Maternal Methylenetetrahydrofolate Reductase C677T Polymorphism and Down Syndrome Risk: A Meta-Analysis from 34 Studies

Vandana Rai1*, Upendra Yadav1, Pradeep Kumar1, Sushil Kumar Yadav1, Om Prakesh Mishra2

1 Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur, India, 2 Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Abstract

Background: Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme of folate metabolic pathway which catalyzes the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. 5-methyltetrahydrofolate donates methyl group for the methylation of homocysteine to methionine. Several studies have investigated maternal MTHFR C677T polymorphism as a risk factor for DS, but the results were controversial and inconclusive. To come into a conclusive estimate, authors performed a meta-analysis.

Aim: A meta-analysis of published case control studies was performed to investigate the association between maternal MTHFR C677T polymorphism and Down syndrome.

Methods: PubMed, Google Scholar, Elsevier, Springer Link databases were searched to select the eligible case control studies using appropriate keywords. The pooled odds ratio (OR) with 95% confidence interval were calculated for risk assessment.

Results: Thirty four studies with 3,098 DS case mothers and 4,852 control mothers were included in the present meta-analysis. The pooled OR was estimated under five genetic models and significant association was found between maternal MTHFR C677T polymorphism and Down syndrome under four genetic models except recessive model (for T vs. C, OR = 1.26, 95% CI = 1.09–1.46, p = 0.001; for TT vs. CC, OR = 1.49, 95% CI = 1.13–1.97, p = 0.008; for CT vs. CC, OR = 1.29, 95% CI = 1.10–1.51, p = 0.001; for TT+CT vs. CC, OR = 1.35, 95% CI = 1.13–1.60, p = 0.0008; for TT vs. CT+CC, OR = 0.76, 95% CI = 0.60–0.94, p = 0.01).

Conclusion: The results of the present meta-analysis support that maternal MTHFR C677T polymorphism is a risk factor for DS-affected pregnancy.

Introduction

Down syndrome (DS) is the most common chromosomal disorder with the prevalence of 1/700–1000 live birth. It is characterized by the trisomy 21, which results from maternal meiotic nondisjunction in majority (90%) of cases. The established risk factor for DS is advanced (>35 years) maternal age at the time of conception. However, a fairly high number of DS children born to younger mothers suggest that risk factors other than advanced maternal age might be involved in predisposing younger mothers to DS-affected pregnancy [1,2]. The molecular and biochemical mechanism of maternal meiotic non-disjunction is still not known. James et al. [3] reported that methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism might be a risk factor for maternal meiotic non-disjunction. Since then several studies have investigated the risk of DS to variants of folate pathway genes like MTHFR, Methionine synthase (MTR) and Methionine synthase reductase (MTRR) in Asian [1,2,4,5] and Caucasian [6–8] populations. Folate deficiency and dysfunctional MTHFR causes abnormal DNA methylation [9,10] and chromosomal segregation [11,12]. Hypomethylation of the centromeric DNA has been suggested as the causative mechanism of meiotic non-disjunction. Abnormal DNA methylation of centromere lead to aberrant kinetochore formation that results into abnormal segregation of chromosomes during meiosis [3,13].

MTHFR is a key enzyme in folate metabolism, which catalyzes the reduction of 5, 10-methylenetetrahydrofolate to the predominant circulating form of folate i.e. 5-methyltetrahydrofolate (5-THF). 5-THF donates methyl group for the conversion of homocysteine to methionine, which is further converted into S-adenosylmethionine (SAM). SAM is the main methyl group donor for all cellular methylation reactions. Folate deficiency and/or dysfunctional MTHFR reduces the conversion of 5, 10-methylene THF to 5-methyl THF, and elevates plasma homocysteine.
concentration. Both folate and MTHFR are involved in many complex biochemical reactions like DNA synthesis, repair and methylation.

There are more than 40 polymorphisms reported in MTHFR gene and among them C677T variant is the most studied and clinically important. The C677T variant (rs 1801133; Ala 222 Val) has been associated with a decreased activity of MTHFR, and increased homocysteine level [14–16]. Mutant homozygous (TT) individuals have a decreased enzymatic activity ~70% and the heterozygote by 40%. A dysfunctional MTHFR leads to lower levels of SAM resulting into DNA hypomethylation. DNA hypomethylation increases the risk of many diseases and disorders like neural tube defects [17], cleft lip and palate [18], Alzheimer disease [19], cardiovascular diseases [14], diabetes [20] and psychiatric disorders [21] etc. Several epidemiological studies have investigated the associations of the maternal MTHFR C677T polymorphism with Down syndrome. However, the results were conflicting and inconclusive. In light of the above facts, we conducted a meta-analysis of published case control studies relating the C677T polymorphism of the maternal MTHFR gene to the risk of having DS offspring.

Materials and Methods

Selection of studies

Electronic searches were conducted using PubMed, Google Scholar, Elsevier and Springer link and all published manuscripts up to January, 2014 were considered in present meta-analysis. The following index terms were used for search ‘MTHFR’ ‘Methylenetetrahydrofolate reductase’, and ‘C677T polymorphism’, ‘maternal risk’ and ‘Down syndrome’. In addition, bibliographies of all articles and reviews were hand searched for additional suitable studies.

![Figure 1. Flow Diagram of Study Searching and Selection Process.](https://doi.org/10.1371/journal.pone.0108552.g001)
Table 1. Characteristics of the eligible studies included in the meta-analysis.

| Study                  | Year | Country | Case | Control | Quality Score | Reference                                                                 |
|------------------------|------|---------|------|---------|---------------|----------------------------------------------------------------------------|
| James et al.           | 1999 | Canada  | 50   | 57      | 7             | Am J Clin Nutr 70:495-50                                                   |
| Hobbs et al.           | 2000 | America | 157  | 140     | 7             | Am J Hum Genet 67:623-630                                                  |
| Chadeaux-Vekemans et al | 2002 | France  | 85   | 70      | 5             | Pediatr Res 51:766-767                                                     |
| O’Leary et al.         | 2002 | Ireland | 41   | 192     | 5             | Am J Med Genet A 107:151–155                                               |
| Stupia et al.          | 2002 | Italy   | 64   | 112     | 7             | Eur J Hum Genet 10:388–390                                                  |
| Boduroglu et al.       | 2004 | Turkey  | 158  | 91      | 5             | Am J Med Genet 127A: 5–10                                                  |
| Acacio et al.          | 2005 | Brazil  | 70   | 88      | 8             | Prenat Diagn 25:1196–1199                                                  |
| Da Silva et al.        | 2005 | Brazil  | 154  | 158     | 7             | Am J Med Genet Part A 135A: 263–267                                       |
| Coppede et al.         | 2006 | Italy   | 79   | 111     | 7             | Am J Med Genet A 140(10): 1083–1091                                        |
| Liang et al.           | 2006 | China   | 30   | 70      | 7             | China J Modern Medicine 20011                                              |
| Rai et al.             | 2006 | India   | 149  | 165     | 6             | Genet Med 8:409-416                                                        |
| Scala et al.           | 2006 | Italy   | 94   | 256     | 8             | Zhonghua Yi Xue Yi Chuan Xue Za Zhi 24:533–537                              |
| Wang et al.            | 2007 | China   | 100  | 100     | 8             | Genet Med Mol Res 7:33–42                                                  |
| Biselli et al.         | 2008 | Brazil  | 82   | 134     | 8             | Downs Syndr Res Pract 12:3.133–137                                        |
| Kohli et al.           | 2008 | India   | 103  | 109     | 6             | Am J Med Genet 146A(11): 1477–1482                                         |
| Martinez-Frias et al.  | 2008 | Spain   | 146  | 188     | 4             | Dis Markers 24:19–26                                                       |
| Meguid et al.          | 2008 | Egypt   | 42   | 48      | 7             | Dis Markers 25:49–157                                                      |
| Santos-Reboucas et al. | 2008 | Brazil  | 103  | 108     | 7             | J Zhejiang Univ Sci B 9(2): 93-99                                         |
| Wang et al.            | 2008 | China   | 64   | 70      | 8             | Indian J Hum Genet 15:60–64                                                 |
| Bandalize et al.       | 2009 | Brazil  | 239  | 197     | 6             | Indian J Hum Genet 15:71–71                                                |
| Coppede et al.         | 2009 | Italy   | 94   | 113     | 8             | Indian J Hum Genet 15:60–64                                                 |
| Cyril et al.           | 2009 | India   | 36   | 60      | 6             | Dis Markers 27:279–285                                                     |
| Kokotas et al.         | 2009 | Denmark | 177  | 984     | 6             | Dis Markers 27:279–285                                                     |
| Pozzi et al.           | 2009 | Italy   | 74   | 184     | 8             | BMC Med Genomics 342                                                       |
| Coppede et al.         | 2010 | Italy   | 29   | 32      | 5             | Yi Chuan 32(5): 461–466                                                    |
| Liao et al.            | 2010 | China   | 60   | 68      | 7             | Yi Chuan 32(5): 461–466                                                    |
| Vranekoviz et al.      | 2010 | Croatia | 111  | 141     | 7             | Dis Markers 28:293–298                                                     |
| Bozovic et al.         | 2011 | Croatia | 112  | 221     | 7             | Pediatri Int 53(4): 546–550                                               |
| Sadiq et al.           | 2012 | Jordan  | 53   | 29      | 6             | Genet Test Mol Biomarker 15:1–7                                            |
| Tayeb                  | 2012 | Saudi Arabia | 30 | 40 | 5 | Egyptian J Med Hum Genet 13(3): 263–268 |
| Zampieri et al.        | 2012 | Brazil  | 105  | 185     | 8             | Dis Markers 32(2): 73–81                                                   |
| Kaur and Kaur          | 2013 | India   | 110  | 111     | 6             | Indian J Hum Genet 19(4): 412–414                                          |
| Pandey et al.          | 2013 | India   | 81   | 99      | 6             | Int J Pharm Bio Sci; 4(2)(B): 44915–19                                     |
| Elsayed et al.         | 2014 | Egypt   | 26   | 61      | 9             | The Egyptian J Med Hum Genet 15(1): 39–44                                  |
Table 2. Distributions of MTHFR C677T genotypes and allele frequencies in DS case mothers and control mothers reported in different included studies.

| Study                        | Country  | CC Case | CC Control | CT Case | CT Control | TT Case | TT Control | C Case | C Control | T Case | T Control |
|------------------------------|----------|---------|------------|---------|------------|---------|------------|--------|-----------|--------|-----------|
| James et al., 1999          | Canada   | 24      | 15         | 22      | 34         | 4       | 8          | 70     | 64        | 30     | 50        |
| Hobbs et al., 2000          | America  | 51      | 67         | 84      | 59         | 22      | 14         | 186    | 193       | 128    | 87        |
| Chadeaux-Vekemans et al., 2002 | France   | 36      | 29         | 42      | 30         | 7       | 11         | 114    | 88        | 56     | 52        |
| O’Leary et al., 2002        | Ireland  | 18      | 90         | 21      | 84         | 2       | 18         | 57     | 264       | 25     | 120       |
| Stuppia et al., 2002        | Italy    | 20      | 27         | 32      | 62         | 12      | 23         | 72     | 116       | 56     | 108       |
| Boduroglu et al., 2004      | Turkey   | 86      | 58         | 55      | 30         | 17      | 3          | 227    | 146       | 89     | 36        |
| Acacio et al., 2005         | Brazil   | 35      | 54         | 30      | 25         | 5       | 9          | 100    | 133       | 40     | 43        |
| Da Silva et al., 2005       | Brazil   | 67      | 84         | 72      | 67         | 15      | 7          | 206    | 235       | 102    | 81        |
| Coppede et al., 2006        | Italy    | 20      | 39         | 43      | 54         | 16      | 18         | 83     | 132       | 75     | 90        |
| Liang et al., 2006          | China    | 7       | 16         | 20      | 34         | 3       | 20         | 34     | 66        | 26     | 74        |
| Rai et al., 2006            | India    | 97      | 124        | 40      | 39         | 12      | 2          | 234    | 287       | 64     | 43        |
| Scala et al., 2006          | Italy    | 31      | 74         | 39      | 125        | 24      | 57         | 101    | 273       | 87     | 239       |
| Wang et al., 2007           | China    | 28      | 48         | 52      | 42         | 20      | 10         | 108    | 138       | 92     | 62        |
| Biselli et al., 2008        | Brazil   | 29      | 100        | 35      | 77         | 8       | 17         | 93     | 229       | 71     | 39        |
| Kohli et al., 2008          | India    | 74      | 71         | 29      | 32         | 0       | 6          | 177    | 174       | 29     | 44        |
| Martinez-Frias et al., 2008 | Spain    | 61      | 76         | 61      | 85         | 24      | 27         | 183    | 237       | 109    | 139       |
| Meguid et al., 2008         | Egypt    | 20      | 33         | 17      | 12         | 5       | 3          | 57     | 78        | 27     | 18        |
| Santos-Reboucas et al., 2008| Brazil   | 51      | 49         | 43      | 47         | 9       | 12         | 145    | 145       | 61     | 71        |
| Wang et al., 2008           | China    | 14      | 36         | 32      | 29         | 18      | 5          | 60     | 101       | 68     | 39        |
| Brandalize et al., 2009     | Brazil   | 94      | 86         | 113     | 93         | 32      | 18         | 301    | 265       | 177    | 129       |
| Coppede et al., 2009        | Italy    | 25      | 40         | 52      | 55         | 17      | 18         | 102    | 135       | 86     | 91        |
| Cyril et al., 2009          | India    | 33      | 60         | 3       | 0          | 0       | 0          | 69     | 120       | 3      | 0         |
| Kokotas et al., 2009        | Denmark  | 92      | 445        | 72      | 449        | 13      | 90         | 256    | 1339      | 98     | 629       |
| Pozzi et al., 2009          | Italy    | 28      | 62         | 30      | 93         | 16      | 29         | 86     | 217       | 62     | 151       |
| Coppede et al., 2010        | Italy    | 5       | 11         | 19      | 17         | 5       | 4          | 29     | 39        | 29     | 25        |
| Liao et al., 2010           | China    | 12      | 23         | 26      | 33         | 22      | 12         | 50     | 79        | 70     | 57        |
| Vaneckovig et al., 2010     | Croatia  | 49      | 66         | 49      | 64         | 13      | 11         | 147    | 196       | 75     | 86        |
| Bozovic et al., 2011        | Croatia  | 46      | 101        | 55      | 97         | 11      | 23         | 147    | 299       | 77     | 143       |
| Sadiq et al., 2011          | Jordan   | 23      | 23         | 27      | 5          | 3       | 1          | 73     | 51        | 33     | 7         |
| Tayeb, 2012                 | Saudi Arabia | 16  | 22         | 10      | 14         | 4       | 4          | 42     | 58        | 18     | 22        |
| Zampieri et al., 2012       | Brazil   | 40      | 94         | 55      | 73         | 10      | 18         | 135    | 261       | 75     | 109       |
| Kaur & Kaur, 2013           | India    | 86      | 89         | 22      | 22         | 2       | 0          | 194    | 200       | 26     | 22        |
| Pandey et al., 2013         | India    | 67      | 87         | 12      | 9          | 2       | 3          | 146    | 183       | 16     | 15        |
| Elsayed et al., 2014        | Egypt    | 11      | 30         | 12      | 24         | 3       | 7          | 34     | 84        | 18     | 38        |

doi:10.1371/journal.pone.0108552.t002
Inclusion criteria

Included studies had to meet the following criteria: (1) article should be published; (2) article should have sufficient data to calculate the odds ratio with 95% CI; (3) article should be case control association study; and (4) author should describe the genotyping protocols.

Data extraction

The following data were extracted from each study: first author's name, publication year, journal name, country name, genotyping method, and different MTHFR genotype numbers.

Meta-analysis

Statistical analysis of maternal MTHFR C677T polymorphism and DS risk was estimated by Odds ratio (ORs) with 95% confidence intervals (CIs). The heterogeneity was tested by the Q-statistics with p-values <0.05. Subgroup analysis was done to know the source of heterogeneity. If higher heterogeneity (I^2 > 50%) would be observed, the random effect model [22] would be applied. Otherwise, fixed-effect model [23] was applied to obtain the summary OR and 95% CI. All p values were two-sided and a p value of less than 0.05 was considered statistically significant. All analyses were performed using the computer program MIX version 1.7 [24]. The control genotypes were tested for Hardy-Weinberg equilibrium (HWE) using the Goodness of fit Chi-square test. The quality of the included studies was measured according to the scoring system for randomized controlled association studies proposed by Clark and Baudouin [25]. Case control studies scoring <5 were defined as low quality study and those ≥5 were defined as high quality study.

Publication bias

Funnel plots of precision by log (OR) and standard error by log (OR) were plotted to determine publication bias and asymmetrical funnel plots represent publication bias. Begg and Mazumdar rank correlation [26] and Egger’s regression intercept [27] tests were adopted to assess the publication bias.

Results

Eligible Studies

With our original search criterion, 85 articles were found. After reviewing each original article, 50 publications were excluded including reviews, case studies, editorials etc. (Figure 1). Following these exclusions, 34 individual case-control studies with a total of
### Table 3. 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), the $I^2$ metric and publication bias p-value (Egger Test) in total studies, Asian, American and European studies.

| Genetic Contrast                        | Fixed effect OR (95% CI), p | Random effect OR (95% CI), p | Heterogeneity p-value (Q test) | $I^2$ (%) | Publication Bias p-value (p of Egger's test) |
|----------------------------------------|-----------------------------|-----------------------------|--------------------------------|----------|---------------------------------------------|
| All Allele Contrast (T vs. C)          | 1.22 (1.13–1.31), <0.0001  | 1.26 (1.09–1.46), 0.001     | <0.0001                       | 69.42    | 0.14                                        |
| Co-dominant (CT vs. CC)                | 1.23 (1.11–1.36), <0.0001  | 1.29 (1.10–1.51), 0.001     | 0.0002                         | 52.49    | 0.02                                        |
| Homozygote (TT vs. CC)                 | 1.44 (1.22–1.69), <0.0001  | 1.49 (1.13–1.97), 0.008     | <0.0001                       | 57.3     | 0.56                                        |
| Dominant (TT+CT vs. CC)                | 1.28 (1.16–1.41), <0.0001  | 1.35 (1.13–1.60), 0.0008    | <0.0001                       | 63.56    | 0.05                                        |
| Recessive (CT+CC vs. TT)               | 0.76 (0.65–0.88), 0.0004   | 0.76 (0.60–0.94), 0.01      | 0.0044                         | 43.68    | 0.926                                       |
| All Allele Contrast (T vs. C)          | 1.53 (1.29–1.82), <0.0001  | 1.52 (1.09–2.1), 0.01       | 0.003                          | 69.43    | 0.82                                        |
| Co-dominant (CT vs. CC)                | 1.52 (1.21–1.91), 0.0003   | 1.57 (1.14–2.14), 0.005     | 0.09                           | 38.05    | 0.11                                        |
| Homozygote (TT vs. CC)                 | 2.41 (1.62–3.59), <0.0001  | 2.21 (1.03–4.76), 0.0411    | 0.0074                         | 60.04    | 0.204                                       |
| Dominant (TT+CT vs. CC)                | 1.64 (1.32–2.0), <0.0001   | 1.70 (1.18–2.4), 0.004      | 0.01                           | 56.67    | 0.30                                        |
| Recessive (CT+CC vs. TT)               | 0.54 (0.37–0.78), <0.0001  | 0.58 (0.29–1.16), 0.12      | 0.0094                         | 58.77    | 0.334                                       |
| All Allele Contrast (T vs. C)          | 1.23 (1.07–1.39), 0.003    | 1.19 (0.99–1.44), 0.06      | 0.06                           | 47.69    | 0.11                                        |
| Co-dominant (CT vs. CC)                | 1.42 (1.17–1.71), 0.0002   | 1.42 (0.97–2.06), 0.066     | 0.005                          | 73.15    | 0.908                                       |
| Homozygote (TT vs. CC)                 | 1.68 (1.24–2.3), 0.0008    | 1.58 (0.84–2.95), 0.148     | 0.007                          | 72.07    | 0.667                                       |
| Dominant (TT+CT vs. CC)                | 1.48 (1.24–1.76), <0.0001  | 1.44 (0.95–2.19), 0.078     | <0.0001                       | 80.11    | 0.782                                       |
| Recessive (CT+CC vs. TT)               | 0.69 (0.51–0.92), 0.0356   | 0.72 (0.44–1.18), 0.203     | 0.0159                         | 59.42    | 0.753                                       |
| All Allele Contrast (T vs. C)          | 1.03 (0.93–1.15), 0.482    | 1.04 (0.93–1.16), 0.451     | 0.3576                         | 8.81     | 0.084                                       |
| Co-dominant (CT vs. CC)                | 0.99 (0.85–1.16), 0.956    | 1.00 (0.85–1.17), 0.992     | 0.3774                         | 6.87     | 0.090                                       |
| Homozygote (TT vs. CC)                 | 1.09 (0.87–1.37), 0.422    | 1.09 (0.85–1.40), 0.455     | 0.3715                         | 7.45     | 0.329                                       |
| Dominant (TT+CT vs. CC)                | 1.02 (0.88–1.17), 0.787    | 1.03 (0.87–1.21), 0.704     | 0.308                          | 13.58    | 0.041                                       |
| Recessive (CT+CC vs. TT)               | 0.90 (0.73–1.10), 0.322    | 0.90 (0.72–1.11), 0.339     | 0.570                          | 0        | 0.948                                       |

doi:10.1371/journal.pone.0108552.t003
3,098 cases and 4,852 controls were found to be suitable for inclusion into meta-analysis and listed in Table 1 (Figure 1). These studies were published between 1999 and 2013. All these thirty four studies were performed in different countries - Brazil [28–33], China [4,34–36], Croatia [8,37], Egypt [38,39], France [40], India [1,5,41–43], Ireland [44], Italy [7,13,45–48], Jordan [49], Netherlands [50], Saudi Arabia [2], Spain [51], Turkey [52] and USA [3,6] (Table 1).

Characteristics of included studies

In thirty four studies included in the present meta-analysis, the smallest case sample size was 26 [39] and highest sample size was 239 [32]. ORs for more than one were reported in twenty four articles [1,2,4–6,8,13,28–30,32,33,35–39,42,43,46–49,51,52]. Except two studies [28,43], control populations of all articles were in Hardy-Weinberg equilibrium.

In all thirty four studies, total cases were 3,098 with CC (1,396), CT (1,326) and TT (376), and controls were 4,852 with CC (2,329), CT (2,015), and TT (508) genotypes. In controls genotypes, percentage of CC, CT and TT were 48.00%, 41.53%, and 10.47% respectively. In total cases, genotype percentage of CC, CT, and TT was 45.06%, 42.8% and 12.14% respectively. Frequencies of CC and CT genotypes were highest in both cases and controls (Table 2). In cases and controls, the allele C was the most common. All five genetic models; allele contrast (T vs C) homozygote (TT vs CC), codominant (CT vs CC), dominant (TT + CT vs CC) and recessive (TT vs CT + CC) models were used to evaluate C677T polymorphism as DS risk.

Meta-analysis

Meta-analysis with allele contrast showed significant association between maternal 677T allele and DS with both fixed effect (OR_{T vs C} = 1.22; 95% CI = 1.13–1.31; p = <0.0001) and random effect models (OR_{T vs C} = 1.26; 95% CI = 1.09–1.45; p = 0.001) (Figure 2) (Table 3). In cumulative meta-analysis using random effect model, the association of maternal T allele with DS turned statistically significant with the addition of study of Wang et al. (2008) and remained significant thereafter.

Table 3 summarizes the ORs with corresponding 95% CIs for association between maternal C677T polymorphism and risk of DS in dominant, recessive, homozygote and co-dominant models. With our primary analysis, there was an increased risk of DS among mutant homozygote variants (TT), with both fixed (OR_{TT vs CC} = 1.44; 95% CI = 1.22–1.69, p = <0.0001) and ran-
dom (OR\textsubscript{TTvs.CC} = 1.49; 95% CI = 1.13–1.97, p = 0.008) effect models with moderate statistical heterogeneity between-study (Figure 3). Association of mutant heterozygous genotype (CT vs. CC) was observed significant with fixed (OR\textsubscript{CTvs.CC} = 1.23; 95% CI = 1.11–1.36; p = <0.0001) and random (OR\textsubscript{CTvs.CC} = 1.29; 95% CI = 1.10–1.51; p = 0.001) effect models. Similarly combined mutant genotypes (TT + CT vs. CC) showed significant association with DS using both fixed (OR\textsubscript{TT+CTvs.CC} = 1.28; 95% CI = 1.16–1.41; p = <0.0001) and random (OR\textsubscript{TT+CTvs.CC} = 1.35; 95% CI = 1.13–1.60; p = 0.0008) effect models (Figure 4).

### Stratified analysis

We also performed sub-group analysis which is based on geographic distribution of population. Out of 34 studies included in present meta-analysis, 11 studies were from Asia, 13 from Europe, 8 from America and 2 from Africa. The subgroup analysis by geographical regions revealed that the significant association between the maternal \textit{MTHFR} C677T polymorphism and DS existed in Asian population (for T vs. C: OR = 1.51; 95% CI = 1.09–2.10; p = 0.01; I\textsuperscript{2} = 69.43%; P\textsubscript{heterogeneity} = 0.0003; P\textsubscript{heterogeneity} = 0.82) (Figure 5; Table 3). Except allele contrast model of American population (T vs. C: OR = 1.23; 95% CI = 1.07–1.39; p = 0.003; I\textsuperscript{2} = 47.69%; P\textsubscript{heterogeneity} = 0.06; P\textsubscript{heterogeneity} = 0.11) (Figure 6) no significant association was found in American and European population (for T vs. C: OR = 1.03; 95% CI = 0.93–1.15; p = 0.482; I\textsuperscript{2} = 8.81%; P\textsubscript{heterogeneity} = 0.357; P\textsubscript{heterogeneity} = 0.084) (Figures 7; Table 3).

### Heterogeneity and Sensitive analysis

A true heterogeneity existed between studies for allele (P\textsubscript{heterogeneity} = <0.0001, Q = 107.92, df = 33, I\textsuperscript{2} = 69.42%, t\textsuperscript{2} = 0.12) and mutant genotypes (P\textsubscript{heterogeneity} = <0.0001, Q = 74.90, df = 32, I\textsuperscript{2} = 57.3%, t\textsuperscript{2} = 0.10) comparisons. The t\textsuperscript{2} value of more than 50% for between studies comparison in both allele and genotype analysis shows high level of true heterogeneity. In Asian (P\textsubscript{heterogeneity} = 0.0003, I\textsuperscript{2} = 67.43%) and American (P\textsubscript{heterogeneity} = 0.0003, I\textsuperscript{2} = 83.25%) allele contrast meta-analysis significant high heterogeneity was observed, in European sub-group meta-analysis low heterogeneity was observed (P\textsubscript{heterogeneity} = 0.357, I\textsuperscript{2} = 8.81) in allele contrast model.

In allele contrast meta-analysis, sensitivity analysis performed by exclusion of the studies in which control population was not in Hardy Weinberg equilibrium, studies with small sample size and studies with high p values. Control population of only two studies...
were not in HW equilibrium and heterogeneity did not decrease after exclusion of these studies (p = 0.0001, I² = 70.00%). Exclusion of seven studies with small sample size, less than 50 (O’Leary et al. [44], n = 41; Liang et al. [34], n = 30; Mequid et al [38], n = 42; Cyril et al. [42], n = 36; Coppede et al. [48], n = 29; Tayeb [2], n = 30; Elsayed et al. [39], n = 26), also did not decrease heterogeneity (P heterogeneity = 0.0001, I² = 72.98%). Similarly exclusion of eleven studies with very high p value (O’Leary et al. [44], p = 0.87; Acacio et al. [28], p = 0.40; Scala et al. [7], p = 0.91; Martinez-Frias et al. [51], p = 0.90; Pozzi et al. [13], p = 0.84; Vranekoviz et al. [37], p = 0.43; Bozovic et al. [8], p = 0.38; Tayeb [2], p = 0.74; Elsayed et al. [39], p = 0.65; Kaur and Kaur [5], p = 0.52; Pandey et al. [43], p = 0.44) did not decrease heterogeneity but increased odds ratio (OR = 1.29, 95% CI = 1.18–1.41, p = <0.0001).

Publication bias

Publication bias was not observed in allele contrast, homozygote, dominant and recessive models (Begg’s p = 0.28, Egger’s p = 0.14 for T versus C; Begg’s p = 0.38, Egger’s p = 0.56 for TT versus CC; Begg’s p = 0.13, Egger’s p = 0.05 for TT+CT versus CC and Begg’s p = 0.19, Egger’s p = 0.05 for TT versus CC+CT) but publication bias was observed in co-dominant model (Begg’s p = 0.04, Egger’s p = 0.02 for CT versus CC) of overall by using Begg’s and Egger’s test (Table 3). Funnel plots were showed in Figures 8 and 9.

Discussion

In 1999, James et al [3] reported that genetic polymorphism of folate and homocysteine pathway enzymes predispose a woman to abnormal chromosome segregation, which act as risk factor for DS pregnancy. In subsequent years, several in vivo studies in humans suggested that chronic folate deficiency has been associated with abnormal DNA methylation [11,53,54], and aberrant chromosome segregation [6,55–59]. Population-based studies have shown that folic acid intake during fetal development has a protective effect, resulting in a significant reduction in the

![Forest plots (Random effect) showed significant association between MTHFR C677T polymorphism and risk of Down syndrome in Asian studies using allele contrast model (T versus C). Results of individual and summary OR estimates and 95% CI of each study were shown. doi:10.1371/journal.pone.0108552.g005](image-url)
Figure 6. Forest plots (Random effect) showed no association between MTHFR C677T polymorphism and risk of Down syndrome in American studies using allele contrast model (T versus C). Results of individual and summary OR estimates and 95% CI of each study were shown. doi:10.1371/journal.pone.0108552.g006

Figure 7. Forest plots (Fixed effect) showed no association between MTHFR C677T polymorphism and risk of Down syndrome in European studies using allele contrast model (T versus C). Results of individual and summary OR estimates and 95% CI of each study were shown. Horizontal lines represented 95% CI, and dotted vertical lines represent the value of the summary OR. doi:10.1371/journal.pone.0108552.g007
occurrence of developmental defects, like neural tube defects (NTD), congenital heart defects, limb defects, and orofacial clefts [60].

Meta-analysis is a powerful tool for analyzing cumulative data with small and low power studies. Several meta-analyses were published accessing *MTHFR* as risk factor to various diseases.
Figure 9. Funnel plots a–f. a. Precision by log odds ratio for additive model; b. standard error by log odds ratio for additive model for Asian studies; c. precision by log odds ratio for additive model; d. standard error by log odds ratio for additive model for American studies; e. precision by log odds ratio for additive model; f. standard error by log odds ratio for additive model for European studies.

doi:10.1371/journal.pone.0108552.g009
disorders like neural tube defects [61,62], cleft lip and palate [63], stroke [64], psychiatric disorders [65]. During literature search, we identified four meta-analyses [66–69] published between 2007 and 2013. They examined the effect of maternal MTHFR C677T as DS risk, but no consistent conclusion was achieved. Zintzaras [66] performed a meta-analysis based on eleven studies and did not find any significant association between the maternal MTHFR polymorphisms and DS risk. Medica et al. [67] aggregated sixteen studies and reported significant relationship between the maternal mutant genotypes (TT+CT vs CC) and risk of DS child. Recently, Wu et al. [68] published a meta-analysis (included twenty eight studies with 2806 cases/4597 controls), and found statistical association with dominant model (OR = 1.305, 95% CI = 1.125–1.514, p = 0, p = 0.003). Yang et al. [69] performed a meta-analysis which was based on twenty six studies (2458 cases/3144 controls) and found statistically significant association in allele contrast model (OR = 1.28; 95% CI: 1.11–1.47) (Table 4). Several newly published studies were not included in the previous published meta-analyses. So authors conducted a comprehensive meta-analysis with the largest number of studies (34 studies). In the present meta-analysis significant association was found between maternal C677T polymorphism and DS risk in total 34 studies using all five genetic models. Whereas in stratified analysis, except allele contrast model in American population, no significant association was observed in European and American population but significant higher risk was found in Asian population. Such phenomenon probably could be ascribed to the folate metabolism profile and dietary structure of different regions.

There are few limitations of the present meta-analysis like: i) we used crude ORs in the pooled analysis without adjustment; ii) the relatively small sample size in some of the included studies, especially those from Asia; iii) we considered only one gene polymorphism (MTHFR C677T) of folate pathway. Present meta-analysis had several advantages/strength to the previous published meta-analyses like: (i) the publication bias was not detected in present meta-analysis, (ii) pooled number of cases and controls from different studies significantly increased the statistical power of the analysis, (iii) largest number of studies (34 studies) with largest sample size (3,098 cases and 4,852 controls) was included in the present meta-analysis, (iv) controls included in the present meta-analysis was mothers of healthy child, (v) distribution of genotypes in control mothers except two studies was in Hardy-Weinberg equilibrium, (vi) significant association was found between maternal MTHFR C677T polymorphism and DS risk in allelic contrast, homozygote, co-dominant and dominant genetic models and (vii) in addition we did sub-group analysis according to geographical regions.

In conclusion, results of present meta-analysis suggest that the maternal MTHFR 677T allele is a risk factor for development of DS pregnancy. However the results of present meta-analysis were based on single gene polymorphism and significant heterogeneity was also observed; hence results should be interpreted with caution.

Supporting Information

Checklist S1 PRISMA checklist.

Author Contributions

Conceived and designed the experiments: VR, PK, OPM. Performed the experiments: UY, SKY. Analyzed the data: VR, UY. Contributed reagents/materials/analysis tools: VR, UY. Wrote the paper: VR.
References

1. Rai AK, Singh S, Mehta S, Kumar A, PandeyLK, et al. (2006) MTHFR C677T and A1298C polymorphisms are risk factors for Down’s syndrome in Indian maternal control study from South India. Indian J Hum Genet 15: 60–64.

2. Tayeb MT (2012) The methylenetetrahydrofolate reductase gene variant (C677T) in risk mothers with Down syndrome. Am J Med Genet 10: 388–390.

3. James SJ, Pogrund M, Pogrund IP, Melnyk S, Hine RJ, et al. (1999) Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 70: 495–500.

4. Wang SS, Qiao F, Feng L, Juan-Juan LV (2005) Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome in China. J Zhejiang Univ Sci B 9: 97–100.

5. Kaur A, Kaur A (2013) Prevalence of methylenetetrahydrofolate reductase C677T polymorphism among mothers of Down syndrome children. Indian J Hum Genet 19(4): 412–414.

6. He HY, CA, Sherman SI, YP, Torri CP, Hine RJ, et al. (2000) Polymorphism in genes involved in folate metabolism as maternal risk factors for Down syndrome. Am J Hum Genet 67: 623–630.

7. Scala I, Granese B, Selliuto M, Salome S, Sammartino A, et al. (2006) Analysis of seven maternal polymorphisms of genes involved in homocysteine/folate metabolism and risk of Down syndrome offspring. Genet Med 8: 409–416.

8. Bozöczi JB, Vranekovicz J, Czigamevic N, Melnyk S, Pogrund M, and Hine RJ (2011) MTHFR C677T and A1298C polymorphisms as a risk factor for congenital heart defects. Pediatr Int 53(4): 546–550.

9. James SJ, Pogrund M, Pogrund S, Pogrund M, and Caudill MA (2002) Elevated levels of S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. J Nutr 132(8 Suppl): 2361S–2366S.

10. Pogrund IP, James SJ, Jernigan SF, Pogribny I, and Yi P, et al. (2001) Homocysteine metabolism in children with Down syndrome: in vitro modulation. Am J Hum Genet 69: 88–95.

11. Parry EM, Parry JM, Corso C, Doherty A, Haddad F, et al. (2002) Detection and characterization of mechanisms of action of aneugenic chemicals. Mutagenesis. 17(6): 509–521.

12. Pozzi E, Vergani P, Dalpra L, Cervini R, Silvestri D, et al. (2009) Maternal polymorphisms for methylenetetrahydrofolate reductase and methionine synthase reductase and risk of children with Down syndrome. Am J Obstet Gynecol 63: e1–e6.

13. Froost PR, Blom HJ, Milos R, Goyette P, et al. (2000) The MTHFR C677T polymorphism in north Indian mothers having babies with Trisomy 21 Down syndrome. DNA Seq 11: 239–244.

14. Bagley TJ, Schuh J (1998) A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of fortylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci USA 95(22): 13217–13220.

15. Brattström L, Wilckén DE, Ohrvik J, Brudin L (1998) Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation 98(23): 2520–2526.

16. van der Put NM, Eik PK, Aap HJH (1997) Is the common 677CT mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. Q J Med 90: 111–113.

17. Blanton SH, Heeny RR, Yuan Q, Mulliken JB, Stal S, et al. (2011) Folate pathway and nonsyndromic cleft lip and palate. Birth Defects Res A Clin Mol Teratol 93: 276–289.

18. Hua Y, Zhao H, Kong Y, Ye M (2011) Association between the MTHFR gene and Alzheimer’s disease: a meta-analysis. Int J Neurosci 121(8): 462–71.

19. Renes F, Kankova M, Muzik J, Grelw M, Benejst et al. (2003) Methylenetetrahydrofolate reductase polymorphism, type II diabetes mellitus, coronary artery disease, and essential hypertension in the Czech population. Mol Genet Metab 73: 188–195.

20. Jonsson EG, Larsson K, Vares M, Hanesen T, Wang AG, et al. (2000) Two methylenetetrahydrofolate reductase polymorphisms, schizophrenia and bipolar disorder: an association study. Am J Med Genet B Neuropsychiatr Genet 147B: 976–982.

21. DerSimovian R, Laird N (1986) Meta-analysis in clinical trials. Controlled Clinical Trials. 7: 177–188.

22. Mantel N, Haenszel W (1959) Statistical analyses of the data from retrospective studies of disease. J Natl Cancer Inst 22: 719–748.

23. Bax L, Yu LM, Ikeda N, Tsuruta H, Moussy KG (2006) Development and validation of a comprehensive free software for meta-analysis of causal research data. BMC Med Res Methodol. 2006: 50.

24. Clark MF, Baudouin SV (2006) A systematic review of the quality of genetic association studies in human sepsis. Intensive Care Med 32(11): 1706–1712.

25. Martinz-Frías ML (2008) The biochemical structure and function of methylenetetrahydrofolate reductase provide the rationale to interpret the
epidemiological results on the risk for infants with Down syndrome. Am J Med Genet A 146A(11): 1477–1482.

52. Boduroğlu K, Alany Y, Köln B, Tunçbilek E (2004) Methylene tetrahydrofolate reductase enzyme polymorphisms as maternal risk for Down syndrome among Turkish women. Am J Med Genet A 127A: 5–10.

53. Balaghi M, Wagner C (1993) DNA methylation in folate deficiency: use of Cpg methylase. Biochemical and Biophysical Research Communications 193: 1184–1190.

54. Fencs M (2001) The role of folic acid and Vitamin B12 in genomic stability of human cells. Mutat Res 475: 57–67.

55. Libbus BL, Borman LS, Ventrone CH, Branda RF (1990) Nutritional folate deficiency in CHO cells: chromosomal abnormalities associated with perturbations in nucleic acid precursors. Cancer Genet Cytogenet 46: 231–242.

56. Leyton BL, Mergudich D, del la Torre D, Sans J (1995) Impaired chromosome segregation in plant anaphase alter moderate hypomethylation of DNA. Cell Prolif 28: 481–496.

57. Pogribny IP, Basnakian AG, Miller BJ, Lopatina NG, Poirier LA, et al. (1995) Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. Cancer Res 55(9): 1894–1901.

58. Chen RZ, Pettersson U, Beared C, Jackson-Gruzby I, Jaenisch R (1998) DNA hypomethylation leads to elevated mutation rates. Nature 393: 89–93.

59. Titienko-Holland N, Jacob RA, Shang N, Balaraman A, Smith MT (1998) Micronuclei in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. Mutat Res 417: 101–114.

60. Boto LD, Yang Q (2000) 5, 10-methylenetetrahydrofolate reductase variants and congenital anomalies: A huge review. Am J Epidemiol 151: 862–877.

61. Zhang T, Lou J, Zhong R, Wu J, Zou L, et al. (2013) Genetic Variants in the Folate Pathway and the Risk of Neural Tube Defects: A Meta-Analysis of the Published Literature. PLoS One 8: e59570.

62. Yadav U, Kumar P, Yadav SK, Mishra OP, Rai V (2014) Polymorphisms in folate metabolism genes as maternal risk factor for Neural Tube Defects: an updated meta-analysis. Metab Brain Dis. [Ahead of Print; DOI: 10.1007/s11011-014-9575-7].

63. Zhao M, Ren Y, Shen L, Zhang Y, Zhou B (2014) Association between MTHFR C677T and A1298C Polymorphisms and NSCL/P Risk in Asians: A Meta-Analysis. PloS One 9(3): e88242.

64. Yadav S, Hasan N, Marjot T, Khan MS, Prasad K, et al. (2013) Detailed Analysis of Gene Polymorphisms Associated with Ischemic Stroke in South Asians. PLoS One 8: e37305.

65. Peerbooms OL, van Oo J, Drukker M, Kenis G, Hoogveld L, et al. (2011) Meta-analysis of MTHFR gene variants in schizophrenia, bipolar disorder and unipolar depressive disorder: evidence for a common genetic vulnerability? Brain Behav Immun 25(8): 1530–1543.

66. Zintzaras E (2007) Maternal gene polymorphisms involved in folate metabolism and risk of Down syndrome offspring: a meta-analysis. J Hum Genet 52: 943–953.

67. Medica I, Maver A, Augusto GF, Peterlin B (2009) Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome: meta-analysis. Cent Eur J Med 4: 395–408.

68. Wu X, Wang X, Guan Y, Jia S, Lao Y, et al. (2013) Folate metabolism gene polymorphisms MTHFR C677T and A1298C and risk for Down syndrome offspring: a meta-analysis. Eur J Obstet Gynecol Reprod Biol 167(2): 154–159.

69. Yang M, Gong T, Lin X, Qi L, Guo Y, et al. (2013) Maternal gene polymorphisms involved in folate metabolism and the risk of having a Down syndrome offspring: a meta-analysis. Mutagenesis 28(6): 661–671.