Review

Fetal MTHFR C677T polymorphism confers no susceptibility to Down syndrome: Evidence from meta-analysis

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A B S T R A C T

Maternal methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism is reported as risk factor for Down syndrome (DS) pregnancy but fetal MTHFR C677T polymorphism was not well studied as risk factor for DS. Some studies were published, but results were controversial. Hence a meta-analysis of all published studies investigated DS case MTHFR polymorphism were performed to explore the association between C677T polymorphism of individual and DS risk. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of association. The analyses were conducted with meta-Analyst and MIX software. Total five case-control studies with 401 DS cases and 529 controls were included in present meta-analysis.

Meta-analysis results suggested that MTHFR C677T polymorphism did not contribute any DS risk in overall population using four genetic models (for T vs. C: OR = 1.56, 95% CI = 0.83–2.89). However, co-dominant model analysis showed significant association between MTHFR C677T polymorphism and DS risk (OR = 1.66; 95% CI = 1.22–2.25; p = 0.001). Less heterogeneity (I^2 = 48.31), so fixed effect model was used. In conclusion, present meta-analysis suggests that MTHFR C677T polymorphism of fetus is not risk factor for DS.

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Contents

1. Introduction

Down syndrome (DS) (MIM 190685), is the most frequent genetic cause of mental retardation, resulting from the trisomy of chromosome 21. The established risk factor for DS is advanced maternal age (>35 years) at the time of conception. Exact mechanism of meiotic non-disjunction is not yet known, however folate deficiency has been well debatable and established cause of chromosomal instability and chromosome aneuploidy [1,2]. Several studies have investigated the risk of DS child to variants of maternal folate pathway genes like MTHFR, Methionine synthase (MTR) and Methionine synthase reductase (MTRR) [3,4] and these genes showed polymorphism worldwide [5,6]. MTHFR is an important enzyme of folate pathway and several studies has been conducted and demonstrated MTHFR polymorphism as risk factor for congenital defects [7]. MTHFR gene is located on the short arm of chromosome 1 (1p36.3) [8]. Several polymorphisms has been reported in MTHFR gene, out of which C677T polymorphism is very well studied and also clinically very important [9]. C677T polymorphism reduces enzyme activity and increases plasma homocysteine concentration [9,10]. Frequency of T allele is well studied and varied greatly worldwide [11–13].

MTHFR enzyme mediates the irreversible conversion of 5, 10-methylenetetrahydrofolate (5, 10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), which remethylates homocysteine to methionine. Methionine is the main precursor for S-adenosylmethionine (SAM), the main methyl donor for DNA, RNA and protein methylation [14]. Insufficient periconceptional folic acid intake and deficient folate metabolism in mothers have been acknowledged as risk factors for DS and several other congenital defects [14,15]. It has been suggested that impaired maternal folate metabolism could promote DNA hypomethylation and meiotic nondisjunction resulting in trisomy 21 [14,15].

Several studies have been investigated maternal MTHFR C677T polymorphism as risk factor for Down syndrome pregnancy [3,14,16–18], whereas very few researchers investigated MTHFR C677T polymorphism in DS cases [19–21]. In order to find out the answer of a question that is fetal MTHFR C677T polymorphism is responsible for DS phenotype or not, authors performed a meta-analysis of published case control studies.

2. Methods

2.1. Study search

Four databases- PubMed, Google Scholar, Science Direct, and Springer Link were searched for case-control association studies published up to January 31, 2017. The search strategy was based on the following keywords: "Down Syndrome", "DS", methylenetetrahydrofolate reductase", "MTHFR" and "C677T".

2.2. Inclusion and exclusion criteria

All eligible relevant studies in this meta-analysis were met the following inclusion criteria: (1) case–control study; (2) analyzed MTHFR C677T polymorphism in DS cases and controls; and (3) sufficient information of MTHFR genotypes in DS cases and control group.

The article exclusion criteria were as follows: (1) only cases studied, lack of control group; (2) maternal MTHFR genotypes analyzed, (3) insufficient genotype details reported; and (4) reviews, comments, letter to editor and abstract of meeting.

2.3. Data extraction

From each study, the following information was extracted: first author's family name, publication year, journal name, ethnic origin of the patients, sample size, genotype/allele numbers/ frequencies etc.

2.4. Statistical analysis

The pooled odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association between the MTHFR C677T polymorphism and DS risk. All five genetic models- allele (T vs. C), homozygote (TT vs. CC), heterozygote (CT vs. CC), dominant (TT + CT vs. CC) and recessive (TT vs. CT + CC) models were applied. The subgroup analysis was not conducted due to less number of studies. Z-test was applied to assess the significance of pooled ORs and $p \leq 0.05$ was considered as statistically significant. According to the heterogeneity, either fixed-effect or random-effect model was used to calculate the pooled odds ratios (ORs) with 95% confidence intervals (CIs). If $I^2 < 50\%$, the fixed-effect model was used to calculate the pooled ORs [22]. Otherwise, the random-effect model was applied [23]. The Hardy–Weinberg equilibrium (HWE) in the control population was assessed by the $\chi 2$ test.

A funnel plot was used to evaluate the publication bias by visual inspection. In addition, Egger's linear regression test was also applied, which is used to assess the asymmetry of a funnel plot using a natural logarithm scale of ORs [24]. All the analyses of data were performed using MetaAnalyst [25] and MIX [26] software.

3. Results

3.1. Study details

With our original search criterion, 85 articles were found. After reviewing each original article, 80 publications were excluded including reviews, case studies, editorials and maternal MTHFR analyzed etc. Following these exclusions, 5 individual case-control studies with a total of 401 cases and 529 controls were found to be suitable for inclusion into meta-analysis [19–21,27,28] (Table 1). These studies were published between 2004 and 2015. All these five studies were performed in France [19] and India [20,21,27,28].

3.2. Characteristics of included studies

In five studies included in the present meta-analysis, the smallest case sample size was 25 [27] and highest sample size was 156 [19]. Except one study [20], control populations of four articles were in Hardy-Weinberg equilibrium. In all five studies, total cases were 401 with CC (223), CT (147) and TT (31), and controls were 529 with CC (373), CT (130), and TT (26) genotypes. In controls genotypes, percentage of CC, CT and TT were 70.5%, 24.57%, and 4.91% respectively. In total cases, genotype percentage of CC, CT, and TT was 55.61%, 36.66% and 7.73% respectively.

3.3. Meta-analysis

Five independent samples with a total of 1860 alleles included in the meta-analysis. The pooled OR using allele contrast model (T vs C) was 1.56, (CI = 0.83–2.89; p = 0.16), indicating that having the T677 allele did not increase the risk for DS in comparison to the C allele (Fig. 1; Table 2). Heterogeneity across studies was significantly high (p = 0.005, $I^2 = 72.82$), so random effect model was adopted.

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

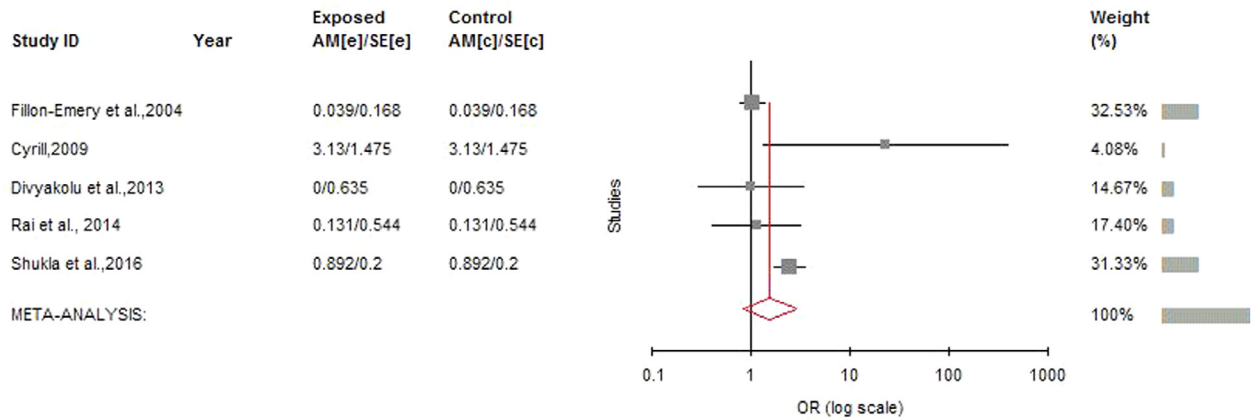| Study	Country	Case	Control	Case genotypes CC/CT/TT	Control genotypes CC/CT/TT	P value (HWE)
Fillon-Emery et al. [19]	France	156	159	61/75/20	67/70/0	0.58
Cyril et al. [20]	India	37	60	32/4/1	60/0/0	0.00
Divyakolu et al. [27]	India	25	50	21/4/0	42/8/0	0.53
Rai et al. [46]	India	32	60	26/6/0	50/10	0.48
Sukla et al. [21]	India	151	200	83/58/10	154/42/4	0.57

Fig. 1. Forest Plot of Allele Contrast (T vs C).

Table 2
Summary estimates for the odds ratio (OR) of MTHFR C677T in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric 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 value of Egger's test)
Allele Contrast (T vs C)	1.46(1.15–1.86),0.001	1.56(0.83–2.89),0.16	0.005	72.82	0.65
Co-dominant (CT vs CC)	1.66(1.22–2.25),0.001	1.60(0.94–2.71),0.08	0.10	48.31	0.80
Homozygote (TT vs CC)	1.54(0.86–2.73),0.14	2.15(0.62–7.35),0.22	0.08	60.44	0.48
Dominant (TT + CT vs CC)	1.69(1.26–2.26),0.0004	1.63(0.89–2.87),0.11	0.04	60.84	0.79
Recessive (TT vs CT + CC)	1.31(0.75–2.25),0.33	1.75(0.58–5.18),0.31	0.11	53.11	0.43
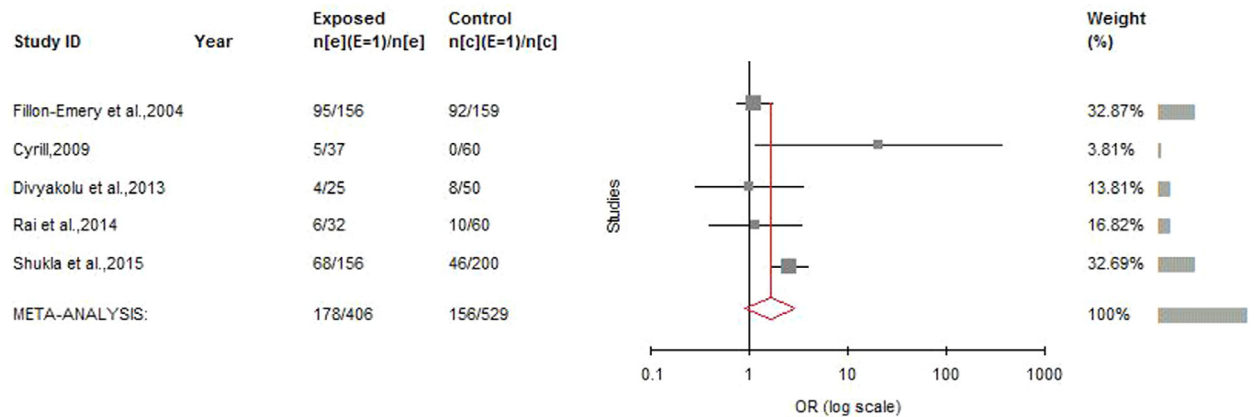
Fig. 2. Forest Plot of dominant model (TT + CT vs CC).

Comparison of mutant homozygote and with CC homozygote (TT vs CC) did not show any association between TT genotype and DS susceptibility (OR = 2.15; 95% CI = 0.62–7.35; p = 0.22). Heterogeneity was observed (p = 0.08; I^2 = 60.44). The pooled OR of dominant model (TT + CT vs CC) was 1.63 (95% CI = 0.89–2.87; p = 0.11) indicated no significant association between DS risk and MTHFR C677T polymorphism (Fig. 2; Table 2). Higher heterogeneity was observed (p = 0.04; I^2 = 60.84%. The pooled OR using recessive model (TT vs CT + CC) was 1.75 (95% CI = 0.58–5.18; p = 0.31), indicating that T allele was not risk for DS. Tests of heterogeneity among studies were not significant (p = 0.11, I^2 = 53.11%). However, co-dominant model analysis showed significant association
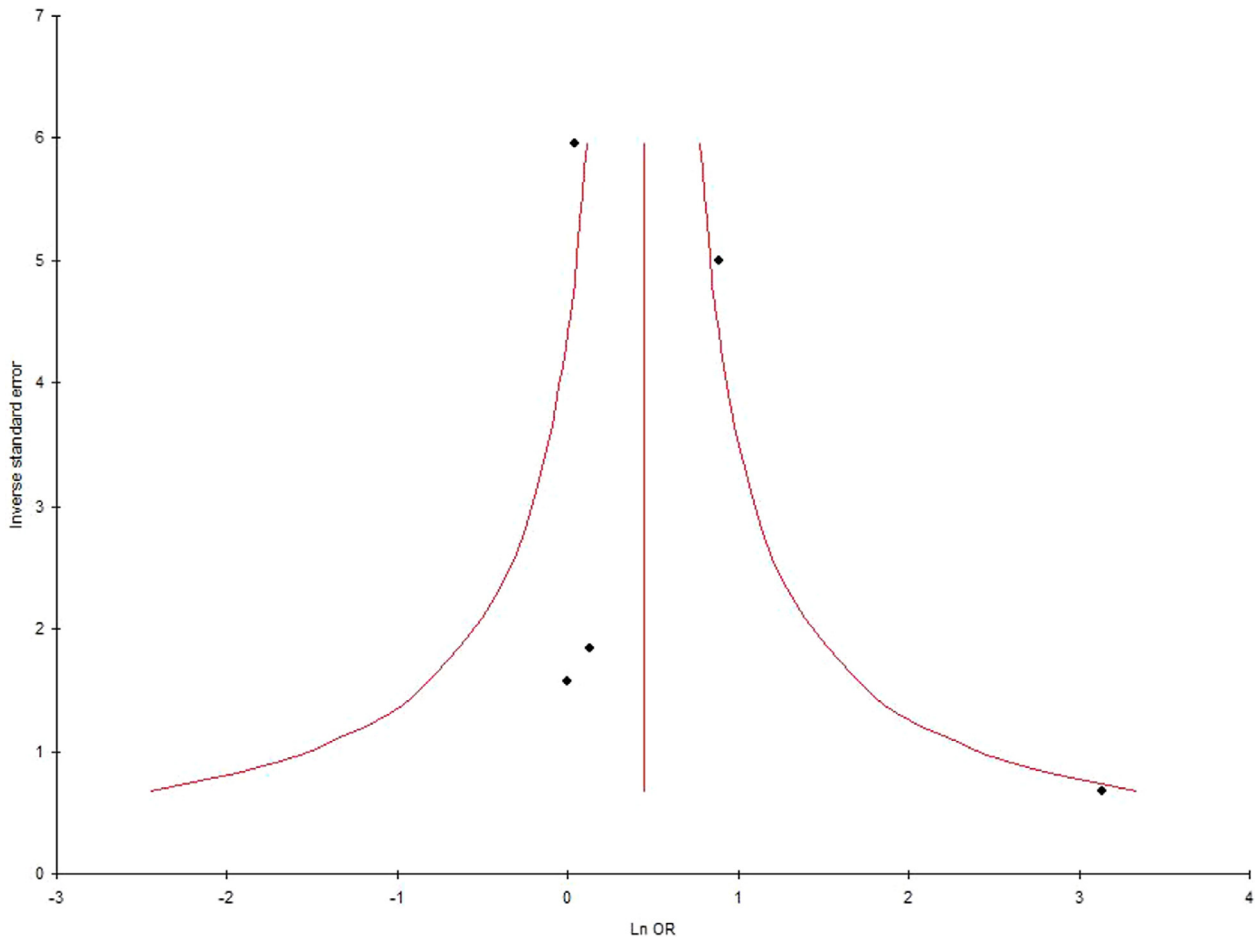
Fig. 3. Funnel plot of precision versus log OR.

between MTHFR C677T polymorphism and DS risk (OR = 1.66; 95% CI = 1.22–2.25; p = 0.001). Less heterogeneity (I^2 = 48.31), so fixed effect model was used.

3.4. Sensitivity analysis

Distribution of control population of one study [20] did not follow Hardy-Weinberg equilibrium, so authors performed sensitivity analysis by excluding this study. Results of sensitivity analysis also did not show any association between MTHFR C677T and DS risk (OR = 1.40; 95%CI = 0.75–2.49; p = 0.05; I^2 = 73.1%).

3.5. Subgroup analysis

Only five studies were included in the present meta-analysis, out of which four studies were conducted in India. Authors performed subgroup analysis also by excluding one study [19] conducted outside India. meta-analysis of three Indian studies [21,27,28] showed no association between MTHFR C677T polymorphism and DS risk (OR = 1.75; 95%CI = 0.96–3.18; p = 0.20; I^2 = 37.2%). However after exclusion of two studies [19,20], significant association was not observed between MTHFR C677T polymorphism and DS risk, but heterogeneity was decreased.

3.6. Publication bias

Funnel plot symmetry showed absence of publication bias (Figs. 3 and 4) and p value of Egger's test was also higher than 0.05 in all five genetic models(allele contrast model p value = 0.65; co-dominant model p value = 0.80; homozygote model p value = 0.48; dominant model p value = 0.79; and recessive model p value = 0.43).

4. Discussion

The results of present meta-analysis did not show any association between fetal MTHFR gene C677T polymorphism and DS risk. The effect (-0.068) size was also calculated and did not reveal any differences between both the groups. Higher heterogeneity between studies was observed in the meta-analysis, which might be due to differences between study design, methodology, sample size and population. It is evident from results of present meta-analysis and previous meta-analysis on case control studies of maternal MTHFR C677T polymorphism [29–32] that maternal MTHFR gene polymorphism is the main cause of DS 21 and three copies of gene on chromosome 21 determine the IQ and etiology of DS.

In the brain, epigenetic programming appears to be most sensitive to environmental and genetic influences in utero and early in postnatal life [33–35]. Methylation reactions are crucial during this time to develop functional neuron networks. Early modifications in DNA methylation that cause cells to deviate from differentiating into their normal lineage can result in significant decreases or expansions of neuron pools that are irreversible [35,36].

meta-analysis is an acceptable and useful statistical method, which can effectively combine data from several case control
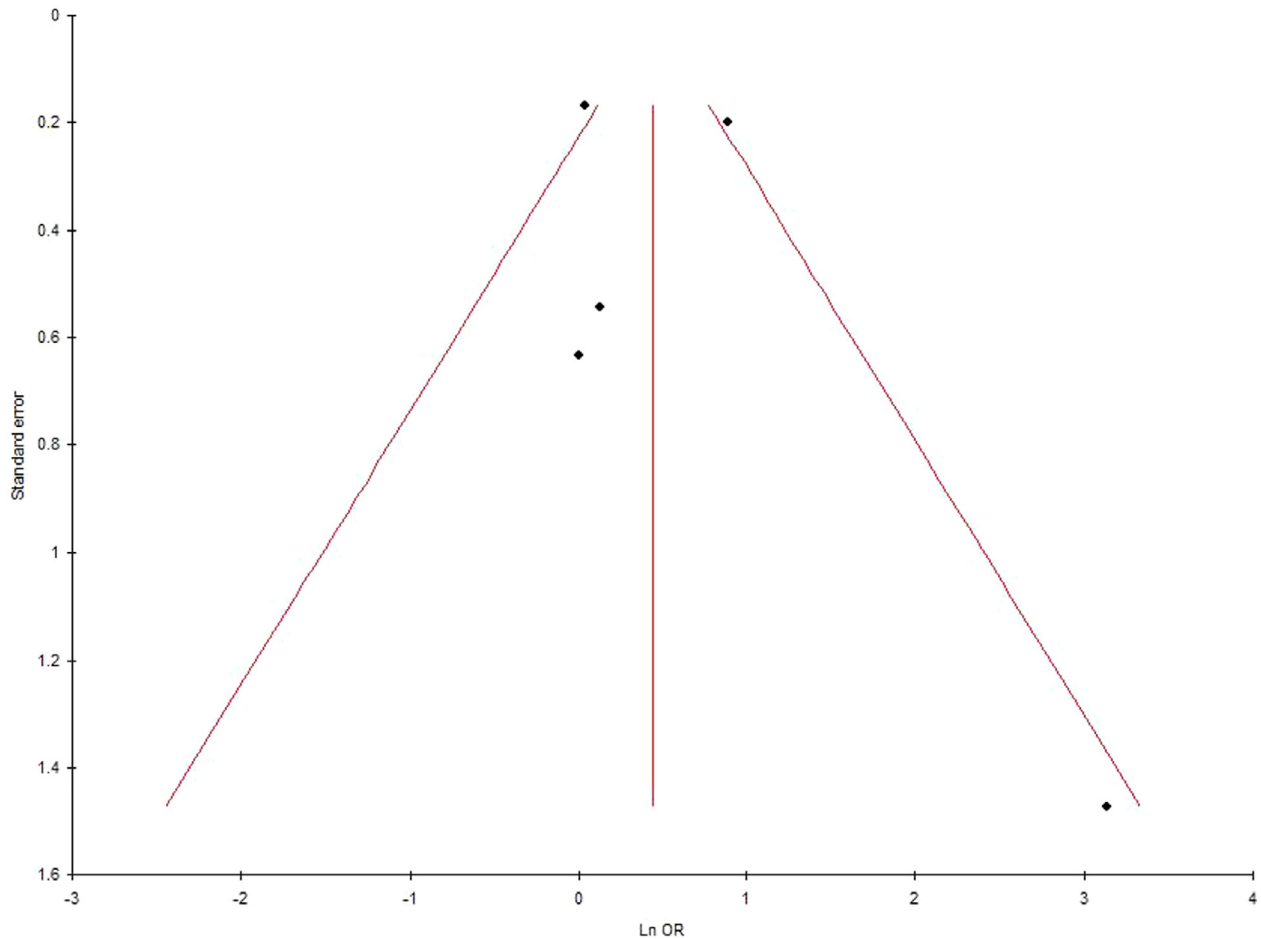
Fig. 4. Funnel plot of SE versus log OR.

studies to obtain reliable results. Several meta-analysis were published which evaluated risk of folate pathway genes polymorphism for different disease and disorders- like Down syndrome [37,38], autism [39], schizophrenia [40], depression [41], Alzheimer's disease [42], pregnancy loss [43] and cancer [44–49] etc.

During literature search, we identified five meta-analysis [29–32,38] published between 2007 and 2014. They examined the effect of maternal MTHFR C677T polymorphism as DS risk but present meta-analysis investigated association between fetal MTHFR C677T polymorphism and DS cases.

The strength of our meta-analysis is that the publication bias was not detected and pooled number of cases and controls from different studies significantly increased the statistical power of the analysis. There are few limitations of the present meta-analysis, which should be acknowledged like- i) Crude ORs without adjustment was used as association measure; ii) Only five studies were included in the meta-analysis; iii) sample size in few studies was very small; iv) Single gene polymorphism (MTHFR C677T) of folate pathway was analyzed and v) In present meta-analysis higher between study heterogeneity was observed.

In conclusion, results of present meta-analysis failed to establish any association between MTHFR C677T polymorphism and DS risk. However co-dominant model analysis showed significant association between C677T polymorphism and DS risk. In present meta-analysis number of included studies were only five and majority of them were from India, so some biases might be present like time bias, population bias etc. which might be influenced the results of present meta-analysis. In future, larger case control studies from different populations are required to confirm the association between MTHFR C677T polymorphism and DS risk. Gene-gene and gene -environmental interactions should also be studies to clarify this association.

Conflict of interest

None

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