Methylenetetrahydrofolate Reductase (MTHFR) Polymorphisms and Susceptibility for Cervical Lesions: A Meta-Analysis

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Abstract

Background: The association between the methylenetetrahydrofolate reductase (MTHFR) C677T/A1298C polymorphisms and the susceptibility to cervical lesions was unclear. This study was designed to investigate their precise association using a large-scale meta-analysis.

Methods: The previous 16 studies were identified by searching PubMed, Embase and CBM databases. The crude odds ratios and their corresponding 95% confidence intervals (CIs) were used to estimate the association between the MTHFR C677T/A1298C polymorphisms and the susceptibility to the cervical lesions. The subgroup analyses were made on the following: pathological history, geographic region, ethnicity, source of controls and source of DNA for genotyping.

Results: Neither of the polymorphisms had a significant association with the susceptibility to the cervical lesions in all genetic models. Similar results were found in the subgroup analyses. No association was found between the MTHFR C677T polymorphism and the cervical lesions in the Asia or the America populations though a significant inverse association was found in the Europe population (additive model: \( P = 0.006, OR = 0.83, 95\% CI = 0.72–0.95 \); CT vs. CC: \( P = 0.05, OR = 0.83, 95\% CI = 0.69–1.00 \); TT vs. CC: \( P = 0.05, OR = 0.73, 95\% CI = 0.53–1.00 \)). Interestingly, women with the MTHFR A1298C polymorphisms had a marginally increased susceptibility to invasive cancer (ICC) when compared with no carriers but no statistically significant difference in the dominant model (\( P = 0.06, OR = 1.21, 95\% CI = 0.99–1.49 \)) and AC vs. AA (\( P = 0.09, OR = 1.21, 95\% CI = 0.97–1.51 \)).

Conclusions: The MTHFR C677T and A1298C polymorphisms may not increase the susceptibility to cervical lesions. However, the meta-analysis reveals a negative association between the MTHFR C677T polymorphisms and the cervical lesions, especially in the European populations. The marginal association between the MTHFR A1298C polymorphisms and the susceptibility to cervical cancer requires a further study.

Introduction

Cervical cancer is the third most frequently encountered cancer and the fourth leading cause of the women’s cancer death in the world, accounting for 9% (529,800) of the total newly-diagnosed cancer cases and 8% (275,100) of the total cancer deaths among females in 2008 [1]. However, cervical cancer is considered a preventable disease because of its relatively long period of precancerous lesions, including cervical intraepithelial neoplasia (CIN). The virological, molecular, clinical and epidemiological studies have provided evidence that cervical cancer is in fact a sequel to a long-term unresolved infection of certain genotypes of the Human Papilloma Virus (HPV) [2,3]. High-risk HPVs are known to infect cervical epithelium, with a subset of these being associated with preneoplastic lesions that can progress to cervical cancer. Nevertheless, despite the extremely high rate of infection by these viruses, the rate of cervical cancer, even in the prescreening area, has been less than one tenth that of exposure [4,5]. Thus, other factors are important for cervical lesion development and progression such as a long-term use of hormonal contraceptives, multiparity, smoking, and some nutritional factors [6–8].

Association between micronutrient depletion, particularly folate deficiency, and cervical lesions has been studied for a long time. Folate deficiency, as a potential risk for cervical cancer, was first reported by some cytopathologists in the 1960s, who had found that the cervical epithelial cells from folate-deficient women had some similarity to the dysplastic cervical cells in cytology [9]. Later on, Whitehead et al. demonstrated that macrocytic changes in the cervical cells of the oral contraceptive users could be reversed with folic acid supplementation [10]. However, conflicting results still
MTHFR Polymorphisms and Cervical Lesion Risk

Search Strategy and Selection Criteria

The computer-based search strategy was comprehensively used to find eligible studies for this meta-analysis. Two investigators (Long, Yang) searched in the PubMed and Emase independently from inception to July 22, 2012, for the studies on the association between the MTHFR C677T polymorphism (rs1801133) and A1298C polymorphism (rs1801131) and the cervical lesions. Following Medical Subject Heading (MeSH) terms and/or text words were used in our search, such as for methylenetetrahydrofolate reductase (“MTHFR” or “methylene tetrahydrofolate reductase”) or Methylene tetrahydrofolate Reductase AND (NADPH2) with terms for genetic variations (“polymorphism” or “variation” or “mutation” or “Single Nucleotide Polymorphism” or Polymorphism, Single Nucleotide” or “SNPs”) and terms for cervical lesions: (“Uterine Cervical Cancer” or “Neoplasms, Cervix” or “Neoplasms, Cervical” or “Cervix Neoplasms” or “Cervix Cancer” or “Cervical Neoplasms” or “Cervical Cancer” or “Cervical Neoplasms” or “Neoplasia, Cervical” or “Intraepithelial Neoplasia, Cervical” or “Cervical Intraepithelial Neoplasms” or “Cervical Intraepithelial Neoplasia” or “Cervical Neoplasms” or “Cervical Intraepithelial Neoplasia” or “Cervical Neoplasms” or “Cervical Intraepithelial Neoplasia” or “Intraepithelial Neoplasia, Cervical” or “Cervical Intraepithelial Neoplasia”). Meanwhile, China Biological Medicine Database (CBM) was also searched for the eligible studies. Full articles published in English or Chinese were considered to be eligible for our study. In addition, reference list of the original research articles and reviews were also manually searched.

The eligible studies must meet the following inclusion criteria: (1) Exploration of associations between the MTHFR genetic polymorphisms (including C677T or A1298C or both) and the susceptibility to cervical cancer or SIL; (2) A case-control study; (3) Provision of information on genotype frequencies of the MTHFR C677T and/or A1298C polymorphism(s) or sufficient data for the calculation. The exclusion criteria were as follows: (1) A review, case report, editorial, or comment; (2) A duplicated study; (3) Laboratory molecular or animal studies. If studies contained overlapping cases and/or controls, the largest study with extractable data was preferred.

Because the data included in this study was taken from literatures, written consent given by the patients and ethical approval acquired by certain committee were not needed in our meta-analysis.

Data Extraction

According to the inclusion and exclusion criteria, extraction from each study was conducted independently by two authors (Long, Yang) and the consensus was achieved for all the data, which were as follows: the first author’s name, year of publication, source of controls, source of DNA for genotyping, country, ethnicity, goodness-in-fitness of Hardy-Weinberg Equilibrium (HWE) in the control group, histological stage of cervical lesions, numbers of cases/patients and controls, and distribution of genotypes in the case and control groups. The patients were recruited into the study regardless of whether they had a first-degree relative with cervical lesions. The controls were recruited regardless of whether they had other diseases, e.g., hysterectomy. For studies with inadequate information, authors of those studies were contacted for further information by E-mail if possible.

These inconclusive results may due to limited sample size, because any single study may be underpowered to detect the precise effects. In addition, there also may be the causes of different characteristics among studies, such as ethnicity, pathological history, sources of controls, and source of DNA for genotyping. Therefore, we have done a meta-analysis on association between MTHFR polymorphisms and cervical lesions using data obtained from the published case-control genetic studies. Our aim was to identify whether the MTHFR polymorphisms affect the susceptibility to SIL or cervical cancer by means of a large-scale meta-analysis. Furthermore, we wanted to summarize the effect size of the polymorphism associated with the susceptibility to the cervical lesions.

Materials and Methods

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These inconclusive results may due to limited sample size, because any single study may be underpowered to detect the precise effects. In addition, there also may be the causes of different characteristics among studies, such as ethnicity, pathological history, sources of controls, and source of DNA for genotyping. Therefore, we have done a meta-analysis on association between MTHFR polymorphisms and cervical lesions using data obtained from the published case-control genetic studies. Our aim was to identify whether the MTHFR polymorphisms affect the susceptibility to SIL or cervical cancer by means of a large-scale meta-analysis. Furthermore, we wanted to summarize the effect size of the polymorphism associated with the susceptibility to the cervical lesions.
Statistical Analysis

Meta-analysis was performed and reported as described previously [36,37]. Crude ORs with 95% CIs were computed to assess the strength of the correlation between the MTHFR C677T/A1298C polymorphisms and the susceptibility to cervical lesions. The pooled ORs were performed for the dominant model (AA+AA vs. AA), recessive model (AA vs. Aa+AA) and additive model (A vs. a). Moreover, the pooled estimates were also calculated for the pair-wise comparisons (allele Aa vs. AA, and allele aa vs. AA). The above-mentioned A and a represented the major and the minor allele respectively. Taking consideration of possible between-study heterogeneity, a statistical test for heterogeneity was performed by the \( \chi^2 \) test or Fisher exact test when appropriate. \( P<0.10 \) or \( I^2 >50\% \) indicated an obvious of the between-study heterogeneity, and OR (95% CI) was calculated by the random-effects model using the DerSimonian and Laird method; otherwise, the fixed-effects model was used by the Mantel-Haenszel method [38,39]. Subgroup analyses were mainly conducted using the corresponding pathological history (ICC, SIL), geographic region (Asia, Europe, United States), ethnicity (Asian, Caucasian, mixed), source of controls (healthy persons, hospital patients and 4 studies from both). 9 studies were performed in Asia; 4 studies performed in Europe; 3 studies performed in America. 5 studies talked about ICC; 3 studies talked about SIL and 8 studies talked about both. For A1298C, all 5 studies performed in Asian; 4 studies recruited controls from healthy persons and 1 study from both healthy persons and hospital patients. I study talked about ICC and 4 studies talked about both ICC and SIL. 14 of the studies presented NS (not significant) were conformed to Hardy Weinberg-Equilibrium (HWE) expectations (\( P>0.05 \)). However, two of the studies [27,35] presented NA (not available) were because we could not perform the HWE test for the subjects (either cases or controls) in those studies, for only the total number of the combined genotypes (CT/TT vs. CC or AC/CC vs. AA) were available. Therefore, this study was included in the analysis on the dominant model, not on other genetic models. Furthermore, the allele and genotype frequencies, at which the MTHFR C677T and the A1298C polymorphisms occurred in case and controls in each of the studies, were also summarized (Table 1, Table 2).

Quantitative Synthesis

Association between the MTHFR C677T polymorphisms and cervical lesions. As for the C677T polymorphism, no association was found between the polymorphism and the susceptibility to cervical lesions in all the genetic models (Table 3, dominant model: OR = 0.99, 95% CI = 0.78–1.26, Figure 2A; recessive model: OR = 1.05, 95% CI = 0.80–1.38; additive model: OR = 0.97, 95% CI = 0.80–1.18; CT vs. CC: OR = 0.97, 95% CI = 0.78–1.20, Figure 2B; TT vs. CC: OR = 1.06, 95% CI = 0.76–1.48, Figure 2C). The heterogeneity was significant in all the genetic models (\( P<0.05 \)) and the random-effects model was used in the meta-analysis. The subgroup analysis of the C677T polymorphisms in the histological stages of the cervical lesions also revealed that the polymorphism was not associated with the risk of ICC or SIL in all the genetic models (Table 3). Although the subgroup analysis of C677T in the geographic regions revealed that no association was found between the C677T polymorphism and the cervical lesions in either the Asia or the America populations, the Europe population showed a significant inverse association in some genetic models (additive model: \( P = 0.006 \), OR = 0.83, 95% CI = 0.72–0.95; CT vs. CC: \( P = 0.05 \), OR = 0.83, 95% CI = 0.69–1.00; TT vs. CC: \( P = 0.05 \), OR = 0.73, 95% CI = 0.53–1.00). The heterogeneity was significantly reduced in the Europe populations in the recessive, additive, C/T vs. C/C, and T/T vs. C/C models. In the sensitivity analysis, the overall association between the MTHFR C677T genotype and the cervical lesions was unchanged after an exclusion of the individual study, including two studies [27,35], which lacked enough data to calculate if it conformed to HWE among the control group. Similar results were found in the sensitivity analyses on the association between the MTHFR C677T genotype and ICC or SIL, indicating that our results were statistically robust. No obvious publication bias was detected according to the shapes of the funnel plots for the C677T polymorphism in all the genetic models (Figure 3). Consistent results of the Egger’s and the Begg’s tests were also obtained in all the genetic models (Table 3). Moreover, neither the funnel plots nor the Begg’s or Egger’s test detected any obvious evidence for the publication bias in the subgroup analyses on all the genetic models (data not shown).

Association between the MTHFR A1298C polymorphisms and cervical lesions. As for the A1298C polymorphism, no association was found between the polymorphism and the cervical lesions in all the genetic models (Table 4, dominant model: OR = 1.21, 95% CI = 0.87–1.690, Figure 4A; recessive model: OR = 0.81, 95% CI = 0.54–1.23; additive model: OR = 0.98, 95% CI = 0.85–1.14; AG vs. AA: OR = 1.02, 95% CI = 0.85–1.24, Figure 4B; CC vs. AA: OR = 0.80, 95% CI = 0.52–1.24, Figure 4C). The heterogeneity was significant in the dominant
Figure 1. Flow diagram of the study selection process.
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Table 1. Characteristics of the included case-control studies on the MTHFR C677T polymorphism in cervical lesions.

| First author [reference] | Year | Source of control | Source of DNA | Country | Ethnicity | HWE | Histology | Sample size | Case control | C T CC CT TT CT TT CT TT |
|--------------------------|------|-------------------|---------------|---------|-----------|------|-----------|-------------|--------------|-----------------------------|
| Prasad [20]              | 2011 | Mixed             | Blood         | India   | Asian     | NS   | ICC       | 62          | 125          | 119 5 57 5 0 5 240 10 116 8 1 9 |
| Mostowska [21]           | 2011 | Healthy persons   | Blood         | Poland  | Caucasian | NS   | ICC       | 124         | 168          | 194 77 56 59 9 68 219 117 69 81 18 99 |
| Tong [26]                | 2011 | Healthy persons   | Blood         | Korea   | Asian     | NS   | LSIL      | 159         | 427          | 186 132 52 82 25 107 502 352 152 198 77 275 |
|                         |      |                    |               |         |           |      | HSIL      | 160         | 427          | 182 138 54 74 32 106 502 352 152 198 77 275 |
|                         |      |                    |               |         |           |      | ICC       | 146         | 427          | 171 121 53 65 28 93 502 352 152 198 77 275 |
| Shekari [32]             | 2008 | Healthy persons   | Blood         | India   | Asian     | NS   | HSIL      | 200         | 200          | 368 128 57 28 5 30 318 82 125 68 7 75 |
|                         |      |                    |               |         |           |      | ICC       | 164         | 231          | 273 55 113 47 4 51 387 75 161 65 5 70 |
| Nandan [27]              | 2008 | Healthy persons   | Blood         | India   | Asian     | NS   | NA        | 80          | 77           | NA NA NA NA NA NA NA NA NA NA NA NA |
|                         |      |                    |               |         |           |      | ICC       | 62          | 77           | NA NA NA NA NA NA NA NA NA NA NA |
|                         |      |                    |               |         |           |      | NA        | 80          | 355          | 134 26 59 16 5 21 562 148 223 116 16 132 |
|                         |      |                    |               |         |           |      | HSIL      | 264         | 592          | 362 166 121 120 23 143 808 376 273 262 57 319 |
|                         |      |                    |               |         |           |      | ICC       | 363         | 592          | 944 328 357 230 49 279 808 376 273 262 57 319 |
|                         |      |                    |               |         |           |      | ICC       | 79          | 74           | 86 72 27 32 20 52 92 56 30 32 12 44 |
|                        |      |                    |               |         |           |      | HSIL      | 40          | 454          | 42 38 10 22 8 30 527 381 153 221 80 301 |
|                         |      |                    |               |         |           |      | ICC       | 40          | 454          | 176 454 190 162 50 90 36 126 527 381 153 221 80 301 |
|                         |      |                    |               |         |           |      | ICC       | 39          | 231          | 67 11 28 11 0 11 387 75 161 65 5 70 |
|                         |      |                    |               |         |           |      | HSIL      | 40          | 454          | 176 454 190 162 50 90 36 126 527 381 153 221 80 301 |
|                         |      |                    |               |         |           |      | LSIL      | 39          | 231          | 67 11 28 11 0 11 387 75 161 65 5 70 |
|                        |      |                    |               |         |           |      | ICC       | 40          | 454          | 176 454 190 162 50 90 36 126 527 381 153 221 80 301 |
| Lambrinou [24]           | 2003 | Healthy persons   | Tissue or cell | Greece  | Caucasian | NS   | LSIL      | 53          | 91           | 68 38 20 28 5 33 121 61 42 37 12 49 |
|                         |      |                    |               |         |           |      | HCIL      | 64          | 91           | 83 45 27 29 8 37 121 61 42 37 12 49 |
|                         |      |                    |               |         |           |      | ICC       | 21          | 91           | 30 12 11 8 2 10 121 61 42 37 12 49 |
|                         |      |                    |               |         |           |      | HSIL      | 150         | 179          | 213 87 73 67 10 77 261 97 93 75 11 86 |
|                         |      |                    |               |         |           |      | ICC       | 25          | 31           | 25 25 6 13 6 19 44 18 16 12 3 15 |
|                         |      |                    |               |         |           |      | HSIL      | 39          | 31           | 45 33 11 23 5 28 44 18 16 12 3 15 |
| Agodi [35]               | 2010 | Healthy persons   | Cell          | Italy   | Caucasian | NS   | SIL       | 123         | 66           | NA NA NA NA NA 5 NA NA NA NA NA |
|                         |      |                    |               |         |           |      | ICC       | 157         | 382          | 229 85 77 5 80 530 234 182 166 34 200 |
| Yang [25]                | 2011 | Mixed             | Blood         | China   | Asian     | NS   | SIL       | 38          | 382          | 60 16 23 14 1 15 530 234 182 166 34 200 |
|                         |      |                    |               |         |           |      | ICC       | 157         | 382          | 229 85 77 5 80 530 234 182 166 34 200 |
| Ma [31]                  | 2006 | Hospital patients | Blood         | China   | Asian     | NS   | ICC       | 111         | 111          | 93 129 20 53 38 91 126 96 33 60 18 78 |

Abbreviations: HWE, Hardy-Weinberg Equilibrium; NA, not available; NS, not significant; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer; SIL, squamous intra-epithelial lesion.

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### Table 2. Characteristics of the included case-control studies on the MTHFR A1298C polymorphism in cervical lesions.

| First author | Source of control | Year | Control | Case | Source of DNA | Country | Ethnicity | HWE | Histology | Sample size |
|--------------|------------------|------|---------|------|---------------|---------|-----------|------|-----------|-------------|
| Tong [26]    | Healthy persons  | 2011 | Blood   | Korea | Asian NS      | NS      | NA        | NA   | SIL       | 160 428     |
|              |                  |      |         |      |               |         |           |      | HSIL      | 273 428     |
|              |                  |      |         |      |               |         |           |      | ICC       | 235 428     |
| Kohaar [22]  | Healthy persons  | 2010 | Tissue or cell | India | Asian NS      | NS      | NA        | NA   | HSIL      | 39 231      |
|              |                  |      |         |      |               |         |           |      | ICC       | 199 231     |
|              |                  |      |         |      |               |         |           |      | NA        | 14 NA       |
|              |                  |      |         |      |               |         |           |      | NA        | 66 NA       |
|              |                  |      |         |      |               |         |           |      | NA        | 37 NA       |
|              |                  |      |         |      |               |         |           |      | NA        | 40 NA       |
| Nandan [27]  | Healthy persons  | 2008 | Blood   | India | Asian NS      | NS      | NA        | NA   | NS        | 80 77       |
|              |                  |      |         |      |               |         |           |      | NA        | 22 NA       |
|              |                  |      |         |      |               |         |           |      | NA        | 42 NA       |
|              |                  |      |         |      |               |         |           |      | NA        | 37 NA       |
|              |                  |      |         |      |               |         |           |      | NA        | 25 NA       |
| Kang [23]    | Healthy persons  | 2005 | Blood   | Korea | Asian NS      | NS      | NA        | NA   | NA        | 62 77       |
|              |                  |      |         |      |               |         |           |      | NA        | 22 55       |
|              |                  |      |         |      |               |         |           |      | NA        | 22 24       |
|              |                  |      |         |      |               |         |           |      | NA        | 14 14       |
|              |                  |      |         |      |               |         |           |      | NA        | 36 14       |
| Yang [25]    | Healthy persons  | 2011 | Blood   | China | Asian NS      | NS      | NA        | NA   | NS        | 38 382      |
|              |                  |      |         |      |               |         |           |      | NA        | 38 382      |
|              |                  |      |         |      |               |         |           |      | NA        | 24 24       |
|              |                  |      |         |      |               |         |           |      | NA        | 24 24       |
|              |                  |      |         |      |               |         |           |      | NA        | 24 24       |
|              |                  |      |         |      |               |         |           |      | NA        | 133 133     |

Abbreviations: HWE: Hardy-Weinberg Equilibrium; NS, not significant; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer; SIL, squamous intra-epithelial lesion.

Discussion

As we know, HPV infection may be necessary but is not sufficient to cause cervical cancer. Other factors may play some important roles in this cancer development. For example, the nutritional factors may affect the persistence of HPV infection and thereby influence progression of early precancerous lesions to invasive cancer. Specifically, folate plays a key role in DNA synthesis, repair, and methylation, and this forms the basis of mechanistic explanations for a putative role for folate in cancer prevention. However, the effect of folate in these processes may be modulated by the genotype for the common C677T or A1298C variants of MTHFR, the homozygosity of which is associated with a lower level of the enzyme activity, lower plasma and red blood cell folate, and elevated plasma homocysteine [42,43]. Several studies investigated the association between the MTHFR polymorphisms and the preinvasive cervical lesions or cervical cancer, but the results were not consistent. Thus, our meta-analysis could better evaluate association between the MTHFR C677T/A1298C polymorphisms and susceptibility to cervical lesions. Our findings demonstrate that there was no association between them. To our knowledge, this is the first meta-analysis on association between the MTHFR C677T/A1298C polymorphisms and susceptibility to cervical lesions, and the largest-scale meta-analysis examining the risk of cervical cancer.

As for the MTHFR C677T, most evidence points to decrease in the susceptibility to colorectal cancer and an increase in the susceptibility to esophagus and gastric cancer [44–48], but the effect on the cervical cancer susceptibility was not consistent. In our meta-analysis, no statistically significant difference was found in the frequency of the MTHFR C677T polymorphism in the patients with cervical lesions when compared with the controls. This finding was consistent with that of one previous meta-analysis [49]. However, 9 new studies [20–22,25–27,32,33,35] have been published since 2006 and all recruited in our study dramatically increased the case number of cervical lesion and controls with genetic information, which indicated that our results could be more reliable. In addition, multiple subgroup analyses made our
### A

| Study or Subgroup | Experimental Events | Control Events | Total | Weight | M-H Ratio M-H, Random, 95% CI | Odds Ratio M-H, Random, 95% CI |
|-------------------|---------------------|----------------|-------|--------|-------------------------------|-------------------------------|
| Agodi 2010        | 5                   | 123            | 11    | 66     | 0.21 [0.07, 0.64]             |                               |
| Goodman 2001      | 77                  | 150            | 86    | 179    | 1.14 [0.74, 1.76]             |                               |
| Kang 2005         | 52                  | 79             | 44    | 74     | 1.31 [0.68, 2.53]             |                               |
| Kohaar 2010       | 62                  | 203            | 70    | 231    | 1.01 [0.67, 1.52]             |                               |
| Lambropoulos 2003 | 80                  | 138            | 49    | 91     | 1.16 [0.69, 2.01]             |                               |
| Ma 2006           | 91                  | 111            | 78    | 111    | 1.93 [1.02, 3.62]             |                               |
| Mostowska 2011    | 68                  | 124            | 99    | 168    | 0.85 [0.53, 1.35]             |                               |
| Nandan 2008       | 72                  | 142            | 24    | 77     | 2.27 [1.27, 4.07]             |                               |
| Pyathilake 2000   | 47                  | 64             | 30    | 62     | 2.95 [1.40, 6.22]             |                               |
| Pyathilake 2007   | 21                  | 80             | 132   | 355    | 0.60 [0.35, 1.03]             |                               |
| Prasad 2011       | 5                   | 62             | 9     | 125    | 1.13 [0.36, 3.53]             |                               |
| Shukari 2008      | 30                  | 200            | 75    | 200    | 0.29 [0.19, 0.49]             |                               |
| Sull 2004         | 329                 | 482            | 301   | 454    | 1.26 [0.95, 1.66]             |                               |
| Tong 2011         | 306                 | 455            | 275   | 427    | 1.06 [0.81, 1.40]             |                               |
| Yang 2011         | 95                  | 196            | 200   | 382    | 0.86 [0.61, 1.22]             |                               |
| Zoodsma 2005      | 422                 | 900            | 319   | 502    | 0.76 [0.61, 0.93]             |                               |

Total (95% CI) 3498 3594 100.0% 0.99 [0.78, 1.26]

Heterogeneity: Tau² = 0.16, Chi² = 66.97, df = 15 (P < 0.00001), I² = 78%

Test for overall effect: Z = 0.06 (P = 0.95)

### B

| Study or Subgroup | Experimental Events | Control Events | Total | Weight | M-H Ratio M-H, Random, 95% CI | Odds Ratio M-H, Random, 95% CI |
|-------------------|---------------------|----------------|-------|--------|-------------------------------|-------------------------------|
| Goodman 2001      | 67                  | 140            | 75    | 168    | 1.14 [0.73, 1.78]             |                               |
| Kang 2005         | 32                  | 59             | 32    | 62     | 1.11 [0.54, 2.27]             |                               |
| Kohaar 2010       | 58                  | 199            | 85    | 226    | 1.02 [0.67, 1.55]             |                               |
| Lambropoulos 2003 | 65                  | 123            | 37    | 79     | 1.27 [0.72, 2.24]             |                               |
| Ma 2006           | 53                  | 73             | 60    | 93     | 1.46 [0.75, 2.84]             |                               |
| Mostowska 2011    | 59                  | 115            | 81    | 150    | 0.90 [0.55, 1.48]             |                               |
| Pyathilake 2000   | 36                  | 53             | 24    | 56     | 2.82 [1.29, 6.18]             |                               |
| Pyathilake 2007   | 16                  | 75             | 116   | 339    | 0.52 [0.29, 0.95]             |                               |
| Prasad 2011       | 5                   | 62             | 9     | 124    | 1.27 [0.40, 4.08]             |                               |
| Shukari 2008      | 28                  | 198            | 68    | 193    | 0.30 [0.18, 0.50]             |                               |
| Sull 2004         | 227                 | 360            | 221   | 374    | 1.16 [0.88, 1.59]             |                               |
| Tong 2011         | 221                 | 380            | 198   | 350    | 1.07 [0.80, 1.43]             |                               |
| Yang 2011         | 89                  | 189            | 166   | 348    | 0.98 [0.68, 1.39]             |                               |
| Zoodsma 2005      | 350                 | 828            | 262   | 535    | 0.76 [0.61, 0.95]             |                               |

Total (95% CI) 2854 3097 100.0% 0.97 [0.78, 1.20]

Heterogeneity: Tau² = 0.10, Chi² = 41.92, df = 13 (P < 0.0001), I² = 69%

Test for overall effect: Z = 0.32 (P = 0.75)

### C

| Study or Subgroup | Experimental Events | Control Events | Total | Weight | M-H Ratio M-H, Random, 95% CI | Odds Ratio M-H, Random, 95% CI |
|-------------------|---------------------|----------------|-------|--------|-------------------------------|-------------------------------|
| Goodman 2001      | 10                  | 83             | 11    | 104    | 1.16 [0.47, 2.88]             |                               |
| Kang 2005         | 20                  | 47             | 12    | 42     | 1.85 [0.76, 4.49]             |                               |
| Kohaar 2010       | 4                   | 145            | 5     | 166    | 0.91 [0.24, 3.47]             |                               |
| Lambropoulos 2003 | 15                  | 73             | 12    | 54     | 0.91 [0.38, 2.13]             |                               |
| Ma 2006           | 38                  | 58             | 18    | 51     | 3.48 [1.58, 7.67]             |                               |
| Mostowska 2011    | 9                   | 65             | 18    | 87     | 0.62 [0.26, 1.48]             |                               |
| Pyathilake 2000   | 11                  | 28             | 6     | 38     | 3.45 [1.09, 10.96]            |                               |
| Pyathilake 2007   | 5                   | 64             | 16    | 239    | 1.18 [0.42, 3.36]             |                               |
| Prasad 2011       | 0                   | 57             | 1     | 117    | 0.68 [0.03, 16.84]            |                               |
| Shukari 2008      | 2                   | 172            | 7     | 132    | 0.21 [0.04, 1.03]             |                               |
| Sull 2004         | 102                 | 236            | 80    | 233    | 1.47 [1.01, 2.13]             |                               |
| Tong 2011         | 85                  | 244            | 77    | 229    | 1.06 [0.72, 1.54]             |                               |
| Yang 2011         | 8                   | 106            | 34    | 216    | 0.32 [0.13, 0.79]             |                               |
| Zoodsma 2005      | 72                  | 550            | 57    | 330    | 0.72 [0.49, 1.06]             |                               |

Total (95% CI) 1927 2038 100.0% 1.06 [0.76, 1.48]

Heterogeneity: Tau² = 0.20, Chi² = 33.71, df = 13 (P = 0.001), I² = 61%

Test for overall effect: Z = 0.35 (P = 0.73)
Figure 2. Forest plot describing the association between the C677T polymorphism and the risk of cervical lesions. (A) Meta-analysis in a random-effects model for CT+TT vs. CC (dominant model). (B) Meta-analysis in a random-effects model for CT vs. CC. (C) Meta-analysis in a random-effects model for TT vs. CC. Each study is shown by the point estimate of the OR (the size of the square is proportional to the weight of each study) and 95% CI for the OR (extending lines).

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| Genetic model | Number of study | Sample Size | Analysis | P (Publication bias test) | Test of Association |
|---------------|----------------|-------------|---------|---------------------------|--------------------|
|               |                | Case | Control | I² (%) | P | OR (95%CI) | Begg's test | Egger's test |
| Total         |                |      |         |        |   |             |             |             |
| Dominant model| 16             | 3498 | 3594    | 0.00   | 0.95 | 0.99 (0.78, 1.26) | 0.558 | 0.626 |
| Recessive model| 14             | 3233 | 3451    | 0.01   | 0.75 | 1.05 (0.80, 1.38) | 0.827 | 0.956 |
| Additive model| 14             | 6177 | 6902    | 0.00   | 0.79 | 0.97 (0.80, 1.18) | 1.000 | 0.659 |
| CT vs. CC     | 14             | 2854 | 3097    | 0.00   | 0.75 | 0.97 (0.78, 1.20) | 0.443 | 0.490 |
| TT vs. CC     | 14             | 1927 | 2038    | 0.00   | 0.73 | 1.06 (0.76, 1.48) | 0.913 | 0.614 |
| Pathological type |            |      |         |        |   |             |             |             |
| ICC           |                |      |         |        |   |             |             |             |
| Dominant model| 12             | 2008 | 2932    | 0.00   | 0.62 | 0.94 (0.72, 1.21) | 0.558 | 0.626 |
| Dominant model*| 11             | 1946 | 2855    | 0.00   | 0.44 | 0.90 (0.69, 1.18) | 0.558 | 0.626 |
| recessive model| 11             | 1946 | 2855    | 0.00   | 0.96 | 1.01 (0.70, 1.45) | 0.558 | 0.626 |
| Additive model| 11             | 3915 | 5710    | 0.00   | 0.51 | 0.92 (0.73, 1.17) | 0.558 | 0.626 |
| CT vs. CC     | 11             | 1731 | 2534    | 0.00   | 0.29 | 0.88 (0.69, 1.12) | 0.558 | 0.626 |
| TT vs. CC     | 11             | 1229 | 1657    | 0.00   | 0.84 | 0.96 (0.62, 1.47) | 0.558 | 0.626 |
| SIL           |                |      |         |        |   |             |             |             |
| Dominant model| 11             | 1490 | 2916    | 0.00   | 0.54 | 1.09 (0.82, 1.45) | 0.558 | 0.626 |
| Dominant model*| 9              | 1287 | 2773    | 0.04   | 0.51 | 1.08 (0.86, 1.35) | 0.558 | 0.626 |
| recessive model| 9              | 1287 | 2773    | 0.04   | 0.51 | 1.08 (0.86, 1.35) | 0.558 | 0.626 |
| Additive model| 9              | 2574 | 5546    | 0.08   | 0.59 | 1.04 (0.90, 1.21) | 0.558 | 0.626 |
| CT vs. CC     | 9              | 1123 | 2475    | 0.06   | 0.27 | 1.09 (0.94, 1.26) | 0.558 | 0.626 |
| TT vs. CC     | 9              | 698  | 1609    | 0.45   | 0.36 | 1.11 (0.88, 1.40) | 0.558 | 0.626 |
| Geographic area|               |      |         |        |   |             |             |             |
| Asian         |                |      |         |        |   |             |             |             |
| Dominant model| 9              | 1919 | 2081    | 0.00   | 0.71 | 1.07 (0.76, 1.49) | 0.558 | 0.626 |
| recessive model| 8              | 1777 | 2004    | 0.00   | 0.74 | 1.08 (0.70, 1.66) | 0.558 | 0.626 |
| Additive model| 8              | 3242 | 4008    | 0.00   | 0.82 | 0.97 (0.71, 1.31) | 0.558 | 0.626 |
| CT vs. CC     | 8              | 1520 | 1770    | 0.00   | 0.72 | 0.95 (0.70, 1.28) | 0.558 | 0.626 |
| TT vs. CC     | 8              | 1064 | 1186    | 0.00   | 0.77 | 1.08 (0.85, 1.30) | 0.558 | 0.626 |
| European      |                |      |         |        |   |             |             |             |
| Dominant model| 4              | 1285 | 917     | 0.05   | 0.18 | 0.77 (0.52, 1.13) | 0.558 | 0.626 |
| recessive model| 3              | 1162 | 851     | 0.00   | 0.89 | 0.79 (0.58, 1.07) | 0.558 | 0.626 |
| Additive model| 3              | 2347 | 1702    | 0.00   | 0.42 | 0.83 (0.62, 0.95) | 0.558 | 0.626 |
| CT vs. CC     | 3              | 1066 | 764     | 0.24   | 0.05 | 0.83 (0.69, 1.00) | 0.558 | 0.626 |
| TT vs. CC     | 3              | 688  | 471     | 0.00   | 0.82 | 0.73 (0.53, 1.00) | 0.558 | 0.626 |
| USA           |                |      |         |        |   |             |             |             |
| Dominant model| 3              | 294  | 596     | 0.00   | 0.62 | 1.22 (0.56, 2.65) | 0.558 | 0.626 |
| recessive model| 3              | 294  | 596     | 0.00   | 0.72 | 1.39 (0.79, 2.45) | 0.558 | 0.626 |
| Additive model| 3              | 588  | 1192    | 0.00   | 0.72 | 1.39 (0.79, 2.45) | 0.558 | 0.626 |
| CT vs. CC     | 3              | 268  | 563     | 0.00   | 0.72 | 1.39 (0.79, 2.45) | 0.558 | 0.626 |
| TT vs. CC     | 3              | 175  | 381     | 0.00   | 1.56 | 1.56 (0.88, 2.77) | 0.558 | 0.626 |

Dominant model: CT+TT vs. CC; Recessive model: TT vs. CC; Additive model: T vs. C; R, Random-effects model; F, fixed-effects model; ICC, invasive cervical cancer; SIL, squamous intra-epithelial lesion; Dominant model*: one study [27] omitted; Dominant model: two studies [27,35] omitted.

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Table 3. Pooled Analysis on Association between the MTHFR C677T polymorphism and the cervical lesion risk.
meta-analysis more convincing too. We meta-analyzed the eligible case-control studies for C677T by geographic regions. No association was found between the C677T polymorphism and the cervical lesions in either in the Asian or in the American populations. However, a significant inverse association was found in the European population. Different genetic backgrounds or environmental conditions could explain the discrepancy. The meta-analysis also stratified by histological stages of cervical lesions showed that there was no association between the MTHFR C677T variants and cervical lesion development. To assess the effect of individual study on the overall meta-analysis estimate, we excluded one study at a time, and the exclusion of any single report did not change the significance of the final conclusion, which indicated that the outcomes were robust. Taken together, we could make a conclusion that cervical lesion were not primarily caused by genetically-determined enzymatic defects in the folate metabolic pathway, which might be different from the pathways supposed for colorectal or gastric carcinogenesis. The effect of those polymorphisms on the cervical cancer susceptibility seems to be further modulated by other cofactors such as infection with the HPV and smoking.

As for MTHFR A1298C, some studies reported a positive association with cervical lesions, which had only borderline significance [25]. More recent studies have revealed no association between the MTHFR A1298C and the cervical lesions [22,23,26,27]. Our meta-analysis confirmed that there is no

![Figure 3. Funnel plot analysis on the detection of the publication bias for the C677T polymorphism.](image)

![Figure 4. Forest plot describing the association between the A1298C polymorphism and the risk of cervical lesions.](image)
Table 4. Pooled Analysis on Association between the MTHFR A1298C polymorphism and the cervical lesion risk.

| Genetic model | Number of study | Sample Size | Analysis Model | \( \tau^2 \) (%) | \( P_h \) | Test of Association | \( \tau \) (Publication bias test) |
|---------------|-----------------|-------------|----------------|-----------------|----------|-------------------|-------------------------------|
|               |                 | Case | Control | P     | OR(95%CI) | Begg's test | Egger's test |
| Total         |                 |      |         |       |          |                |                 |
| Dominant      | 5               | 1087 | 1202    | R     | 68       | 0.26          | 1.21(0.87, 1.69) | 0.462 | 0.290 |
| Recessive     | 4               | 945  | 1125    | F     | 42       | 0.16          | 0.33(0.54, 1.23) | 1.000 | 0.992 |
| Additive      | 4               | 1890 | 2250    | F     | 0        | 0.81          | 0.82(0.85, 1.14) | 1.000 | 0.587 |
| AC vs. AA     | 4               | 912  | 1066    | F     | 0        | 0.81          | 0.80(0.85, 1.24) | 1.000 | 0.930 |
| CC vs. AA     | 4               | 597  | 717     | F     | 37       | 0.19          | 0.80(0.52, 1.24) | 1.000 | 0.971 |
| Pathological type |             |     |         |       |          |                |                 |
| ICC           |                 |      |         |       |          |                |                 |
| Dominant      | 5               | 610  | 1202    | F     | 0        | 0.63          | 0.06(0.99, 1.49) | 1.000 | 0.81 |
| Recessive     | 4               | 548  | 1125    | R     | 51       | 0.10          | 0.46(0.24, 1.93) | 1.000 | 0.81 |
| Additive      | 4               | 1096 | 2250    | F     | 0        | 1.00          | 0.43(0.90, 1.27) | 1.000 | 0.81 |
| AC vs. AA     | 4               | 520  | 1066    | F     | 0        | 0.62          | 0.09(0.97, 1.51) | 1.000 | 0.81 |
| CC vs. AA     | 4               | 319  | 717     | F     | 43       | 0.15          | 0.46(0.49, 1.38) | 1.000 | 0.81 |
| SIL           |                 |      |         |       |          |                |                 |
| Dominant      | 4               | 477  | 1118    | R     | 83       | 0.00          | 0.49(0.63, 2.60) | 1.28(0.63, 2.60) | 1.000 | 0.81 |
| Recessive     | 3               | 397  | 1041    | F     | 0        | 0.85          | 0.43(0.76, 1.44) | 1.000 | 0.81 |
| Additive      | 3               | 794  | 2082    | F     | 0        | 0.90          | 0.14(0.85, 1.06) | 1.000 | 0.81 |
| AC vs. AA     | 3               | 382  | 983     | F     | 0        | 0.75          | 0.25(0.85, 1.12) | 1.000 | 0.81 |
| CC vs. AA     | 3               | 278  | 658     | F     | 0        | 0.86          | 0.34(0.74, 1.38) | 1.000 | 0.81 |

Dominant model: CC+AC vs. AA; Recessive model: CC vs. AC+AA; Additive model: C vs. A; R, Random-effects model; F, fixed-effects model; ICC, invasive cervical cancer; SIL, squamous intra-epithelial lesion.
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Figure 5. Influence analysis of the summary odds ratio coefficients on the association between the A1298C polymorphism and cervical cancer in dominant model. The results were computed by omitting each study (left column) in turn. Bars, 95% CIs.
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association between the A1298C polymorphism and cervical lesions, similar to that found by the subgroup analysis on the ethnic groups and the histological stages of cervical lesions. No association was found between the A1298C polymorphism and SILs, but the ICC showed a marginally positive association though with no statistically significant difference. This result suggested that a probably higher risk for cervical cancer was linked to the A1298C variants, implying their important role in later stages of cervical carcinogenesis but not in SILs. Sensitivity analyses revealed that the overall association between the MTHFR A1298C genotype and cervical lesions could be changed after excluding one study [27] which lacked sufficient data to calculate whether it conformed to HWE among or not in the control group. In contrast, the results were virtually unchanged after the exclusion of any other individual study. To sum up, it is possibly indicated that the study by Nandtan et al. could be the main source of the observed heterogeneity across the studies in this meta-analysis. Alternatively, the study may had limitations or because of other unknown factors.

To some extent, several limitations of this meta-analysis should be addressed. One limitation of the present study was that the sample size of A1298C mutation involved is not big enough. We need more original researches to make our conclusions more reliable and accurate. The studies on the A1298C variant had reported only 5 articles, and their participants were entirely Asians with no population variation in minor allele frequency. So, the subgroup meta-analysis on this gene polymorphism was not possible by race. Another limitation was that significant heterogeneity in the studies was mainly present in overall analyses and subgroup analyses. Though several possible sources of the between-study heterogeneity were investigated, including pathological history, geographic region, ethnicity, source of controls, and source of DNA for genotyping ethnicity (data not shown), none of them could sufficiently explain the heterogeneity. The effect estimates might depend on some unidentified sources of heterogeneity. Besides, part of the exposure information was still lacking in the available studies, e.g., HPV infection status, smoking status or nutritional status (particularly folate intake or level). Therefore, effects of environment exposure or lifestyle on association between MTHFR variants and cervical lesions could not be determined by this meta-analysis.

In summary, despite the above-mentioned limitations, the present study provides evidence that the MTHFR C677T and A1298C polymorphisms may not increase the susceptibility to cervical cancer development. However, our meta-analysis reveals a negative association between the MTHFR C677T mutations and cervical lesions, especially in the European populations. The marginal association between the MTHFR A1298C polymorphisms and the susceptibility for cervical cancer need to be further studied.

Supporting Information

Table S1 PRISMA checklist.

Author Contributions

Conceived and designed the experiments: SL XY PY. Performed the experiments: SL XY XL. Analyzed the data: SL XY XL. Contributed reagents/materials/analysis tools: SL XY XL. Wrote the paper: SL XY. Helped edit the manuscript: XL PY.

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