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REVIEW

MTHFR 1298A>C Substitution is a Strong Candidate for Analysis in Recurrent Pregnancy Loss: Evidence from 14,289 Subjects

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Abstract

We undertook meta-analyses on *MTHFR* 1298A>C substitution for critically evaluating its association with recurrent pregnancy loss (RPL). *MTHFR* genotype data for 5888 cases and 8401 controls from 39 studies were pooled to perform this meta-analyses. Genotype data were screened, scrutinized, pooled, analysed and subjected to sensitivity analysis to carefully evaluate the association between *MTHFR* 1298A>C and recurrent pregnancy loss. Genetic associations were sought using dominant, recessive and co-dominant models of genetic testing with odds ratio and 95% Confidence interval (CI) as the effect measures. Further analyses were undertaken by classifying the studies into Caucasian and East Asian sub-groups. Genetic heterogeneity was tested before pooling the data across studies. For assessing publication bias, Egger's intercept test was undertaken. We found a significant association of 1298A>C substitution with increased risk of RPL in the dominant (P=0.000; OR = 1.58; 95% CI =1.25–1.99) as well as recessive (P=0.000; OR = 1.66; 95% CI =1.25–2.20) models. In sub-group analysis, we observed a significant association of the polymorphism with RPL in the Caucasian populations using dominant (P=0.000; OR = 1.98; 95% CI =1.42–2.76) and recessive (P=0.000; OR = 2.20; 95% CI =1.49–3.24) models. However, this substitution showed no association with RPL in the East Asian populations (P=0.149; OR = 1.187; 95% CI =0.94–1.50). *MTHFR* 1298A>C substitution shows association with the risk of recurrent pregnancy loss. The association is in a population-specific manner with the substitution being a strong risk factor only in the Caucasian populations.

Keywords MTHFR · Polymorphism · Recurrent pregnancy loss · Genetic testing

Introduction

The spontaneous loss of a pregnancy before the foetus reaches viability is known as a miscarriage. By definition, "recurrent" pregnancy loss (RPL) is defined as the loss of two or more pregnancies occurring within 20–24 weeks of conception. RPL generally occurs with an incidence as high as 3–5% of all pregnancies [1, 2]. The cause of RPL in as high as 50% of the patients cannot be ascertained [3, 4]. Miscarriage is a loss of a newborn for most people and their families as well as the loss of dreams and aspirations they believe in [4, 5]. It is a traumatic event which affects every woman differently, but can lead to grief, anxiety, depression, and even symptoms of post-traumatic stress disorder (PTSD) [6]. Pregnancy loss can also affects male partners equally significantly [7].

Folic acid is essential for pregnant women as it is critical for the synthesis of DNA, RNA and metabolism of amino acids. Single carbon derivatives of folate participate in DNA synthesis [8]. After three consequent reductions, folate forms 5,10-methylenetetrahydrofolate by the addition of a methylene bridge from formate, serine or glycine. Subsequently, 5,10-methylenetetrahydrofolate is irreversibly reduced to 5-methyltetrahydrofolate by the action of methylenetetrahydrofolate reductase (MTHFR). 5,10-Methylenetetrahydrofolate is used by methionine synthase to transform homocysteine (Hcy) into methionine, which retains and preserves homocysteine in the blood at a normal level [9]. A high level of homocysteine, termed hyperhomocysteinemia, has been reported to associate with complications in pregnancy [10] and adverse events in the cardiovascular system [11]. This suggests

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an important role of folic acid and one carbon cycle in foetal development and the success of pregnancy.

Several studies found that *MTHFR* (methylenetetrahydrofolate reductase) gene mutations increase the risk of recurrent pregnancy loss (RPL) before completion of 20 weeks of the gestation period [12]. *MTHFR* polymorphisms 677C>T (rs1801133) and 1298A>C (rs1801131) have been widely studied in RPL and found to increase RPL risk [13, 14]. Elevated homocysteine levels and low folic acid levels can lead to complications of pregnancy, including miscarriage, preeclampsia, and other congenital disabilities [15–17]. *MTHFR* 677C>T or 1298A>C polymorphisms have also been correlated with hyperhomocysteinemia. Eventually, *MTHFR* 677C>T has been shown to significantly increase the risk of miscarriage in Asians, which was also supported by meta-analyses [18–21]. Second important polymorphism in this gene, 1298A>C, has been considered to be a risk factor for pregnancy loss [22, 23].

In a previous study, we reported highly significant association of 1298A>C substitution with RPL [22]. With the availability of human genome sequence, comprehensive genetic testing of mothers would be an attractive option. However, in RPL, most of the studies have focussed on the foetal causes of pregnancy loss, which emphasizes on chromosomal aneuploidies as a major contributor [24, 25]. Nonetheless, maternal causes would also contribute significantly to pregnancy loss and genetic testing of the mother can provide significant clues for the management of RPL. While genetic testing panels for a number of human diseases have been launched, RPL in this regard is in infancy [26–28]. In order to rigorously evaluate the candidature of *MTHFR* 1298A>C polymorphism for genetic testing in RPL, we undertook this meta-analysis on 39 studies with 5888 cases and 8401 controls. We found that 1298A>C substitution increases the risk of RPL significantly and this variant could be adopted for analysis in clinical settings. Interestingly, this can help in appropriate management of the RPL cases by adjusting the folate and vitamin B12 dosage of the patients.

Material and Methods

Search Strategy

A comprehensive screening of published studies in the online repositories of Medline (PubMed), GoogleScholar, EmBase, ScienceDirect, Ovid Science Citation Index (SCI) and Cochrane Library was conducted for identifying studies published until October 2019. Keywords MTHFR, persistent pregnancy loss, 1298A>C polymorphism, methylenetetrahydrofolate reductase, miscarriage and spontaneously aborted embryos were searched in different combinations. All articles thus identified were manually reviewed for the identification of additional citations. This was followed by acquiring full text of relevant articles.

Inclusion and Exclusion Criteria

Studies fulfilling the following criteria were included: (i) case-control studies analysing *MTHFR* 1298A>C polymorphism in recurrent pregnancy loss; (ii) genotype analysis should involve the use of standard genotyping methods; (iii) sufficient details of genotype data were provided for calculation of the odds ratio (ORs) and confidence interval (CI). We omitted articles including review, meta-analysis and those on other pregnancies complications such as recurrent implant failure, foetal death and spontaneous abortion with foetal chromosomal aneuploidy. We also excluded those studies which were covered under broad title of adverse pregnancy outcomes.

Data Extraction

The following information were extracted from each of the full text articles: first author's name, year of publication, country of origin, race/ethnicity, number of cases/controls and genotypes. Information was independently extracted by two authors (PM and RV) to avoid errors. In the event of a dispute, the senior author (SR) was consulted. Data extracted from each included study are detailed in Table 1. Genotype frequencies of control samples for each study were checked for their goodness of fit in the Hardy-Weinberg equilibrium. The genotype frequencies and controls was compared using 95% CI, OR and chi-square. $P < 0.05$ was judged to be significant.

Data Analysis

We used odds ratio (ORs) and 95% confidence interval for assessment of the association of 1298A>C substitution with RPL. The association was tested using dominant (AA vs AC+CC), recessive (AA+AC vs CC) and co-dominant (AC vs AC; AA vs CC; AC vs CC) models of analysis. Since different studies were conducted under different settings, significant heterogeneity across studies may affect the results of pooled analysis. For this, we analysed data using fixed effect and random effects models of pooled data analysis. The heterogeneity between the studies was quantitatively assessed using Q statistics, considering P value less than 0.10 as statistically significant. Heterogeneity index (I^2) value < 25% means no heterogeneity, 50% means moderate heterogeneity and 75% and above corresponds to high heterogeneity [67]. Pooled OR was calculated using both the fixed effect and random effects models and high-resolution plots (forest plot) were generated. The inference was made using fixed or random effects model of analysis based upon the level of heterogeneity.

Table 1 Characteristics of the studies included in meta-analyses

Study name	Country	Ethnicity	Cases			Controls			HWE *P* value	Ref.
			AA	AC	CC	AA	AC	CC		
Hohlagschwandter et al., 2003	Austria	Caucasian	63	67	15	35	50	16	0.791	[29]
Li et al., 2004	China	East Asian	33	21	3	29	18	3	0.926	[30]
Mtiraoui et al.,2006	Tunisia	Caucasian	108	65	27	130	62	8	0.858	[31]
Wang et al.,2006	China	East Asian	103	35	10	60	20	2	0.829	[32]
Callejon et al., 2007	Spain	Caucasian	209	89	44	248	149	37	0.036	[33]
Ren et al., 2007	China	East Asian	0	17	54	1	6	86	0.037	[34]
Bae et al., 2009	Korea	East Asian	144	68	9	75	43	4	0.466	[35]
Ciacci et al., 2009	Italy	Caucasian	22	0	56	52	0	92	3.553e-33	[36]
Rodriguez-Guillen et al., 2009	Mexico	Caucasian	18	5	0	60	14	0	0.369	[37]
Bagheri et al., 2010	Iran	Caucasian	24	28	9	21	24	8	0.7912	[38]
Kim et al., 2011	Korea	East Asian	23	10	0	113	38	4	0.709	[39]
Settin et al., 2011	Egypt	Caucasian	15	49	6	36	97	3	1.808e-09	[40]
Tehrani et al., 2011	Iran	Caucasian	69	27	4	94	6	0	0.757	[41]
Dissanayake et al., 2012	Sinhalese	Unknown	74	78	43	72	79	46	0.009	[42]
Idali et al., 2012	Iran	Caucasian	40	46	20	94	6	0	0.757	[43]
Ozdemir et al., 2012	Turkey	Caucasian	201	257	85	71	35	0	0.042	[44]
Poursadegh et al., 2012	Iran	Caucasian	35	46	8	13	34	3	0.003	[45]
Sheikhha et al., 2012	Iran	Caucasian	8	45	7	34	26	0	0.032	[46]
Nair et al., 2013	India	Caucasian	48	68	13	116	80	6	0.073	[22]
Parveen et al., 2013	India	Caucasian	88	92	20	157	127	16	0.133	[47]
Boas et al.,2014	Brazil	Unknown	57	27	5	80	62	8	0.364	[48]
Cao et al., 2013	China	East Asian	49	31	2	132	31	3	0.462	[49]
Hu et al., 2014	China	East Asian	33	12	7	12	3	1	0.248	[50]
Hubacek et al., 2014	Czech	Caucasian	209	212	43	1145	1066	275	0.258	[51]
Khaleghprast et al., 2014	Iran	Caucasian	11	13	6	5	2	3	0.065	[52]
Luo et al., 2015	China	East Asian	82	40	3	78	54	3	0.068	[53]
Yousefian et al., 2014	Iran	Caucasian	98	81	25	68	39	9	0.317	[54]
Farahmand et al., 2015	Iran	Caucasian	134	152	44	329	20	1	0.251	[55]
Lino et al., 2015	Brazil	Unknown	71	32	9	52	43	3	0.092	[56]
Zhu., 2015	China	East Asian	48	58	12	76	88	10	0.017	[57]
Al Achkar et al., 2017	Syria	Caucasian	53	40	8	65	40	1	0.053	[58]
Lopez et al., 2016	Mexico	Caucasian	42	13	1	37	13	0	0.291	[59]
Najafian et al., 2016	Iran	Caucasian	30	48	36	58	56	0	0.0005	[60]
Hwang et al., 2017	Korea	East Asian	209	86	7	210	93	12	0.672	[61]
Wolski et al., 2017	Poland	Caucasian	123	143	37	179	172	49	0.442	[62]
Bigdeli et al., 2018	Iran	Caucasian	142	48	10	171	28	1	0.899	[63]
Kim et al., 2018	Korea	East Asian	25	14	2	65	25	2	0.823	[64]
Wolski et al., 2019	Poland	Caucasian	25	42	9	179	172	49	0.442	[65]
Xu et al., 2019	China	East Asian	155	58	5	214	44	6	0.05	[66]
Total			2921	2263	704	4666	2965	770		

The publication bias was tested by funnel plot asymmetry and the Egger's Regression Intercept Test. Sensitivity analysis was conducted by eliminating one study at a time and re-estimating the OR on the remaining data sets. We also conducted sensitivity analysis on studies using three or more abortions as the criteria defining recurrent pregnancy loss.

For sub-group analysis, ethnicity was classified as East Asian, Caucasian and others, but sub-group meta-analysis was conducted only in the groups having more than three

studies. We identified that 16 studies were conducted on East Asian populations, 20 were conducted on Caucasian populations, and three studies did not fit in any major ethnic group.

Results

Since the objective of this analysis was to test the association of 1298A>C polymorphism with RPL, we selected only those studies which had clearly stated analysis on recurrent pregnancy loss cases only. After applying inclusion and exclusion criteria, data from 5888 cases and 8401 controls from 39 studies were included in the analysis (Fig. 1).

1298 A>C Substitution Increases the Risk of RPL

In the dominant model (AA vs AC+CC), the I^2 value was 87.44, showing a high level of heterogeneity (Q =302.56; P=0.000; I^2 = 87.44; t^2 = 0.44; SE =0.15). Therefore, the random effects model was applied for drawing the inference (Table 2). The pooled analysis using random effects model showed that the mutant genotype was a significant risk factor for recurrent pregnancy loss (P=0.000; OR = 1.58; 95% CI =1.25–1.99) (Fig. 2). An almost symmetric distribution of studies on the funnel plot suggested the absence of publication bias (Supplementary Fig. 1). Egger's test for intercept confirmed no publication bias (intercept = 1.85; SE = 1.06; 95% CI = −0.30–4.00; $P_{2\text{-tailed}}$=0.08) (Table 2).

In the recessive model (AA+ AC versus CC), the I^2 value was 62.14, showing a high level of heterogeneity (Q =97.73; P=0.000; I^2 = 62.14; t^2 = 0.36; SE =0.17). Therefore, we used the random effects model for drawing the inference (Table 2). The pooled analysis using random effects model showed a highly significant increase in the risk of recurrent pregnancy loss in mutation carriers (P=0.000; OR = 1.66; 95% CI =1.25–2.20) (Fig. 3). Funnel plot showed asymmetrical distribution of the studies, suggesting publication bias (Supplementary Fig. 1), which was confirmed by Egger's test for intercept (intercept = 1.64; SE = 0.41; 95% CI = 0.81–2.50; $P_{2\text{-tailed}}$=0.000) (Table 2).

1298 A>C Substitution Increases the Risk of RPL in Caucasians

A total of 24 studies were conducted on Caucasian (Austrian, Czech Republican, Egyptian, Iranian, Indian, Italian,

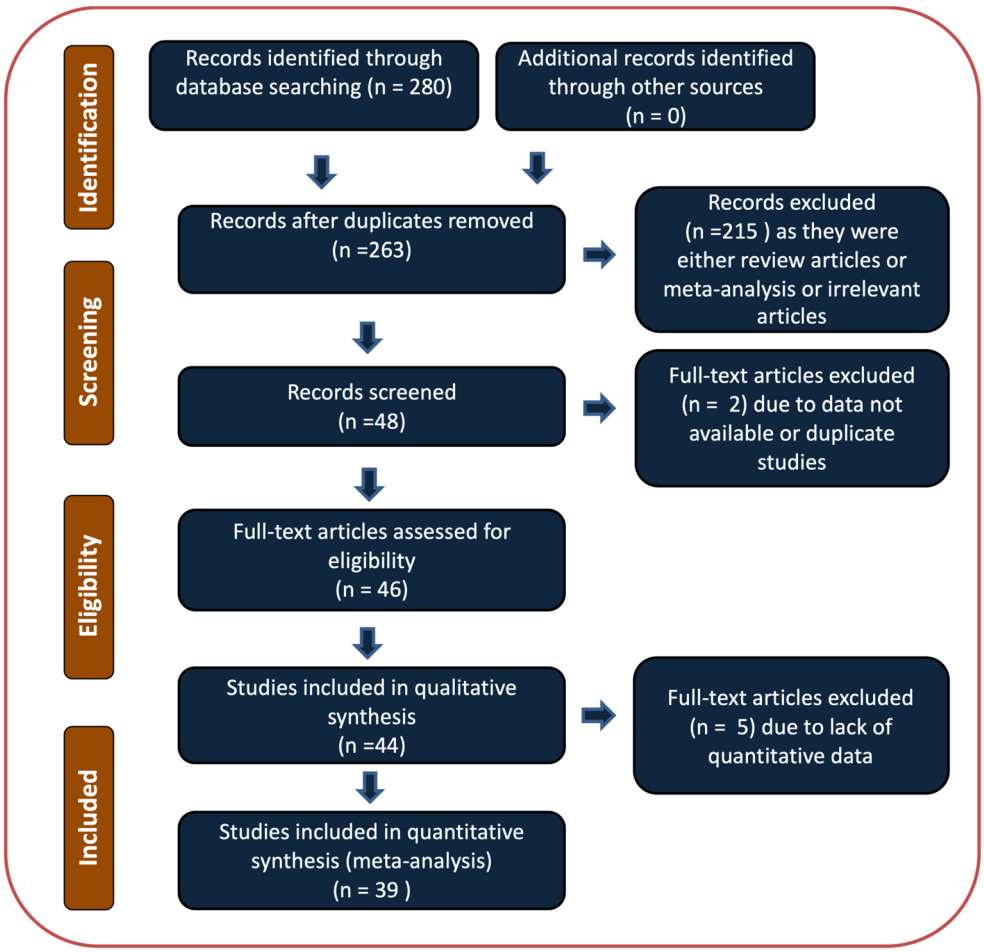

Fig. 1 PRISMA flow diagram showing screening of studies for inclusion in the meta-analysis

Table 2 Summary of pooled data analysis for various groups using various genetic models

Comparison model	No. of studies	Test model	Type of association			Test of heterogeneity			Publication bias
			OR	95% CI	*P*	*Q*	*P*	I^2	Egger's *P*
All populations									
Dominant		Fixed	1.37	1.27–1.48	0.000	302.56	0.000	87.44	0.089
AA vs AC+CC		Random	1.58	1.25–1.99	0.000				
Recessive		Fixed	1.28	1.10–1.47	0.001	97.73	0.000	62.14	0.0002
AA+AC vs CC		Random	1.66	1.26–2.20	0.000				
Co-dominant		Fixed	1.30	1.19–1.41	0.000	248.17	0.000	85.09	0.117
AA vs AC		Random	1.47	1.170–1.85	0.000				
AA vs CC		Fixed	1.39	1.19–1.63	0.000	102.31	0.000	63.83	0.0001
		Random	1.93	1.42–2.61	0.000				
AC vs CC		Fixed	0.85	0.71–0.99	0.037	71.98	0.000	49.88	0.0067
		Random	0.72	0.55–0.94	0.014				
Caucasians									
Dominant		Fixed	1.56	1.42–1.71	0.000	253.24	0.000	90.92	0.0585
AA vs AC+CC		Random	1.98	1.42–2.76	0.000				
Recessive		Fixed	1.38	1.16–1.63	0.000	76.65	0.000	71.3	0.00006
AA+AC vs CC		Random	2.19	1.49–3.24	0.000				
Co-dominant		Fixed	1.46	1.32–1.61	0.000	202.51	0.000	89.14	0.0906
AA vs AC		Random	1.80	1.30–2.49	0.000				
AA vs CC		Fixed	1.49	1.25–1.79	0.000	91.801	0.000	76.035	0.00005
		Random	2.71	1.75–4.21	0.000				
AC vs CC		Fixed	0.79	0.65–0.95	0.012	50.55	0.000	58.453	0.00138
		Random	0.58	0.40–0.83	0.003				
East Asians									
Dominant		Fixed	1.15	0.98–1.35	0.097	19.823	0.048	44.508	0.3428
AA vs AC+CC		Random	1.19	0.94–1.49	0.149				
Recessive		Fixed	1.03	0.71–1.49	0.882	15.895	0.145	30.796	0.2779
AA+AC vs CC		Random	1.09	0.68–1.75	0.714				
Co-dominant		Fixed	1.14	0.96–1.34	0.130	20.981	0.034	45.572	0.2826
AA vs AC		Random	1.18	0.92–1.51	0.198				
AA vs CC		Fixed	1.26	0.84–1.92	0.268	6.127	0.865	0.000	0.4814
		Random	1.27	0.84–1.92	0.268				
AC vs CC		Fixed	1.14	0.77–1.69	0.502	14.620	0.201	24.761	0.5364
		Random	1.11	1.11–0.69	0.660				

Mexican, Polish, Spanish, Syrian, Tunisian and Turkish) populations, consisting of 3953 cases and 6251 controls.

In the dominant model (AA vs AC+CC), the I^2 value was 90.91, suggesting a high level of heterogeneity (Q =253.22; P=0.000; I^2 = 90.91; t^2 = 0.582; SE =0.25). Therefore, we used the random effects model for drawing the inference (Table 2). The pooled analysis using random effects model showed a highly significant association of 1298A>C substitution with increased risk of recurrent pregnancy loss (P=0.000; OR = 1.98; 95% CI =1.42–2.76) (Fig. 4). An almost symmetric distribution of studies on the funnel plot shows the absence of publication bias (Supplementary Fig. 1). Egger's test for intercept confirmed no publication bias (intercept = 2.91; SE = 1.46; 95% CI = −0.11–5.95; $P_{2\text{-tailed}}$=0.58) (Table 2).

In the recessive model (AA+AC vs CC), the I^2 value was 71.30, suggesting a high level of heterogeneity (Q =76.65; P=0.000; I^2 = 71.30; t^2 = 0.50; SE =0.07). Therefore, we used random effects model for overall inference (Table 2). The pooled analysis using random effects model showed a highly significant association of 1298A>C substitution with increased risk of recurrent pregnancy loss (P=0.000; OR = 2.20; 95% CI =1.49–3.24) (Fig. 5). Funnel plot showed asymmetric distribution of the studies (Supplementary Fig. 1), suggesting publication bias, which was confirmed by Egger's test for intercept (intercept = 2.35; SE = 0.50 95% CI = 1.37–3.33; $P_{2\text{-tailed}}$=0.000) (Table 2).

1298 A>C Substitution Does Not Affect RPL Risk in East Asians

A total of 12 studies were conducted on East Asian (Chinese and Korean) populations, consisting of 1468 cases and 1664

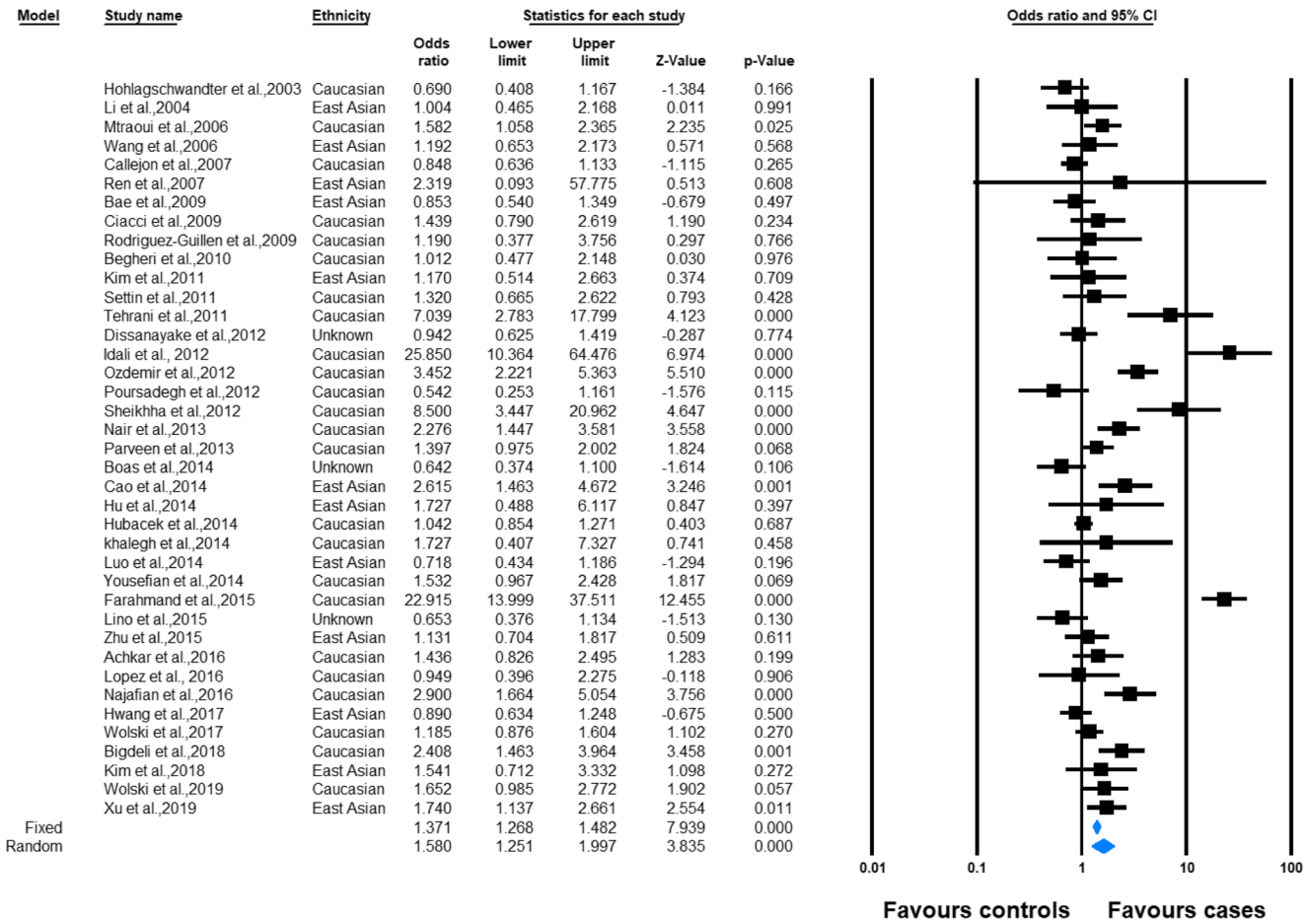

Fig. 2 Forest plot for association of *MTHFR* 1298A>C polymorphism with RPL risk using the dominant (AA vs AC+CC) model in all populations. The *Z* value shows the degree and direction of relationship, whereas the *P* value shows the significance of the relationship. The horizontal bar shows the 95% CI with OR in the centre. The direction of projection of the horizontal bar shows the direction of association. The diamond-shaped box shows the pooled OR and its width indicates the 95% CI

controls. In the dominant model (AA vs AC+CC), the I^2 value was 44.50, suggesting a moderate, but significant level of heterogeneity (Q =19.82; P=0.048; I^2 = 44.50; t^2 = 0.07; SE =0.07). Therefore, we used the random effects model for inference (Table 2). The pooled analysis using random effects model showed no significant association between 1298A>C substitution and the risk of recurrent pregnancy loss (P=0.149; OR = 1.187; 95% CI =0.94–1.50) (Fig. 6). An almost symmetric distribution of studies on the funnel plot suggested the absence of publication bias (Supplementary Fig. 1). Egger's test for intercept confirmed no publication bias (intercept = 0.99; SE = 1.00; 95% CI = −1.23–3.23; $P_{2\text{-tailed}}$=0.34) (Table 2).

In the recessive model (AA vs AC+CC), the I^2 value was 30.79 in the East Asian group, suggesting no significant heterogeneity (Q =15.90; P=0.14; I^2 = 30.79; t^2 = 0.20; SE =0.30). Therefore, we used the fixed effect model for inference (Table 2). The pooled analysis using fixed effect model showed no significant association of 1298A>C with the risk of recurrent pregnancy loss (P=0.88; OR = 1.03; 95% CI =0.71–1.50) (Fig. 7). An almost symmetric distribution of studies on the funnel plot shows the absence of publication bias (Supplementary Fig. 1). Egger test for intercept confirmed no publication bias (intercept = 1.22; SE = 1.10 95% CI = −1.15–3.60; $P_{2\text{-tailed}}$=0.28) (Table 2).

Sensitivity Analysis

Since the presence of sensitive studies can affect the overall inference, we conducted sensitivity analysis using two methods. The studies failing to fit in the Hardy-Weinberg equilibrium and those with very low sample size (<100 in either group) were excluded, followed by re-analysis of data each time. However, this did not change the results significantly and there was no change in the inference (Tables 3 and 4). Hence, no study was considered to be sensitive for inclusion in the pooled analysis.

In another set of sensitivity analysis, we undertook meta-analyses on studies recruiting cases with three or more pregnancy losses as the defining criteria. This also showed a

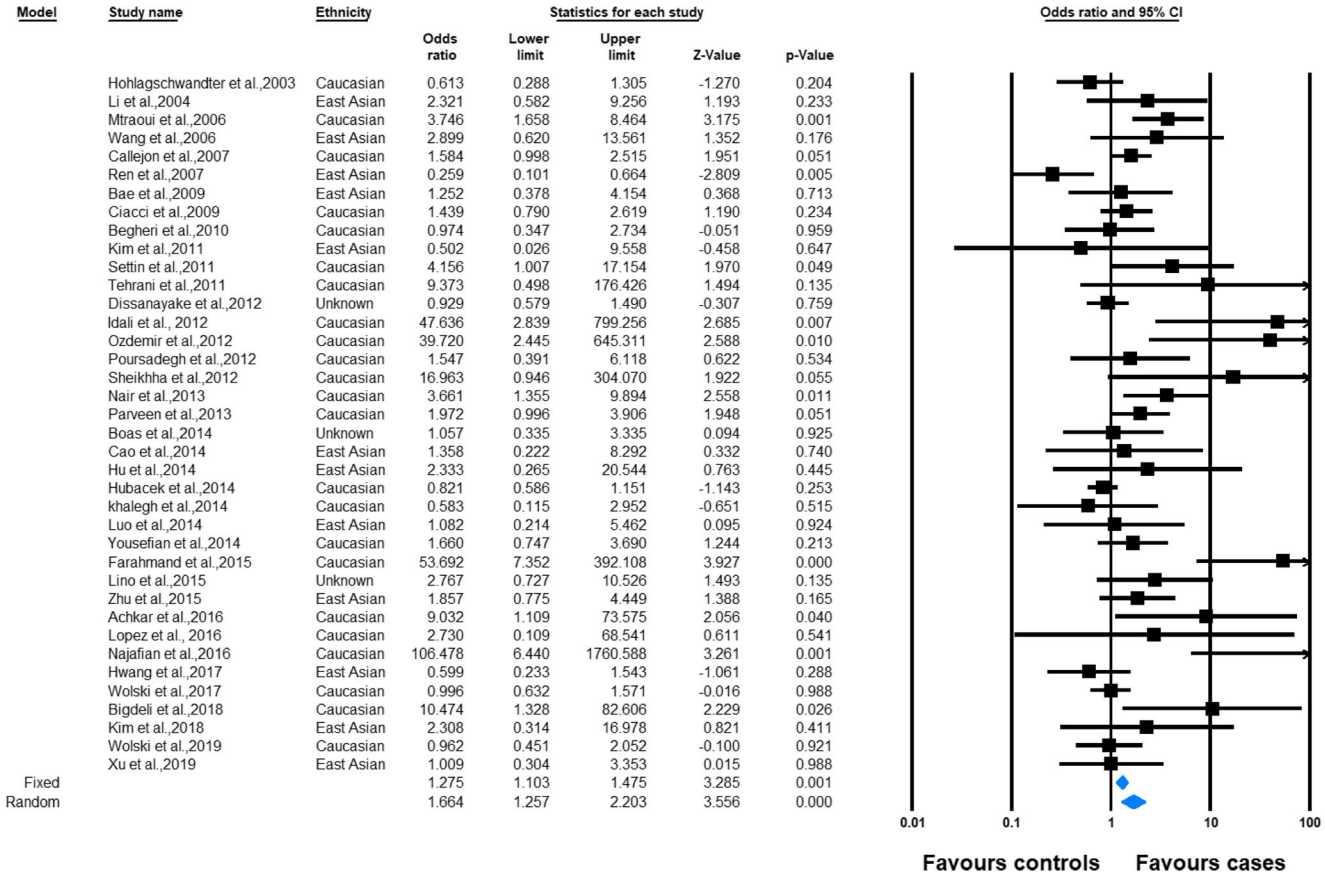

Fig. 3 Forest plot for association of *MTHFR* 1298A>C polymorphism with RPL risk using the recessive (AA+AC vs CC) model in all populations

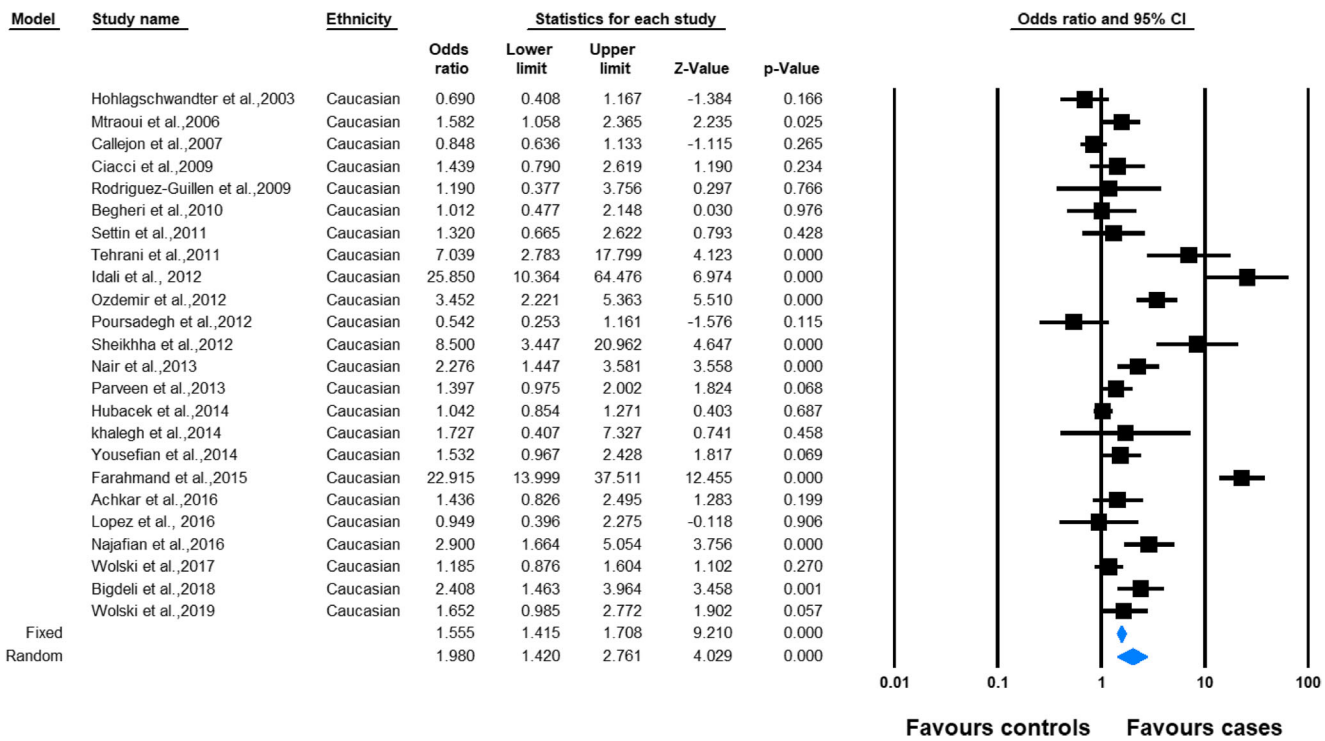

Fig. 4 Forest plot for association of *MTHFR* 1298A>C polymorphism with RPL risk in Caucasians using the dominant (AA vs AC+CC) model

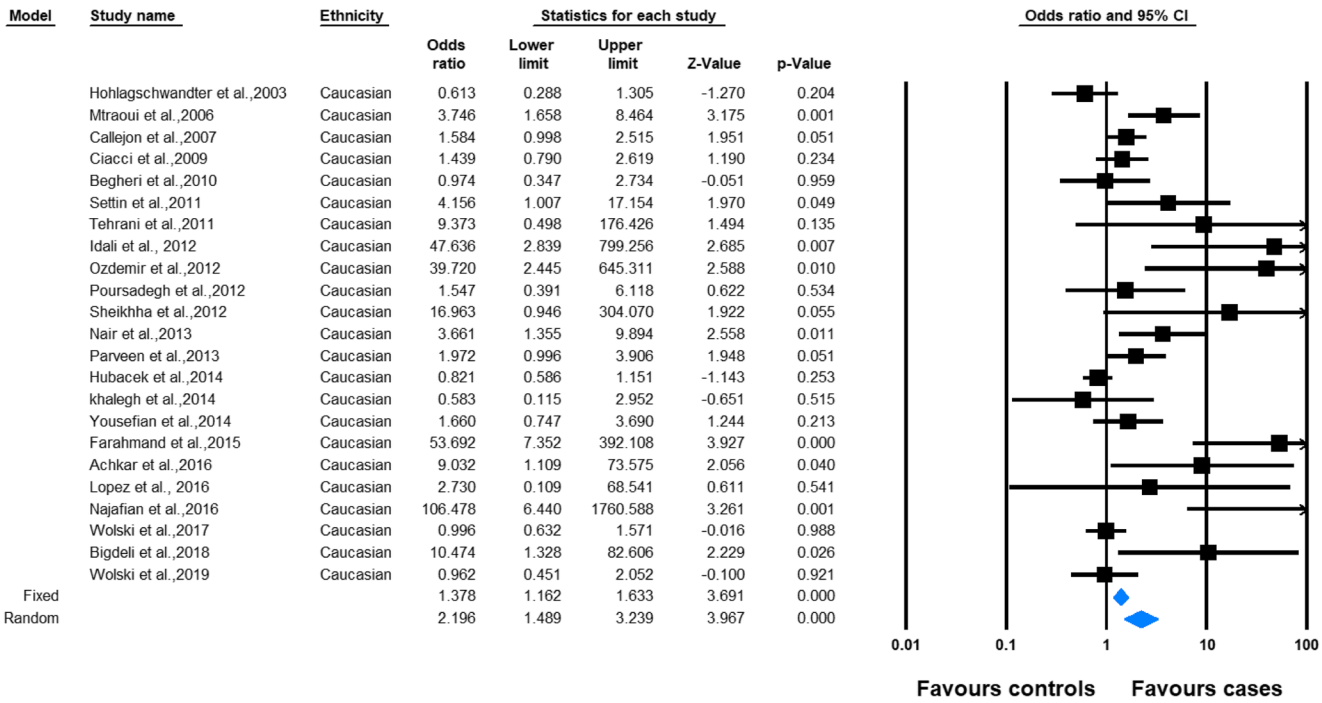

Fig. 5 Forest plot for association of *MTHFR* 1298A>C polymorphism with RPL risk in Caucasians using the recessive (AA+AC vs CC) model

significant association of 1298A>C polymorphism with recurrent pregnancy loss in both dominant and recessive models of genetic analyses (Fig. 8)

Discussion

Post-fertilization development requires massive cellular proliferation and differentiation to support the rapid pace of foetal development. DNA replication during this process demands nucleotide synthesis, which is critically dependent upon the folate cycle. The importance of folate in pregnancy has been studied since long with its first ever study published in the year 1964 [68]. Folic acid is taken from diet, which is converted to its active form, i.e. 5-MTHF. Dietary folate upon conversion to intermediate products regulates the synthesis of precursor molecules for DNA synthesis, protein synthesis and DNA methylation and gene imprinting (Fig. 9). DNA methylation is essential for regulating gene expression, which is critical for early development [69]. Methylene tetrahydrofolate enzyme is central to the folate cycle, one carbon cycle and methionine cycle (Fig. 9). Functional polymorphism, 677C>T causing Ala222Val substitution, results in a thermolabile enzyme with reduced activity [70]. Similarly, another functional

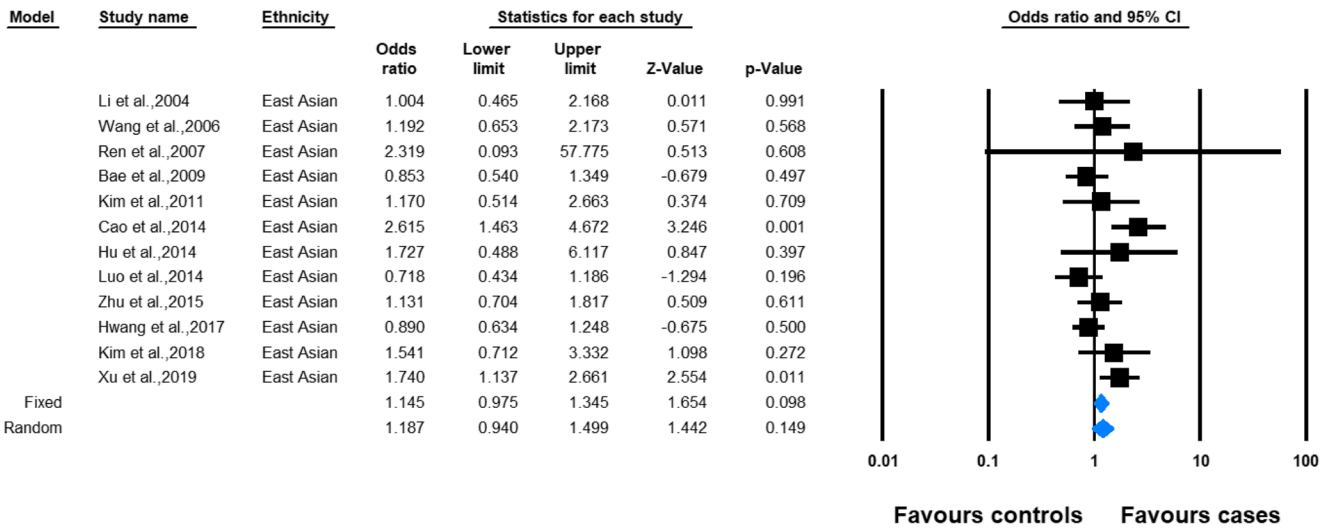

Fig. 6 Forest plot for association of *MTHFR* 1298A>C polymorphism with RPL risk in East Asians using the dominant (AA vs AC+CC) model

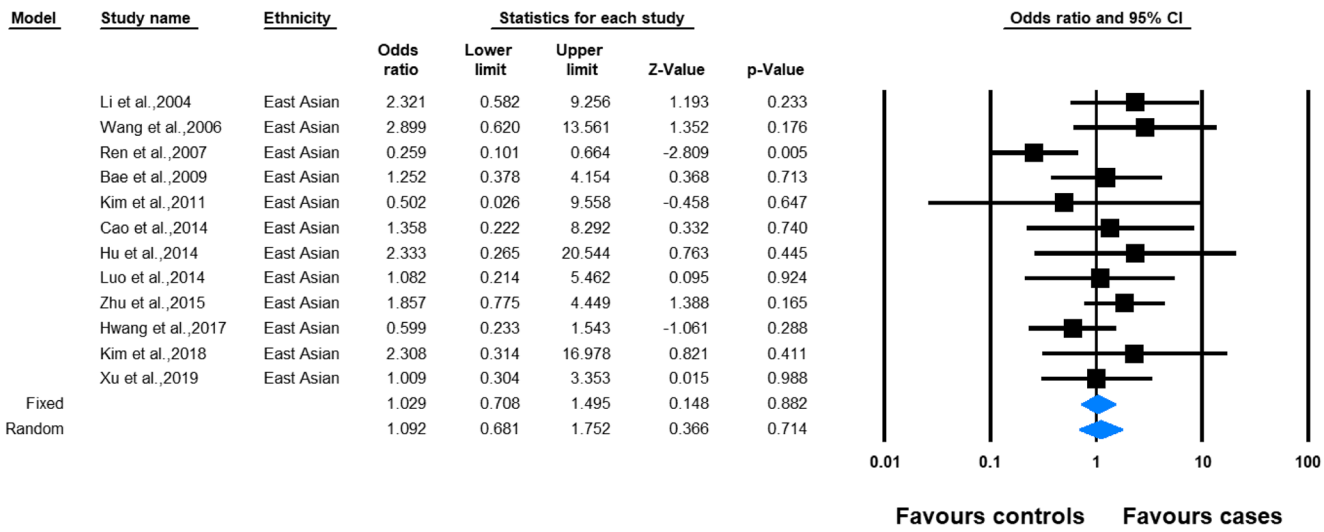

Fig. 7 Forest plot for association of *MTHFR* 1298A>C polymorphism with RPL risk in East Asians using the recessive (AA+AC vs CC) model

polymorphism, 1298A>C causing Glu429Ala substitution, results in decreased enzyme activity [71]. As a result of disturbed folate cycle, *MTHFR* mutations have also been linked to chromosomal anomalies in the developing foetus [39, 72], hypo-methylation during trophoblast development [73] and pregnancy loss or neural tube defects in live birth cases [74]. Foetal chromosomal abnormalities and developmental defects are a major cause of recurrent pregnancy loss [24, 25]. Therefore, these polymorphisms have a mechanistic link with pregnancy loss.

Due to its undisputed importance, folic acid is prescribed before and during pregnancy. According to the Center for Disease Control and Prevention (CDC), 400 mcg/day of folic acid is required, which is critical during pregnancy as it can permanently affect the foetal development. However, the use is indiscriminate in the lack of ways to figure out individual requirements. The need for genetic testing before prescribing medicine is the key to the personalized medicine. Dietary folate is converted to active folate depending upon the availability and activity of the MTHFR enzyme. Individuals homozygous for the variant allele of 677C>T polymorphism are poor folate metabolizers because of only 30% enzyme activity available as compared to the wild-type enzyme and heterozygotes have 65% of enzyme activity [75]. The individuals with

Table 3 Meta-analysis of pooled genotype data after excluding studies failing HW equilibrium

Comparison model	No. of studies	Test model	Type of association			Test of heterogeneity			Publication bias
			OR	95% CI	P	Q	P	I^2	Egger's *P*
All populations									
Dominant		Fixed	1.381	1.263–1.510	0.000	242	0.000	88.462	0.1848
AA vs AC+CC		Random	1.581	1.196–2.089	0.001				
Recessive		Fixed	1.250	1.047–1.493	0.014	62.025	0.000	56.469	0.00182
AA+AC vs CC		Random	1.641	1.187–2.267	0.003				
Caucasians									
Dominant		Fixed	1.581	1.419–1.762	0.000	197.59	0.000	91.903	0.1218
AA vs AC+CC		Random	2.061	1.364–3.116	0.001				
Recessive		Fixed	1.241	1.019–1.512	0.032	55.140	0.000	72.797	0.0027
AA+AC vs CC		Random	1.949	1.226–3.098	0.005				
East Asians									
Dominant		Fixed	1.145	0.965–1.358	0.121	19.635	0.020	54.163	0.3840
AA vs AC+CC		Random	1.197	0.913–1.569	0.193				
Recessive		Fixed	1.213	0.765–1.923	0.411	5.423	0.796	0.000	0.2104
AA+AC vs CC		Random	1.213	0.765–1.923	0.411				

Table 4 Meta-analysis of pooled genotype data after excluding studies failing HW equilibrium and those with low sample size

Comparison model	No. of studies	Test model	Type of association			Test of heterogeneity			Publication bias
			OR	95% CI	*P*	*Q*	*P*	I^2	Egger's *P*
All populations									
Dominant		Fixed	1.401	1.276–1.538	0.000	239.99	0.000	91.66	0.0828
AA vs AC+CC		Random	1.712	1.223–2.396	0.002				
Recessive		Fixed	1.228	1.020–1.479	0.030	58.102	0.000	65.578	0.0039
AA+AC vs CC		Random	1.693	1.158–2.474	0.007				
Caucasians									
Dominant		Fixed	1.613	1.444–1.803	0.000	194.55	0.000	93.832	0.0303
AA vs AC+CC		Random	2.377	1.478–3.822	0.000				
Recessive		Fixed	1.264	1.032–1.549	0.024	53.834	0.000	77.709	0.00044
AA+AC vs CC		Random	2.252	1.337–3.793	0.002				
East Asians									
Dominant		Fixed	1.18	0.924–1.353	0.252	18.472	0.002	72.932	0.5565
AA vs AC+CC		Random	1.174	0.800–1.723	0.413				
Recessive		Fixed	0.895	0.517–1.552	0.694	1.436	0.920	0.000	0.66662
AA+AC vs CC		Random	0.895	0.517–1.552	0.694				

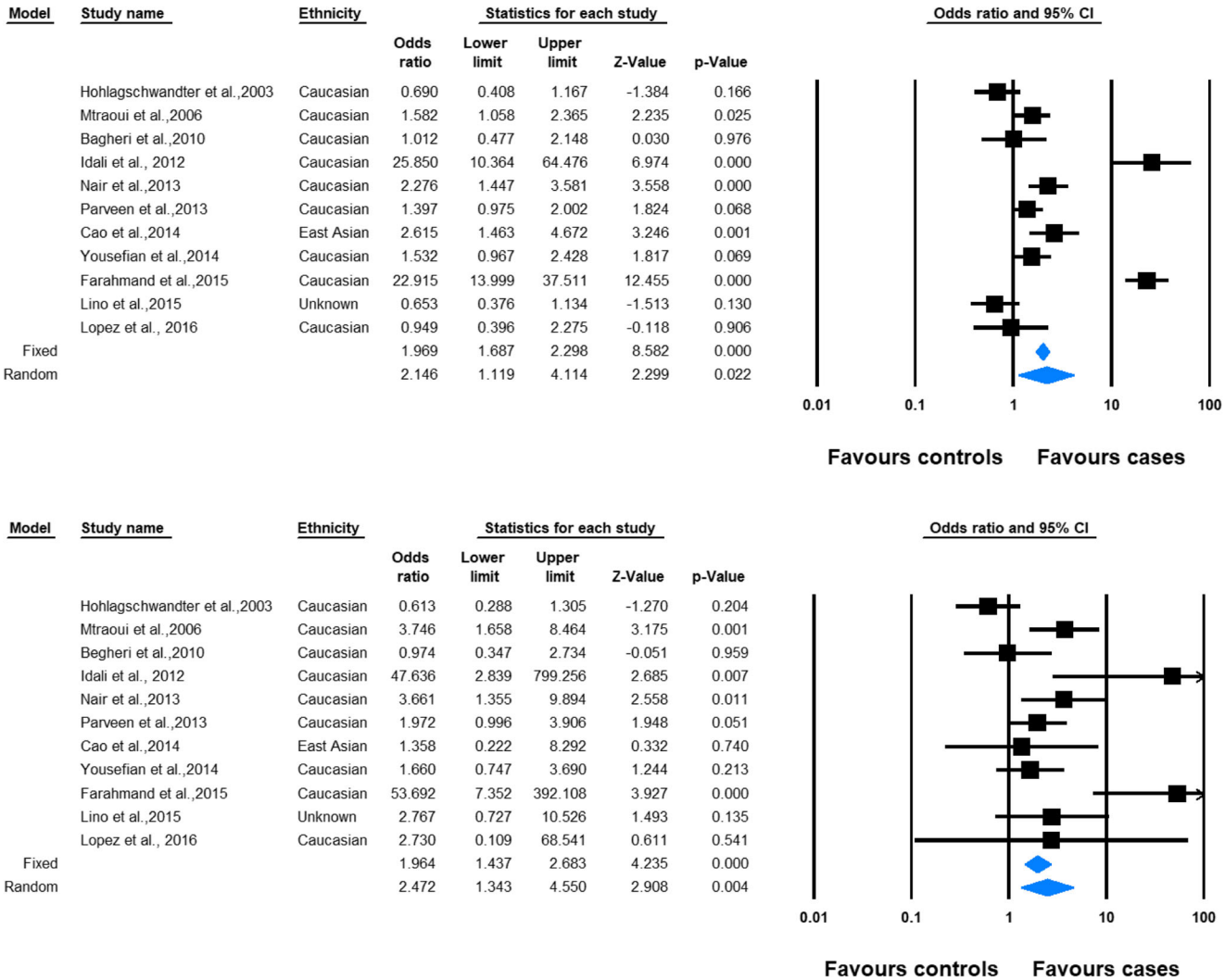

Fig. 8 Forest plot for sensitivity analysis excluding studies using two or more criteria of recurrent pregnancy loss. Data analysis was undertaken using the dominant (upper panel) and the recessive (lower panel) models of genetic analyses

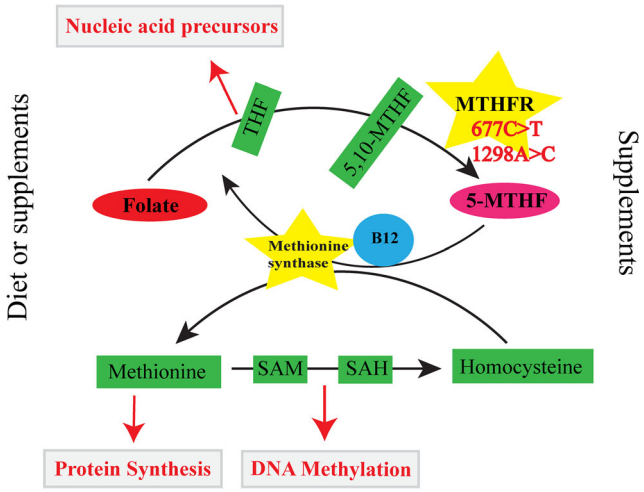

Fig. 9 Folate metabolism cycle. (THF: tetrahydrofolate; 5,10-MTHF: methyl tetrahydrofolate; MTHFR: methyl tetrahydrofolate reductase; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine)

1298A>C homozygous substitution have about 68% of enzyme activity [76]. The presence of both these polymorphisms would further reduce enzyme activity. Thus, these polymorphisms dictate the level of active folate. A decreased enzyme activity of MTHFR can reduce the transition of homocysteine to methionine, resulting in the deposition of homocysteine in the blood vessels [77]. Hyperhomocysteinemia can cause endothelial vascular damage and lead to blood clots during pregnancy [10, 78]. Inadequate foetal blood flow and villous necrosis due to placental artery embolization may ultimately lead to pregnancy loss [79].

The availability of complete human genome sequence has boosted genetic diagnosis of a number of health conditions. In a previous meta-analysis, we reported a significant association of 1298A>C polymorphism with RPL risk, suggesting it to be a good candidate for genetic analysis in RPL cases. But *MTHFR* polymorphisms have not yet been adopted in clinical testing of RPL. A number of recent studies have further suggested its importance in RPL. The present meta-analysis on 5888 cases and 8401 controls established 1298A>C to be a significant risk factor for RPL in an ethnic-specific manner. This meta-analysis suggested 1298A>C to be a significant risk factors in the Caucasians, but not in East Asians. The latter is in agreement with previous studies on the East Asian populations [19, 20]. In contrast, 677C>T polymorphism affects RPL risk in East Asians only [19–21, 49]. There were only two studies on Brazilian populations and only one on Sinhalese, which forced their exclusion from sub-group analysis. Since both these polymorphisms affect MTHFR significantly, some studies have also analysed both the polymorphisms, showing a significant association of compound heterozygote combination (677C>T/1298A>C) with RPL [51, 55, 58, 66]. Variations in the association across ethnic populations could be partly due to the dietary variations which affect folate availability.

To date, there have been a few meta-analyses for association between *MTHFR* 1298 A>C polymorphism and the risk of recurrent pregnancy loss. Most of the meta-analyses till date have been conducted on Asian populations, showing no association of 1298A>C substitution with RPL [19, 49, 80]. Similarly, another meta-analysis also showed no significant association of 1298A>C with RPL in Asians [81]. Later on, a Chinese group conducted a meta-analysis on 2924 maternal cases, 375 foetal cases and 327 paternal cases and found that the maternal and paternal *MTHFR* 677C>T and 1298A>C polymorphisms are associated with RPL [23]. Previously, we performed a meta-analysis on 1,080 maternal cases and 709 controls and 375 case and 384 control samples of spontaneously aborted embryos, which suggested a significant association of this polymorphism with increased risk of pregnancy loss in the carriers of AC and CC genotypes [22]. Similarly, in the case of Iranian populations, 1298A>C showed a significant association with RPL in all genetic models [82]. Most of these studies suggest a significant association of *MTHFR* 1298A>C substitution with an increased risk of RPL in populations other than Asians.

There is evidence that *MTHFR* genotyping can help in the management of RPL. The individuals with *MTHFR* mutations have low enzyme activity to convert folate into its active form, thus slowing the whole pathway. A few recent studies have undertaken *MTHFR* genotyping in the course of RPL management. Servy et al. (2018) prescribed folate (5 mg/day) to women with *MTHFR* mutations, but without any improvement; however, administration of the active form of folate, i.e. 5-MTHF (600mcg/day), resulted in spontaneous pregnancies [83]. Some other studies have tried different combinations of medicines for RPL management in consultation with *MTHFR* genotyping. For example, Merviel et al. (2017) recruited *MTHFR* 677C>T mutant females to study their response to treatment. Folate (5 mg/day) and aspirin with or without enoxaparin (0.4 mg/day) were administered. The authors observed a much higher delivery rate in the group receiving anti-coagulant (79.7%) in comparison to those receiving only folate and aspirin (46.3%) [84]. Though the exact reason for better efficacy of the treatment regimen incorporating the anti-coagulant could be complex, this may be related to blood clots in fine vasculature as a result of *MTHFR* mutations and hyper-homocysteinemia [85]. Similarly, another case of RPL benefitted from *MTHFR* testing. A patient with a history of several miscarriages and hypothyroidism was treated with low dose aspirin, enoxaparin, LT4 (levothyroxine) and folic acid (5 mg/day), but she still faced two miscarriages at the 14th week of gestation. *MTHFR* testing identified the patient to be 1298A>C homozygous, and a change from folate to 5-MTHF and cobalamin in this patient resulted in a successful birth [86]. From these evidences, it is clear that *MTHFR* genotyping can help in the management of RPL and also in ensuring successful birth in pregnancy loss patients.

Heterogeneity across the studies and small sample size in some of the studies could be considered as the limitation of this meta-analysis. We tried to overcome the limitations of heterogeneity by selecting random effects model wherever applicable, and the issue of small sample size was taken care by undertaking a sensitivity analysis. Overall, the present analysis suggests that *MTHFR* 1298A>C has a significant association with the risk of recurrent pregnancy loss. Similarly, *MTHFR* 677C>T has already been established to be a significant risk factor for RPL. This suggests the advantage of genotyping of these two polymorphisms before planning a pregnancy or in infertility clinics for better management of infertility and pregnancy loss. Since genetic polymorphisms show a population-specific penetration, population-wise analysis is the key to the personalized medicine. 1298A>C is a significant risk factor only in the Caucasian populations, while 677C>T polymorphism is a significant risk factor only in the East Asian populations. This meta-analysis in view of previous studies on *MTHFR* gene polymorphisms emphasizes that *MTHFR* 1298A>C and 677C>T analysis could be included in the clinical workup during pregnancy, if not before. This can be of great help in reducing the burden of pregnancy loss and offer higher success rate in infertility patients who had difficulty in conceiving. *MTHFR* genotyping in clinical management of pregnancy loss is in infancy. Identification of other similar risk factors would pave the way to the development of a genetic screening panel for pregnancy loss to guide its management.

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