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REVIEW

Null association of maternal *MTHFR* A1298C polymorphism with Down syndrome pregnancy: An updated meta-analysis

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KEYWORDS

Down syndrome;
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A1298C;
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Abstract *Background:* Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme of folate/homocysteine pathway and is essential for DNA synthesis and methylation. *MTHFR* gene polymorphisms have been reported as risk factors for congenital defects and several metabolic and neurological disorders. Several studies have investigated an association between maternal *MTHFR* A1298C polymorphism and Down syndrome (DS) child. However, results have been inconclusive.

Aim: A meta-analysis of published case–control studies up to December, 2015 was performed to investigate this association.

Methods: Electronic databases were searched for case–control studies and odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess the association. Total twenty-one case–control studies with 2004 cases and 2523 controls were included in the present meta-analysis.

Results: Results of meta-analysis showed a significant association between maternal A1298C polymorphism and DS pregnancy with homozygote model (CC vs. AA: OR = 1.26, 95% CI = 1.01–1.58, $p = 0.04$), but no such association was found in any other genetic models (C vs. A: OR = 1.07, 95% CI = 0.93–1.23, $p = 0.32$; CC + AC vs. AA: OR = 1.08, 95% CI = 0.96–1.23, $p = 0.18$; CC vs. AC + AA: OR = 1.11, 95% CI = 0.90–1.36, $p = 0.30$; AC vs. AA: OR = 1.06, 95% CI = 0.93–1.21, $p = 0.34$).

Conclusion: Subgroup and sensitivity analysis results showed that this polymorphism is a risk factor for DS pregnancy in Asian populations but not in Caucasian population as well as in overall meta-analysis.

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Contents

1. Introduction

Down syndrome (DS) is the commonest chromosome abnormality in humans, characterized by trisomy 21. It is a major cause of abortion and fetal mental retardation, with an incidence of 1–2/1000 live birth [1]. Advanced maternal age is the only well-reported risk factor for maternal nondisjunction [2], while the underlying mechanism remains unexplained. Numerous studies have suggested an association between DS and maternal folate pathway gene polymorphism. In 1999, James et al. [3] were the first to propose the hypothesis that abnormal DNA methylation patterns resulting from aberrant folate metabolism may increase DNA hypomethylation in centromeric regions, increasing the risk of trisomy 21 [4]. Folate plays an important role in genetic material distribution during cell division, because of its part in the cellular methylation reactions, which, epigenetically regulate chromosome segregation [5,6]. 5,10-Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme of folate pathway and several studies reported significant association between maternal *MTHFR* polymorphisms and DS [3,7–10], whereas some others studies could not find any association [11–13].

MTHFR enzyme catalyzes the synthesis of 5-methylenetetrahydrofolate, which remethylates homocysteine to methionine. Methionine is the main precursor for S-adenosylmethionine (SAM), the main methyl donor for DNA, RNA and protein methylation [1]. Insufficient periconceptional folic acid intake on one hand and deficient folate metabolism in mothers and fetuses on the other hand have been acknowledged as risk factors for DS and several other congenital defects [7,14,15]. It has been suggested that genetic predisposition to impaired folate metabolism in mothers could promote DNA hypomethylation and meiotic nondisjunction resulting in trisomy 21 [7,14].

Several polymorphisms have been reported in *MTHFR* gene, out of which C677T and A1298C are clinically important [16,17]. C677T polymorphism makes MTHFR enzyme thermolabile. A cytosine to thymine nucleotide substitution at 677 position (C677T) reduces MTHFR enzyme activity and increases plasma homocysteine concentration [16,18,19]. The second polymorphism A1298C involving alanine to cytosine nucleotide substitution in *MTHFR* gene has also been reported to reduce enzyme activity [17]. Mutant allele (C) frequency differs greatly in various ethnic groups of the world. The prevalence of the A1298C homozygote variant (CC) ranges from 7% to 12% in the White populations of North America and Europe. Lower frequencies have been reported in Hispanics (4–5%), and Asian populations (1–4%) [20,21]. Several studies have been conducted and demonstrated *MTHFR* polymorphism as a risk factor for congenital defects like NTD [22], oral clefts [23], congenital heart defects [24], adult disease conditions like cardiovascular and cerebrovascular diseases [20]. The present meta-analysis was carried out to assess the association of maternal *MTHFR* A1298C polymorphism with Down syndrome pregnancy.

2. Method

2.1. Selection of studies

Studies were identified by a search of PubMed, Google Scholar, Elsevier, and Springer Link databases up to July, 2015. The following terms were used: 'methylenetetrahydrofolate reductase', 'MTHFR', 'A1298C', and 'Down syndrome' to identify eligible articles for meta-analysis. The distribution of the genotypes in the control group was tested for the Hardy–Weinberg equilibrium (HWE).

2.2. Inclusion and exclusion criteria

Included studies had to meet the following criteria (i) study should be a case–control association study, (ii) study should have reported the genotypes of *MTHFR* A1298C

Table 1 Distributions of *MTHFR* A1298C genotypes and allele number for cases and controls.

Study	Ethnicity	Total number of cases	Total number of controls	Genotype						Allele				HWE (*p*)
				AA		AC		CC		A		C		
				Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	
Bosco et al. (2003)	Caucasian	63	72							88	100	38	44	NA
Boduroglu et al. (2004)	Caucasian	152	91	44	21	97	60	11	10	185	102	119	80	0.001*
Acacio et al. (2005)	Caucasian	70	88	2	6	37	32	30	50	41	44	97	132	0.77
Chango et al. (2005)	Caucasian	197	119	59	52	49	56	11	12	167	160	71	80	0.58
da Silva et al. (2005)	Caucasian	154	158	99	101	49	50	6	7	247	252	61	64	0.79
Rai et al. (2006)	Asian	89	70	28	28	39	37	22	5	95	93	83	47	0.12
Scala et al. (2006)	Caucasian	94	264	38	128	39	108	17	25	115	364	73	158	0.74
Biselli et al. (2008)	Caucasian	72	194	40	108	27	74	5	12	107	290	37	98	0.88
Martinez-Frias et al. (2008)	Caucasian	146	188	76	91	57	78	13	19	209	260	83	116	0.7
Meguid et al. (2008)	Caucasian	42	48	8	18	20	29	14	1	36	65	48	31	0.008*
Santos-Reboucas et al. (2008)	Caucasian	103	108	58	57	40	49	5	2	156	163	50	53	0.01*
Brandalize et al. (2009)	Caucasian	239	197	143	113	84	76	12	8	370	302	108	92	0.27
Cyrill et al. (2009)	Asian	36	60	14	26	19	21	3	13	47	73	25	47	0.03*
Coppede et al. (2010)	Caucasian	29	32	14	13	15	19	0	0	43	45	15	19	0.01*
Vranekovic et al. (2010)	Caucasian	111	141	48	63	56	68	7	10	152	194	70	88	0.14
Bozovic et al. (2011)	Caucasian	107	221	55	101	52	98	5	22	162	300	62	142	0.8
Sadiq et al. (2011)	Asian	53	29	24	10	29	18	0	1	77	38	29	20	0.04*
Zampieri et al. (2012)	Caucasian	105	183	51	101	48	73	6	9	150	275	60	91	0.36
Pandey et al. (2013)	Asian	80	100	27	60	31	22	23	17	85	142	77	56	0.0001*
Izci Ay et al. (2015)	Caucasian	47	49	16	16	24	23	7	10	56	55	38	43	0.74
Sukla et al. (2015)	Asian	151	186	69	104	68	65	14	17	206	273	96	99	0.15

HWE (*p*) *p* value for the Hardy–Weinberg equilibrium in control group.
NA = not applicable.
* *p* values < 0.05 in the exact test for the Hardy–Weinberg equilibrium.

Table 2 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, subgroup analyses.

	Genetic contrast	Fixed effects OR (95% CI), *p*	Random effects OR (95% CI), *p*	Heterogeneity *p*-value (*Q* test)	I^2 (%)
All	Allele contrast (C vs. A)	1.06 (0.97–1.17), 0.15	1.07 (0.93–1.23), 0.32	0.002	52
	Dominant (CC + AC vs. AA)	1.08 (0.96–1.23), 0.18	1.09 (0.93–1.28), 0.26	0.06	34
	Homozygote (CC vs. AA)	1.26 (1.01–1.58), 0.04	1.20 (0.85–1.71), 0.28	0.009	48.29
	Co-dominant (AC vs. AA)	1.06 (0.93–1.21), 0.34	1.06 (0.92–1.23), 0.38	0.24	16.97
	Recessive (AA + AC vs. CC)	1.11 (0.90–1.36), 0.30	1.07 (0.78–1.47), 0.64	0.008	48.44
Asian	Allele contrast (C vs. A)	1.40 (1.14–1.71), 0.001	1.31 (0.89–1.92), 0.15	0.01	68
	Dominant (CC + AC vs. AA)	1.56 (1.19-2.06), 0.001	1.49 (0.96–2.31), 0.07	0.07	53.87
	Homozygote (CC vs. AA)	1.78 (1.15-2.76), 0.009	1.52 (0.64–3.61), 0.34	0.02	64.77
	Co-dominant (AC vs. AA)	1.52 (1.13–2.04), 0.005	1.48 (0.94–2.32), 0.08	0.09	50
	Recessive (AA + AC vs. CC)	1.42 (0.95–2.13), 0.08	1.22 (0.52-2.84), 0.63	0.01	66.87
Caucasian	Allele contrast (C vs. A)	0.99 (0.90–1.10), 0.96	1.00 (0.88–1.13), 0.97	0.12	30
	Dominant (CC + AC vs. AA)	0.99 (0.86–1.13), 0.89	0.98 (0.85–1.13), 0.86	0.63	0
	Homozygote (CC vs. AA)	1.11 (0.85–1.45), 0.42	1.08 (0.75–1.54), 0.67	0.08	35.57
	Co-dominant (AC vs. AA)	0.97 (0.84–1.12), 0.74	0.97 (0.84–1.12), 0.72	0.89	0
	Recessive (AA + AC vs. CC)	1.02 (0.80–1.29), 0.86	0.99 (0.71–1.38), 0.99	0.68	37.93

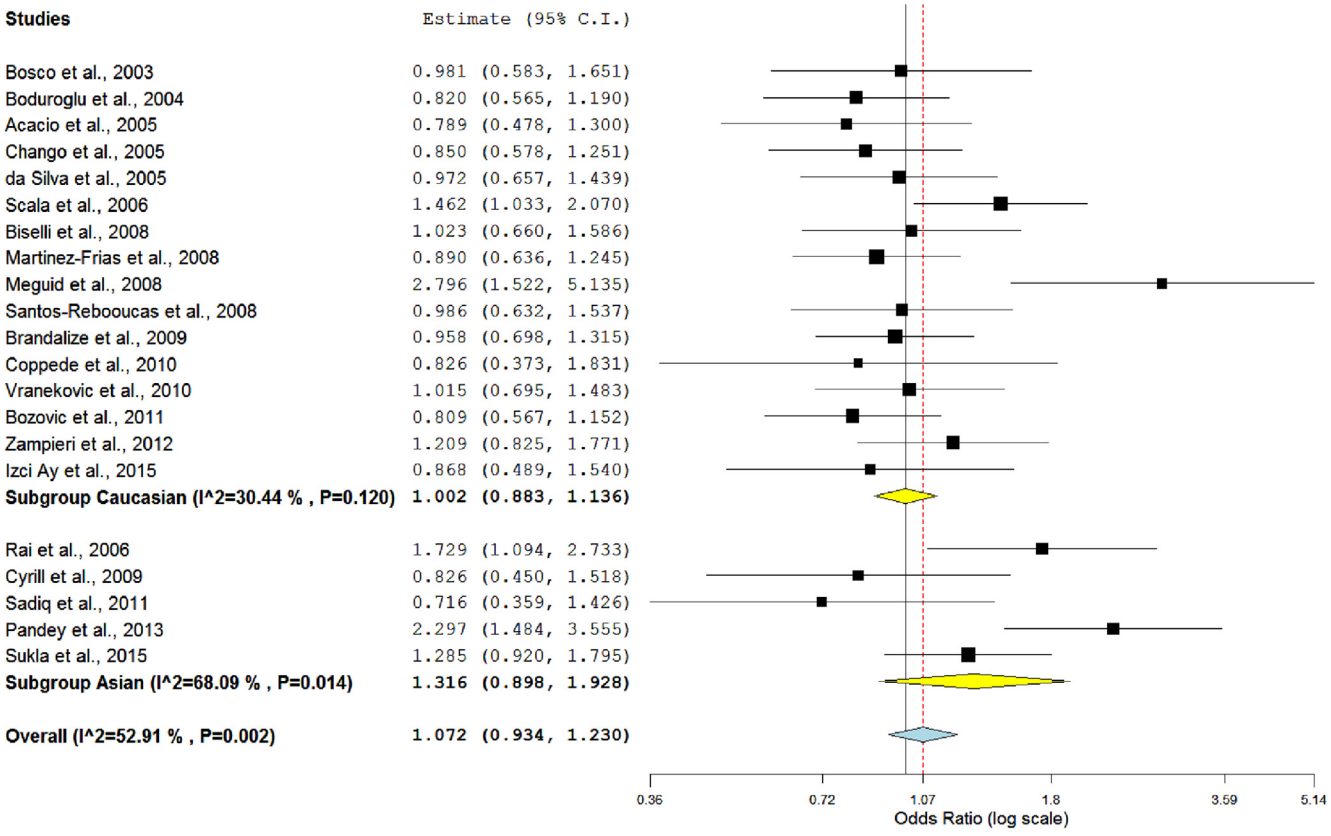

Figure 1 Forest plots (random effects) show insignificant association between *MTHFR* A1298C polymorphism (C vs. A) and maternal risk of Down syndrome. Results of individual and summary OR estimates, 95% CI, and weights of each study are shown. Horizontal lines represent 95% CI, and dotted vertical lines represent the value of the summary OR.

polymorphism in cases and controls and other information essential for estimation of odds ratio with 95% confidence interval (CI), and (iii) study should be published. Following criteria were used for exclusion of studies (i) only cases studied, (ii) review articles, case reports and editorials and (iii) studies that contained duplicate data.

2.3. Data extraction

From each study, the following information was extracted: first author's family name, journal name, country name, year of publication, and the number of A1298C genotypes in cases and controls. The allele numbers were calculated from the corresponding genotype distributions.

2.4. Meta-analysis

The meta-analysis examined the overall association of maternal C allele as a risk for DS relative to allele A. The association was measured as odds ratios (OR) with 95% confidence interval (CI). Heterogeneity between studies was tested using the Q-statistic [25,26] and was quantified with the I^2 metric. I^2 takes values between 0% and 100% with higher values denoting greater heterogeneity [27,28]. The pooled OR was estimated using fixed effects (FE) [29] and random effects [30] models. When there is higher heterogeneity, then the random effects model is preferably adopted [31]. All analyses were performed using the computer program open meta-analyst [32]. A p value less than 0.05 was considered statistically significant, and all the p values were two sided.

2.5. Sub-group analysis

The subgroup analysis was performed on the basis of ethnicity i.e. Asian and Caucasian. The sub-group meta-analysis cannot be performed by the genotyping method because out of twenty-one studies, nineteen studies were performed on the basis of PCR-RFLP and one study each on the basis of sequencing and Taq Man probe.

2.6. Publication bias

Publication bias was assessed by Egger's test and visual observation of funnel plot [33]. $p < 0.05$ was considered statistically significant publication bias.

3. Results

3.1. Characteristics of included studies

Twenty-one studies were found suitable for inclusion in the present meta-analysis [9,10,13,19,34–50]. The studies were car-

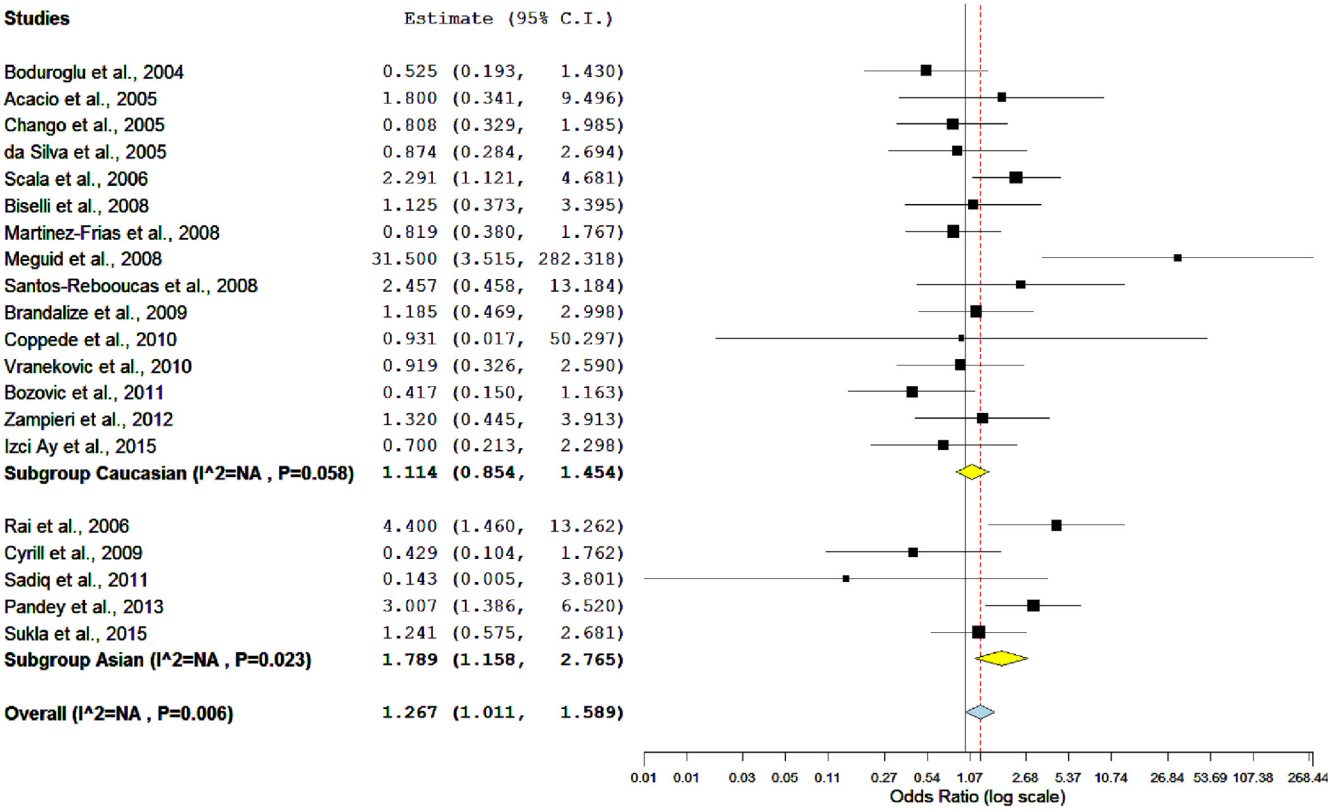

Figure 2 Forest plots (fixed effects) show significant association between *MTHFR* A1298C polymorphism (CC vs. AA) and maternal risk of Down syndrome. Results of individual and summary OR estimates, 95% CI, and weights of each study are shown. Horizontal lines represent 95% CI, and dotted vertical lines represent the value of the summary OR.

ried out in India [36,42,48,50], Brazil [9,35,37,40,41,47], Italy [10,34,43], France [19], Croatia [44,45], Egypt [39], Jordan [46], Spain [38] and Turkey [13,49]. Details of included twenty-one studies are summarized in Table 1.

3.2. Statistical details

In all twenty-one studies, total cases were 2004 with AA (913), AC (880) and CC (211), and controls were 2523 with AA (1217), AC (1056), and CC (250). In controls genotype percentage of AA, AC and CC was 48.24%, 41.85% and 9.91% respectively. In total cases genotype percentage of AA, AC, and CC was 45.56%, 43.91% and 10.53% respectively. Frequencies of AA genotype were the highest in both cases and controls. Genotypes in control samples of seven studies were not in the Hardy–Weinberg equilibrium [13,39,40,42,43,46,48] (Table 1).

3.3. Meta-analysis

In allele contrast meta-analysis, maternal mutant C allele did not show significant association with DS in both fixed effects (OR = 1.06, 95% CI = 0.97–1.17, $p = 0.15$, $P_{hetero} = 0.002$, $I^2 = 52\%$) and random effects (OR = 1.07, 95% CI = 0.93–1.23, $p = 0.32$) models (Table 2, Fig. 1).

Homozygote (CC vs. AA) meta-analysis showed significant association with DS adopting fixed effects model (OR = 1.26, 95% CI = 1.01–1.58, $p = 0.04$, $P_{hetero} = 0.009$, $I^2 = 48.29\%$) (Fig. 2). Association of mutant heterozygous genotype (AC vs. AA; co-dominant model) was observed insignificant with both fixed (OR = 1.06; 95% CI = 0.93–1.21; $p = 0.34$; $I^2 = 16.97\%$; $P_{hetero} = 0.24$) and random (OR = 1.06; 95% CI = 0.92–1.23; $p = 0.38$) effects models. Similarly combined maternal mutant genotypes (CC + AC vs. AA; dominant model) also did not show any association with DS using both fixed (OR = 1.08; 95% CI = 0.96–1.23; $p = 0.18$; $I^2 = 34\%$; $P_{hetero} = 0.06$) and random (OR = 1.09; 95% CI = 0.93–1.28; $p = 0.26$) effects models (Fig. 3; Table 2).

3.4. Subgroup analysis

We also performed sub-group analysis which is based on ethnicity. Out of 21 studies included in this meta-analysis, five studies were on Asian and 16 were on Caucasian. In Asian populations, allele contrast meta-analysis showed statistically insignificant association with random effects model (OR = 1.31, 95% CI = 0.89–1.92, $p = 0.15$) with high heterogeneity ($I^2 = 68\%$), whereas combined mutant genotypes showed significant association adopting random effects model (OR = 1.49, 95% CI = 0.96–2.31, $p = 0.07$, $I^2 = 53.87\%$). In this group heterogeneity between studies was high with the absence of publication bias (Table 2; Fig. 1).

In Caucasian population, allele contrast meta-analysis showed no association with fixed effects model (OR = 0.99, 95% CI = 0.90–1.10, $p = 0.96$, $P_{hetero} = 0.12$, $I^2 = 30\%$)

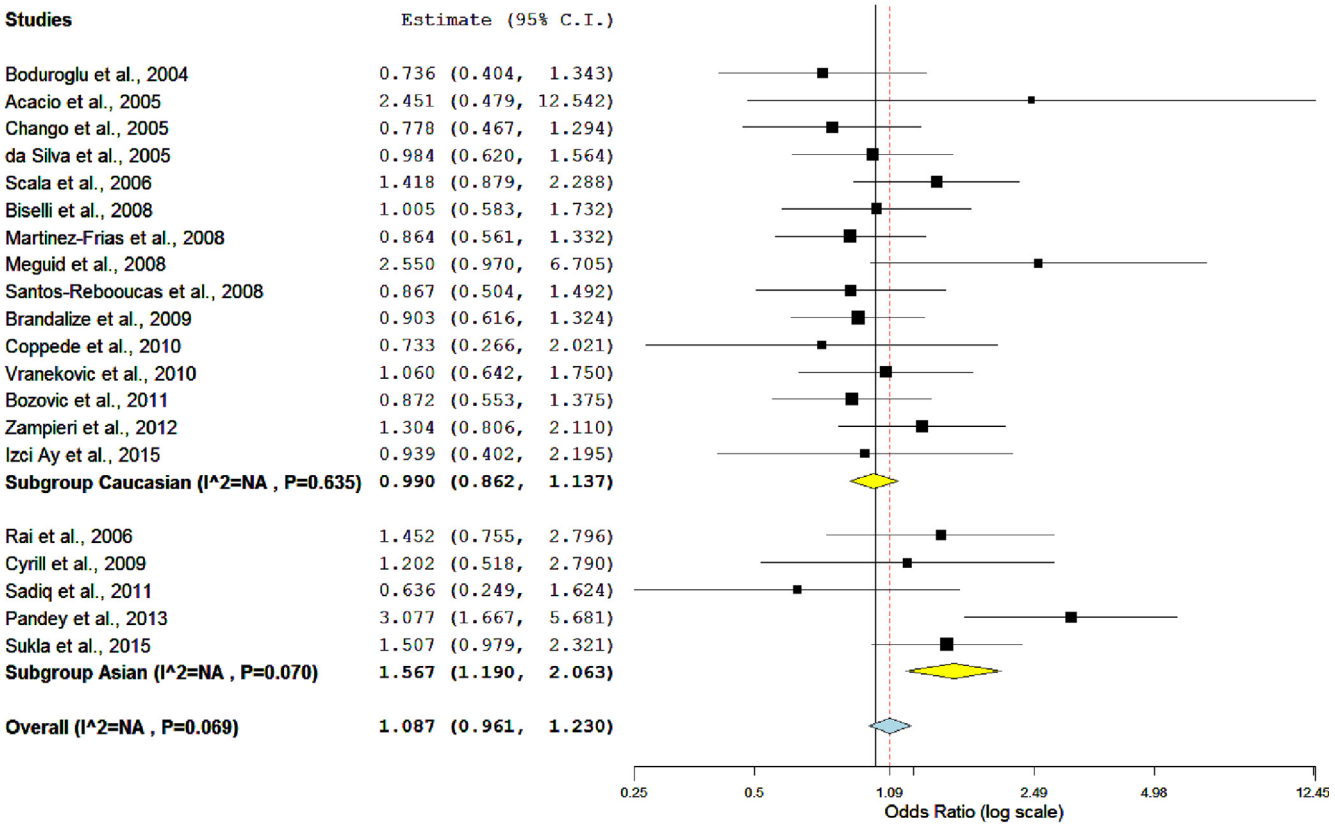

Figure 3 Forest plots (fixed effects) show no association between *MTHFR* A1298C polymorphism (CC + AC vs. AA) and maternal risk of Down syndrome. Results of individual and summary OR estimates, 95% CI, and weights of each study are shown. Horizontal lines represent 95% CI, and dotted vertical lines represent the value of the summary OR.

and combined mutant genotypes also showed no association with fixed (OR = 0.99, 95% CI = 0.86–1.13, $p = 0.89$, $P_{hetero} = 0.63$, $I^2 = 0\%$) effects models (Table 2; Fig. 1).

3.5. Sensitivity analysis

In allele contrast meta-analysis, sensitivity analysis performed by exclusion of the studies in which control population was not in the Hardy–Weinberg equilibrium. In overall analysis, after exclusion of seven studies [13,39,40,42,43,46,48], heterogeneity was decreased ($I^2 = 20.58\%$, $p = 0.23$) but odds ratio remained non-significant (OR = 1.04, 95% CI = 0.93–1.15, $p = 0.46$). Similar effect was also seen in Caucasian subgroup meta-analysis, after elimination of studies in which control population was not in HWE [13,39,40,43] heterogeneity was decreased ($I^2 = 0\%$) OR remains non-significant (OR = 0.98, 95% CI = 0.87–1.10, $p = 0.77$). In Asian studies, control population of three studies [42,46,48] was not in HWE. After elimination of these three studies heterogeneity was decreased and OR was increased (OR = 1.42, 95% CI = 1.08–1.86, $p = 0.01$; $P_{hetero} = 0.30$; $I^2 = 4.92\%$).

3.6. Publication bias

Funnel plots were symmetrical and did not show any evidence of publication bias. Results of Egger's test also suggested the absence of publication bias ($p = 0.83$) for C vs. A (Fig. 4).

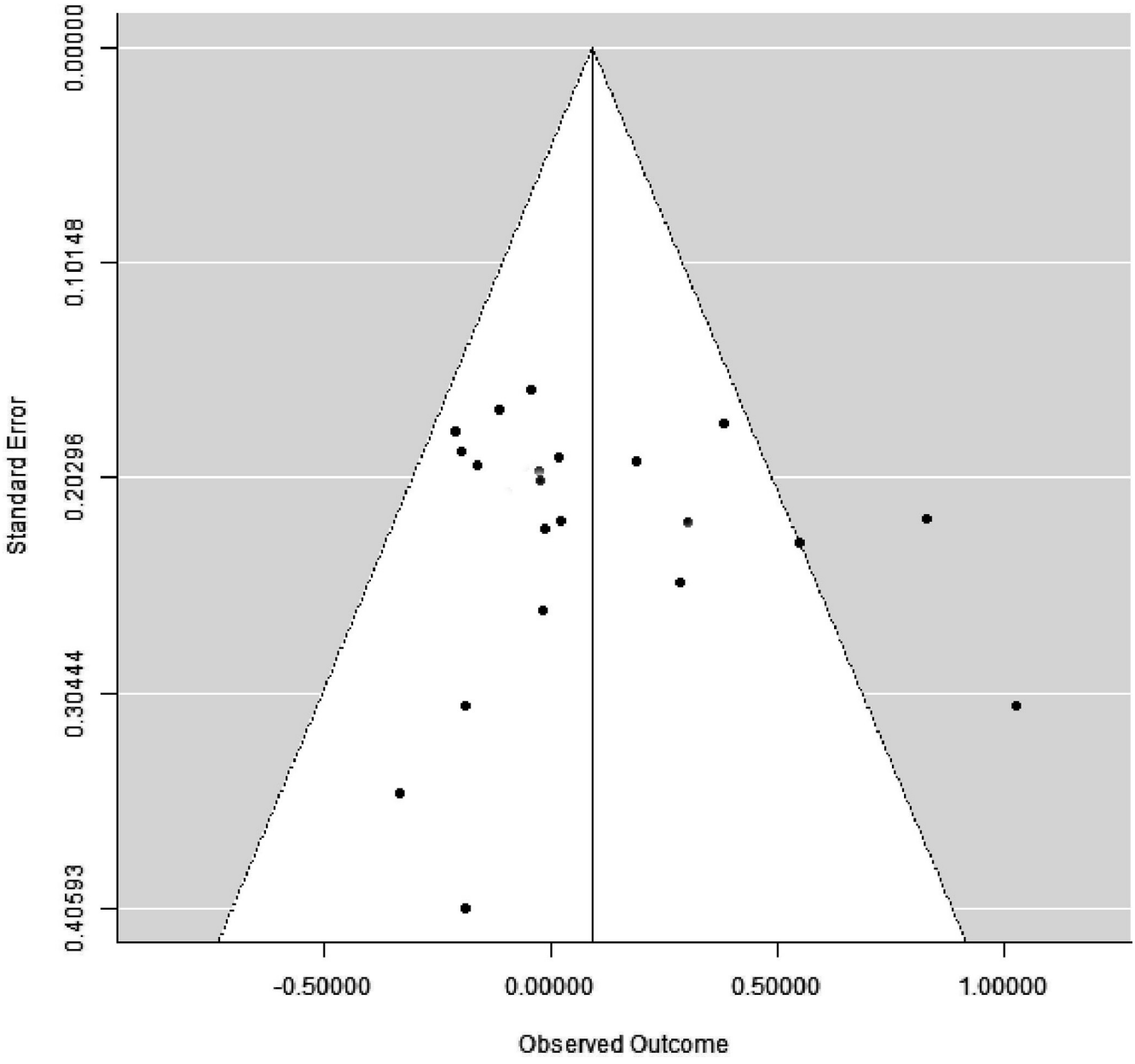

Figure 4 Funnel plot of allele contrast model.

4. Discussion

The present meta-analysis was designated to evaluate the role of *MTHFR* A1298C gene polymorphism in the Down syndrome pregnancy. A total of twenty-one studies (with 2004 cases and 2523 controls) were incorporated in this study. In overall analysis the homozygote model (CC vs. AA) showed a significant association (OR = 1.26; 95% CI: 1.01–1.58; $p = 0.04$) while no such association was observed in any other genetic model. Similarly when sub-group analysis based on ethnicity was performed no significant association was found in any genetic model.

Several *in vivo* and *in vitro* studies have reported that folate deficiency in culture media, or inadequate folate dietary intake, results in DNA hypomethylation, and abnormal chromosome segregation [47]. For this reason it has been postulated that impaired folate/homocysteine metabolism due to genetic polymorphisms of folate pathway genes could predispose an individual to chromosome damage events and might act as a risk factor for a DS pregnancy [3]. Since 1999, several case–control studies have investigated maternal folate pathway gene polymorphism as a risk factor for DS offspring and positive association has been observed [3,7,8,10,34,35,51]. *MTHFR* variants due to less enzymatic activity lead to hypomethylation of centromeric DNA, which may be the major cause of missegregation of chromosomes during meiosis and results in trisomy 21. Hassold et al. [2] analyzed polymorphism of *MTHFR* and *MTRR* (methionine synthase reductase) maternal genes in trisomy of several chromosomes and compared the distribution of genotypes to those of control populations and observed a significant increase in the *MTHFR* polymorphisms in mothers of trisomy cases. Low or inadequate intake of folic acid is involved in the disruption of methionine metabolism, because methylenetetrahydrofolate, the primary form of folate in the circulation, acts as the carbon donor for homocysteine remethylation to yield methionine and tetrahydrofolate [52]. Several population-based studies and animal model based studies have shown that folic acid intake during fetal development has a protective effect, resulting in a significant reduction in the occurrence of developmental defects, including neural tube defects (NTD), congenital heart defects (CHD), limb defects, and orofacial clefts [20].

Meta-analysis is a powerful tool for analyzing cumulative data of studies where the sample sizes of individual studies are small and the statistical power is low. During past decade several meta-analyses were published assessing *MTHFR* as a risk factor to various diseases/disorders like NTD [53], cleft lip and palate [54], congenital heart defects [55], recurrent pregnancy loss [56], stroke [57], Down syndrome [58], bipolar disorder [59], depression [60] and cancer [61,62]. Four meta-analyses were published regarding maternal *MTHFR* polymorphism and DS risk [13,58,63,64], out of which only two meta-analyses investigated maternal *MTHFR* A1298C polymorphism as a risk factor for DS [13,63]. Zintzaras [13] carried out the first meta-analysis of *MTHFR* A1298C polymorphism and DS risk, and reported that the allele contrast C versus A showed significant heterogeneity among studies ($p = 0.04$, $I^2 = 56\%$) and the association was insignificant: FE OR = 1.02 (95% CI: 0.81–1.29). A meta-analysis of 10 retrospective studies (1007 case mothers and 1318 controls) was carried out by Medica et al. [63] and reported no association (1.06 (95% CI: 0.85–1.31)). There are several newly published studies available but not included in the previous meta-analyses. So we conducted a comprehensive meta-analysis with the largest number of studies to date to investigate the possible relationship between maternal *MTHFR* A1298C polymorphism and the risk of having DS child.

The present meta-analysis has some limitations also like (i) crude odds ratio was used, (ii) studies with small sample sizes [42,43] were included, (iii) meta-analysis was restricted to single polymorphism (A1298C), other gene polymorphism of folate pathway should also be considered, and (iv) except genetic polymorphism, other important factors such as maternal age, folate intake and homocysteine concentration should also be considered. The present meta-analysis also had some strength along with limitations. The main strengths of our meta-analysis were the absence of publication bias and pooled number of cases and controls from different studies significantly increased the power of the study.

In conclusion, the present study did not support any association between maternal *MTHFR* gene A1298C polymorphism and Down syndrome. However, the present meta-analysis was based on relatively a small number of studies and participants, and only one polymorphism was considered, hence case–control studies that investigate gene-gene and gene-environment interaction might well elucidate genetics of Down syndrome.

Compliance with ethical standards

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Conflict of interest: The authors Vandana Rai, Upendra Yadav, and Pradeep Kumar declare that they have no conflict of interest to declare.

Research involving human participants and/or animals: This is a systematic review, human participants are not involved.

Informed consent: Since this is a review article, there is no need for taking informed consent.

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