Association of the Maternal MTHFR C677T Polymorphism with Susceptibility to Neural Tube Defects in Offsprings: Evidence from 25 Case-Control Studies

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Abstract

Background: Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism and is involved in DNA methylation, DNA synthesis, and DNA repair. In addition, it is a possible risk factor in neural tube defects (NTDs). The association of the C677T polymorphism in the MTHFR gene and NTD susceptibility has been widely demonstrated, but the results remain inconclusive. In this study, we performed a meta-analysis with 2429 cases and 3570 controls to investigate the effect of the MTHFR C677T polymorphism on NTDs.

Methods: An electronic search of PubMed and Embase database for papers on the MTHFR C677T polymorphism and NTD risk was performed. All data were analysed with STATA (version 11). Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess the association. Sensitivity analysis, test of heterogeneity, cumulative meta-analysis, and assessment of bias were performed in our meta-analysis.

Results: A significant association between the MTHFR C677T polymorphism and NTD susceptibility was revealed in our meta-analysis (TT versus CC: OR = 2.022, 95% CI: 1.508, 2.712; CT+TT versus CC: OR = 1.303, 95% CI: 1.089, 1.558; TT versus CC+CT: OR = 1.716, 95% CI: 1.448, 2.033; 2TT+CT versus 2CC+CT: OR = 1.330, 95% CI: 1.160, 1.525). Moreover, an increased NTD risk was found after stratification of the MTHFR C677T variant data by ethnicity and source of controls.

Conclusion: The results suggested the maternal MTHFR C677T polymorphism is a genetic risk factor for NTDs. Further functional studies to investigate folate-related gene polymorphisms, periconceptional multivitamin supplements, complex interactions, and the development of NTDs are warranted.

Introduction

Neural tube defects (NTDs) are a group of severe congenital malformations with an average worldwide birth prevalence of 1 in 500 [1], occurring due to incomplete closure of the neural tube between days 22 and 26 (somite stage 10–12) during embryo development [2]. These birth defects can cause lifelong disability or death.

Although the cause of NTDs is still poorly understood, accumulated evidence has suggested that genetic and/or environmental factors may contribute to NTD aetiology. Among these factors, maternal nutritional status is a key determinant of pregnancy outcome, and attention has been focused on folic acid, a water-soluble B vitamin that acts as a cofactor in one-carbon transfer reactions and plays a central role in DNA methylation, synthesis, and repair [3,4]. It has been shown that the occurrence and recurrence risk of NTDs is reduced by 50–70% with folic acid supplementation during the periconceptional period [5]. However, the underlying mechanisms by which folic acid protects against NTDs are still unknown. In addition, it is not known why some women who take folic acid supplements during the periconceptional period still have offspring with NTDs [6]. Therefore, candidate genes that encode enzymes involved in folate metabolism or receptors involved in folate transport have been analysed.

The enzyme MTHFR plays a key role in the folate metabolism pathway and regulates the intracellular folate pool for synthesis and methylation of DNA [7,8]. The MTHFR gene
Table 1. Characteristics of the association studies on maternal MTHFRC677T polymorphism and the risk of Neural tube defects (NTDs).

| ID  | first author          | year | Source of control | Region  | Ethnicity   | total | Case genotypes | Control genotypes | HWE |
|-----|-----------------------|------|-------------------|---------|-------------|-------|----------------|-------------------|-----|
| 1   | Arbour L [17]         | 2002 | mixed             | Canada  | Caucasian   | 175   | 32/31/11       | 52/38/11          | 0.319 |
| 2   | Candito M [18]        | 2008 | HB                | France  | Caucasian   | 138   | 25/40/12       | 26/29/6           | 0.610 |
| 3   | Ceyhan ST [19]        | 2008 | HB                | Turkey  | Caucasian   | 64    | 9/14/6         | 20/12/3           | 0.544 |
| 4   | Christensen B [20]    | 1999 | HB                | Canada  | Caucasian   | 152   | 24/27/11       | 44/36/10          | 0.526 |
| 5   | Dalal A [21]          | 2007 | PB                | India   | Caucasian   | 143   | 56/21/6        | 45/12/3           | 0.095 |
| 6   | Deb R [22]            | 2011 | PB                | India   | Caucasian   | 333   | 80/25/6        | 149/64/9          | 0.524 |
| 7   | Félix TM [23]         | 2004 | HB                | Brazil  | Caucasian   | 85    | 19/15/7        | 16/22/6           | 0.718 |
| 8   | Godbole K [24]        | 2011 | HB                | India   | Caucasian   | 989   | 238/62/5       | 521/158/5         | 0.059 |
| 9   | Houcher B [25]        | 2009 | HB                | Algeria | African     | 174   | 35/42/15       | 33/35/14          | 0.375 |
| 10  | Lacasana M [26]       | 2012 | HB                | Mexico  | Mixed       | 189   | 11/45/42       | 20/49/22          | 0.460 |
| 11  | Li K [27]             | 2000 | HB                | China   | Asian       | 51    | 1/17/9         | 5/16/3            | 0.093 |
| 12  | Lucock M [28]         | 2000 | PB                | UK      | Caucasian   | 50    | 8/9/2          | 11/17/3           | 0.330 |
| 13  | Martínez de Villarreal LE [29] | 2001 | PB                | Mexico  | Mixed       | 69    | 11/12/15       | 12/16/3           | 0.479 |
| 14  | Molloy AM [30]        | 1998 | HB                | Ireland | Caucasian   | 343   | 34/35/13       | 119/121/21        | 0.200 |
| 15  | Munoz JB [31]         | 2007 | HB                | Mexico  | Mixed       | 230   | 14/54/50       | 25/57/30          | 0.833 |
| 16  | Naushad SM [32]       | 2010 | PB                | India   | Caucasian   | 130   | 33/11/6        | 64/16/0           | 0.320 |
| 17  | Parle-McDermott A [33] | 2003 | HB                | Ireland | Caucasian   | 529   | 102/138/34     | 126/103/26        | 0.469 |
| 18  | Perez AB [34]         | 2003 | HB                | Brazil  | Mixed       | 257   | 67/55/9        | 70/54/2           | 0.019*|
| 19  | Relton CL [35]        | 2004 | HB                | UK      | Caucasian   | 698   | 86/78/22       | 191/254/67        | 0.222 |
| 20  | Relton CL [36]        | 2004 | HB                | UK      | Caucasian   | 251   | 31/36/15       | 66/88/15          | 0.058 |
| 21  | Shang Y [37]          | 2008 | HB                | China   | Asian       | 118   | 14/20/4        | 25/38/17          | 0.718 |
| 22  | Shields DC [38]       | 1999 | HB                | Ireland | Caucasian   | 460   | 80/108/30      | 114/108/20        | 0.426 |
| 23  | Ubbink JB [39]        | 1999 | PB                | Africa  | African     | 107   | 42/11/0        | 43/11/0           | 0.405 |
| 24  | Wang F [40]           | 2008 | HB                | China   | Asian       | 198   | 14/50/35       | 34/48/17          | 0.990 |
| 25  | Yu J [41]             | 2000 | PB                | China   | Asian       | 66    | 2/25/15        | 5/16/3            | 0.093 |

*P<0.05.
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Materials and Methods

Study eligibility

Potentially relevant reports were selected by searching Embase and PubMed (the last search update was performed on January 10, 2012) using the main search terms “methylene-tetrahydrofolate reductase,” “MTHFR,” and “neural tube defects,” “NTD”. All studies were published in English or in the Chinese language, and were human studies only. The related reference articles were searched to identify other relevant publications. Unpublished data and further information were also obtained from the authors.

Validity assessment

Potential studies were selected following inclusion criteria: 1) MTHFR C677T polymorphism and NTDs; 2) human case-control design; 3) sufficient maternal genotype data for estimating an odds ratio (OR) with a 95% confidence interval (CI); and 4) published in English or Chinese. The criteria for the exclusion of studies are as follows: 1) not related to the MTHFR C677T polymorphism and NTDs; 2) not a primary case-control study; 3) no usable or sufficient maternal genotype data reported; and 4) controls are not mothers with at least one healthy birth.
Data extraction

Two investigators independently extracted the data from all eligible studies using the selection criteria listed above. Any disagreement was resolved by discussion. We extracted the following information: the first author’s name, year of publication, the country in which the study was conducted, the ethnicities of the individuals involved, the source of control groups (population-based or hospital-based controls or mixed), the sample size, number of cases and controls with the CC, CT, and TT genotypes.

Data synthesis

All statistical analyses were performed using the STATA software (version 11). Two-sided $P$ values less than 0.05 were considered statistically significant. For the control groups of each study, the observed genotype frequencies of the $MTHFR C677T$ polymorphism were assessed for Hardy-Weinberg equilibrium.

The strength of the association between the $MTHFR C677T$ polymorphism and NTD risk was evaluated by the odds ratios (ORs) with 95% confidence intervals (CIs). The pooled ORs were calculated for the homozygote comparison (TT versus CC), heterozygote comparison (CT versus CC), dominant model (CT + TT versus CC), recessive model (TT versus CT + CC), and an additive model (2TT + CT versus 2CC + CT). Subgroup analyses were performed based on the source of controls and ethnicity if the data permitted.

The evaluation of the meta-analysis results included a test for heterogeneity, an analysis of the sensitivity, and an examination for bias. The chi-squared test-based Q-statistic was calculated to test the heterogeneity between studies and detect the source of heterogeneity by ethnicity, publication year, control source, and sample size. The model used for the analysis of the pooled ORs depends on the $P$ value. If the heterogeneity test result was $P<0.1$, the pooled ORs were analysed using the random-effects model (the DerSimonian and Laird method) [14]; otherwise, the fixed-effects model was used (the Mantel-Haenszel method) [15]. Additionally, sensitivity analyses were performed after sequential removal of each study. Finally, the Begg’s funnel plot and Egger’s test were performed to statistically analyse the publication bias [16].

Results

Study characteristics

We included 25 eligible studies [17–41] in our meta-analysis. The studies contained data on 5999 mothers (2429 case mothers and 3570 control mothers) who had an $MTHFR C677T$ polymorphism in the case-control design. The characteristics of all reports on the association between the $MTHFR C677T$ polymorphism and NTDs are shown in Table 1. In our meta-analysis, 7 studies were population-based controls, 17 studies were hospital-based controls, 1 did not provide detailed information regarding the source of the controls were mixed; 4 studies included Asian populations, 15 studies were Caucasian population, 2 studies were African population, and 4 studies were mixed population (white and non-white). The genotype distributions in the controls for all studies were consistent with Hardy-Weinberg equilibrium, except for the controls in a study by Perez et al.[34]. Figure 1 shows the study selection procedure.

Evidence synthesis

In our meta-analysis the CC genotype was used as the reference group. The maternal $MTHFR C677T$ polymorphism showed pooled odds ratios for homozygote comparison (TT versus CC: OR = 2.022, 95% CI: 1.508, 2.712, $P<0.001$), for dominant model comparison (CT + TT versus CC: OR = 1.303, 95% CI: 1.089, 1.558, $P=0.004$), for recessive model comparison (TT versus CT + CC: OR = 1.716, 95% CI: 1.448, 2.033, $P<0.001$), and for additive model comparison (2TT + CT versus 2CC + CT: OR = 1.330, 95% CI: 1.160, 1.525, $P<0.001$). Overall, there was
a significant association between the maternal MTHFR C677T polymorphism and NTDs. The forest plot is shown in Figure 2.

Subgroup analysis

We also performed subgroup analysis stratified by ethnicity and study design. We found that the variant genotypes were associated with a significantly increased NTD risk in Asian, Caucasian and mixed populations. In heterozygote comparison (CT versus CC), the pooled odds ratio was 1.933 (95% CI: 1.167, 3.202, \(P = 0.010\)) for Asian population. The pooled odds ratios were 1.524 (95% CI: 1.227, 1.893, \(P < 0.001\)), and 2.514 (95% CI: 1.720, 3.676, \(P < 0.001\)) for Caucasian and mixed populations, respectively, under the recessive model. However, we did not find an association between the C677T polymorphism and NTD risk in African groups in any genetic models. The meta-analysis results for the other genetic models are listed in Table 2.

Significantly increased risks were also found in the subgroup analysis stratified by the source of the controls. The pooled odds ratios were 2.756 (95% CI: 1.528, 4.970, \(P = 0.001\)) in the population-based control subgroups and 1.951 (95% CI: 1.404, 2.713, \(P = 0.001\)) in the hospital-based control subgroups by homozygote comparison. The meta-analysis results for the other genetic models are listed in Table 2.

Test for heterogeneity

There was significant heterogeneity in four genetic models: TT versus CC: \(P_{\text{heterogeneity}} = 0.006\); CT versus CC: \(P_{\text{heterogeneity}} = 0.033\); CT + TT versus CC: \(P_{\text{heterogeneity}} = 0.002\); and 2TT + CT versus 2CC + CT: \(P_{\text{heterogeneity}} < 0.001\). Data are listed in Table 2. We assessed the source of heterogeneity by ethnicity, publication year, control source, and sample size. However, we did not observe any sources that contribute to the substantial heterogeneity.

Sensitivity analysis and cumulative meta-analysis

Sensitivity analyses were conducted to ascertain the primary origin of the heterogeneity. Two independent studies by Relton CL[35] and Wang F[40] affected the heterogeneity in maternal case-control studies. The heterogeneity was effectively decreased by the exclusion of the two studies: Heterogeneity chi-squared = 48.33, \(P_{\text{heterogeneity}} = 0.002\) and heterogeneity chi-squared = 29.94, \(P_{\text{heterogeneity}} = 0.120\), before and after removal, respectively. Furthermore, no single study qualitatively changed the pooled ORs, suggesting that the results of this meta-analysis were stable. In the cumulative meta-analysis, the pooled ORs tended to be stable, and the associations tended towards significant associations with the accumulation of more data over time.
| Contrast | Variables | Comparisons    | OR   | 95% CI      | P      | P* heterogeneity |
|----------|-----------|----------------|------|-------------|--------|------------------|
| TT vs CC | Overall   | 24             | 2.022 | 1.508–2.712 | <0.001 | 0.006            |
|          | Study design |                |      |             |        |                  |
|          | PB        | 6              | 2.756 | 1.528–4.970 | 0.001  | 0.112            |
|          | HB        | 17             | 1.951 | 1.404–2.713 | <0.001 | 0.006            |
|          | Mixed     | 1              | 1.625 | 0.632–4.179 | 0.314  | -                |
|          | Ethnicity  |                |      |             |        |                  |
|          | Asian     | 4              | 3.750 | 0.732–19.217| 0.113  | 0.003            |
|          | Caucasian | 15             | 1.596 | 1.271–2.005 | <0.001 | 0.280            |
|          | African   | 1              | 1.010 | 0.423–2.411 | 0.982  | -                |
|          | Mixed     | 4              | 3.595 | 2.139–6.042 | <0.001 | 0.888            |
| CT vs CC | Overall   | 25             | 1.154 | 0.982–1.357 | 0.083  | 0.053            |
|          | Study design |                |      |             |        |                  |
|          | PB        | 7              | 0.991 | 0.717–1.370 | 0.955  | 0.525            |
|          | HB        | 17             | 1.188 | 0.973–1.451 | 0.014  | 0.017            |
|          | Mixed     | 1              | 1.326 | 0.694–2.532 | 0.393  | -                |
|          | Ethnicity  |                |      |             |        |                  |
|          | Asian     | 4              | 1.933 | 1.167–3.202 | 0.010  | 0.198            |
|          | Caucasian | 15             | 1.080 | 0.886–1.316 | 0.445  | 0.039            |
|          | African   | 2              | 1.095 | 0.641–1.872 | 0.740  | 0.864            |
|          | Mixed     | 4              | 1.248 | 0.877–1.775 | 0.218  | 0.566            |
| CT+TT vs CC | Overall  | 25             | 1.303 | 1.089–1.558 | 0.004  | 0.002            |
|          | Study design |                |      |             |        |                  |
|          | PB        | 7              | 1.174 | 0.867–1.588 | 0.299  | 0.210            |
|          | HB        | 17             | 1.318 | 1.060–1.639 | 0.013  | 0.001            |
|          | Mixed     | 1              | 1.393 | 0.762–2.546 | 0.282  | -                |
|          | Ethnicity  |                |      |             |        |                  |
|          | Asian     | 4              | 2.455 | 0.886–6.803 | 0.084  | 0.026            |
|          | Caucasian | 15             | 1.186 | 0.968–1.454 | 0.100  | 0.011            |
|          | African   | 2              | 1.075 | 0.644–1.792 | 0.783  | 0.994            |
|          | Mixed     | 4              | 1.576 | 1.125–2.208 | 0.008  | 0.450            |
| TT vs CT+CC | Overall | 24             | 1.716 | 1.448–2.033 | <0.001 | 0.134            |
|          | Study design |                |      |             |        |                  |
|          | PB        | 6              | 2.818 | 1.62–4.882 | <0.001 | 0.214            |
|          | HB        | 17             | 1.631 | 1.359–1.958 | <0.001 | 0.147            |
|          | Mixed     | 1              | 1.429 | 0.583–3.498 | 0.435  | -                |
|          | Ethnicity  |                |      |             |        |                  |
|          | Asian     | 4              | 1.949 | 0.752–5.056 | 0.170  | 0.033            |
|          | Caucasian | 15             | 1.524 | 1.227–1.893 | <0.001 | 0.632            |
|          | African   | 1              | 0.946 | 0.426–2.102 | 0.892  | -                |
|          | Mixed     | 4              | 2.514 | 1.720–3.876 | <0.001 | 0.412            |
| 2TT+CT vs 2CC +CT | Overall | 25             | 1.330 | 1.160–1.525 | <0.001 | 0.001            |
|          | Study design |                |      |             |        |                  |
|          | PB        | 7              | 1.462 | 1.006–2.124 | 0.047  | 0.045            |
|          | HB        | 17             | 1.305 | 1.116–1.525 | 0.001  | 0.001            |
|          | Mixed     | 1              | 1.320 | 0.840–2.074 | 0.228  | -                |
|          | Ethnicity  |                |      |             |        |                  |
|          | Asian     | 4              | 1.633 | 0.908–2.939 | 0.102  | 0.009            |
|          | Caucasian | 15             | 1.223 | 1.049–1.427 | 0.010  | 0.013            |
|          | African   | 2              | 1.029 | 0.698–1.516 | 0.886  | 0.985            |
|          | Mixed     | 4              | 1.653 | 1.331–2.052 | <0.001 | 0.470            |

*: Random-effects model was used when P value for heterogeneity test < 0.10; otherwise, fix-effects model was used.
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Association.

Each point represents a separate study for the indicated association. Despite the potential implication of MTHFR and low plasma folate levels, it contributes to NTDs [47].

Overall, due to its potential role in lowering plasma folate and vitamin B12 levels and increased homocysteine levels [45,46]. Additionally, individuals with the TT variant also have lower normal enzyme activity and 10% lower red blood cell folate levels [43,44].

Overall, due to its potential role in decreasing MTHFR activity, causing high plasma homocysteine and low plasma folate levels, it contributes to NTDs [47].

Because heterogeneity is a potential problem when interpreting the results of all meta-analyses, we detected the source of heterogeneity by ethnicity, publication year, control source, and sample size and found that none substantially contributed to the heterogeneity. One possible reason might be the matching status.

It is assumed that MTHFR genetic polymorphisms play an important role in the development of NTDs; however, only 13% of NTDs were attributed to the MTHFR C677T mutation [51], suggesting that the MTHFR C677T polymorphism alone cannot be responsible for NTDs. Thus, potential gene-gene, maternal-foetal, genetic-nutritional interactions [48], and other SNPs in the MTHFR gene may have an association with NTD risk.

1) **Gene-gene interactions.** Folate metabolism is complex and involves several regulatory mechanisms. Genetic variations affecting protein function at any step may alter the balance of metabolites, and gene-gene interactions [52]. The combination of MTHFR and cystathionine-β-synthase (CBS) mutations was reported to have a fivefold increase in the risk for spina bifida compared with each variant alone [53], indicating the presence of gene-gene interactions.

2) **Maternal-foetal interactions.** Maternal variant genotypes were associated with NTD risk, indicating possible maternal-foetal interactions. Using family-based approaches, researchers have found that the OR increased to 4.1 (95% CI: 1.5, 11.1) if the mother had a TT genotype and her child a CT genotype and to 6.1 (95% CI: 1.0, 35.5) if both the mother and her child had TT genotypes [54].

3) **Genetic-nutritional interactions.** The combination of MTHFR mutations and low folate concentrations could lead to a hypomethylation of homocysteine to methionine, enhancing the impairment of folate metabolism and increasing the risk for NTDs [1], suggesting a strong genetic-nutritional interaction. This interaction was found in a previous study that showed the combination of MTHFR TT genotype and RBC folate level in the lowest quartile conferred an odds ratio of 13.43 (95% CI: 2.49, 72.33) for an NTD case and an odds ratio of 3.28 (95% CI: 0.84, 12.85) for having offspring with NTDs [20].

Moreover, the pooled odds ratios of mixed populations were higher than those of Caucasian populations and the overall populations in mothers (TT versus CC: OR = 3.595, 1.596, and 2.022, respectively). Many factors may contribute to the finding that the same polymorphism affects different ethnic populations to a different extent. First, the frequency of the T-allele varies in different ethnicities with different genetic backgrounds [49].

Second, different populations may have different dietary patterns, such as intake of folic acid, vitamin B12, and vitamin B6, some of which may affect NTD development. Finally, analysis of the data from the various ethnic groups might eliminate some bias caused by language because only papers written in English or Chinese were included. Thus, large-scale studies should be performed to validate ethnic differences in the effect of this functional polymorphism on NTD risk.

When stratified by study design, significantly increased risks were also found in both population-based and hospital-based studies. Nevertheless, population-based studies have a higher risk than hospital-based studies. Hospital-based studies usually have a high risk of producing unreliable results because hospital-based controls may not always accurately represent the general population, especially when the genotypes under investigation are expected to affect disease conditions that might be observed in the hospital-based controls [50].

Discussion

The folate metabolism pathway plays an important role in DNA methylation, DNA synthesis, cell division, and tissue growth, especially in the rapidly developing cells [42]. Thus, a defective folate metabolism could result in an impaired DNA synthesis or DNA methylation involved in the neurulation process. MTHFR is a key enzyme in the folate metabolism pathway. Although several single nucleotide polymorphisms (SNPs) in the MTHFR gene have been characterised, the C677T polymorphism is a widely described mutation. Heterozygotes (CT) for the polymorphism have 65% of the normal enzyme activity and 10% lower red blood cell folate level; patients with the homozygous variant (TT) have only 30% of normal enzyme activity and 18% lower red blood cell folate levels [43,44].

Overall, due to its potential role in decreasing MTHFR activity, causing high plasma homocysteine and low plasma folate levels, it contributes to NTDs [47]. Despite the potential implication of MTHFR C677T in the pathogenesis of NTDs [18–21,25–27,29–34,37,38,40,41], the association between the MTHFR C677T polymorphism and NTDs remains unclear.

Our meta-analysis, which included 2429 cases and 3570 controls, explored the associations between the maternal MTHFR C677T polymorphism and susceptibility to NTDs. Overall, we found that mothers with the homozygous TT genotype showed a significantly increased NTD risk compared with homozygous CC genotype carriers (with pooled odds ratio 2.022; 95% CI: 1.508, 2.712; P<0.001). Our results were consistent with a previous report [48] that showed an overall odds ratio of 2.04 (TT versus CC; 95% CI: 1.49, 2.81).

In subgroup analysis stratified by ethnicity, we found that the variant genotypes were associated with a significantly increased NTD risk in Asian, Caucasian and mixed populations. However, we did not find this association in African groups in any genetic model, possibly due to the limited studies and a small sample size.
Although these studies were hampered by small sample sizes, they illustrate the existence of potential interactions. Thus, further large-scale studies focusing on these complex interactions with NTDs should be performed.

**4 Other SNPs in the MTHFR gene.** Some researchers have demonstrated that other SNPs in MTHFR gene showed increased NTD risks, such as A1298C [53,56], C677T [57], G1799A [57], and were linkage disequilibrium with C677T polymorphism. All these suggest these SNPs can be additional genetic factors for NTDs.

**Limitations**

Several potential limitations of this meta-analysis should be discussed. 1) Although the funnel plot and Egger’s test showed no publication bias, selection bias may have occurred because only studies in English or Chinese were selected. 2) Our results were based on unadjusted estimates due to the absence of available studies in English or Chinese were selected. 2) Our results were publication bias, selection bias may have occurred because only large-scale studies focusing on these complex interactions with NTDs. Thus, further functional studies to investigate folate-related gene polymorphisms, periconceptional multivitamin supplements, complex interactions and their role in development of NTDs are warranted.

**Author Contributions**

Conceived and designed the experiments: LY AG. Performed the experiments: PZ GJ AG. Analyzed the data: LY LZ AG. Contributed materials/analysis tools: LY LZ PZ. Wrote the paper: LY AG. Proof read and revised the manuscript: YL.

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