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Methylenetetrahydrofolate reductase gene A1298C polymorphism and susceptibility to recurrent pregnancy loss: a meta-analysis

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Abstract

Environmental and genetic factors are thought to be involved in the pathogenesis of recurrent pregnancy loss (RPL)/spontaneous abortions (SA), which include endocrine, anatomical abnormalities within the genital organs, autoimmune diseases and some gene variants. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme of the folate/methionine metabolic pathway and it is well established fact that folate deficiency causes pregnancy complications like recurrent pregnancy loss, preeclempsia and birth defects affected pregnancies. MTHFR A1298C polymorphism reduces the enzymatic activity and mimics as folate deficiency. To date, many studies have investigated the association between MTHFR A1298C polymorphism and RPL risk; however, the result is still controversial and inconclusive. The aim of the present study was to address the association of MTHFR A1298C polymorphism with RPL risk by meta-analysis. By searching electronic databases, total seventeen studies were identified for present meta-analysis. Crude odds ratios (OR) with 95 % confidence intervals (CIs) was used to assess the strength of association between A1298C polymorphism and RPL. The results indicate that the A1298C polymorphism is not associated with RPL ($OR_{Cvs\ A}$ = 1.13 ,95 % CI= 0.87-1.46, P = 0.36 ; $OR_{ACvs\ AA}$ = 1.22 ,95 % CI= 0.94- 1.6, P = 0.13; OR_{CCvsAA} =1.35, 95 % CI= 76-2.36, P = 0.30; $OR_{CC+AC\ vs\ AA}$ = 1.15, 95 % CI= 88 -1.49, P = 0.29; $OR_{CCvs\ AC+AA}$ = 1.29, 95 % CI= 76 -2.12, P = 0.34). Further prospective studies were needed to confirm the precise relationship between the MTHFR A1298C polymorphism and RPL.

Key words: Meta-analysis, MTHFR, A1298C, recurrent early pregnancy loss, Spontaneous abortion, hyperhomocysteinemia.

Introduction

Recurrent pregnancy loss (RPL) or spontaneous abortions (SA) is defined as three or more consecutive miscarriages occurring before 20 weeks post-menstruation (1-3). RPL is a major concern in gynecology, affecting about 1–5% of couples(2,4,5) and frequently accompanied by maternal morbidity as well as a considerable psychological burden. The risk of recurrence increases with the maternal age and number of successive losses (6,7). Despite intense anatomic, endocrinologic, and immunologic screening efforts, up to 30–50% of RPL remain unexplained (8).

Mild-to-moderate hyperhomocysteinemia is a risk factor for arterial and venous thrombosis. It has been suggested as a possible risk factor in women suffering from habitual abortions or placental abruption (9). Increased levels of homocysteine may lead to premature vascular disease, i.e., early damage to decidual or chorionic vessels that may cause disturbed implantation of the conceptus (10). Hyperhomocysteinemia and the associated vascular disease are probably caused by an interaction between nutritional and genetic factors. Malnutrition and malabsorption of folate and vitamin B_{12} or an inherited enzymatic defect, such as methylenetetrahydrofolate reductase (MTHFR) deficiency, may result in hyperhomocysteinemia.

Methylenetetrahydrofolate reductase (MTHFR) is a vital enzyme catalyzing the reduction of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which serves as cofactor in methylation of homocysteine to methionine. Several allelic variants of MTHFR are reported with ~30 to 70% decreased enzymatic activity. In individuals having genetic variants of MTHFR, decreased enzymatic activity leads to increase plasma concentration of homocysteine. Two clinically important mutations of the MTHFR gene namely C677T and A1298C are reported to be associated with various pathological conditions including cardiovascular diseases (11), cancer (12,13), Down syndrome (14), neural tube defects (15), and male infertility (16). The C677T polymorphism is very well characterized and clinically associated with several diseases. The second common mutation for the MTHFR gene is produced by an A to C tranversion at nucleotide 1298 (A1298C), leading to a glutamate to alanine substitution in the MTHFR protein, and resulting finally in a 40% reduction in the activity of the MTHFR enzyme. In contrast to C677T, where homozygosity (TT) results in significant increase in total plasma homocysteine levels, homocysteine concentrations do not significantly elevated with the 1298CC genotype (17,18). A1298C allele frequency differs greatly in various ethnic groups of the world. The prevalence of the A1298C homozygote variant genotype ranges from 7 to 12% in White populations from North America and Europe. Lower frequencies have been reported in Hispanics (4 to 5 %), Chinese (1 to 4 %) and other Asian populations (1 to 4%) (19,20). During past years several case control studies were investigated the association between MTHFR A1298C polymorphism and RPL but the results were inconclusive. Hence in the present study a meta-analysis of all published case control studies investigating A1298C polymorphism as risk factor for RPL, was carried out to shed some lights on conclusive

role of A1298C polymorphism in RPL.

Methods

All research articles that investigate the association of the MTHFR A1298C SNP with the risk of recurrent pregnancy loss published before December, 2013 were extracted by electronic search of 'Pubmed', 'Google Scholar', 'Elsevier'and 'Springer Link' databases. The search terms MTHFR, Methylenetetrahydrfolate reductase, A1298C, recurrent miscarriage, recurrent spontaneous abortion, and recurrent pregnancy loss were used.

Data Extraction

Relevant information were extracted from all selected studies like- author name, journal name, year of publication, country name and number of cases and controls for each A1298C genotypes (AA, AC and CC genotypes). Allelic frequencies for the cases and controls were calculated from corresponding genotypes.

Statistical methods

Pooled odds ratios (Ors) together with 95 % confidence intervals (CIs) were calculated by fixed effects model or random effects model according to the heterogeneity. Heterogeneity was checked by the Q test and a P>0.05 indicates a lack of heterogeneity among studies (21). The pooled OR was estimated using fixed effects (FE) (22) and random effects (23) models. Random effects modeling assume a genuine diversity in the results of various studies, and it incorporates to the calculations a between-study variance. Hence, when there is heterogeneity between studies, then the pooled OR is preferably estimated using the random effects model. Any study with controls not in Hardy– Weinberg equilibrium (HWE) was excluded from the meta-analysis.

The potential publication bias was tested using Egger's test (24). P<0.05 considered to be representative of a statistical significance. All of the statistical analyses were performed using MIX version 1.7 (25). A p value less than 0.05 was considered statistically significant, and all the p values were two sided.

Results

Characteristics of included studies

Seventeen studies were found suitable for the inclusion in present meta-analysis (26-42) (Mitraoui et al, 2006;Sotiriadus et al,2006; Wang et al.,2006; Callijon et al, 2007; Ciacci et al.,2009; Jeddi Tehrani et al.,2011; Kim et al.,2011; Settin et al.,2011;Dissanayke et al.,2012; Ozdemir et al.,2012; Torabi et al.,2012; Zonouzi et al.,2012;Herodez et al.,2013; Nair et al.,2013; Parveen et al.,2013; Hefler et al.,2014; Lino et al.,2014). The studies were carried out in Austria (41), Behrain (26), Brazil (42), China (28), Egypt (33), Greece (27), India (39,40), Iran (31,36,37), Italy (30), Korea (32), Slovenia (38), Spain (29), Srilanka (34), and Turkey (35). Genotypes were in Hardy-Weinberg equilibrium in all controls. Details of seventeen studies were summarized in Table 1.

Statistical analysis

In all seventeen studies, total controls were 2588 with AA (1414), AC (936) and CC (238), and cases were 2338 with AA (1203), AC (906), and CC (229). In controls genotypes percentage of AA, AC and CC were 54.64%, 36.17% and 9.2% respectively. In total cases genotype percentage of AA, AC, and CC was 51.45%, 38.75% and 9.79 % respectively (Table 2). Frequency of AA genotype and A alleles were highest in both cases and controls, and number of A and C alleles were calculated and presented in table 2.

Meta-analysis

The meta-analysis was carried out using all five genetic models- allele contrast (C vs A), co-dominant (AC vs AA), homozygote (CC vs AA), dominant (CC+AC vs AA), and recessive (CC vs AC+AA) models. Higher heterogeneity was observed so random effect model was adopted for risk assessment. The results of overall meta-analysis did not indicate any significant association between A1298C polymorphism with RPL ($OR_{Cvs A}$ = 1.13 ,95 % CI= 0.87-1.46, P = 0.36 ; $OR_{ACvs\ AA}$ = 1.22 ,95 % CI= 0.94- 1.6, P = 0.13; OR_{CCvsAA} =1.35,

Table 1. Characteristics of seventeen studies included in the present meta-analysis.

Study	Year	Country	Control	Case	Reference
Mtiraoui et al.,2006	2006	Behrain	200	200	Reproduction,131:395–401
Sotiriadis et al.,2006	2006	Greece	90	88	Am J Reprod Immunol, 57:133–141
Wang et al.,2006	2006	China	82	148	Intern J Gynec Obstet, 92: 264-265
Callejon et al.,2007	2007	Spain	434	314	Human Reproduction,22: 3249–3254
Ciacci et al.,2009	2009	Italy	72	39	Digestive and Liver Disease, 41: 717–720
Jeddi-Tehrani et al.,2011	2011	Iran	100	100	Am J Reprod Immunol, 66: 149–156
Kim et al.,2011	2011	Korea	155	33	Am J Reprod Immunol 66: 252–258
Settin et al.,2011	2011	Egypt	136	70	Genet Test Mol Biomarkers,15(12):887–892
Dissanayke et al.,2012	2012	Srilanka	197	195	J. Obstet. Gynaecol. Res.,38: 1168–1176,
Ozdemir et al.,2012	2012	Turkey	104	327	Genet Test Mol Biomarkers 16:279–86
Torabi et al.,2012	2012	Iran	166	100	J Reprod Infertil, 13(2):89-94
Zonouzi et al.,2012	2012	Iran	50	89	ISRN Obstetrics and Gynecology article ID 945486.
Herodez et al.,2013	2013	Slovenia	108	100	BJMG, 16:31-40
Nair et al.,2013	2013	India	202	129	Fertility and Sterility, 99: 0015-0282
Parveen et al.,2013	2013	India	300	200	Arch Gynecol Obstet (2013) 288:1171–1177
Hefler et al.,2014	2014	Austria	94	94	J Soc Gynecol Investig,11: 43
Lino et al.,2014	2014	Brazil	98	112	Clin App Thromb/Hemostasis 1-8

Table 2. The distributions of MTHFR A1298C genotypes and allele frequencies for RPL cases and controls.

Study ID	Genotype						Alleles			
	AA		AC		CC		A		C	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Mtiraoui,2006	108	130	65	62	27	8	281	322	119	78
Sotiriadis,2006	44	45	37	39	7	6	125	129	51	51
Wang,2006	103	60	35	20	10	2	241	140	55	24
Callejon ,2007	209	248	89	149	16	37	507	645	121	223
Ciacci,2009	18	29	20	34	1	9	56	92	22	52
Jeddi-Tehrani,2011	69	94	27	6	4	0	165	194	35	6
Kim,2011	23	113	10	38	0	4	56	264	10	46
Settin,2011	15	36	49	97	6	3	79	169	61	103
Dissanayke,2012	74	72	78	79	43	46	226	223	164	171
Ozdemir,2012	144	69	145	35	38	0	433	173	221	35
Torabi,2012	69	94	27	6	4	66	165	194	35	138
Zonouzi,2012	35	13	46	34	8	3	116	60	62	40
Herodez,2013	36	43	48	47	16	18	120	133	80	83
Nair,2013	48	116	68	80	13	6	164	312	94	92
Parveen,2013	88	157	92	127	20	16	268	441	132	159
Hefler,2014	49	43	38	40	7	11	136	126	52	62
Lino,2014	71	52	32	43	9	3	174	147	50	49

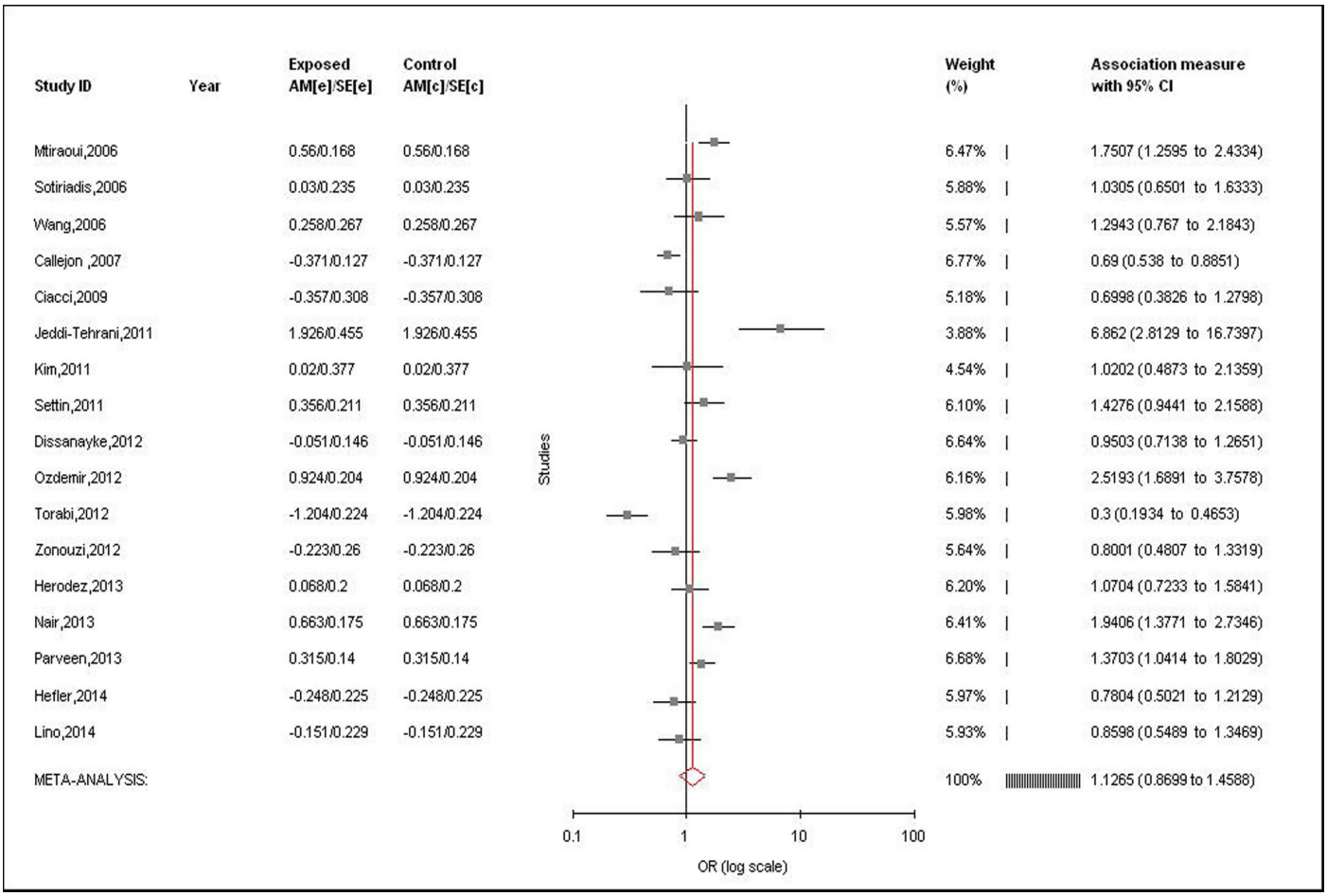

Figure 1. Forest plots for the association between MTHFR A1298C polymorphism and RPL for allele contrast model (C vs A) with random effect model in overall studies.

95 % CI= 0.76 -2.36, P = 0.3; $OR_{CC+AC\ vs\ AA}$ = 1.15, 95 % CI= 0.88 -1.49, P = 0.29; $OR_{CCvs\ AC+AA}$ = 1.29, 95 % CI= 0.76 -2.12, P = 0.34) (Figure 1). However higher heterogeneity was observed in overall meta-analysis (For C vs A: I^2= 85.54%, P_{hetero}<0.0001; For AC vs AA: I^2= 72.53% ,P_{hetero}<0.001; For CC vs AA: I^2= 77.51, P_{hetero}<0.001; For CC+AC vs AA: I^2=76.53, P_{hetero}<0.0001 ; For CC vs AC+AA: I^2=76.97, P_{hetero}<0.0001) and to identify the source of heterogeneity subgroup analysis was done according to ethnicity.

Subgroup analysis

Out of 17 studies included in the present meta-analysis, 9 studies were carried out on Asian population, and 8 studies were carried out on Caucasian population. The subgroup analysis by ethnicity also revealed that the no significant association was found between MTHFR A1298C polymorphism and RPL in Asian

population (for C vs. A: OR=1.228 ; 95% CI= 0.81-1.85; p= 0.32; I^2= 88.85%; $P_{heterogeneity}$= <0.0001; P_{Pb}= 0.91; for AC vs AA: OR= 1.513; 95% CI=1.02-2.23 ; p= 0.04; I^2= 75.39%; $P_{heterogeneity}$= <0.0001; P_{Pb}= 0.; for CC vs. AA: OR= 1.496; 95% CI= 0.62-3.6; p= 0.37 I^2= 83.83%; $P_{heterogeneity}$=<0.0001 P_{Pb}= 0.82; for CC+AC vs. AA: OR= 1.303; 95% CI= 0.90-1.89; p= 0.16; I^2=77.88 ; $P_{heterogeneity}$= <0.0001; P_{Pb}= 0.89; for CC vs AC+AA: OR= 1.385; 95% CI= 0.58-3.27;p=0.45; I^2= 84.4%; $P_{heterogeneity}$=<0.0001 P_{Pb}= 0.80) (Table 4) (Figure 2A), and Caucasian population (for C vs. A: OR=1.03 ; 95% CI= 0.75-1.43; p= 0.82; I^2= 80%; $P_{heterogeneity}$= <0.001; P_{Pb}= 0.47; for AC vs AA: OR= 0.978; 95% CI= 0.71-1.33; p= 089.; I^2= 60.21%; $P_{heterogeneity}$= 0.0.014; P_{Pb}= 0.56; for CC vs. AA: OR= 1.153; 95% CI= 0.57-2.3; p= 0.69 I^2= 66.98%; $P_{heterogeneity}$=0.003 P_{Pb}= 0.13; for CC+AC vs. AA: OR=1.005 ; 95% CI=0.69-1.44 ; p= 0.97; I^2= 73.13%; $P_{heterogeneity}$= 0.0005; P_{Pb}= 0.52; for CC vs. AC+AA: OR= 1.154; 95% CI= 0.60-2.21; p= 0.66; I^2= 63.35; $P_{heterogeneity}$= 0.007; P_{Pb}= 0.14) (Table 5) (Figure 2B).

Publication bias

Publication bias was not observed in five genetic models (Begg's p= 0., Egger's p= 066. for C vs. A; Begg's p= 0., Egger's p= 0.38 for CC vs. AA; Begg's p= 0., Egger's p= 0.22 for AC vs. AA; Begg's p= 0., Egger's p= 0.58 for CC+AC vs. AA; and Begg's p= 0., Egger's p= 0.41 for CC vs. AC+AA) of overall as well as stratified sub group meta-analysis by using of Begg's and Egger's test (Table 3-5) (Figure 3A-F).

Discussion

MTHFR C677T and A1298C SNPs were implicated in several pregnancy-related complications (43-45), including placental anomalies (46) and preeclampsia

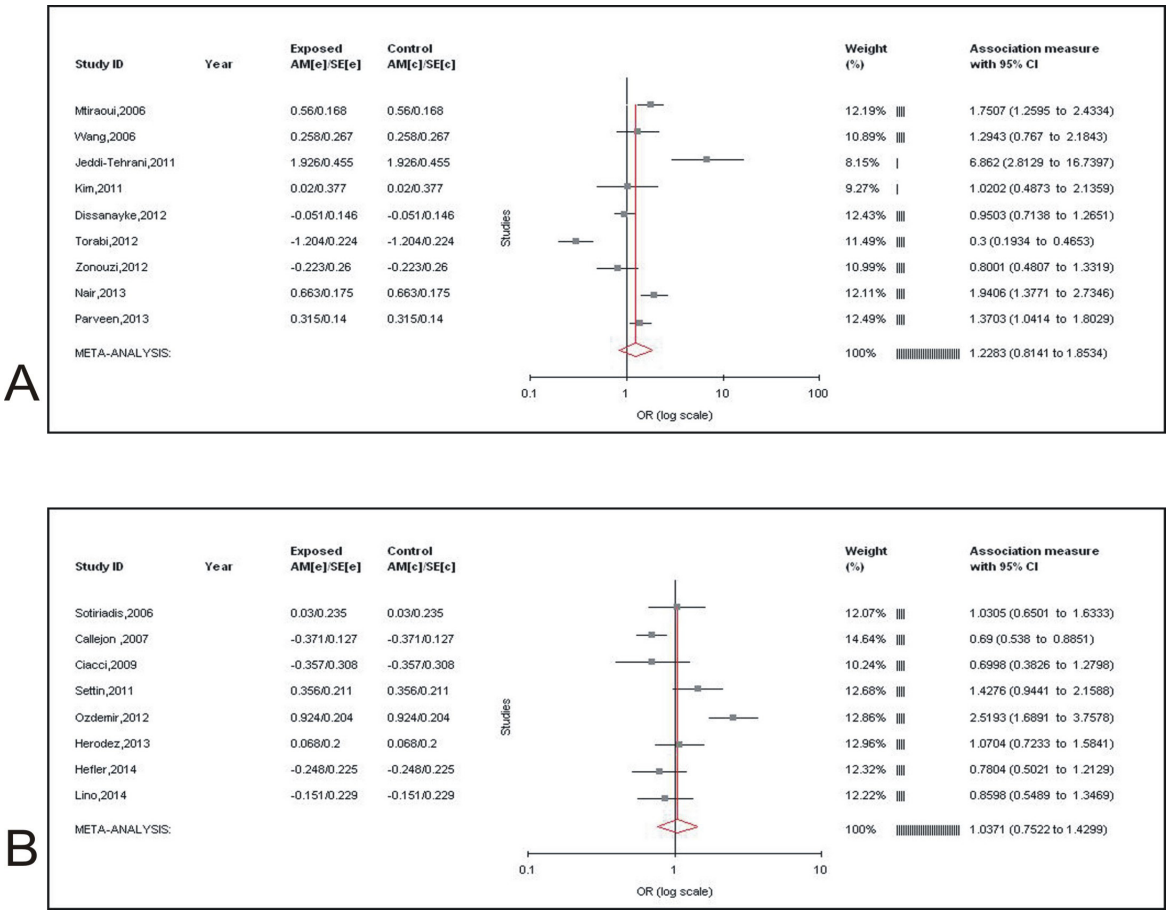

Figure 2. Forest plots for the association between MTHFR A1298C polymorphism and RPL for allele contrast model (C vs A) with random effect model, **A** Asian population, **B** Caucasian population.

Table: 3. Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric: overall analysis, and publication bias p-value (Egger Test).

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (C vs A)	1.09(1.0-1.2),0.06	1.13(0.87-1.46),0.36	<0.0001	85.54	0.66
Co-dominant (AC vs AA)	1.166(1.02-1.32),0.02	1.22(0.94-1.6),0.13	<0.0001	72.53	0.22
Homozygote (CC vs AA)	1.14(0.92-1.4),0.23	1.35(0.76-2.36),0.3	<0.0001	77.51	0.38
Dominant (CC+AC vs AA)	1.13(1.00-1.27),0.04	1.15(0.88-1.49),0.29	<0.0001	76.53	0.58
Recessive (AA+AC vs CC)	1.06(0.87-1.29),0.56	1.29(0.76-2.12),0.34	<0.0001.	76.97	0.41

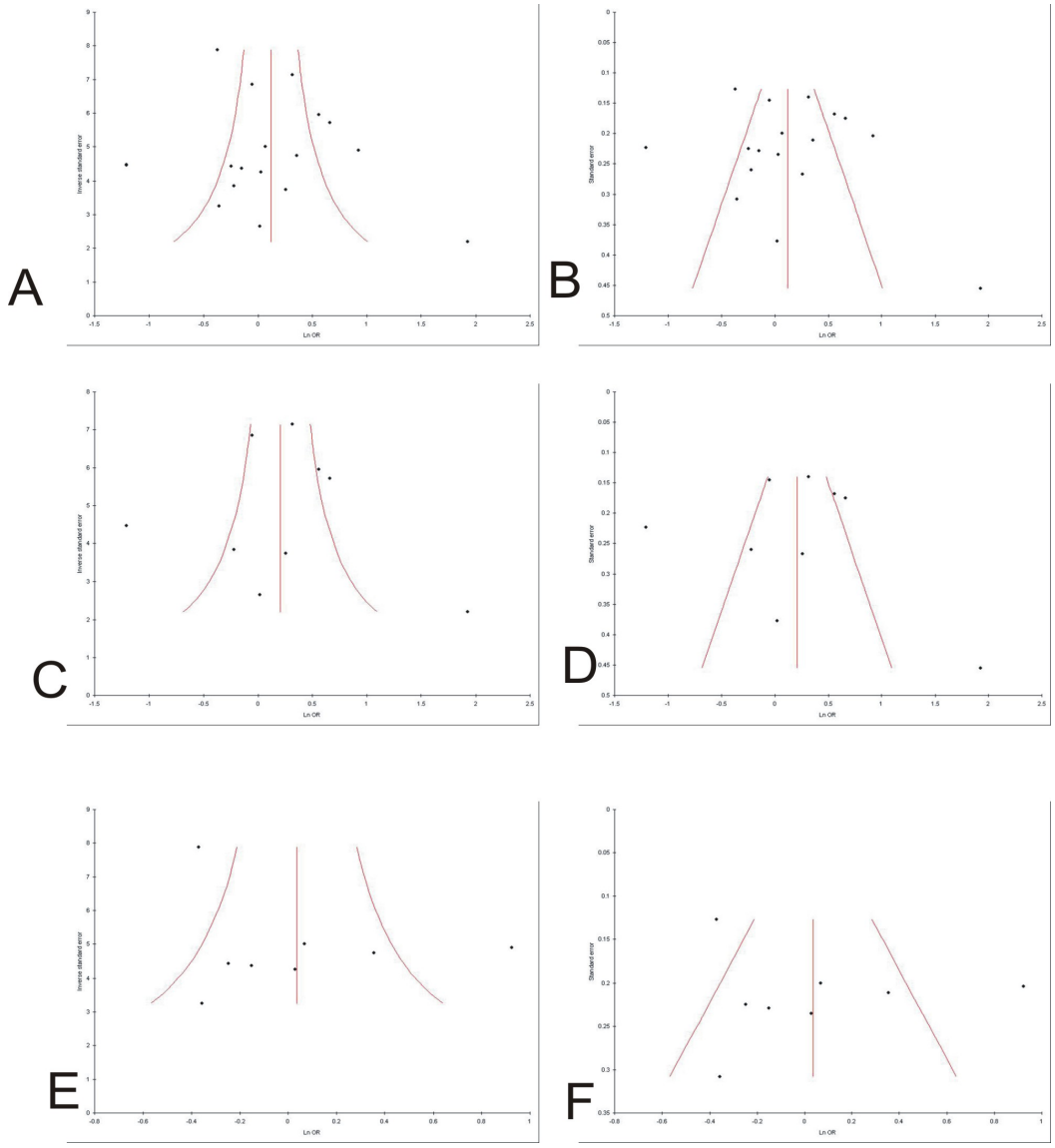

Figure 3. Funnel plots (C vs A). precision versus OR in overall studies, B. standard error versus OR in overall studies, C. precision versus OR in Asian studies, D. standard error versus OR in Asian studies, E. precision versus OR in Caucasian studies, F. standard error versus OR in Caucasian studies.

Table 4. Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric: Asian studies analysis, and publication bias p-value (Egger Test).

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (C vs A)	1.198(1.05-1.36),0.006	1.228(0.81-1.85),0.32	<0.0001	88.85	0.91
Co-dominant (AC vs AA)	1.419(1.18-1.69),0.0001	1.513(1.02-2.23),0.04	<0.0001	75.39	0.335
Homozygote (CC vs AA)	1.1534(0.87-1.51),0.3	1.495(0.62-3.6),0.37	<0.0001	83.83	0.82
Dominant (CC+AC vs AA)	1.31(1.11-1.54),0.001	1.303(0.9-1.89),0.16	<0.0001	77.88	0.89
Recessive (AA+AC vs CC)	1.03(0.79-1.33),0.81	1.385(0.58-3.27),0.45	<0.0001	84.4	0.80

Table 5. Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric: Caucasian studies analysis, and publication bias p-value (Egger Test).

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (C vs A)	0.9896(0.86-1.13),0.88	1.03(0.75-143),0.82	<0.0001	80	0.47
Co-dominant (AC vs AA)	0.947(0.78-1.13),0.55	0.978(0.72-1.33),0.89	0.014	60.21	0.56
Homozygote (CC vs AA)	1.1145(0.80-1.54),0.51	1.153(0.57-2.3),0.69	0.003	66.98	0.13
Dominant (CC+AC vs AA)	0.96(0.80-1.14),0.64	1.005(0.69-1.44),0.97	0.0005	73.13	0.52
Recessive (AA+AC vs CC)	1.108(0.80-1.52),0.53	1.154(0.60-2.21),0.66	0.007	63.35	0.14

(47,48). Scientists speculated that maternal and paternal homozygosity for MTHFR A1298C mutation might interfere with embryonic development, thus causing miscarriage, as it has been reported that C/C homozygosity results in a global reduction of DNA methylation and may also interfere with the methylation of RNA, proteins and lipids (49). It is well evidenced from the literature that maternal homocystenemia has been associated with early pregnancy loss and a number of other adverse pregnancy outcomes associated with placental insufficiency, such as intrauterine growth restriction, preeclampsia, abruptio placenta and fetal death (50-53).

Meta-analysis provides a tool to analyze the data from several studies jointly and several meta-analysis studies illustrate the utility of met-analytic techniques in identification of risk association of MTHFR polymorphism with disease/disorders like- Down syndrome (54), Retinopathy in type 2 diabetes (55), gastric cancer (56), breast cancer (57), Schizophrenia(58), and acute lymphoblastic leukaemia (59) etc. Author identified one meta-analysis (39) concerning similar topic during the literature search. Nair et al. (39) included only five studies included in their meta-analysis with 1,080 case and 709 control subjects. They reported both fixed effect (P=.000; OR=¼ 1.90; 95% CI = 1.53–2.37) and random effects (P=.020; OR= 1.99; 95% CI=1.11–3.55) models of meta-analysis showed significantly increased RPL risk in the presence of mutant genotypes (CC+AC versus AA).

The main factors influencing the present meta-analysis may be (i) the small number of studies, (ii) the small sample size, (iii) the different ethnic backgrounds of the individuals included in the study, (iv) crude ORs were used for calculation and (v) and the inconsistent definition of recurrent miscarriage.

The sample size was rather small and further studies are necessary for confirming the result of this meta-analysis that MTHFR A1298C polymorphism is a risk factor for recurrent miscarriage. Further studies, including meta-analysis with large number of subjects is needed for the thorough understanding of the contribution and significance of MTHFR A1298C polymorphism and homocysteinemia in recurrent miscarriage.

RPL is a serious disease influencing the reproduction of humans. To forecast it by detecting MTHFR A1298C polymorphism may be a way to decrease the incidences. Based on the studies available, this meta-analysis demonstrated that the MTHFR A1298C polymorphism is not associated with RPL.

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