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# Association between Maternal and Fetal MTHFR C677T and MTRR A66G Polymorphisms with the Risk of NTDs: A Systematic Review and Meta-Analysis Study

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**Abstract**

**Background:** Neural tube defects (NTDs) are classed as multifactorial birth defects of the brain and spinal cord that arise during embryonic development. Although the etiology is not well understood, NTDs are reported to be prevented by maternal folic acid supplementation before and during early pregnancy.

**Objectives:** This meta-analysis study aimed to assess the association between fetal and maternal methylenetetrahydrofolate reductase (MTHFR) C677T and methionine synthase reductase (MTRR) A66G polymorphisms with the risk of NTDs.

**Methods:** The PubMed, Scopus, and Springer Link databases were searched (from March 2000 to November 2020) for the literature on the association between MTHFR C677T and MTRR A66G polymorphisms with the risk of NTDs.

**Results:** In total, 33 studies were reviewed in the present study, and it was revealed that, unlike MTRR A66G polymorphism, MTHFR C677T was statistically associated with the risk of NTDs in the overall population. The results of subgroup analysis showed that the Indian subcontinent subgroup with maternal MTHFR C677T polymorphism and the European subgroup with fetal MTHFR C677T polymorphism was significantly susceptible to NTDs.

**Conclusion:** The obtained results revealed that, unlike MTRR A66G, maternal and fetal MTHFR C677T polymorphisms were significantly associated with NTDs. Subgroup analysis also demonstrated that folic acid deprivation can be considered the main cause of MTHFR C677T polymorphism in some areas.

*Keywords:* Methionine synthase reductase, Methylenetetrahydrofolate reductase, Neural tube defects, Polymorphism

## 1. Background

The neural tube defects (NTDs) are among the most important congenital malformations occurring during the early stage of embryogenesis. NTDs occurs by a failure in neural tube closure and its prevalence is about 1 per 1000 birth (1, 2). The NTDs are classified into two major groups including anencephaly and spina bifida. The precise mechanism of NTDs is not fully elucidated; however, it has been suggested that environmental and genetic factors are involved in its incidence (3). The results of previous studies revealed that folic acid deficiency and high levels of homocysteine might be associated with the risk of NTDs (4, 5). Based on epidemiological studies, hyperhomocysteinemia is an emerging risk factor for cardiovascular diseases and neural tube abnormalities. In addition, the consumption of folic acid supplementation by pregnant women reduces the risk of NTDs in fetuses. Considering the role of folic acid in the occurrence of NTDs, the enzymes involved in folate metabolism might be important in NTDs pathogenesis and single nucleotide polymorphisms (SNPs) (4, 6, 7). One of these enzymes is methylenetetrahydrofolate reductase (MTHFR) which prompts the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and has a key role in folic acid metabolism (8). This enzyme has also a pivotal role in the synthesis of DNA and RNA, and protein metabolism (9). MTHFR gene is located on chromosome 1 (1p36.3) and has been documented for 14 rare and one common mutation (C677T, rs1801133) (10). C677T polymorphism is a point mutation at nucleotide 677 of the MTHFR gene which triggers the conversion of C nucleotide to T nucleotide and substitution of alanine with valine in MTHFR protein (8, 11).

Methionine synthase reductase (MTRR) is another enzyme maintaining methylcobalamin (cofactor of methionine synthase) in sufficient levels. This enzyme catalyzes the methylation of cobalamin using S-adenosylmethionine as a methyl donor and ultimately restores methionine synthase activity (12, 13). MTRR gene is mapped on chromosome 5p15.2-p15.3 (14). A66G is a single nucleotide polymorphism (rs1801394) in the MTRR gene that provokes the substitution of isoleucine with methionine at position 22 (13). It has been previously documented that MTRR polymorphism increases the risk of NTDs (15-17).

## 2. Objectives

Regarding the potential role of mentioned polymorphisms on folate metabolism pathway and level, and considering the existing controversy in previous studies, the present meta-analysis study aimed to investigate the association of maternal and fetal MTHFR C677T and MTRR A66G with the risk of NTDs.

## 3. Methods

*3.1. Search strategy*

The association of maternal or fetal MTHFR C677T and maternal or fetal MTRR A66G polymorphisms with the risk of NTDs was evaluated independently by two authors who performed a comprehensive literature search in PubMed, Scopus, and Springer Link databases. The keywords used for systematic search in databases included MTHFR C677T, methylenetetrahydrofolate reductase, polymorphism OR variant OR SNPs, MTRR A66G, methionine synthase reductase, NTDs, neural tube defect, and spina bifida. Two authors independently reviewed literature from each study according to the name of the first author, the year of publication, a sample size of case and control groups, and allelic and genotype frequencies. Any conflict between the two authors was resolved by consultation with a third reviewer.

*3.2. Inclusion criteria*

The inclusion criteria in the present meta-analysis study included case-control studies which assessed the association of maternal or fetal MTHFR C677T and maternal or fetal MTRR A66G polymorphisms with NTDs risk. The literature was supposed to provide enough data for genotype or allelic frequencies in case and control groups. Eventually, only full texts studies with human subjects written in English were included in the present meta-analysis study.

*3.3. Exclusion criteria*

The exclusion criteria in the present study included 1) Studies conducted before 2000; 2) Animal studies; 3) Studies with insufficient information for allelic and genotype frequencies in case and control groups; 4) Non-English articles; and 5) Letters, reviews, hypothesis studies, and short communication articles.

*3.4. Statistical analysis*

The data were analyzed using STATA software (Version 16.0). The odds ratio (OR) with a 95% confidence interval (CI) was used to assess the association of maternal and fetal MTHFR C677T and MTRR A66G polymorphism with susceptibility to NTDs. The pooled ORs were calculated by the Random-effects model (Mantel-Hansel method) for allelic, homozygous, heterozygous, dominant, and recessive models. The heterogeneity in the present meta-analysis study was evaluated using I2, $\chi 2$, and $\tau 2$. The Forest plots were used to visualize the overall effects, and the Egger'test, Beggs's test (18), and funnel plot were adopted for the assessment of publication bias. Eventually, the literature was geographically subdivided into different sub-groups.

## 4. Results

*4.1. Literature characteristics*

The 573 articles were identified in PubMed, Scopus, and Springer link databases. According to the inclusion and exclusion criteria, authors reviewed the records based on their titles and their abstracts, and 531 literatures were excluded from the study. The selected studies were reviewed according to their full text and nine studies were removed subsequently. Ultimately, 33 articles were included in the present meta-analysis study (Figure 1). Tables 1 and 2 present the main characteristics of the included studies.

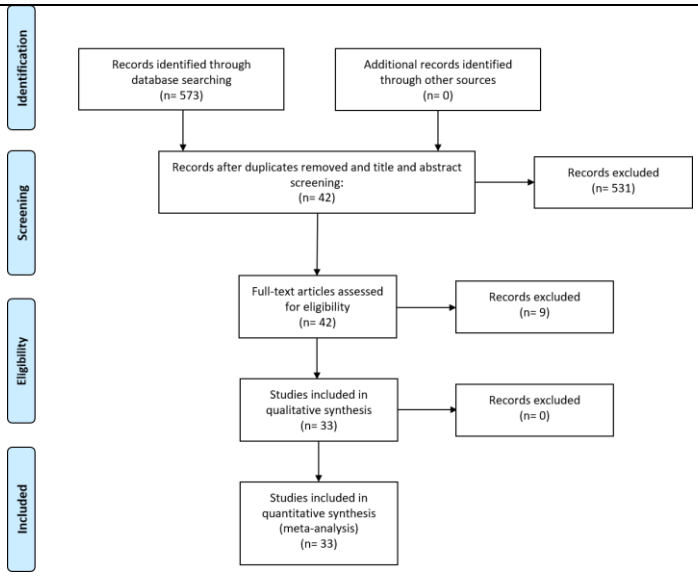


**Figure 1**. Flowchart of the procedure for literature search

**Table 1.** Characteristics of included studies for the association of maternal and fetal MTHFR C677T with neural tube defect risk

| Characteristics of included studies on the association of maternal and fetal MTHFR C677T with neural tube defect risk | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Author's name** | **Year** | **Country** | **Case (N)** | **Control (N)** | **CC** | **Case CT** | **TT** | **CC** | **Control CT** | **TT** |
| **Da´ valos (34)** | 2000 | Mexico | 68 | 101 | 19 | 35 | 14 | 31 | 51 | 19 |
| **Lucock (15)** | 2000 | United Kingdom | 19 | 31 | 8 | 9 | 2 | 11 | 17 | 3 |
| **Lucock (35)** | 2001 | United Kingdom | 21 | 28 | 9 | 9 | 3 | 12 | 13 | 3 |
| **martinez (36)** | 2001 | Mexico | 38 | 31 | 11 | 12 | 15 | 12 | 16 | 3 |
| **Garcia-Fragoso(37)** | 2002 | Puerto | 37 | 100 | 7 | 23 | 7 | 41 | 50 | 9 |
| **Arbour (38)** | 2002 | Canada | 74 | 101 | 32 | 31 | 11 | 52 | 38 | 11 |
| **Parle-McDermott (27)** | 2003 | Ireland | 277 | 255 | 105 | 138 | 36 | 125 | 104 | 26 |
| **Alvarez Perez (39)** | 2003 | Brazil (white) | 81 | 52 | 37 | 38 | 6 | 30 | 16 | 6 |
| **Alvarez Perez (39)** | 2003 | Brazil (nonwhite) | 50 | 74 | 37 | 12 | 1 | 51 | 20 | 3 |
| **Relton (40)** | 2004 | United Kingdom | 186 | 512 | 86 | 78 | 22 | 191 | 254 | 67 |
| **Felix (41)** | 2004 | Brazil | 41 | 44 | 19 | 15 | 7 | 16 | 22 | 6 |
| **Grandone (42)** | 2006 | Italy | 57 | 143 | 10 | 35 | 12 | 38 | 79 | 26 |
| **Dalal (43)** | 2007 | India | 101 | 60 | 56 | 21 | 26 | 45 | 12 | 3 |
| **Houcher (44)** | 2009 | Algeria | 48 | 147 | 14 | 25 | 9 | 67 | 59 | 21 |
| **Erdogan (45)** | 2010 | Turkey | 26 | 48 | 5 | 14 | 7 | 18 | 21 | 9 |
| **Naushad (46)** | 2010 | India | 50 | 80 | 33 | 11 | 6 | 64 | 16 | 0 |
| **Godbole (47)** | 2011 | India | 305 | 684 | 238 | 62 | 5 | 521 | 158 | 5 |
| **Husna (25)** | 2013 | Malaysia | 24 | 14 | 15 | 8 | 1 | 11 | 3 | 0 |
| **Zhi-zhen (48)** | 2013 | China | 51 | 66 | 10 | 25 | 16 | 22 | 22 | 7 |
| **Kondo (49)** | 2014 | Japan | 230 | 9034 | 47 | 56 | 12 | 1763 | 2060 | 694 |
| **Wang (50)** | 2015 | China | 144 | 300 | 31 | 73 | 40 | 96 | 159 | 45 |
| **Bourouba (51)** | 2018 | Algeria | 48 | 82 | 25 | 17 | 6 | 33 | 35 | 14 |
| **Nasri (32)** | 2018 | Tunisia | 77 | 71 | 21 | 41 | 15 | 31 | 34 | 6 |
| **Nauman (52)** | 2018 | Pakistan | 109 | 100 | 67 | 31 | 11 | 72 | 26 | 2 |
| **Cai (53)** | 2019 | China | 61 | 61 | 5 | 30 | 26 | 15 | 27 | 19 |
| Characteristics of included studies on the association of fetal MTHFR C677T with neural tube defect risk | | | | | | | | | | |
| **Garcia-Fragos (37)** | 2002 | USA | 31 | 100 | 10 | 18 | 3 | 41 | 50 | 9 |
| **Parle-McDermott (27)** | 2003 | Ireland | 279 | 255 | 108 | 118 | 53 | 125 | 104 | 26 |
| **Alvarez Perez (39)** | 2003 | Brail (white) | 81 | 51 | 35 | 38 | 8 | 29 | 15 | 7 |
| **Alvarez Perez (39)** | 2003 | Brazil (nonwhite) | 50 | 75 | 32 | 16 | 2 | 52 | 22 | 1 |
| **Relton (40)** | 2004 | United Kingdom | 200 | 578 | 92 | 78 | 30 | 267 | 247 | 64 |
| **Grandone (42)** | 2006 | Italy | 15 | 143 | 0 | 11 | 4 | 38 | 79 | 26 |
| **Erdogan (45)** | 2010 | Turkey | 33 | 48 | 13 | 16 | 4 | 18 | 21 | 9 |
| **Behunova (54)** | 2010 | Slovakia | 93 | 290 | 47 | 36 | 9 | 164 | 106 | 20 |
| **ESER(55)** | 2010 | Turkey | 39 | 34 | 18 | 14 | 7 | 19 | 17 | 8 |
| **Husna MZ (25)** | 2013 | Malaysia | 24 | 13 | 18 | 6 | 0 | 11 | 2 | 0 |
| **Fang (56)** | 2018 | China | 152 | 169 | 22 | 67 | 64 | 40 | 77 | 52 |

**Table 2.** Characteristics of included studies on the association of maternal and fetal MTRR A66G with neural tube defect risk

| Characteristics of included studies on the association of maternal MTRR A66G with neural tube defect risk | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Author's name** | **Year** | **Country** | **Case N** | **Control N** | **AA** | **Case AG** | **GG** | **AA** | **Control AG** | **GG** |
| **LUCOCK (35)** | 2001 | United Kingdom | 21 | 28 | 5 | 10 | 6 | 8 | 14 | 6 |
| **Relton (40)** | 2004 | United Kingdom | 203 | 532 | 208 | 107 | 68 | 58 | 263 | 211 |
| **Relton (57)** | 2004 | United Kingdom | 89 | 176 | 12 | 42 | 35 | 20 | 82 | 74 |
| **O'Leary (24)** | 2005 | Ireland | 447 | 476 | 149 | 215 | 83 | 178 | 222 | 76 |
| **Van an derLinden(58)** | 2006 | Netherlands | 116 | 264 | 18 | 45 | 53 | 53 | 135 | 76 |
| **Candito (24)** | 2008 | France | 77 | 61 | 16 | 39 | 22 | 22 | 25 | 14 |
| **Naushad(46)** | 2010 | India | 50 | 80 | 0 | 33 | 17 | 0 | 52 | 28 |
| **Abbas (31)** | 2016 | Algeria | 38 | 67 | 10 | 20 | 8 | 15 | 43 | 9 |
| **Nasri (32)** | 2018 | Tunisia | 62 | 64 | 16 | 34 | 12 | 7 | 44 | 13 |
| **Cai (53)** | 2019 | China | 61 | 61 | 22 | 31 | 8 | 33 | 24 | 4 |
| Characteristics of included studies on the association of fetal MTRR A66G with neural tube defect risk | | | | | | | | | | |
| **Relton (40)** | 2004 | Tunisia | 201 | 601 | 23 | 125 | 53 | 28 | 265 | 308 |
| **O'Leary (26)** | 2005 | Ireland | 470 | 476 | 149 | 240 | 81 | 178 | 222 | 76 |
| **Wang (59)** | 2015 | China | 165 | 280 | 45 | 91 | 29 | 105 | 139 | 36 |
| **Abbas (31)** | 2016 | Algeria | 48 | 66 | 20 | 25 | 3 | 14 | 43 | 9 |
| **Fang (56)** | 2018 | China | 151 | 169 | 66 | 67 | 18 | 95 | 62 | 11 |

*4.2. Evaluation of the association between maternal MTHFR C677T polymorphism and NTDs risk*

In total, 25 literature, including 2,225 cases and 12,204 controls were analyzed in the present meta-analysis study to assess the association of maternal MTHFR C677T polymorphism with the risk of NTDs. The random-effects model was used to evaluate the pooled OR. As indicated in Table 3 and Figure 2A, alleles and genotypes of maternal MTHFR C677T polymorphism were statically associated with NTDs. The allelic (log (OR)=1.344, 95% CI=1.147, 1.574) (P<0.001), homozygous (log (OR)=1.880, 95%

CI=1.360, 2.598) (P<0.001) and heterozygous (log (OR) =1.237, 95% CI=1.036, 1.477) (P=0.019) models showed significant correlation with the risk of NTDs. On the other hand, same results were observed in dominant (log (OR)=1.384, 95% CI=1.136, 1.689) (P<0.001) and recessive models (log (OR)=1.536, 95% CI=1.194, 1.977) (P<0.001).

*4.3. Evaluation of the association of fetal MTHFR C677T polymorphism with NTDs risk*

In total, 11 studies with 997 cases and 1,756 controls were included in the present meta-analysis to evaluate the association of fetal MTHFR C677T polymorphism with risk of NTDs. As presented in Table 3 and Figure 2 B, allelic (log (OR)=1.261, 95% CI=1.072, 1.485) (P=0.005), homozygous (log (OR)=1.6644, 95% CI=1.263, 2.140) (P<0.001), and heterozygous models (log (OR)=1.212, 95% CI=1.008, 1.456) (P=0.040) were associated with the risk of NTDs. In addition, the dominant (log (OR)=1.297, 95% CI=1.092, 1.540) (P=0.003) and recessive models (log (OR)=1.467, 95% CI=1.163, 1.850) (P<0.001) were significantly associated with the risk of NTDs.

*4.4. Evaluation of the association between maternal and fetal MTRR A66G polymorphism with NTDs risk*

In total, 10 literatures with 1,164 cases and 1,809 controls were included to assess the maternal MTRR A66G polymorphism with NTD risk. In the following, five articles with 1,035 cases and 1,592 controls were included in the present study to analyze fetal MTRR A66G polymorphism with NTD risk. As presented in Table 4 and Figure 3 A and B, it was revealed that alleles and genotypes of maternal and fetal MTRR A66G had no significant association with NTD risk.

**Table 3.** Meta-Analysis of pooled association of maternal and fetal MTHFR C677T polymorphism with neural tube defect risk

| Meta-Analysis of the pooled association between maternal MTHFR C677T polymorphism with the risk of neural tube defect | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variation** | **Number of Studies** | **Case frequency** | **Control frequency** | **OR (95% CI)** | **OR P-value** | **$I^2$, heterogeneity P-value** | **Publication Bias (Begg's Test, P-value; Egger's Test, P-value)** |
| **C** | 25 | 2738 | 10041 | - | | - | - |
| **T** | 25 | 1482 | 5323 | - | | - | - |
| **CC** | 25 | 947 | 3368 | - | | - | - |
| **CT** | 25 | 849 | 3312 | - | | - | - |
| **TT** | 25 | 316 | 1007 | - | | - | - |
| **T vs. C** | 25 | - | - | 1.344 (1.147, 1.574) | <0.001 | 64.4%, P< 0.001 | (z= -.28,P-value =0.005, z=.76, P-value =0.083) |
| **TT vs. CC** | 25 | - | - | 1.880 (1.360, 2.598) | <0.001 | 54.4%, P< 0.001 | (z=-.39,P-value =0.003, z=1.2,P-value =0.140) |
| **CT vs. CC** | 25 | - | - | 1.237 (1.036, 1.477) | 0.019 | 38.5%, P= 0.027 | (z=1.12,P-value =0.088, z=.86,P-value =0.088) |
| **TT+CT vs. CC** | 25 | - | - | 1.384 (1.136, 1.689) | <0.001 | 55.8%, P< 0.001 | (z=1.24P-value =0.021, z=-.98P-value =0.047) |
| **TT vs. CT+CC** | 25 | - | - | 1.536 (1.194, 1.977) | <0.001 | 40.5%, P= 0.020 | (z=1.43,P-value <0.001, z=.46,P-value =0.200) |
| Meta-Analysis of the pooled association between fetal MTRR A66G polymorphism and neural tube defect risk | | | | | | | |
| **C** | 11 | 1206 | 2348 | - | | - | - |
| **T** | 11 | 797 | 1208 | - | | - | - |
| **CC** | 11 | 395 | 804 | - | | - | - |
| **CT** | 11 | 418 | 740 | - | | - | - |
| **TT** | 11 | 184 | 222 | - | | - | - |
| **T vs. C** | 11 | - | - | 1.261 (1.072, 1.485) | 0.005 | 31.2%, P= 0.150 | (z=1.53,P-value =0.534, z=-1.37P-value =0.533) |
| **TT vs. CC** | 11 | - | - | 1.644 (1.263, 2.140) | < 0.001 | 0.0%, P= 524 | (z=1.69,P-value =0.527, z=-1.32P-value =0.429) |
| **CT vs. CC** | 11 | - | - | 1.212 (1.008, 1.456) | 0.040 | 0.0%, P= 0.601 | (z=1.45.P-value =0.321, z=1.62,P-value =0.125) |
| **TT+CT vs. CC** | 11 | - | - | 1.297 (1.092, 1.540) | 0.003 | 0.0%, P= 0.569 | (z=-.78,P-value =0.706, z=.88,P-value =0.460) |
| **TT vs. CT+CC** | 11 | - | - | 1.467 (1.163, 1.850) | < 0.001 | 0.0%, P= 0.700 | (z=1.3,P-value =0.016, z=.6,P-value =0.090) |

Log (OR): Logarithm of odds ratio, I2: relative heterogeneity, OR P<0.05 was considered statistically significant

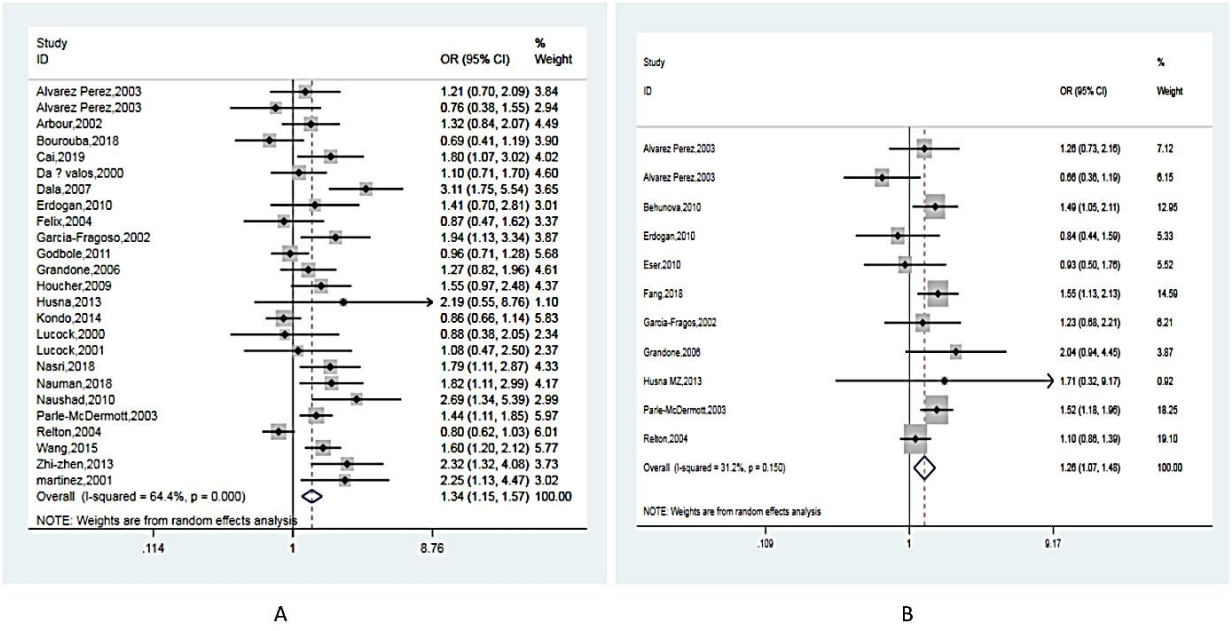


**Figure 2.** Forest plot of the association between maternal (A) and fetal (B) MTHFR C677T polymorphism with neural tube defect risk using T versus C allelic model in total population

*4.5. Subgroup analysis of the pooled association between maternal and fetal MTHFR C677T and MTRR A66G polymorphisms with NTDs risk*

As specified in Table 5, maternal MTHFR C677T polymorphism in Indian subcontinent subgroup had a significant association with NTDs risk in allelic (log(OR)=1.875, 95% CI=1.023, 3.434) (P=0.042), homozygous (log(OR)=4.920, 95% CI=2.387, 10.13) (P<0.001), and recessive models (log(OR)=4.745, 95% CI=2.313, 9.734) (P<0.001).

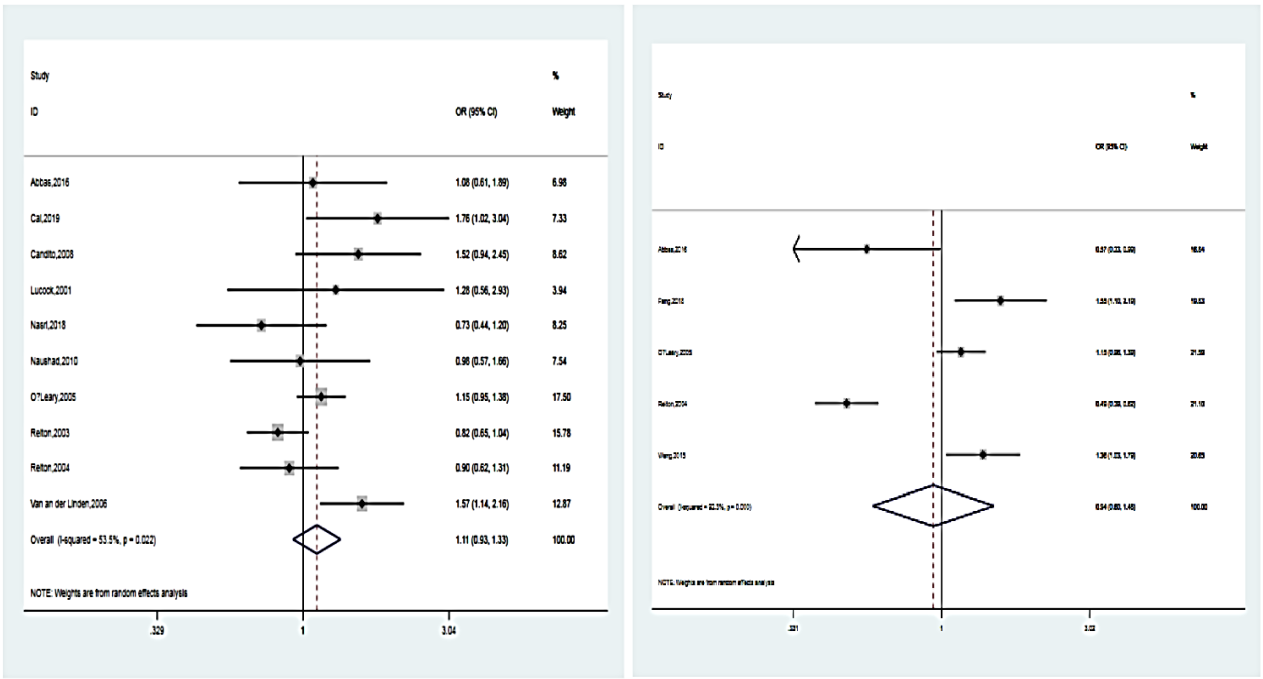


**Figure 3**. Forest plot of association of maternal (A) and fetal (B) MTRR A66G polymorphism with neural tube defect risk using A versus G (allelic model) in total population

**Table 4.** Meta-analysis of the pooled association between maternal and fetal MTRR A66G polymorphism with neural tube defect risk

| Meta-Analysis of the pooled association between maternal MTRR A66G polymorphism and neural tube defect risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variation** | **Number of Studies** | **Case frequency** | **Control frequency** | **OR (95% CI)** | **OR P-value** | **$I^2$, heterogeneity P-value** | **Publication Bias (Begg's Test, *P*-value; Egger's Test, *P*-value)** |
| **A** | 10 | 1126 | 1692 | - | | - | - |
| **G** | 10 | 1198 | 1926 | - | | - | - |
| **AA** | 10 | 456 | 394 | - | | - | - |
| **AG** | 10 | 576 | 904 | - | | - | - |
| **GG** | 10 | 312 | 511 | - | | - | - |
| **G vs. A** | 10 | - | - | 1.110 (0.926, 1.331) | 0.259 | 53.5%, P= 0.022 | (z=.7, P-value =0.027, z=-.88, P-value =0.127) |
| **GG vs. AA** | 10 | - | - | 0.947 (0.362, 2.474) | 0.912 | 93.3%, P<0.001 | (z=1.47, P-value =0.323, z=2.03, P-value =0.220) |
| **AG vs. AA** | 10 | - | - | 0.772 (0.353, 1.690) | 0.518 | 92.7%, P<0.001 | (z=1.5, P-value =0.405, (z=-.001,P-value =0.461) |
| **GG+AG vs. AA** | 10 | - | - | 0.825 (0.356, 1.911) | 0.653 | 94.5%, P<0.001 | (z=1.36, P-value =0.351, z=-0.16, P-value =0.408) |
| **GG vs. AG+AA** | 10 | - | - | 1.106 (0.675, 1.813) | 0.689 | 85.0%, P<0.001 | (z=2.06, P-value =0.273, z=1.002, P-value=0.129) |
| Meta-Analysis of the pooled association between fetal MTRR A66G polymorphism and neural tube defect risk | | | | | | | |
| **A** | 5 | 1572 | 1154 | - | | - | - |
| **G** | 5 | 1610 | 916 | - | | - | - |
| **AA** | 5 | 420 | 303 | - | | - | - |
| **AG** | 5 | 731 | 548 | - | | - | - |
| **GG** | 5 | 440 | 184 | - | | - | - |
| **G vs. A** | 5 | - | - | 0.940 (0.599, 1.477) | 0.790 | 92.3%, P<0.001 | (z=.13,P-value =0.982, z=.29, P-value =0.849) |
| **GG vs. AA** | 5 | - | - | 0.831 (0.334, 2.068) | 0.691 | 89.5%, P<0.001 | (z=1.2, P-value =0.789, z=-1.26, P-value =0.480) |
| **AG vs. AA** | 5 | - | - | 1.033 (0.683, 1.562) | 0.879 | 73.2%, P= 0.05 | (z=1.4, P-value =0.349, z=1.28, P-value =0.162) |
| **GG+AG vs. AA** | 5 | - | - | 0.933 (0.548, 1.588) | 0.798 | 85.3%, P<0.001 | (z=1.29, P-value =0.621, (z=-1.85, P-value=0.170) |
| **GG vs. AG+AA** | 5 | - | - | 0.873 (0.427, 1.786) | 0.710 | 88.9%, P<0.001 | (z=1.88, P-value =0.834, (z=3.2, P-value =0.551) |

Log (OR): Logarithm of odds ratio, I2: relative heterogeneity, OR P<0.05 was considered statistically significant

**Table 5.** Subgroup meta-analysis of the pooled association between maternal and fetal MTHFR and MTRR polymorphisms and neural tube defect risk

| Subgroup meta-analysis of the pooled association between maternal MTHFR C677T polymorphism with neural tube defect risk | | | | | |
|---|---|---|---|---|---|
| | **T vs. C OR (95%CI), P-value** | **TT vs. CC OR (95%CI), P-value** | **CT vs. CC OR (95%CI), P-value** | **TT+CT vs. CC OR (95%CI), P-value** | **TT vs. CT+CC OR (95%CI), P-value** |
| **Africa** | 1.260 (0.727, 2.186), 0.410 | 1.631 (0.571, 4.657), 0.361 | 1.338 (0.667, 2.685), 0.413 | 1.383 (0.638, 2.996), 0.411 | 1.370 (0.674, 2.786), 0.385 |
| **East Asia** | 1.489 (0.959, 2.312), 0.076 | 2.290 (0.860, 6.093), 0.097 | 1.528 (0.965, 2.420), 0.071 | 1.807 (0.985, 3.316), 0.056 | 1.545 (0.794, 3.007), 0.200 |
| **Europe** | 1.090 (0.797, 1.490), 0.589 | 1.177 (0.765, 1.812), 0.458 | 1.075 (0.655, 1.763), 0.776 | 1.097 (0.671, 1.794), 0.712 | 1.103 (0.799, 1.523), 0.552 |
| **Indian subcontinent** | 1.875 (1.023, 3.434), 0.042 | 4.920 (2.387, 10.13), <0.001 | 1.011 (0.778, 1.314), 0.935 | 1.547 (0.917, 2.609), 0.102 | 4.745 (2.313, 9.734), <0.001 |
| **South America** | 1.138 (0.832, 1.557), 0.419 | 1.258 (0.650, 2.433), 0.496 | 1.032 (0.688, 1.547), 0.880 | 1.099 (0.773, 1.543), 0.598 | 1.289 (0.609, 2.727), 0.506 |
| **Mixed** | 1.544 (1.141, 2.089), 0.005 | 2.361 (1.239, 4.498), 0.009 | 1.784 (1.121, 2.840), 0.015 | 1.859 (1.200, 2.878), 0.005 | 1.681 (0.950, 2.977), 0.075 |
| Subgroup meta-analysis of the pooled association between fetal MTHFR C677T polymorphism and neural tube defect risk | | | | | |
| **Europe** | 1.375 (1.107, 1.707), 0.004 | 1.784 (1.235, 2.576), 0.002 | 1.147 (0.859, 1.531), 0.353 | 1.274 (0.944, 1.720), 0.114 | 1.643 (1.210, 2.231), 0.001 |
| **Mixed** | 1.121 (0.864, 1.455), 0.390 | 1.444 (0.932, 2.236), 0.100 | 1.407 (1.022, 1.939), 0.036 | 1.407 (1.042, 1.899), 0.026 | 1.258 (0.880, 1.797), 0.208 |
| Subgroup meta-analysis of the pooled association between maternal MTRR A66G polymorphism and neural tube defect risk | | | | | |
| | G vs. A OR (95%CI), P-value | GG vs. AA OR (95%CI), P-value | AG vs. GG OR (95%CI), P-value | GG+AG vs. G OR (95%CI), P-value | GG vs. AG+AA OR (95%CI), P-value |
| **Europe** | 1.132 (0.905, 1.417), 0.278 | 0.902 (0.253, 3.221), 0.874 | 0.781 (0.269, 2.266), 0.649 | 0.831 (0.266, 2.602), 0.751 | 1.026 (0.524, 2.011), 0.940 |
| **Mixed** | 1.067 (0.740, 1.539), 0.728 | 1.066 (0.406, 2.801), 0.896 | 0.792 (0.319, 1.963), 0.614 | 0.855 (0.341, 2.146), 0.739 | 1.186 (0.747, 1.885), 0.469 |

Log (OR): Logarithm of odds ratio, P<0.05 was considered statistically significant

In addition, maternal MTHFR C677T polymorphism in the mixed subgroup was connected to NTDs risk in allelic (log (OR)=1.544, 95% CI=1.141, 2.089) (P=0.001) model. Same results were associated with the risk of NTDs in homozygous (log (OR)=2.361, 95% CI=1.239, 4.498) (P=0.009), heterozygous (log (OR) =1.784, 95% CI=1.121, 2.840) (P=0.015), and dominant models (log (OR)=1.859, 95% CI=(1.200, 2.878) (P=0.005).

As for the fetal MTHFR C677T polymorphism in the Europe sub group, a significant association with NTDs risk was observed in the allelic (log (OR)=1.375, 95% CI=(1.107, 1.707) (P=0.004), homozygous (log(OR)=1.784, 95% CI=1.235, 2.576) (P=0.002) and recessive models (log(OR)=1.634, 95% CI=(1.210, 2.231) (P=0.001).

No significant association was observed in the maternal MTRR A66G polymorphism with the risk of NTDs in different subgroups (Table 5).

*4.6. Publication bias*

The Egger's test and Begg's funnel plot were performed to evaluate the publication bias between the studies. However, since Egger's test could indicate publication bias better than Begg's test, the results of Egger's test were assessed to determine the publication bias. Based on the results of Egger's test, no publication bias was detected in different genetic models, except for the maternal MTHFR C677T dominant model (TT+CT vs. CC P=0.047) (Table 3 and 4).

## 5. Discussion

The NTD, as a congenital malformation of the central nervous system, occurs in the early stage of embryogenesis (about 28 days after conception) and is caused by a defect in neural tube closure (19, 20). Different major types of NTDs include anencephaly, encephalocele, and spina bifida. Anencephaly is the fetal type of NTDs, while spina bifida causes severe disability in affected children (3). The results of previous studies revealed that the consumption of folic acid supplementation during pregnancy reduced the risk of NTDs in the offspring of these women. This indicates the crucial function of folic acid in the pathogenesis of NTDs (4, 6). As a coenzyme for DNA synthesis and DNA methylation enzymes, folic acid has a central role in the neurulation process during embryogenesis (21-23). Regarding the role of folic acid in the pathogenesis of NTDs, the enzymes involved in the folic acid metabolism can be targeted for NTDs-related studies. MTHFR and MTRR are among the enzymes that are involved in folate metabolism (8, 16). The SNPs in the genes of these enzymes can change their activity and cause NTDs by altering folate levels. The results of previous studies revealed that MTHFR C677T and MTRR A66G polymorphisms might be related to NTDs incidence (24-27). Considering the statistical power of the meta-analysis, the present study aimed to investigate the relation of maternal and fetal MTHFR C677T and MTRR A66G with susceptibility to NTDs.

The results of 25 literatures with 2,225 cases and 12,204 controls were combined to assess the association of maternal MTHFR C677T with the NTDs risk. With no publication bias, it was revealed that maternal MTHFR C677T polymorphism was linked to the occurrence of NTDs in newborns. In addition, the results of subgroup analysis indicated that there was a significant correlation between the maternal MTHFR C677T polymorphism and NTDs risk in the Indian subcontinent and mixed subgroups.

In the present meta-analysis study, the association of fetal MTHFR C677T polymorphism with the risk of NTDs was investigated in 11 studies with 997 cases and 1,756 controls that have been selected based on the inclusion and exclusion criteria. The findings showed that fetal MTHFR C677T polymorphism was associated with susceptibility to NTDs in the overall population and the European subgroup. This result was in line with the result of the study performed by Yang et al. (28), which showed that maternal and fetal MTHFR C677T polymorphism was associated with the risk of NTDs. In the same line, Yadav et al. also reported a positive association between maternal MTHFR C677T polymorphism and MTRR A66G polymorphism with the NTDs risk (29). According to this study, MTHFR C677T polymorphism was strongly associated with NTDs in Asian, European, and American subgroups. These results might be associated with variations in folate, vitamin B12, and vitamin B6 intake in different geographical areas. These findings were in line with those obtained in this study, except that in the present study a positive relation was observed between the fetal MTHFR C677T polymorphism in the European subgroup. The results of the present study indicated that there was no association between maternal and fetal MTHFR C677T polymorphism with the risk of NTDs in the East Asian population. Our results opposed those obtained in the previous studies which have verified the association of MTHFR polymorphism and the risk of NTDs in the Asian population (28, 30). This contradiction might be due to the omission of non-English language studies in this meta-analysis study which included only original articles and excluded letters, short communication articles, and reports.

Previous studies have introduced MTRR A66G polymorphism as another mutation contributing to both maternal and fetal NTDs (31, 32). This theory was assessed with a combination of the results of 10 literatures (including 1,164 cases and 1,809 controls) and 5 studies (including 1,035 cases and 1,592 controls). The obtained results reported a negative correlation of the maternal and fetal MTRR A66G polymorphisms with NTDs risk in offspring. In contrast to the results of this study, Yadav et al. (29)

showed that maternal MTRR A66G was associated with the occurrence of NTD. Although the adopted inclusion and exclusion criteria in this study were similar to those used in other studies, the discrepancies in the results could be attributed to the number of original publications included in this study.

In another meta-analysis study conducted by Shengrong Ouyang et al., 10 studies (including 1,358 cases and 2,169 controls) were analyzed to explore the association of MTRR A66G polymorphisms and NTDs risks in Caucasian children. The result of this study reflected a negative association between the MTRR A66G polymorphisms and NTDs risks (33). These results could be related to the limited number of publications in the Caucasus area. The results of this study were consistent with these results; however, a negative relation between the MTRR A66G polymorphisms and NTDs risk during embryonic development was observed in this meta-analysis study.

The contradictory results in different meta-analysis studies can be explained by limited publications, different inclusion and exclusion criteria, and various folate supplementation in different countries. In addition, gene to gene interaction, epigenetic factors, and family history are important factors that were not involved in assessing the link of MTHFR and MTRR polymorphisms with the NTDs risks. All previous studies agreed on the importance of folate supplementation before and during the pregnancy and confirmed its relation with MTHFR and MTRR polymorphisms.

## 6. Conclusion

The present meta-analysis study revealed the possible association of maternal and fetal MTHFR polymorphisms and MTRR polymorphisms with NTDs risk by pooling the available data. Based on the obtained results in the present meta-analysis study, maternal and fetal MTHFR C677T polymorphism is associated with susceptibility to NTDs. Moreover, the results of the association of maternal and fetal MTRR A66G with the risk of NTDs suggested that there was no significant statistical association between maternal and fetal MTRR A66G with the risk of NTDs.

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## Footnotes

**Authors' contributions:** Dr. Reza Mahdian Joibari designed and wrote the manuscript draft. Dr. Abolfazl Movafagh revised the draft, and Alireza Molaie performed the statistical analysis.

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## References

1. Copp AJ, Greene ND, Murdoch JN. The genetic basis of mammalian neurulation. *Nat. Rev. Genet* 2003;4(10):784-93. doi: 10.1038/nrg1181. [PubMed: 13679871].
2. Ouyang S, Liu Z, Li Y, Ma F, Wu J. Cystathionine beta-synthase 844ins68 polymorphism is unrelated to susceptibility to neural tube defects. *Gene*. 2014;**535**(2):119-23. doi: 10.1016/j.gene.2013.11.052. [PubMed: 24316487].
3. Au KS, Ashley-Koch A, Northrup H. Epidemiologic and genetic aspects of spina bifida and other neural tube defects. *Dev. Disabil. Res. Rev*. 2010;**16**(1):6-15. doi: 10.1002/ddrr.93. [PubMed: 20419766].
4. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, et al. Prevention of neural-tube defects with folic acid in China. *N Engl*. 1999;**341**(20):1485-90. doi: 10.1056/NEJM199911113412001. [PubMed: 10559448].
5. Gaughan DJ, Kluijtmans LA, Barbaux S, McMaster D, Young IS, Yarnell JW, et al. The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. *Atherosclerosis*. 2001;**157**(2):451-6. doi: 10.1016/s0021-9150(00)00739-5. [PubMed: 11472746].
6. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl*. 1992;**327**(26):1832-5. doi: 10.1056/NEJM199212243272602. [PubMed: 1307234].
7. Smithells R, Sheppard S, Schorah C, Seller M, Nevin N, Harris R, et al. Possible prevention of neural-tube defects by periconceptional vitamin supplementation. *Lancet*. 1980;**315**(8164):339-40. doi: 10.1016/s0140-6736(80)90886-7. [PubMed: 6101792].
8. Frosst P, Blom H, Milos R, Goyette P, Sheppard CA, Matthews R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet*. 1995;**10**(1):111-3. doi: 10.1038/ng0595-111. [PubMed: 7647779].
9. Liew S-C, Gupta ED. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. *Eur J Med Genet*. 2015;**58**(1):1-10. doi: 10.1016/j.ejmg.2014.10.004. [PubMed: 25449138].
10. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet*. 1994;**7**(2):195-200. doi: 10.1038/ng0694-195. [PubMed: 7920641].
11. Rosenberg N, Murata M, Ikeda Y, Opare-Sem O, Zivelin A, Geffen E, et al. The frequent 5, 10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in whites, Japanese, and Africans. *Am. J. Hum. Genet*. 2002;**70**(3):758-62. doi: 10.1086/338932. [PubMed: 11781870].
12. Leclerc D, Wilson A, Dumas R, Gafuik C, Song D, Watkins D, et al. Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. *Proc. Natl. Acad. Sci. U.S.A*. 1998;**95**(6):3059-64. doi: 10.1073/pnas.95.6.3059. [PubMed: 9501215].
13. Olteanu H, Banerjee R. Human methionine synthase reductase, a soluble P-450 reductase-like dual flavoprotein, is sufficient for NADPH-dependent methionine synthase activation. *J Biol Chem*. 2001;**276**(38):35558-63. doi: 10.1074/jbc.M103707200. [PubMed: 11466310].
14. Rai V, Yadav U, Kumar P. MTRR A66G polymorphism among two caste groups of Uttar Pradesh (India). *Indian J Med Sci*.

2012;**66**(5/6):136. doi: 10.4103/0019-5359.114200. [PubMed: 23806987].
15. Lucock M, Daskalakis I, Briggs D, Yates Z, Levene M. Altered folate metabolism and disposition in mothers affected by a spina bifida pregnancy: influence of 677c→ t methylenetetrahydrofolate reductase and 2756a→ g methionine synthase genotypes. *Mol. Genet. Metab.* 2000;**70**(1):27-44. doi: 10.1006/mgme.2000.2994. [PubMed: 10833329].
16. Wilson A, Platt R, Wu Q, Leclerc D, Christensen B, Yang H, et al. A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. *Mol. Genet. Metab*. 1999;**67**(4):317-23. doi: 10.1006/mgme.1999.2879. [PubMed: 10444342].
17. Zhu H, Wicker NJ, Shaw GM, Lammer EJ, Hendricks K, Suarez L, et al. Homocysteine remethylation enzyme polymorphisms and increased risks for neural tube defects *Mol. Genet. Metab*. 2003;**78**(3):216-21. doi: 10.1006/mgme.1999.2879. [PubMed: 10444342].
18. Lin L, Chu H. Quantifying publication bias in meta-analysis. *Biometrics*. 2018;**74**(3):785-94. doi: 10.1111/biom.12817. [PubMed: 29141096].
19. Liu J, Qi J, Yu X, Zhu J, Zhang L, Ning Q, et al. Investigations of single nucleotide polymorphisms in folate pathway genes in Chinese families with neural tube defects. *J. Neurol. Sci.* 2014;**337**(1-2):61-6. doi: 10.1016/j.jns.2013.11.017. [PubMed: 24326202].
20. Obeid R, Holzgreve W, Pietrzik K. Is 5-methyltetrahydrofolate an alternative to folic acid for the prevention of neural tube defects? *J Perinat Med*. 2013;**41**(5):469-83. doi: 10.1515/jpm-2012-0256. [PubMed: 23482308].
21. Molloy AM, Daly S, Mills JL, Kirke PN, Whitehead AS, Ramsbottom D, et al. Thermolabile variant of 5, 10-methylenetetrahydrofolate reductaseassociated with low red-cell folates: implications for folate intake recommendations. *Lancet*. 1997;**349**(9065):1591-3. doi: 10.1016/S0140-6736(96)12049-3. [PubMed: 9174561].
22. Molloy AM, Mills JL, Kirke PN, Ramsbottom D, McPartlin JM, Burke H, et al. Low blood folates in NTD pregnancies are only partly explained by thermolabile 5, 10-methylenetetrahydrofolate reductase: low folate status alone may be the critical factor. *Am. J. Med. Genet.* 1998;**78**(2):155-9. [PubMed: 9674907].
23. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thromb. Haemost.* 1997;**78**(01):523-6. [PubMed: 9198208].
24. Candito M, Rivet R, Herbeth B, Boisson C, Rudigoz RC, Luton D, et al. Nutritional and genetic determinants of vitamin B and homocysteine metabolisms in neural tube defects: a multicenter case–control study. *Am. J. Med. Genet. A* . 2008;**146**(9):1128-33. doi: 10.1002/ajmg.a.32199. [PubMed: 18386810].
25. Husna M, Endom I, Ibrahim S, Selvi NA, Fakhrurazi H, Htwe RO, et al., editors. Screening of polymorphisms for MTHFR and DHFR genes in spina bifida children and their mothers. AIP Conference Proceedings; 2013: American Institute of Physics.
26. O'Leary VB, Mills JL, Pangilinan F, Kirke PN, Cox C, Conley M, et al. Analysis of methionine synthase reductase polymorphisms for neural tube defects risk association. *Mol Genet Metab*. 2005;**85**(3):220-7. doi: 10.1016/j.ymgme.2005.02.003. [PubMed: 15979034].
27. Parle-McDermott A, Mills JL, Kirke PN, O'Leary VB, Swanson DA, Pangilinan F, et al. Analysis of the MTHFR 1298A→ C and 677C→ T polymorphisms as risk factors for neural tube defects. *J. Hum. Genet.* 2003;**48**(4):190-3. doi: 10.1007/s10038-003-0008-4. [PubMed: 12730722].
28. Yang Y, Chen J, Wang B, Ding C, Liu H. Association between MTHFR C677T polymorphism and neural tube defect risks: A comprehensive evaluation in three groups of NTD patients, mothers, and fathers. *Birth Defects Res. Part A Clin Mol Teratol*. 2015;**103**(6):488-500. doi: 10.1002/bdra.23361. [PubMed: 25808073].
29. Yadav U, Kumar P, Yadav SK, Mishra OP, Rai V. Polymorphisms in folate metabolism genes as maternal risk factor for neural tube defects: an updated meta-analysis. *Metab. Brain Dis*. 2015;**30**(1):7-24. doi: 10.1007/s11011-014-9575-7. [PubMed: 25005003].
30. Tabatabaei RS, Fatahi-Meibodi N, Meibodi B, Javaheri A, Abbasi H, Hadadan A, et al. Association of Fetal MTHFR C677T Polymorphism with Susceptibility to Neural Tube Defects: A Systematic Review and Update Meta-Analysis. *Fetal and Pediatric Pathology*. 2020:1-17. doi: 10.1080/15513815.2020.1775734. [PubMed: 32536242].
31. Abbas A, Sifi K, Naimi D, Benmebarek K, Abadi N. Genetic Polymorphisms in Methionine Synthase and Methionine Synthase Reductase, their Metabolic Effects, and Risk of Neural Tube Defects in Algerian Population.
32. Nasri K, Midani F, Kallel A, Jemaa NB, Aloui M, Boulares M, et al. Association of MTHFR C677T, MTHFR A1298C, and MTRR A66G Polymorphisms with Neural Tube Defects in Tunisian Parents. *Pathobiology*. 2019;**86**(4):190-200. doi: 10.1159/000499498. [PubMed: 31238314].
33. Ouyang S, Liu Z, Li Y, Wu J. Meta-analyses on the association of MTR A2756G and MTRR A66G polymorphisms with neural tube defect risks in Caucasian children. *J. Matern.-Fetal Neonatal Med.* 2013;**26**(12):1166-70. doi: 10.3109/14767058.2013.777699. [PubMed: 23425389].
34. Dávalos I-P, Olivares N, Castillo Ma-T, Cantú J-M, Ibarra B, Sandoval L, et al., editors. The C677T polymorphism of the methylenetetrahydrofolate reductase gene in Mexican mestizo neural-tube defect parents, control mestizo and native populations. Annales de genetique; 2000: Elsevier. doi: 10.1016/s0003-3995(00)90012-1. [PubMed: 10998450].
35. Lucock M, Daskalakis I, Hinkins M, Yates Z. An examination of polymorphic genes and folate metabolism in mothers affected by a spina bifida pregnancy. *Mol. Genet. Metab.* 2001;**73**(4):322-32. doi: 10.1006/mgme.2001.3205. [PubMed: 11509014].
36. de Villarreal LEMn, Delgado-Enciso I, Valdéz-Leal R, Ortíz-López R, Rojas-Martínez A, Limón-Benavides C, et al. Folate levels and N 5, N 10-methylenetetrahydrofolate reductase genotype (MTHFR) in mothers of offspring with neural tube defects: a case-control study. *Arch. Med. Res.* 2001;**32**(4):277-82. doi: 10.1016/s0188-4409(01)00292-2. [PubMed: 11440783].
37. Garcia-Fragoso L, García-García I, de la Vega A, Renta J, Cadilla CL. Presence of the 5, 10-methylenetetrahydrofolate reductase C677T mutation in Puerto Rican patients with neural tube defects. *J. Child Neurol*. 2002;**17**(1):30-2. doi: 10.1177/088307380201700107. [PubMed: 11913566].
38. Arbour L, Christensen B, Delormier T, Platt R, Gilfix B, Forbes P, et al. Spina bifida, folate metabolism, and dietary folate intake in a Northern Canadian aboriginal population. *Int J Circumpolar Health*. 2002;**61**(4):341-51. doi: 10.3402/ijch.v61i4.17492. [PubMed: 12546192].
39. Perez ABA, D'Almeida V, Vergani N, de Oliveira AC, de Lima FT, Brunoni D. Methylenetetrahydrofolate reductase (MTHFR): incidence of mutations C677T and A1298C in Brazilian population and its correlation with plasma homocysteine levels in spina bifida. *Am. J. Med. Genet.* 2003;**119**(1):20-5. doi: 10.1002/ajmg.a.10059. [PubMed: 12707953].
40. Relton C, Wilding C, Pearce M, Laffling A, Jonas P, Lynch S, et al. Gene–gene interaction in folate-related genes and risk of neural tube defects in a UK population. *J. Med. Genet.* 2004;**41**(4):256-60. doi: 10.1136/jmg.2003.010694. [PubMed: 15060097].
41. Félix TM, Leistner S, Giugliani R. Metabolic effects and the methylenetetrahydrofolate reductase (MTHFR) polymorphism associated with neural tube defects in southern Brazil. *Birth Defects Res. Part A Clin. Mol. Teratol.* 2004;**70**(7):459-63. doi: 10.1002/bdra.20011. [PubMed: 15259035].
42. Grandone E, Corrao AM, Colaizzo D, Vecchione G, Girgenti CD, Paladini D, et al. Homocysteine metabolism in families from southern Italy with neural tube defects: role of genetic and nutritional determinants. *Prenat. Diag*. 2006;**26**(1):1-5. doi: 10.1002/pd.1359. [PubMed: 16374895].
43. Dalal A, Pradhan M, Tiwari D, Behari S, Singh U, Mallik G, et al. MTHFR 677C→ T and 1298A→ C polymorphisms: evaluation of maternal genotypic risk and association with level of neural tube defect. *Gynecol. Obstet. Invest.* 2007;**63**(3):146-50. doi: 10.1159/000096735. [PubMed: 17085942].
44. Houcher B, Bourouba R, Djabi F, Yilmaz E, Eğin Y, Akar N. Polymorphisms of 5, 10-Methylenetetrahydrofolate Reductase and Cystathionine β-Synthase Genes as a Risk Factor for Neural Tube Defects in Sétif, Algeria. *Pediatr. Neurosurg*. 2009;**45**(6):472-7. doi: 10.1159/000283086. [PubMed: 20160465].

45. Erdogan M, Yildiz S, Solak M, Eser O, Cosar E, Eser B, et al. C677T polymorphism of the methylenetetrahydrofolate reductase gene does not affect folic acid, vitamin B *Genet Mol Res*. 2010;**9**(2):1197-203. doi: 10.4238/vol9-2gmr816. [PubMed: 20589617].
46. Naushad SM, Devi ARR. Role of parental folate pathway single nucleotide polymorphisms in altering the susceptibility to neural tube defects in South India. *J Perinat Med*. 2010;**38**(1):63-9. doi: 10.1515/jpm.2009.119. [PubMed: 20047525].
47. Godbole K, Gayathri P, Ghule S, Sasirekha BV, Kanitkar-Damle A, Memane N, et al. Maternal one-carbon metabolism, MTHFR and TCN2 genotypes and neural tube defects in India. *Birth Defects Res. Part A Clin. Mol. Teratol.* 2011;**91**(9):848-56. doi: 10.1002/bdra.20841. [PubMed: 21770021].
48. Liu Z-z, Zhang J-t, Dan L, Hao Y-h, Chang B-m, Jun X, et al. Interaction between maternal 5, 10-methylenetetrahydrofolate reductase C677T and methionine synthase A2756G gene variants to increase the risk of fetal neural tube defects in a Shanxi Han population. *Chin. Med. J.* 2013;**126**(5):865-9. [PubMed: 23489792].
49. Kondo A, Fukuda H, Matsuo T, Shinozaki K, Okai I. C677T mutation in methylenetetrahydrofolate reductase gene and neural tube defects: Should J apanese women undergo gene screening before pregnancy? *Congenit Anom*. 2014;**54**(1):30-4. doi: 10.1111/cga.12026. [PubMed: 24588777].
50. Wang Y, Liu Y, Ji W, Qin H, Wu H, Xu D, et al. Variants in MTHFR gene and neural tube defects susceptibility in China. *Metab. Brain Dis.* 2015;**30**(4):1017-26. doi: 10.1007/s11011-015-9662-4. [PubMed: 25855017].
51. Bourouba R, Houcher B, Akar N. Risk factors of neural tube defects: A reality of Batna region in Algeria. *Egypt. J. Medical Hum. Genet*. 2018;**19**(3):225–9. doi: 10.1016/j.ejmhg.2017.10.003
52. Nauman N, Jalali S, Shami S, Rafiq S, Große G, Hilger AC, et al. Low maternal folate concentrations and maternal MTHFR C677T polymorphism are associated with an increased risk for neural tube defects in offspring: a case-control study among Pakistani case and control mothers. *Asia Pac J Clin Nutr*. 2018;**27**(1):253. doi: 10.6133/apjcn.032017.10. [PubMed: 29222906].
53. Cai C-Q, Fang Y-L, Shu J-B, Zhao L-S, Zhang R-P, Cao L-R, et al. Association of neural tube defects with maternal alterations and genetic polymorphisms in one-carbon metabolic pathway. *Ital. J. Pediatr*. 2019;**45**(1):37. doi: 10.1186/s13052-019-0630-1. [PubMed: 30867013].
54. Behunova J, Klimcakova L, Zavadilikova E, Potocekova D, Sykora P, Podracka L. Methylenetetrahydrofolate reductase gene polymorphisms and neural tube defects epidemiology in the Slovak population. *Birth Defects Res. Part A Clin. Mol. Teratol.* 2010;**88**(8):695-700. doi: 10.1002/bdra.20692. [PubMed: 20672355].
55. Eser B, Cosar M, Eser O, Erdogan MO, Aslan A, Yildiz H, et al. 677C> T and 1298A> C polymorphisms of methylenetetrahydropholate reductase gene and biochemical parameters in Turkish population with spina bifida occulta. *Turk Neurosurg.* 2010;**20**(1):9-15. [PubMed: 20066615].
56. Fang Y, Zhang R, Zhi X, Zhao L, Cao L, Wang Y, et al. Association of main folate metabolic pathway gene polymorphisms with neural tube defects in Han population of Northern China. *Childs Nerv Syst.* 2018;**34**(4):725-9. doi: 10.1007/s00381-018-3730-0. [PubMed: 29392422].
57. Relton CL, Wilding CS, Laffling AJ, Jonas PA, Burgess T, Binks K, et al. Low erythrocyte folate status and polymorphic variation in folate-related genes are associated with risk of neural tube defect pregnancy. *Mol. Genet. Metab.* 2004;**81**(4):273-81. doi: 10.1016/j.ymgme.2003.12.010. [PubMed: 15059614].
58. Van der Linden IJ, den Heijer M, Afman LA, Gellekink H, Vermeulen SH, Kluijtmans LA, et al. The methionine synthase reductase 66A> G polymorphism is a maternal risk factor for spina bifida. *J. Mol. Med.* 2006;**84**(12):1047-54. doi: 10.1007/s00109-006-0093-x. [PubMed: 17024475].
59. Wang Y, Liu Y, Ji W, Qin H, Wu H, Xu D, et al. Analysis of MTR and MTRR polymorphisms for neural tube defects risk association. *Medicine.* 2015;**94**(35). doi: 10.1097/MD.0000000000001367. [PubMed: 26334892].