recent research has reported such placental epigenetics are regulated with imprinted genes such as *IGF2/H19*, *PEG10* on paternal chromosome and *PHLDA2*, *CDKN1C* on maternal chromosome [33].

MTHFR polymorphisms induce structural changes of MTHFR protein. For instance, C677T causes alanine to be substituted for valine and A1298C causes glutamate to be substituted for alanine. The thermolability and reduction of enzyme activity lead to elevated concentration of homocysteine [34]. Hcy concentration is also controlled through folate-independent pathways including CBS (cystathionine β-synthase) and BHMT (betaine-homocysteine methyltransferase) in liver and kidney [35]. The compensatory regulations fail to remove Hcy accumulated in pregnant women with MTHFR deficiency, as the peak demand cannot be

Table 6 Begg's test and Egger's test on publication bias of primary case-control studies

Begg's test												
	Allele		Dominant		Recessive		Recessive (6 included)		Homogeneous		Heterogeneous	
	z	*p*	*z*	*p*	*z*	*p*	*z*	*p*	*z*	*p*	*z*	*p*
C677T												
Asian	1.12	0.265	1.66	0.096	2.82	0.005*	2.82	0.005*	3.02	0.002*	0.39	0.696
Caucasian	1.13	0.26	2.17	0.03*	1.63	0.103	1.31	0.189	1.81	0.071	1.9	0.058
Total	1.91	0.057	2.38	0.017*	3.17	0.002*	2.86	0.004*	3.4	0.001*	1.34	0.182
A1298C												
Asian	0.79	0.428	0.89	0.373	0.99	0.322			0.79	0.428	1.63	0.102
Caucasian	0.11	0.913	0	1	1.53	0.127			1.4	0.161	0.55	0.583
Total	0.54	0.586	1.03	0.302	1.36	0.173			1.36	0.173	1.34	0.179
Egger's test												
	Allele		Dominant		Recessive		Recessive (6 included)		Homogeneous		Heterogeneous	
	t	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
C677T												
Asian	2.02	0.05	1.81	0.078	3.16	0.003*	3.16	0.003*	3.99	0.001*	0.32	0.754
Caucasian	0.35	0.73	1.35	0.189	1.02	0.32	0.62	0.54	1.2	0.24	1.14	0.264
Total	1.74	0.087	2.53	0.014*	3.09	0.003*	2.58	0.012*	3.48	0.001*	1.42	0.161
A1298C												
Asian	0.92	0.374	1.48	0.161	0.92	0.374			1.14	0.273	2.04	0.062
Caucasian	0.54	0.596	0	0.999	2.09	0.061			1.74	0.11	−0.43	0.678
Total	0.98	0.337	1.19	0.243	1.48	0.15			1.82	0.081	1.58	0.126

*In Begg's test or Egger's test, p value < 0.05

reached [36, 37]. High level of homocysteine leads to RPL with pathway of Hcy toxicity such as homocysteinylation, oxidative stress induction, and biotoxicity itself [38].

While numerous primary studies and systematic reviews have investigated the correlation between MTHFR polymorphisms and RPL, the outcome of this research remains unclear, and even controversial when comparing across different authors [39]. When comparing OR and 95%CI in Allele model conducted in this current study with the results from previous systematic reviews, it was evident that the C677T polymorphism could represent a risk marker for RPL, especially in Asian population. Results from Homozygous and Recessive models further indicated that TT genotype could represent a significant risk marker for RPL. Despite the moderate level of

Table 7 AMSTAR scores of 11 systematic reviews included

Author	Year	1. A priori design	2. Duplicate selection	3. Literature search	4. Status of publication	5. List of studies	6. Characteristics of included	7. Quality of included	8. Scientific quality used	9. Appropriate methods	10. Likelihood of bias	11. Conflict of interest
Nelen	2000	+	+	+	+	–	–	–	–	+	–	+/–
Wiwanitkit	2005	+	–	–	+	–	+	–	–	–	–	+/–
Ren	2006	+	+	+	+	–	+	–	–	+	+	+/–
Wu	2012	+	+	+	+	–	+	–	–	+	+	+/–
Cao	2012	+	+	+	+	+	+	–	–	+	+	+/–
Nair	2013	+	+/–	+	+	–	+	–	–	+	+	+/–
Parveen	2013	+	+/–	–	+	–	–	–	–	+/–	+	+/–
Rai	2014	+	–	+	+	–	+	–	–	+	+	+/–
Chen	2015	+	+	+	+	–	+	–	–	+	+	+/–
Yang	2015	+	+	+	+	–	+	–	–	+	+	+/–
Rai	2016	+	–	+	+	–	+	–	–	+	+	+/–

+: yes; −: no; +/−: partially answered or unclear

Table 8 OQAQ scores of 11 systematic reviews included

Author	Year	1. Search methods stated	2. Search comprehensive	3. Inclusion criteria reported	4. Selection bias avoided	5. Validity criteria reported	6. Validity assessed appropriately	7. Combining methods reported	8. Finding combined appropriately	9. Conclusions supported by the data
Nelen	2000	+	+	+	+	–	–	+	+	+
Wiwanitkit	2005	–	–	–	–	–	–	–	–	+
Ren	2006	+	+	+	+	–	–	+	+	+
Wu	2012	+	+	+	+	–	–	+	+	+
Cao	2012	+	+	+	+	–	–	+	+	+
Nair	2013	+	+	+	+	–	–	+	+	+
Parveen	2013	–	+/–	–	+/–	–	–	+/–	+	+
Rai	2014	+	+	–	–	–	–	+	+	+
Chen	2015	+	+	+	+	–	–	+	+	+
Yang	2015	+	+	+	+	–	–	+	+	+
Rai	2016	+	+	+	–	–	–	+	+	+

+: yes; –: no; +/–: partially answered or unclear

risk shown in Heterozygous model, there may be no relationship between A1298C and RPL.

Paternal MTHFR gene influence may have contributed to discrepancies in A1298C, resulting aneuploidy in embryo [40]. Despite chromosome abnormality, paternal MTHFR polymorphisms could also lead to destruction in sperm nucleus DNA as well [41]. Cornet [42] reported that, in population included (18 homozygous, 77 heterozygous, 1405 control) with SDI (sperm nucleus decondensation index) above 20%, the homozygote group

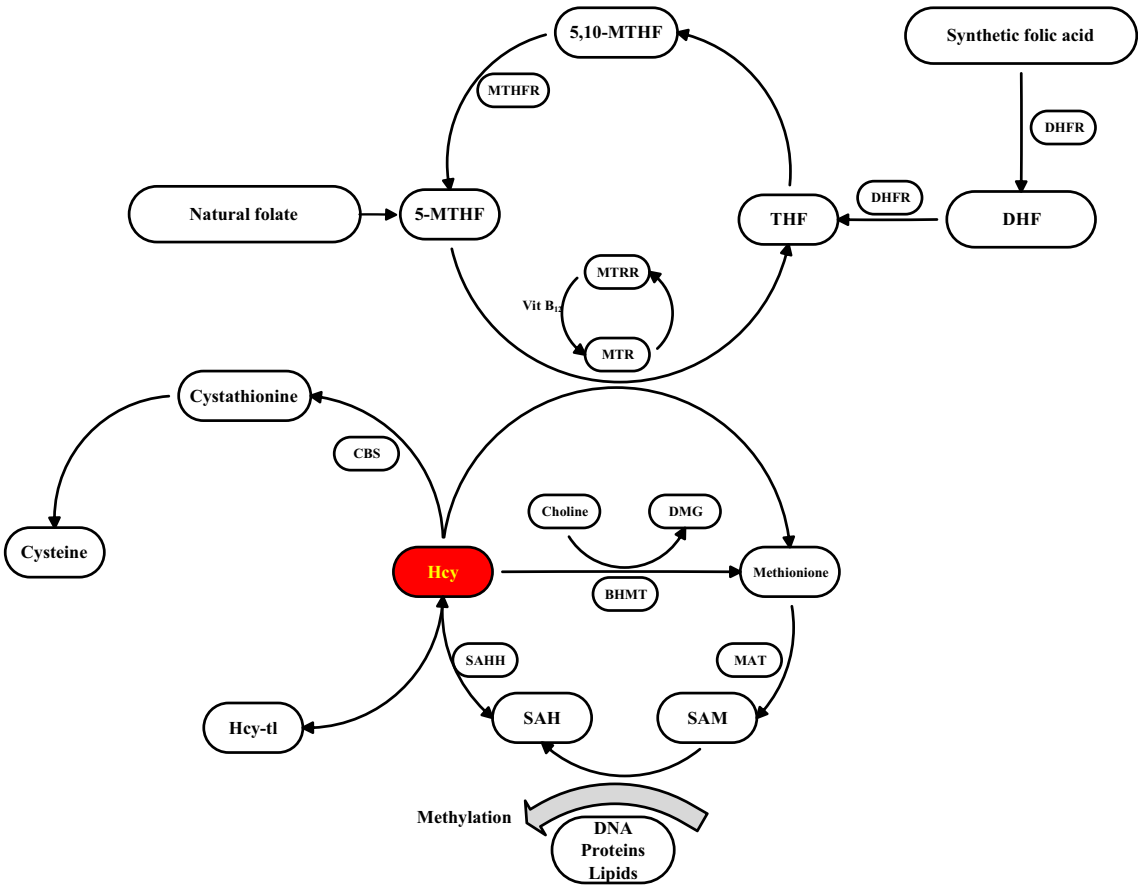

Fig. 4 Mechanism of MTHFR in recurrent pregnancy loss (RPL)

presented with 67% comparing to 23% in the control group and 30% in heterozygote group. The selection of primary studies from systematic reviews and meta-analyses most likely contributed to the discrepancies evident in the published conclusions from different authors; this was evident in the selection matrix.

AMSTAR and OQAQ scales showed moderate quality overall in 11 systematic reviews and low quality in 2 systematic reviews. A common deficiency of the 11 systematic reviews with moderate quality was the lack of validity criteria or the nature of the validity criteria used, which is commonly evaluated with the NOS (Newcastle-Ottawa Scale) [43] for case-control studies. The lack of qualification for primary studies may therefore have contributed to the heterogeneity and controversy evident in the published conclusions.

The tendency of stability was demonstrated by the cumulative meta-analysis of MTHFR polymorphisms in RPL. The meta-analyses relating to C677T, and based upon early primary studies, did not show any correlation with RPL; this was in accordance with the views of previous systematic reviews and meta-analyses which were published prior to 2005. However, as the number of primary studies increased, the OR and 95%CI of the C677T polymorphism changed to positive and became stable over the next few years in Allele, Recessive, and Homozygous models. The trend for the A1298C polymorphism in Allele model remains unclear; despite the positive change appearing in 2011, the data was mainly based on recent primary studies in Asian population.

The mild tendency of A1298C in Heterozygous model might attribute to composite C677T and A1298C. Xu [44] has found that compound 677/1298 heterozygous genotype is a risk factor in RPL. In 218 in RPL group and 264 in control group, no composite homozygote genotype appeared; however, patients with composite heterozygote (677CT-1298AC) presented with higher risk compared with the control group (OR, 95%CI 4.996, 1.65–15.129). Paternal effect may also have contributed to the compound MTHFR genotype in embryo [45].

There are some limitations in this present study which need to be considered. First of all, heterogeneity was observed in each genotype model for C677T and A1298C polymorphisms; thus, results based on primary studies should be interpreted with caution. Second, the NOS is not used to investigate the quality of primary studies and this might be the underlying factor responsible for the overall high heterogeneity. Finally, there are some factors which could have influenced our results but were not considered, such as age, body mass index (BMI), smoking, alcohol and drug abuse, and environmental factors [46].

In conclusion, MTHFR C677T polymorphism appears to represent a risk marker for RPL, especially in Asian population. The TT genotype of C677T appears more significant than the other genotypes, particularly in term of RPL. However, there is no significant correlation between A1298C and RPL, except for in Heterozygous model, which indicates moderate risk. We believe that differences in the selection of primary studies lead to the controversial and inconsistent conclusions made by different authors in the systematic reviews and meta-analyses considered in our present study. More comprehensive and rigorous systematic reviews and meta-analyses should now be performed, which incorporate quality-controlled primary case-control studies. Future investigations also need to consider different ethnicities, gene-gene interaction, and gene-environment interaction in order to fully investigate the influence of MTHFR polymorphisms on RPL.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Page JM, Silver RM. Genetic causes of recurrent pregnancy loss. J Clin Obstet Gynecol. 2016;59(3):498–508. https://doi.org/10.1097/grf.0000000000000217.
2. Pritchard AM, Hendrix PW, Paidas MJ. Hereditary thrombophilia and recurrent pregnancy loss. J Clin Obstet Gynecol. 2016;59(3):487–97. https://doi.org/10.1097/grf.0000000000000226.
3. Nelen WL, Blom HJ, Steegers EA, den Heijer M, Thomas CM, Eskes TK. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. J Obstet Gynecol. 2000;95(4):519–24.
4. Moll S, Varga EA. Homocysteine and MTHFR mutations. J Circulation. 2015;132(1):e6–9. https://doi.org/10.1161/circulationaha.114.013311.
5. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg (London, England). 2010;8(5):336–41. https://doi.org/10.1016/j.ijsu.2010.02.007.
6. Jr Faggion CM. Critical appraisal of AMSTAR: challenges, limitations, and potential solutions from the perspective of an assessor. J BMC Med Res Methodol 2015;15:63. doi:https://doi.org/10.1186/s12874-015-0062-6.
7. Delaney A, Bagshaw SM, Ferland A, Laupland K, Manns B, Doig C. The quality of reports of critical care meta-analyses in the Cochrane database of systematic reviews: an independent appraisal. J Crit Care Med. 2007;35(2):589–94. https://doi.org/10.1097/01.Ccm.0000253394.15628.Fd.
8. Cao Y, Xu J, Zhang Z, Huang X, Zhang A, Wang J, et al. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: a meta-analysis. J Gene. 2013;514(2):105–11.
9. Chen H, Yang X, Lu M. Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss in China: a systematic review and meta-analysis. J Arch Gynecol Obstet. 2016;293(2):283–90.
10. Nair RR, Khanna A, Singh R, Singh K. Association of maternal and fetal MTHFR A1298C polymorphism with the risk of pregnancy

loss: a study of an Indian population and a meta-analysis. J Fertil Steril. 2013;99(5):1311–8e4.
11. Nelen WLDM, Blom HJ, Steegers EAP, Den Heijer M, Eskes TKAB. Hyperhomocysteinemia and recurrent early pregnancy loss: a meta-analysis. J Fert Steril. 2000;74(6):1196–9.
12. Rai V. Methylenetetrahydrofolate reductase gene A1298C polymorphism and susceptibility to recurrent pregnancy loss: a meta-analysis. J Cell Mol Biol. 2014;60(2):27–34.
13. Ren A, Wang J. Methylenetetrahydrofolate reductase C677T polymorphism and the risk of unexplained recurrent pregnancy loss: a meta-analysis. J Fertil Steril. 2006;86(6):1716–22.
14. Wu X, Zhao L, Zhu H, He D, Tang W, Luo Y. Association between the MTHFR C677T polymorphism and recurrent pregnancy loss: a meta-analysis. J Genet Test Mol Biomarkers. 2012;16(7):806–11.
15. Yang Y, Luo Y, Yuan J, Tang Y, Xiong L, Xu MM, et al. Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: a comprehensive evaluation. J Arch Gynecol Obstet. 2016;293(6):1197–211.
16. Simcox LE, Ormesher L, Tower C, Greer IA. Thrombophilia and pregnancy complications. J Int J Mol Sci. 2015;16(12):28418–28.
17. Clement A, Cornet D, Cohen M, Jacquesson L, Amar E, Clement P, et al. Impact of MTHFR iso form C667T on fertility through sperm DNA fragmentation index (DFI) and sperm nucleus decondensation (SDI). J Hum Reprod. 2017;32:i149–i50.
18. Bogdanova N, Markoff A. Hereditary thrombophilic risk factors for recurrent pregnancy loss. J Community Genet. 2010;1(2):47–53.
19. Cohen H. Inherited thrombophilia and pregnancy loss-epidemiology. J Thromb Res. 2005;115(SUPPL):13–7.
20. Moll S. Thrombophilias - practical implications and testing caveats. J J Thromb Thrombolysis. 2006;21(1):7–15.
21. Rambaldi MP, Mecacci F, Paidas MJ. Evaluation and management of recurrent pregnancy loss. J Placenta. 2011;32:5278.
22. Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. J Placenta. 1999;20(7):519–29.
23. Yamada H, Sata F, Saijo Y, Kishi R, Minakami H. Genetic factors in fetal growth restriction and miscarriage. J Semin Thromb Hemost. 2005;31(3):334–45.
24. Chen J, Chen L, Zhu LH, Zhang ST, Wu YL. Association of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism with preterm delivery and placental abruption: a systematic review and meta-analysis. J Acta Obstet Gynecol Scand. 2016;95(2):157–65. https://doi.org/10.1111/aogs 12789 / (c) 2015 Nordic Federation of Societies of Obstetrics and Gynecology.
25. Di Nisio M, Rutjes AWS, Ferrante N, Tiboni GM, Cuccurullo F, Porreca E. Thrombophilia and outcomes of assisted reproduction technologies: a systematic review and meta-analysis. J Blood. 2011;118(10):2670–8.
26. Wiwanitkit V. Roles of methylenetetrahydrofolate reductase C677T polymorphism in repeated pregnancy loss. J Clin Appl Thromb/Hemost. 2005;11(3):343–5.
27. Parveen F, Tuteja M, Agrawal S. Polymorphisms in MTHFR, MTHFD, and PAI-1 and recurrent miscarriage among north Indian women. J Arch Gynecol Obstet. 2013;288(5):1171–7. https://doi.org/10.1007/s00404-013-2877-x.
28. Rai V. Methylenetetrahydrofolate reductase C677T polymorphism and recurrent pregnancy loss risk in Asian population: a meta-analysis. J Indian J Clin Biochem. 2016;31(4):402–13.
29. Naderi N, House JD. Recent developments in folate nutrition. Adv Food Nutr Res. 2018;83:195–213. https://doi.org/10.1016/bs.afnr.2017.12.006.
30. Quere I, Mercier E, Bellet H, Janbon C, Mares P, Gris JC. Vitamin supplementation and pregnancy outcome in women with recurrent early pregnancy loss and hyperhomocysteinemia. J Fertil Steril. 2001;75(4):823–5.
31. Serman L, Dodig D. Impact of DNA methylation on trophoblast function. Clin Epigenetics. 2011;3:7. https://doi.org/10.1186/1868-7083-3-7.
32. Branco MR, King M, Perez-Garcia V, Bogutz AB, Caley M, Fineberg E, et al. Maternal DNA methylation regulates early trophoblast development. Dev Cell. 2016;36(2):152–63. https://doi.org/10.1016/j.devcel.2015.12.027.
33. Koukoura O, Sifakis S, Spandidos DA. DNA methylation in the human placenta and fetal growth (review). Mol Med Rep. 2012;5(4):883–9. https://doi.org/10.3892/mmr.2012.763.
34. Ozdemir O, Yenicesu GI, Silan F, Koksal B, Atik S, Ozen F, et al. Recurrent pregnancy loss and its relation to combined parental thrombophilic gene mutations. J Genet Test Mol Biomarkers. 2012;16(4):279–86. https://doi.org/10.1089/gtmb.2011.0191.
35. Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. Nutr Metab. 2017;14:78. https://doi.org/10.1186/s12986-017-0233-z.
36. Imbard A, Benoist JF, Esse R, Gupta S, Lebon S, de Vriese AS, et al. High homocysteine induces betaine depletion. Biosci Rep. 2015;35(4). https://doi.org/10.1042/bsr20150094.
37. Hiraoka M, Kagawa Y. Genetic polymorphisms and folate status. Congenit Anom. 2017;57(5):142–9. https://doi.org/10.1111/cga.12232.
38. Skovierova H, Vidomanova E, Mahmood S, Sopkova J, Drgova A, Cervenova T, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int J Mol Sci. 2016;17(10). https://doi.org/10.3390/ijms17101733.
39. Dutra CG, Fraga LR, Nacul AP, Passos EP, Goncalves RO, Nunes OL, et al. Lack of association between thrombophilic gene variants and recurrent pregnancy loss. J Hum Fertil (Camb). 2014;17(2):99–105. https://doi.org/10.3109/14647273.2014.882022.
40. Enciso M, Sarasa J, Xanthopoulou L, Bristow S, Bowles M, Fragouli E, et al. Polymorphisms in the MTHFR gene influence embryo viability and the incidence of aneuploidy. Hum Genet. 2016;135(5):555–68. https://doi.org/10.1007/s00439-016-1652-z.
41. Servy EJ, Jacquesson-Fournols L, Cohen M, Menezo YJR. MTHFR isoform carriers. 5-MTHF (5-methyl tetrahydrofolate) vs folic acid: a key to pregnancy outcome: a case series. J Assist Reprod Genet. 2018. https://doi.org/10.1007/s10815-018-1225-2.
42. Cornet D, Cohen M, Clement A, Amar E, Fournols L, Clement P, et al. Association between the MTHFR-C677T isoform and structure of sperm DNA. J Assist Reprod Genet. 2017;34(10):1283–8. https://doi.org/10.1007/s10815-017-1015-2.
43. Lo CK, Mertz D, Loeb M. Newcastle-Ottawa Scale: comparing reviewers' to authors' assessments. J BMC Med Res Methodol. 2014;14:45. https://doi.org/10.1186/1471-2288-14-45.
44. Xu Y, Ban Y, Ran L, Yu Y, Zhai S, Sun Z, et al. Relationship between unexplained recurrent pregnancy loss and 5,10-methylenetetrahydrofolate reductase polymorphisms. Fertil Steril. 2019. https://doi.org/10.1016/j.fertnstert.2018.11.011.
45. Govindaiah V, Naushad SM, Prabhakara K, Krishna PC, Radha Rama Devi A. Association of parental hyperhomocysteinemia and C677T methylene tetrahydrofolate reductase (MTHFR) polymorphism with recurrent pregnancy loss. Clin Biochem. 2009;42(4–5):380–6. https://doi.org/10.1016/j.clinbiochem.2008.12.003.
46. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. J Fertil Steril. 2012;98(5):1103–11. doi:https://doi.org/10.1016/j.fertnstert.2012.06.048