Association between *MTHFR* gene 1298A>C polymorphism and breast cancer susceptibility: a meta-analysis based on 38 case-control studies with 40,985 subjects

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**Abstract**

**Background:** Studies investigating the association between the *methyleneetetrahydrofolate reductase* (*MTHFR*) gene 1298A>C polymorphism and the risk of breast cancer have reported inconsistent results. So, we performed this updated meta-analysis and tried to give a more precise estimation of association between *MTHFR* gene 1298A>C polymorphism and breast cancer susceptibility.

**Methods:** Relevant studies published before 1 January 2016 were identified by searching PubMed and EMBASE. The strength of relationship between the *MTHFR* gene 1298A>C polymorphism and breast cancer susceptibility was assessed using odds ratio (OR) and corresponding 95% confidence interval (95% CI). The meta-analysis was performed using Stata 11.0 software.

**Results:** A total number of 38 case-control studies including 18,686 cases and 22,299 controls were identified. No association was found in five genetic models (dominant model: OR = 0.99, 95% CI 0.99–1.00, *P* = 0.218; recessive model: OR = 1.00, 95% CI 0.97–1.02, *P* = 0.880; homozygote genetic model: OR = 0.99, 95% CI 0.98–1.01, *P* = 0.390; heterozygote genetic model: OR = 0.99, 95% CI 0.97–1.00, *P* = 0.138; and allele contrast genetic model: OR = 0.99, 95% CI 0.98–1.01) for *MTHFR* gene 1298 A>C polymorphism and breast cancer susceptibility. In the subgroup analysis stratified by source of control, decreased risk of breast cancer was found in studies with hospital-based controls in dominant model (OR = 0.98, 95% CI 0.96–1.00, *P* = 0.037).

**Conclusions:** Our meta-analysis suggested that there is no significant association between *MTHFR* gene 1298A>C polymorphism and breast cancer susceptibility for overall population.

**Keywords:** *MTHFR* gene 1298A>C polymorphism, Breast cancer, Gene polymorphism, Meta-analysis, One-carbon metabolism, Variant

**Abbreviations:** MTHFR, Methylenetetrahydrofolate reductase; OR, Odds ratio; SNPs, Single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium

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Background
Breast cancer is the most frequently diagnosed cancer among women, which contributed to 25 % of all cancer cases in women worldwide, and it is the leading cause of female cancer-related death [1]. In UK, 48,034 women were diagnosed as breast cancer holders in 2008, and in USA, more than 2.8 million women suffered from breast cancer in 2015 [2, 3]. In China, breast cancer mortality have also raised quickly in recent years, from 3.53/100,000 in 1990–1992 to 4.25 in 2012 [4]. The high morbidity and mortality of the disease lead to increasing global public health burden gradually. It is widely accepted that several factors, such as hormonal, environmental, and genetic factors as well as their interactions contribute to the onset of breast cancer [5, 6]. In 1993, mutations in breast cancer (BRCA1) gene were suggested to be linked with high incidence of breast cancer in some families [7]. Since then, many susceptible genes involved in initiation and evolution of breast cancer have been researched, and one of them, the methylenetetrahydrofolate reductase (MTHFR) gene has been widely studied.

The MTHFR locus locates on chromosome 1 at the end of short arm (1p36.6), which encodes enzymes relevant to folates metabolism. The enzyme encoded by MTHFR gene takes part in the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which plays a crucial role in homocysteine remethylation to methionine [8]. Previous studies have indicated that functional single nucleotide polymorphisms (SNPs) of MTHFR gene participate in the folate-metabolizing genetic pathway and are fundamental during the synthesis, repair, and methylation process of DNA, RNA, and protein, which may affect folate and vitamin B12 level [9, 10]. Of these SNPs, 1298A>C polymorphism is caused by A to C transition in exon 7 and results in alanine in substitution of glutamine at codon 429 of the protein [11]. Subjects with mutated MTHFR 1298A>C genetic polymorphisms have higher plasma level of homocysteine [12] and may be more susceptible to different kinds of cancers, including breast cancer.

Many studies have investigated the association between MTHFR gene 1298A>C polymorphism and breast cancer risk. However, the results are inconsistent, with some studies found significant association [13, 14], while others were not [15, 16]. Although previous meta-analysis has tried to clarify the association [17], recently, several new case-control studies have been published [18–20]. In order to avoid the limitations of single case-control studies and provide renewed evidence, we performed this updated meta-analysis and tried to give a more precise and comprehensive estimation of association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility.

Methods

Data sources
Two databases were electronically searched, including PubMed and EMBASE, to retrieve studies analyzing the association between breast cancer susceptibility and MTHFR gene 1298A>C polymorphism until January 1, 2016. Searching terms were “breast cancer” or “breast neoplasm”, in combination with “methylene tetrahydrofolate reductase” or “MTHFR” or “MTHFR A1298C” or “MTHFR 1298A>C” or “rs1801131” or “Glu429Ala”, and in combination with, “polymorphism” or “variant” or “genotype” or “allele”. We also hand-checked the reference lists of all the included studies to make sure no study was missed. Two researches conducted the searches independently. If several publications carried out among same patients and controls, we only included one study with the most complete data.

Inclusion criteria
We first performed initial screening of titles and abstract. A second round screening was based on full-text reviews. Studies were considered eligible if they met the following criteria: (1) it was a case-control study in design; (2) it evaluated the MTHFR gene 1298 A>C polymorphism and breast cancer susceptibility; (3) breast cancer was pathologically confirmed for all of the patients; (4) sample sizes and individual genotype frequencies in cases and controls were available; and (5) cases and controls should be matched.

Exclusion criteria
Researches were excluded if they met any one of the following criteria: (1) data came from reviews or abstracts; (2) genotype and allele frequencies were both unavailable; (3) subjects with other malignant tumor were included in controls; (4) repeatedly published literature; (5) not breast cancer susceptibility outcome; and (6) controls were chosen from women with a family history of breast cancer or with other kinds of malignant tumors.

Data extraction and quality assessment
Two reviewers independently searched and selected literature, and then, extracted relevant data according to a data extraction form. Disagreements were solved by discussion until consensus was made. The extracted data including the first author, year of publication, country of origin, ethnicity of the study population, source of control, sample size, the genotype and allele frequencies of the MTHFR gene 1298A>C polymorphism, and information of Hardy-Weinberg equilibrium (HWE) in control groups. Different ethnicity descents were categorized as Caucasian, Asian, African, and if studies were with more than one ethnicity, they were categorized as mixed ethnicity.

For each included study, the quality assessment was conducted according to the STrengthening the REporting
of Genetic Association (STREGA) studies). If the study met all or most of the criteria in this approach, it would be classified as “+++” or “high quality”. For study in which some of the criteria were fulfilled and the others were not likely to change the results and conclusions, it would be graded as “++” or “moderate quality”. For studies fulfilled few or no criteria and the results were thought to be with non-ignorable bias, it would be classified as “++” or “low quality” [21].

Statistical analysis
Data analysis was conducted using STATA 11.0 software (Stata Statistical software, College Station, TX, USA, www.stata.com). Odds ratio (OR) and its corresponding 95% confidence intervals (95% CI) were used to evaluate the strength of association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility. Heterogeneity among included studies was tested using chi-square-based Q test and I² test. \( p_{het} < 0.05 \) or \( I^2 > 50 \% \) were considered as statistically significant for heterogeneity. The Mantel-Haenszel method was used for fix-effect model if no heterogeneity was found. Otherwise, the DerSimonian-Laird random-effect model was used. Fix-effect model considers that across all studies, the genetic factors have similar effects on genetic disorder susceptibility and the observed differences among studies are caused just by chance [22]. Random-effect model considers that different studies may have substantial diversity, and it calculates within- as well as between-study difference [23]. Five comparison genetic models were used to assess the association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility. We assessed the dominant model (AA + AC vs. CC), recessive model (AA vs. AC + CC), allele contrast genetic model (A vs. C), the heterozygote comparison (AC vs. CC), and the homozygote comparison (AA vs. CC). \( P < 0.05 \) showed the statistical significance. HWE was tested for included studies if no relevant information was provided in original research. Sensitivity analyses were conducted by omitting individual studies sequentially. Moreover, we performed subgroup analysis stratified by ethnicity, source of control, and deviation from HWE. Publication bias was quantitatively assessed by Egger's linear regression test [24] and visual inspection of Begg’s funnel plots.

Results
Literature search
We initially identified 373 potentially relevant studies from searching the two databases and the reference lists of relevant studies. Firstly, we eliminated duplications, and after this procedure, 248 studies were retained. After reading the titles and abstracts, we excluded 193 studies. Among them, 89 were not case-control studies, 91 were irrelevant to MTHFR polymorphism or breast cancer susceptibility, and 13 were reviews or meta-analysis. Then, we read the full texts of the 55 retained articles and 17 were excluded. Of them, 11 was irrelevant to 1298A>C polymorphism, four focused on breast cancer mortality, one conducted among the same patients and controls with another study, but provided less completed data, and for one study, the controls were chosen from BRCA1 carriers. We finally identified 38 case-control studies eligible for the meta-analysis [13–16, 18–20, 25–55], including 18,686 cases and 22,299 controls. A flow chart of data selection was presented in Fig. 1.

Main characteristics of included studies
Table 1 presents the main characteristics and genotype frequencies of the included studies. Of the 38 studies, 15 studies were carried out among Asians, 13 among Caucasians, and 10 among mixed populations. All studies included were case-control studies in design, and all patients with breast cancer fulfilled the pathological diagnosis. The number ranged from 35 to 1986 for cases, and 33 to 2414 for controls. In 21 studies, controls were normal healthy people randomly recruited from general population, and in 15 studies, controls were recruited from hospital among women with benign disease or through women going to hospital for routine physical examinations, but in the two studies, we were unable to find out the source of controls. In most of the included studies, controls were matched with cases in ethnicity and age. In quality assessment, 17 studies included were categorized as “high quality,” and 21 as “moderate quality” (Table 1). In eight studies, the genotype distributions in control groups were deviated from HWE (Table 1).

Quantitative data analysis
Association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility
The results of the five genetic models testing MTHFR gene 1298A>C polymorphism and breast cancer susceptibility are presented in Table 2. In the dominant model (AA + AC vs. CC), \( P \) value for heterogeneity was 0.000, and \( I^2 \) was 50.5%, indicating significant heterogeneity among studies. Thus, random-effect model was used. The overall effect \( Z \) value was 1.12 (\( P = 0.218 \)) and OR was 0.99 (95% CI 0.99–1.00), suggesting that no association was found in the dominant model. The Egger’s linear regression test indicated that there was some evidence of publication bias in this model (Egger, \( P = 0.01 \)). Other four genetic models were also performed (Table 2), but no association was found. In subgroup analyses stratified by source of control, a significant decrease in breast cancer susceptibility was found in hospital-based controls in dominant model (OR = 0.98, 95% CI 0.96–1.00, \( P = 0.037 \)), but not in allele contrast genetic model (OR = 0.97, 95% CI 0.94–1.00, \( P = 0.092 \))
Moreover, the results showed that in subgroups of Asians and population-based studies, the heterogeneity among studies was significantly reduced. Figure 2 shows the forest plot of the dominant model testing the association between MTHFR 1298A>C polymorphism and breast cancer risk, stratified by ethnicity. Figure 3 shows the forest plot of the dominant model testing the association between MTHFR 1298A>C polymorphism and breast cancer risk, stratified by source of control.

**Sensitivity analysis and publication bias**
Sensitivity analyses were conducted by omitting each dataset sequentially, and the result did not change under any genetic model. Sensitivity analysis suggested that for all of the five genetic comparisons of MTHFR gene 1298A>C polymorphism and breast cancer susceptibility, the results were statistically robust.

Visual inspection of Begg’s funnel plots identified the substantial asymmetry for dominant model, the allele contrast genetic model, the heterozygote comparison, and the homozygote comparison. The Egger’s linear regression test also indicated the similar results ($P < 0.05$ for all models tested except the recessive genetic model) (Table 2). Figure 4 shows the Begg’s funnel plot under dominant model of MTHFR 1298A>C polymorphism.
Discussion

*MTHFR* is an essential gene in the one-carbon metabolism pathway. During the past few years, many meta-

analyses assessing the association between *MTHFR* gene polymorphism and cancer risks have been published, including liver cancer, ovary cancer, lung cancer, gastric

| First author       | Year | Ethnicity | Source of controls | Cases | Controls | Deviation from HWE | Quality grade |
|--------------------|------|-----------|--------------------|-------|----------|-------------------|---------------|
| Aram               | 2012 | Caucasian | HB                 | 35    | 55       | 20                | AA            |
| Awwad              | 2015 | Asian     | PB                 | 68    | 61       | 17                | AA            |
| Carvalho Barbosa Rde | 2012 | Mixed     | PB                 | 68    | 80       | 17                | AA            |
| Chen               | 2005 | Mixed     | PB                 | 558   | 417      | 87                | AA            |
| Cheng              | 2008 | Asian     | HB                 | 207   | 125      | 19                | A             |
| Chou               | 2006 | Asian     | HB                 | 104   | 30       | 8                 | A              |
| Ergul              | 2003 | Caucasian | HB                 | 50    | 48       | 20                | A              |
| Ericson            | 2009 | Caucasian | PB                 | 242   | 242      | 57                | A              |
| Forsti             | 2004 | Caucasian | NA                 | 94    | 102      | 27                | A              |
| Gao                | 2009 | Asian     | PB                 | 446   | 165      | 9                 | A              |
| He                 | 2014 | Asian     | HB                 | 138   | 132      | 40                | A              |
| Hosseini           | 2011 | Caucasian | HB                 | 36    | 96       | 162               | A              |
| Inoue              | 2008 | Asian     | PB                 | 225   | 139      | 16                | A              |
| Justenhoven        | 2005 | Caucasian | PB                 | 273   | 256      | 53                | A              |
| Kakkoura           | 2015 | Mixed     | PB                 | 138   | 465      | 468               | A              |
| Kotsopoulos        | 2008 | Caucasian | HB                 | 466   | 390      | 85                | A              |
| Lajin              | 2012 | Caucasian | HB                 | 44    | 52       | 23                | A              |
| Le Marchand        | 2004 | Mixed     | PB                 | 741   | 372      | 77                | A              |
| Lissowska          | 2007 | Caucasian | PB                 | 892   | 874      | 220               | A              |
| Liu                | 2013 | Asian     | HB                 | 206   | 176      | 53                | A              |
| Lopez-Cortes       | 2015 | Mix       | PB                 | 110   | 3        | 1                 | A              |
| Lu                 | 2015 | Asian     | HB                 | 369   | 172      | 19                | A              |
| Ma                 | 2009 | Mixed     | HB                 | 269   | 168      | 21                | A              |
| Mir                | 2008 | Asian     | NA                 | 15    | 19       | 1                 | A              |
| Ozen               | 2013 | Mix       | PB                 | 17    | 29       | 5                 | A              |
| Papandreou         | 2012 | Caucasian | HB                 | 129   | 135      | 36                | A              |
| Platek             | 2009 | Mix       | PB                 | 443   | 402      | 83                | A              |
| Qi                 | 2004 | Asian     | PB                 | 155   | 58       | 4                 | A              |
| Sangrajrang        | 2010 | Asian     | HB                 | 302   | 223      | 38                | A              |
| Sharp              | 2002 | Caucasian | PB                 | 27    | 25       | 3                 | A              |
| Shrubsole          | 2004 | Asian     | PB                 | 768   | 311      | 42                | A              |
| Stevens            | 2007 | Mixed     | PB                 | 224   | 228      | 42                | A              |
| Vainer             | 2010 | Caucasian | HB                 | 398   | 353      | 80                | A              |
| Weiwei             | 2014 | Asian     | HB                 | 135   | 129      | 32                | A              |
| Wu                 | 2012 | Asian     | PB                 | 37    | 32       | 6                 | A              |
| Xu                 | 2007 | Mixed     | PB                 | 558   | 417      | 87                | A              |
| Zhang              | 2015 | Asian     | PB                 | 98    | 87       | 31                | A              |
| Ziva Cerne         | 2011 | Caucasian | PB                 | 258   | 219      | 47                | A              |

PB population-based study, HB hospital-based study, NA not available, HWE Hardy-Weinberg equilibrium
cancer, pancreatic cancer, cervical cancer, and esophageal cancer [56–60]. Genetic variation in enzymes and proteins involved in folate metabolism is also a rational candidate for studying the genetic of breast cancer. Therefore, the interest in MTHFR gene 1298A>C polymorphism and breast cancer susceptibility has existed for a long time. In 2002, Sharp et al. for the first time published a case-control study estimating the association between MTHFR gene 1298A>C polymorphism and breast cancer risk. Their result suggested that risk was significantly lower for the 1298CC genotype compared to AA genotype (OR = 0.24, 95 % CI 0.06–0.97) [49]. However, after that, a number of subsequent studies were conducted and their results were inconsistent, with some studies showed significant associations while others were not. The inconsistency may be caused by several reasons. First of all, although in vitro, the variant genotype is associated with a substantial decrease in enzymatic activity [11], this functional polymorphism may be an important but not the exclusive influencing factor in etiology and pathogenesis of breast cancer. Special lifestyle and environmental factors, such as tea drinking [61], dietary intake of folate, vitamin B6 and B12 [62], physical activities [63], long-term oral contraceptive use [64], and hormone replacement therapy use [65], are possibly confounding factors taking part in the disease etiology. Moreover, differences in patient choosing criteria, ethnicity, sample size, and sources of control could contribute to inconsistency. Hence, it is necessary to conduct a meta-analysis providing quantitative approach for pooling the results of all studies with the same purpose and explaining the overall estimation as well as the diversity.

Our study has important strengths. All original studies used a case-control study design, which is a useful tool to identify gene and disease associations. However, individual genotype case-control studies could not be based on a large number of subjects or contain patients in different ethnicities, and thus has insufficient statistical power. Our meta-analysis based on case-control studies involving 40,985 subjects brings to light that there is no significant association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility for overall population, with ORs from 0.99 to 1.00 and narrow 95 % CIs for all of the five genetic models. Moreover, in our study, no association was found in different ethnicities or in population-based studies, which thereby strengthened this association. As shown in our meta-analysis, studies with hospital-based design or controls deviated from HWE had a weak, but statistical significant decreased association with breast cancer in dominant model. However, in these two kinds of studies, the controls may not represent the whole population and thereby, the results from them should be interpreted

### Table 2

| Genetic model | OR (95 % CI) | Z  | P value | $P^2$ % | $P_{het}$ | Effect model | Egger’s test |
|---------------|-------------|----|---------|---------|-----------|--------------|--------------|
| AA + AC vs. CC | 0.99 (0.99–1.00) | 1.23 | 0.218 | 50.5 | 0.000 | R | -2.72 | 0.010 |
| AA vs. AC + CC | 1.00 (0.97–1.02) | 0.15 | 0.880 | 35.9 | 0.016 | R | -1.45 | 0.155 |
| AA vs. CC | 0.99 (0.98–1.01) | 0.86 | 0.390 | 43.8 | 0.002 | R | -2.75 | 0.014 |
| AC vs. CC | 0.99 (0.97–1.00) | 1.48 | 0.138 | 41.2 | 0.005 | R | -2.55 | 0.015 |
| A vs. C | 0.99 (0.98–1.01) | 0.92 | 0.360 | 55.5 | 0.000 | R | -2.27 | 0.029 |

OR: odds ratio, CI: confidence interval, R: random-effect model, $P_{het}$: P value for heterogeneity

### Table 3

| Stratified by | Comparison | Number of datasets | Dominant genetic model | Allele contrast |
|--------------|------------|--------------------|------------------------|-----------------|
| Ethnicity    | Asian      | 15                 | 1.00 (0.99–1.00)       | 1.01 (0.99–1.02) |
|              | Caucasian  | 13                 | 0.98 (0.95–1.01)       | 0.97 (0.93–1.00) |
|              | Mixed      | 10                 | 0.99 (0.99–1.00)       | 0.99 (0.98–1.01) |
| Source of control | PB | 21                 | 1.00 (0.99–1.01)       | 1.00 (0.99–1.02) |
|              | HB         | 15                 | 0.98 (0.96–1.00)       | 0.97 (0.94–1.00) |
|              | NA         | 2                  | 0.99 (0.94–1.04)       | 0.99 (0.91–1.08) |
| Deviation from HWE | Yes | 8                  | 0.98 (0.95–1.00)       | 0.98 (0.95–1.01) |
|              | No         | 30                 | 1.00 (0.99–1.01)       | 1.00 (0.98–1.01) |

PB: population-based study, HB: hospital-based study, NA: not available
with caution. Overall, our meta-analysis based on 38 case-control studies provided reliable and comprehensive estimations. The association in the five genetic models sustained unchanged in the sensitivity analysis, which further confirmed the results of main analysis.

It is also important to mention the heterogeneity existed in this study. For all genetic models in the main analysis, \( P \) value for heterogeneity was less than 0.05, indicating significant heterogeneity among the included studies. Finding the potential sources of heterogeneity is an important part of meta-analysis, which can greatly influence the results of the research. To detect the possible source of heterogeneity in our meta-analysis, we performed the subgroup analysis stratified by ethnicity, source of control, and deviation from HWE. When stratified by ethnicity and source of control, the heterogeneity was significantly decreased in Asian and population-based subgroups. Therefore, the different ethnicity and source of control may contribute to the overall heterogeneity. However, heterogeneity still existed in Caucasian, mixed ethnicity, and hospital-based control subgroups, suggesting that ethnicity and source of control did not fully explain the heterogeneity among studies. Further studies may try to explore in interactions between different factors and to minimize the heterogeneity in subgroups.

Several previous meta-analyses have been published to analyze the association between \( \text{MTHFR} \) gene polymorphisms and breast cancer susceptibility, and the majority of them concerned on \( 677C>T \) polymorphism [66–69]. Two studies published in 2014 have detected the \( 1298A>C \) polymorphism [17, 70]. The main result of our study was consistent with the previous meta-analyses. Comparing with these two studies, our study has some important improvements. In 2014–2015, some new studies were published and they were included in our meta-analysis. Through strict methodological process, we provided a more comprehensive view of included studies. The abovementioned meta-analyses only stratified by ethnicity to test if there existed differences in variant ethnicities. In present study, we also
Fig. 3 shows the forest plot of the dominant model testing the association between MTHFR 1298A>C polymorphism and breast cancer risk, stratified by source of control.

**Table:**

| Study ID | OR (95% CI) | Weight |
|----------|-------------|--------|
| HB       |             |        |
| Aram (2012) | 0.86 (0.78, 0.94) | 0.68 |
| Cheng (2008) | 0.98 (0.95, 1.01) | 0.96 |
| Chou (2008) | 1.01 (0.96, 1.06) | 1.00 |
| Ergul (2003) | 0.92 (0.83, 1.01) | 0.73 |
| He (2014) | 1.01 (0.95, 1.07) | 0.57 |
| Hosseini (2011) | 0.95 (0.89, 1.01) | 0.29 |
| Kotsopoulos (2008) | 1.00 (0.97, 1.03) | 0.60 |
| Lajin (2012) | 0.96 (0.91, 1.00) | 0.58 |
| Liu (2013) | 0.98 (0.94, 1.02) | 0.58 |
| Lu (2015) | 1.01 (0.98, 1.04) | 0.46 |
| Ma (2009) | 1.00 (0.97, 1.03) | 0.77 |
| Papandreou (2012) | 0.95 (0.93, 1.00) | 0.58 |
| Sangrajrang (2010) | 0.98 (0.95, 1.01) | 0.65 |
| Vainer (2010) | 1.00 (0.97, 1.03) | 0.42 |
| Weiwei (2014) | 0.97 (0.92, 1.02) | 0.89 |
| Subtotal (I-squared = 71.4%, p = 0.000) |             |        |
| PB       |             |        |
| Awad (2015) | 0.98 (0.90, 1.06) | 0.94 |
| Carvalho Barbosa Rde (2012) | 0.95 (0.83, 1.01) | 0.40 |
| Chen (2005) | 1.02 (0.99, 1.05) | 0.02 |
| Erdos (2009) | 0.98 (0.96, 1.03) | 0.11 |
| Gao (2009) | 1.00 (0.97, 1.02) | 0.64 |
| Inoue (2008) | 1.02 (0.99, 1.05) | 0.76 |
| Justenhoven (2005) | 1.03 (0.99, 1.07) | 0.83 |
| Kakkoura (2015) | 0.98 (0.91, 1.00) | 0.11 |
| Le Marchand (2004) | 0.98 (0.97, 1.00) | 0.21 |
| Lisovska (2007) | 1.00 (0.98, 1.03) | 0.71 |
| Lopez-Cortes (2015) | 1.00 (0.98, 1.01) | 0.67 |
| Ozen (2013) | 0.96 (0.92, 0.99) | 0.71 |
| Platek (2009) | 1.01 (0.99, 1.04) | 0.18 |
| Qi (2004) | 1.00 (0.97, 1.02) | 0.34 |
| Sharp (2002) | 1.16 (1.01, 1.33) | 0.36 |
| Shrubsole (2004) | 1.00 (0.96, 1.04) | 0.47 |
| Stevens (2007) | 1.00 (0.99, 1.03) | 0.77 |
| Wu (2012) | 0.99 (0.96, 1.03) | 0.77 |
| Xu (2007) | 1.02 (0.99, 1.05) | 0.02 |
| Zhang (2015) | 0.98 (0.91, 1.05) | 0.68 |
| Ziva Germe (2011) | 0.99 (0.94, 1.04) | 0.37 |
| Subtotal (I-squared = 25.4%, p = 0.141) |             |        |
| NA       |             |        |
| Fosti (2004) | 1.01 (0.94, 1.08) | 0.33 |
| Mir (2009) | 0.97 (0.90, 1.05) | 0.96 |
| Subtotal (I-squared = 0.0%, p = 0.445) |             |        |
| Overall (I-squared = 50.5%, p = 0.000) | 0.99 (0.99, 1.00) | 100.00 |

**NOTE:** Weights are from random effects analysis.

Fig. 4 Begg's funnel plot under dominant model of MTHFR 1298A>C polymorphism
conducted subgroup study stratified by source of control and deviation from HWE in control group, to analyze if there were differences between subgroups.

We should also pay attention to the several limitations in our study, which may affect the result. Firstly, we only included published studies meeting our inclusion criteria from two databases, similar studies in other databases and unpublished researches may have been missed, and this is also the main reason for the publication bias we found in four of the five genetic models. Secondly, the control groups in some of the included studies were deviated from HWE, which may fail to represent the whole population and have some effects on the overall estimation. Thirdly, although the results from subgroup and sensitivity analyses were quite similar to the main analysis, significant heterogeneity was detected in all five genetic models of MTHFR gene 1298A>C polymorphism and breast cancer susceptibility. Different characteristics in study population and study design may contribute to the heterogeneity. Considering that meta-analysis is a kind of retrospective research and may easily be affected by methodological deficiencies of the included studies, we developed a detailed protocol before conducting this analysis, to ensure the quality of our research.

Conclusions

From the combination results of currently included studies, our meta-analysis suggested that there is no significant association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility for overall population.

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Availability of data and materials

This research is a meta-analysis, and all data and materials are available in database of PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and EMBASE (http://www.embase.com/).

Authors’ contributions

JHZ and GML wrote the paper. JHZ and JLZ analyzed the data. GML organized the whole work. All authors read and approved the final manuscript.

Competing interests

All the authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.

2. CancerStats Incidence 2008-UK. Available from: http://info.cancerresearchuk.org/prod_consump/groups/ct_common/invenesta/documents/generalcontent/cr_072111.pdf Accessed date 2 Feb 2016.

3. McGuire A, Brown JA, Malone C, McLaughlin R, Kerin MJ. Effects of age on the detection and management of breast cancer. Cancers. 2015;7(2):908–29.

4. Yearbook of Chinese health statistics, 2013. Available from: http://www.nhfpc.gov.cn/htmlfiles/wbkqztpjtx/year2013/index2013.html Accessed date 2 Feb 2016.

5. Hankinson SE, Colditz GA, Willett WC. Towards an integrated model for breast cancer etiology: the lifelong interplay of genes, lifestyle, and hormones. Breast Cancer Research. BCR. 2004;6(5):213–8.

6. Durmitrescu RG, Cotarla I. Understanding breast cancer risk—where do we stand in 2005? J Cell Mol Med. 2005;9(1):208–21.

7. Weber BL, Abel KJ, Couch FJ, Merajver SD, Chandrasekharappa SC, Castilla L, et al. Progress toward isolation of a breast cancer susceptibility gene, BRCA1. Cold Spring Harb Symp Quant Biol. 1994;59:531–6.

8. Xu X, Gammon MD, Zeisel SH, Lee YL, Wtemur JG, Teitelbaum SL, et al. Choline metabolism and risk of breast cancer in a population-based study. FASEB J. Official Publication of the Federation of American Societies for Experimental Biology. 2000;24(2):405–52.

9. Wei LK, Sutherland H, Au A, Camilleri E, Haupt LM, Gan SH, et al. A potential epigenetic marker mediating serum folate and vitamin B12 levels contributes to the risk of ischemic stroke. BioMed Research International. 2015;2015:167976.

10. Xu X, Chen J. One-carbon metabolism and breast cancer: an epidemiological perspective. Journal of Genetics and Genomics = Yi chuan xue bao. 2009;36(4):203–14.

11. Weihsberg J, Tran P, Christiansen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab. 1998;64(3):169–72.

12. Lee YS, Han DH, Jeon CM, Lyoo IK, Na C, Chae SL, et al. Serum homocysteine, folate level and methylenetetrahydrofolate reductase 677, 1298 gene polymorphism in Korean schizophrenic patients. Neuroreport. 2006;17(7):743–6.

13. Ergul E, Sazci A, Ufkan Z, Canturk NZ. Polymorphisms in the MTHFR gene are associated with breast cancer. Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine. 2003;24(6):286–90.

14. Stevens VL, McCullough ML, Pavluck AL, Talbot JT, Feigelson HS, Thun MJ, et al. Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology. 2007;16(6):1140–7.

15. Ericson U, Sonestedt E, Ivarsson M, Gullberg B, Carlson J, Olsson H, et al. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmo Diet and Cancer cohort. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology. 2009;18(4):1101–10.

16. Justenhoven C, Hamanni U, Pfeil CB, Rabstein S, Pesch B, Harth V, et al. One-carbon metabolism and breast cancer risk: no association of MTHFR, MTR, and TYMS polymorphisms in the GENICa study from Germany. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology. 2005;14(2):3015–8.

17. Li K, Li W, Dong X. Association of 677 C>T (rs1801133) and 1298 A>C (rs1801133) polymorphisms in the MTHFR gene and breast cancer susceptibility: a meta-analysis based on 57 individual studies. PLoS One. 2014;9(6):e91290.

18. Zhang XF, Liu T, Li Y, Li S. Association between MTHFR 677C>T and 1298A/C gene polymorphisms and breast cancer risk. Genetics and Molecular Research: GMR. 2015;14(4):16425–30.

19. Lu Q, Jiang K, Li Q, Ji YJ, Chen WL, Xue XH. Polymorphisms in the MTHFR gene are associated with breast cancer risk and prognosis in a Chinese population. Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;36(5):3757–62.

20. Awad N, Yousef AM, Abuhalima A, Abdalla I, Yousef M. Relationship between genetic polymorphisms in MTHFR (C677T, A1298C and their haplotypes) and the incidence of breast cancer among Jordanian females—case-control study. Asian Pacific Journal of Cancer Prevention: APJCP. 2015;16(12):5007–11.

21. Little J, Bradley L, Bray MS, Clyne M, Dormian J, Ellsworth DL, et al. Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations. Am J Epidemiol. 2002;156(4):300–10.
22. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177–88.
23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539–58.
24. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629–34.
25. Akram M, Malik FA, Kayani MA. Mutational analysis of the MTHFR gene in breast cancer patients of Pakistani population. Asian Pacific Journal of Cancer Prevention: APJCP. 2012;13(4):1599–603.
26. Carvalho Barbosa Rde C, Menezes DC, Freire TF, Sales DC, Alencar VH, Rabenhorst SH. Associations of polymorphisms of folate cycle enzymes and risk of breast cancer in a Brazilian population are age dependent. Mol Biol Rep. 2012;39(4):849–807.
27. Chen J, Gammom MD, Chan W, Palomeque C, Wetmur JG, Kabat GC, et al. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res. 2005;65(4):1606–14.
28. Cheng CW, Yu JC, Huang CS, Sheih JC, Fu YP, Wang HW, et al. Polymorphism of cytosolic serine hydroxymethyltransferase, estrogen and breast cancer risk among Chinese women in Taiwan. Breast Cancer Res Treat. 2008;111(1):45–55.
29. Chou YC, Wu MH, Yu JC, Lee MS, Yang T, Shih HL, et al. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: a case-control study in Taiwan. Carcinogenesis. 2006;27(11):2295–300.
30. Forsti A, Angelini S, Festa F, Sanyal S, Zhang Z, Grzybowska E, et al. Single nucleotide polymorphisms in breast cancer. Oncol Rep. 2004;11(4):917–22.
31. Gao CM, Tang JH, Cao HX, Ding JH, Wu JZ, Wang J, et al. MTHFR polymorphisms, dietary folate intake and breast cancer risk in Chinese women. J Hum Genet. 2009;54(7):414–8.
32. He JM, Pu YD, Wu YJ, Qin R, Zhang QJ, Sun YS, et al. Association between dietary intake of folate and MTHFR and MTR genotype with risk of breast cancer. Genetics and Molecular Research: GMR. 2014;13(4):8925.
33. Hosseini M, Houshmand M, Ebrahimi A. MTHFR polymorphisms and breast cancer risk. Archives of Medical Science. 2011;7(1):134–7.
34. Inoue M, Robien K, Wang R, Van Den Berg DJ, Koh WP, Yu MC. Green tea intake, MTHFR/TYMS genotype and breast cancer risk: results from the Shanghai breast cancer study. Cancer Epidemiology, Biomarkers & Prevention. 2011;20(11):2871–90.
35. Kakkoura MG, Demetriou CA, Loizidou MA, Loucaides G, Neophytou I, Marcou AA, et al. Evidence of association between methylenetetrahydrofolate reductase gene and breast cancer susceptibility in Kashmiri women. International Journal of Health Sciences. 2008;2(1):3–14.
36. Ozcan F, Erdi E, Sik E, Silan F, Uludag A, Ozdemir O. Germ-line MTHFR C677T, FV H1299R and PAI-1 5G/AG variations in breast carcinoma. Asian Pacific Journal of Cancer Prevention: APJCP. 2013;14(5):2903–8.
37. Papandreou CN, Donaxi C, Zdukopoulos N, Vlachostergios PJ, Hatziakos E, Bakalos G, et al. Evidence of association between methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: a candidate-gene association study in a South-eastern European population. DNA Cell Biol. 2012;31(2):193–8.
38. Platek ME, Shields PG, Marian C, McCann SE, Bonner MR, Nie J, et al. Alcohol consumption and genetic variation in methylenetetrahydrofolate reductase and S-methyltetrahydrofolate-homocysteine methyltransferase in relation to breast cancer risk. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(9):2453–9.
39. Q J, Miao XP, Tan W, Yu CY, Liang G, Lu WF, et al. Association between genetic polymorphisms in methylenetetrahydrofolate reductase and risk of breast cancer. Zhonghua zhong liu za zhi (Chinese Journal of Oncology). 2004;26(5):287–9.
40. Sangrajrang S, Sato Y, Sakamoto H, Ohnami S, Khupaprema T, Yoshida T. Genetic polymorphisms in folate and alcohol metabolism and breast cancer risk: a case-control study in Thai women. Breast Cancer Res Treat. 2010;123(3):885–93.
41. Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, et al. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett. 2002;181(1):65–73.
42. Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Hebert JR, et al. MTHFR polymorphisms, dietary folate intake, and breast cancer risk results from the Shanghai breast cancer study. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2004;13(2):190–6.
43. Vainer AS, Boizankish UA, Voronina EN, Seleznева IA, Sinkina TV, Lazarev AF, et al. Polymeric variants of folate metabolizing genes (C677T and A1298C) in breast cancer patients of Pakistani population. Asian Pacific Journal of Cancer Prevention: APJCP. 2012;13(9):2450–6.
44. Weiwei Z, Liping C, Dequan L. Association between dietary intake of folate, vitamin B6, B12 & MTHFR, MTR genotype and breast cancer risk. Pakistan Journal of Medical Sciences. 2014;30(1):106–10.
45. Wu XY, Ni J, Xu WJ, Zhou T, Wang X. Interactions between MTHFR C677T/A1298C variants and folic acid deficiency affect breast cancer risk in a Chinese population. Asian Pacific Journal of Cancer Prevention: APJCP. 2012;13(9):2193–7.
46. Xu X, Gammom MD, Zhang H, Wetmur JG, Rao M, Teitelbaum SL, et al. Polymorphisms of one-carbon-metabolizing genes and risk of breast cancer in a population-based study. Carcinogenesis. 2007;28(7):1504–9.
47. Ziva Cerne J, Stegel V, Gersak K, Novakovic S. Lack of association between methylenetetrahydrofolate reductase genetic polymorphisms and postmenopausal breast cancer risk. Mol Med Rep. 2011;4(1):175–79.
48. Gao LN. Methylenetetrahydrofolate reductase C677T polymorphism and cervical cancer risk: a meta-analysis. Asian Pacific Journal of Cancer Prevention: APJCP. 2012;13(5):2193–7.
49. Lanson SC, Giovannucci E, Wilk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. Gastroenterology. 2006;131(4):1271–83.
50. Hou XH, Huang YM, Mi YY. Methylenetetrahydrofolate reductase gene C677T polymorphism and lung cancer: an updated meta-analysis. Asian Pacific Journal of Cancer Prevention: APJCP. 2012;13(5):2025–9.
51. Ma C, Liu Y, Zhang W, Liu P. The association between MTHFR C677T polymorphism and ovarian cancer risk: a meta-analysis of 18,628 individuals. Mol Biol Rep. 2013;40(3):2601–8.
52. Qi X, Sun X, Xu J, Wang Z, Zhang J, Peng Z. Associations between methylenetetrahydrofolate reductase polymorphisms and hepatocellular carcinoma risk in Chinese population. Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(3):1757–62.
53. Gao Y, Huang YB, Liu XO, Chen C, Dai HJ, Song FJ, et al. Tea consumption, alcohol drinking and physical activity associations with breast cancer risk
among Chinese females: a systematic review and meta-analysis. Asian Pacific Journal of Cancer Prevention: APJCP. 2013;14(12):7543–50.

62. Lajous M, Lazzcano-Ponce E, Hernandez-Avila M, Willett W, Romieu I. Folate, vitamin B6, and vitamin B(12) intake and the risk of breast cancer among Mexican women. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(3):443–8.

63. Pozo C, Boniol M, Mullie P, Koechlin A, Boniol M, Boyle P, et al. Physical activity, hormone replacement therapy and breast cancer risk: a meta-analysis of prospective studies. Eur J Cancer. 2016;52:138–54.

64. Zhu H, Lei X, Feng J, Wang Y. Oral contraceptive use and risk of breast cancer: a meta-analysis of prospective cohort studies. The European Journal of Contraception & Reproductive Health Care: The Official Journal of the European Society of Contraception. 2012;17(6):402–14.

65. Sillero-Arenas M, Delgado-Rodriguez M, Rodrigues-Canteras R, Bueno-Cavanillas A, Galvez-Vargas R. Menopausal hormone replacement therapy and breast cancer: a meta-analysis. Obstet Gynecol. 1992;79(2):286–94.

66. Langsenlehner T, Renner W, Yazdani-Biuki B, Langsenlehner U. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. Breast Cancer Res Treat. 2008;107(3):459–60.

67. Macis D, Maisonneuve P, Johansson H, Bonanni B, Botteri E, Iodice S, et al. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. Breast Cancer Res Treat. 2007;106(2):263–71.

68. Yu L, Chen X. Association of MTHFR Ala222Val (rs1801133) polymorphism and breast cancer susceptibility: an update meta-analysis based on 51 research studies. Diagn Pathol. 2012;7:171.

69. Zhang J, Qiu LX, Wang ZH, Wu XH, Liu XJ, Wang BY, et al. MTHFR C677T polymorphism associated with breast cancer susceptibility: a meta-analysis involving 15,260 cases and 20,411 controls. Breast Cancer Res Treat. 2010;123(2):549–55.

70. Rai V. Methylenetetrahydrofolate reductase A1298C polymorphism and breast cancer risk: a meta-analysis of 33 studies. Annals of Medical and Health Sciences Research. 2014;4(6):841–51.