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# MTHFR C677T polymorphism associated with breast cancer susceptibility: a meta-analysis involving 15,260 cases and 20,411 controls

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**Abstract** Published data on the association between MTHFR C677T polymorphism and breast cancer risk are inconclusive. To derive a more precise estimation of the relationship, a meta-analysis was performed. Medline, PubMed, Embase, and Web of Science were searched. Crude ORs with 95% CIs were used to assess the strength of association between the MTHFR C677T polymorphism and breast cancer risk. The pooled ORs were performed with co-dominant model (CT vs. CC, TT vs. CC), dominant model (CT + TT vs. CC), and recessive model (TT vs. CC + CT), respectively. A total of 37 studies including 15,260 cases and 20,411 controls were involved in this meta-analysis. Overall, significantly elevated breast cancer risk was associated with TT variant genotype in homozygote comparison and dominant genetic model when all studies were pooled into the meta-analysis (TT vs. CC: OR = 1.11, 95% CI = 1.01–1.23; dominant model: OR = 1.04, 95% CI = 1.00–1.09). In the subgroup analysis by ethnicity, significantly increased risks were found for TT allele carriers among Asians (TT vs. CC: OR = 1.18, 95% CI = 1.04–1.35; recessive model: OR = 1.15, 95% CI = 1.03–1.29). When stratified by study design, statistically significantly elevated risk was found in hospital-based studies (TT vs. CC: OR = 1.18, 95% CI = 1.02–1.38; recessive model: OR = 1.17, 95% CI = 1.05–1.29). In the subgroup analysis by menopausal status, statistically significantly increased risk was found among postmenopausal women (CT vs. CC: OR = 1.12, 95% CI = 1.02–1.23; dominant model: OR = 1.11, 95% CI = 1.01–1.22). In conclusion, this meta-analysis suggests that the MTHFR T allele is a low-penetrant risk factor for developing breast cancer.

**Keywords** MTHFR · Polymorphism · Breast cancer · Susceptibility · Meta-analysis

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## Introduction

In 2009, breast cancer was expected to account for 27% (192,370) of all new cancer cases among women in the United States [1]. Breast cancer is still a major challenge for women's health. While the exact etiology of the disease is poorly understood, there are some recognized risk factors that may contribute to the development of breast cancer including age, ethnicity, reproductive events, exogenous hormones, lifestyle, bone density, as well as genetic factors [2]. It has been suggested that low-penetrance susceptibility genes combining with environmental factors may be important in the development of cancer [3]. In recent years, several common low-penetrant genes have been identified as potential breast cancer susceptibility genes, one of which is 5,10-methylenetetrahydrofolate reductase (MTHFR) gene. It encodes a critical enzyme for intracellular folate homeostasis and metabolism, which catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methylene-THF). The latter is the predominant form of folate in plasma and provides the methyl group for de novo methionine synthesis through homocysteine remethylation [4]. A common

polymorphism in the MTHFR gene has been identified in which a C to T substitution at nucleotide position 677 (C677T) results in an alanine to valine substitution and the production of a thermolabile variant of the MTHFR enzyme that has approximately 30% of the activity of the wild-type enzyme [5, 6]. This polymorphism to breast cancer risk has been a research focus in scientific community and has drawn increasing attention. Forty original studies have reported the role of MTHFR C677T polymorphism in breast cancer risk [7–46], but the results are inconclusive, partially because of the possible small effect of the polymorphism on breast cancer risk and the relatively small sample size in each of published studies. Therefore, we performed this meta-analysis to derive a more precise estimation of these associations.

## Methods

### Publication search

Medline, PubMed, Embase, and Web of Science were searched (last search was done on Jan 22, 2010, using the search terms: "methylenetetrahydrofolate reductase," "MTHFR," "polymorphism," and "breast"). All searched studies were retrieved, and their bibliographies were checked for other relevant publications. Review articles and bibliographies of other relevant studies identified were hand-searched to find additional eligible studies. Only published studies with full text articles were included. When more than one of the same patient population was included in several publications, only the most recent or complete study was used in this meta-analysis.

### Inclusion criteria

The inclusion criteria were: (a) evaluation of the MTHFR C677T polymorphism and breast cancer risk, (b) case–control studies, and (c) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI).

### Data extraction

Information was carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors. If these two authors could not reach a consensus, another author was consulted to resolve the dispute and a final decision was made by the majority of the votes. The following data were collected from each study: first author's name, publication date, ethnicity, study design, menopausal status, total number of cases and controls, and numbers of cases and controls with the MTHFR C677T genotypes, respectively. Different ethnicities were categorized as Caucasian, Asian, mixed or not-stated. Study design was stratified into population-based studies and hospital-based studies. Menopausal status was divided into premenopausal and postmenopausal. We did not define any minimum number of patients to be included in our meta-analysis.

### Statistical methods

Crude ORs with 95% CIs were used to assess the strength of association between the MTHFR C677T polymorphism and breast cancer risk. The pooled ORs were performed with co-dominant model (CT vs. CC, TT vs. CC), dominant model (CT + TT vs. CC), and recessive model (TT vs. CC + CT), respectively. Heterogeneity assumption was checked with the Chi-square-based $Q$-test [47]. A $P$ value greater than 0.10 for the $Q$-test indicates a lack of heterogeneity among studies, so the pooled OR estimate of the each study was calculated with the fixed-effects model (the Mantel–Haenszel method) [48]. Otherwise, the random-effects model (the DerSimonian and Laird method) was used [49]. Subgroup analyses were performed by ethnicity, study design, and menopausal status. Sensitivity analysis was performed to assess the stability of the results. A single study involved in the meta-analysis was deleted each time to define the influence of the individual data-set to the pooled ORs [50]. An estimate of potential publication bias was carried out by the funnel plot, in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was assessed by the method of Egger's linear regression test, a linear regression approach to measure funnel plot asymmetry on the natural logarithm scale of the OR. The significance of the intercept was determined with the $t$-test suggested by Egger ($P < 0.05$ was considered representative of statistically significant publication bias) [51]. All the statistical tests were performed with STATA version 10.0 (Stata Corporation, College Station, TX).

## Results

### Study characteristics

A total of 40 publications met the inclusion criteria [7–46]. Studies of Ericson et al. [16], Tao et al. [44], and Xu et al. [45] were excluded because the subjects had been included by Ericson et al. [15], Platek et al. [35], and Chen et al. [10], respectively. Hence, a total of 37 studies including

15,260 cases and 20,411 controls were used in the meta-analysis. Table 1 lists the studies identified and their main characteristics. Of the 37 studies, sample sizes ranged from 93 to 4,256. There were 13 studies of Caucasians, 12 studies of Asians, 8 studies of mixed populations, and 4 studies of unstated populations. Almost all of the cases were pathologically confirmed. Controls were mainly healthy populations and matched for age. Among these studies, 15 were population-based and 22 were hospital-based.

## Main results

Table 2 lists the main results of this meta-analysis. Overall, significantly elevated breast cancer risk was associated with MTHFR T allele when all studies were pooled into the meta-analysis (TT vs. CC: OR = 1.11, 95% CI = 1.01–1.23; dominant model: OR = 1.04, 95% CI = 1.00–1.09). In the subgroup analysis by ethnicity, significantly increased risk was only found for Asians (TT vs. CC: OR = 1.18, 95% CI = 1.04–1.35; recessive model:

**Table 1** Main characteristics of all studies included in the meta-analysis

| First author | Year | Location | Ethnicity | Source | Cases | Controls |
|---|---|---|---|---|---|---|
| Sharp L | 2002 | UK | Not Stated | HB | 54 | 57 |
| Campbell IG | 2002 | UK | Caucasian | HB | 335 | 233 |
| Semenza JC | 2003 | USA | Caucasian | HB | 105 | 247 |
| Ergul E | 2003 | Turkey | Caucasian | HB | 118 | 193 |
| Langsenlehner U | 2003 | Austria | Caucasian | HB | 494 | 495 |
| Grieu F | 2004 | Australia | Mixed | HB | 334 | 551 |
| Lee SA | 2004 | Korea | Asian | HB | 186 | 147 |
| Marchand LL | 2004 | USA | Mixed | PB | 1189 | 2414 |
| Shrubsole MJ | 2004 | China | Asian | PB | 1112 | 1160 |
| Forsti A | 2004 | Finland | Caucasian | PB | 223 | 298 |
| Lin WY | 2004 | Taiwan | Asian | PB | 88 | 342 |
| Qi J | 2004 | China | Asian | HB | 217 | 218 |
| Deligezer U | 2005 | Turkey | Mixed | HB | 189 | 223 |
| Justenhoven C | 2005 | Germany | Caucasian | PB | 584 | 633 |
| Chen J | 2005 | USA | Mixed | PB | 1063 | 1104 |
| Kalemi TG | 2005 | Greece | Caucasian | HB | 42 | 51 |
| Chou YC | 2006 | Taiwan | Asian | HB | 142 | 285 |
| Kalyankumar Ch | 2006 | India | Not Stated | PB | 88 | 95 |
| Stevens VL | 2007 | USA | Mixed | PB | 494 | 494 |
| Yu CP | 2007 | Taiwan | Asian | PB | 109 | 420 |
| Lissowska J | 2007 | Poland | Caucasian | PB | 1974 | 2282 |
| Macis D | 2007 | Italy | Caucasian | PB | 46 | 80 |
| Hekim N | 2007 | Turkey | Not Stated | HB | 40 | 68 |
| Reljic A | 2007 | Croatia | Caucasian | HB | 93 | 65 |
| Inoue M | 2008 | Singapore | Asian | PB | 380 | 662 |
| Suzuki T | 2008 | Japan | Asian | HB | 454 | 909 |
| Kotsopoulos J | 2008 | Canada | Caucasian | HB | 944 | 680 |
| Cheng CW | 2008 | Taiwan | Asian | HB | 349 | 530 |
| Platek ME | 2009 | USA | Mixed | PB | 994 | 1802 |
| Ma E | 2009 | Japan | Asian | HB | 388 | 387 |
| Ma E | 2009 | Brazil | Mixed | HB | 458 | 458 |
| Cam R | 2009 | Turkey | Not Stated | HB | 110 | 95 |
| Jin ZG | 2009 | China | Asian | HB | 247 | 100 |
| Ericson U | 2009 | Sweden | Caucasian | PB | 540 | 1074 |
| Henriguez-Hernandez | 2009 | Spain | Caucasian | HB | 135 | 292 |
| Maruti SS | 2009 | USA | Mixed | PB | 318 | 647 |
| Gao CM | 2009 | China | Asian | HB | 624 | 620 |

*PB* Population-based study, *HB* hospital-based study

**Table 2** Main results of pooled ORs in the meta-analysis

| | CT vs. CC | | TT vs. CC | | Dominant model | | Recessive model | |
|---|---|---|---|---|---|---|---|---|
| | OR (95% CI) | $P_h$ | OR (95% CI) | $P_h$ | OR (95% CI) | $P_h$ | OR (95% CI) | $P_h$ |
| Total | 1.04(0.99–1.08) | 0.56 | 1.11(1.01–1.23) | 0.01 | 1.04(1.00–1.09) | 0.29 | 1.09(0.99–1.20) | 0.02 |
| Ethnicity | | | | | | | | |
| Caucasian | 1.02(0.95–1.10) | 0.39 | 0.96(0.85–1.08) | 0.27 | 1.01(0.94–1.08) | 0.71 | 0.99(0.83–1.19) | 0.06 |
| Asian | 1.02(0.94–1.12) | 0.45 | 1.18(1.04–1.35) | 0.13 | 1.05(0.97–1.15) | 0.13 | 1.15(1.03–1.29) | 0.43 |
| Source | | | | | | | | |
| PB | 1.05(0.99–1.12) | 0.91 | 1.05(0.93–1.19) | 0.07 | 1.05(0.99–1.11) | 0.69 | 1.03(0.91–1.16) | 0.05 |
| HB | 1.01(0.93–1.09) | 0.22 | 1.18(1.02–1.38) | 0.07 | 1.04(0.97–1.12) | 0.11 | 1.17(1.05–1.29) | 0.15 |
| Menopausal status | | | | | | | | |
| Premenopausal | 0.90(0.77–1.06) | 0.64 | 1.11(0.87–1.41) | 0.23 | 0.95(0.82–1.11) | 0.82 | 1.15(0.92–1.44) | 0.12 |
| Postmenopausal | 1.12(1.02–1.23) | 0.97 | 1.14(0.86–1.51) | 0.01 | 1.11(1.01–1.22) | 0.63 | 1.07(0.83–1.39) | 0.01 |

$P_h$ *P* value of *Q*-test for heterogeneity test, *PB* population-based study, *HB* hospital-based study

OR = 1.15, 95% CI = 1.03–1.29). When stratified by study design, statistically significantly elevated risk was found in hospital-based studies (TT vs. CC: OR = 1.18, 95% CI = 1.02–1.38; recessive model: OR = 1.17, 95% CI = 1.05–1.29). In the subgroup analysis by menopausal status, statistically significantly increased risk was found among postmenopausal women (CT vs. CC: OR = 1.12, 95% CI = 1.02–1.23; dominant model: OR = 1.11, 95% CI = 1.01–1.22).

### Sensitivity analysis

A single study involved in the meta-analysis was deleted each time to define the influence of the individual data-set to the pooled ORs, and the corresponding pooled ORs were not materially altered (data not shown), indicating that our results were statistically robust.

### Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of literatures. The shape of the funnel plot did not reveal any evidence of obvious asymmetry (figures not shown). Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias ($P = 0.76$ for CT vs. CC; $P = 0.14$ for TT vs. CC; $P = 0.32$ with dominant model; and $P = 0.16$ with recessive model).

## Discussion

The present meta-analysis, including 15,260 cases and 20,411 controls, explored the association between the MTHFR C677T polymorphism and breast cancer risk. The results from our meta-analysis indicate that the MTHFR T allele is a low-penetrant risk factor for developing breast cancer.

This finding may be biologically plausible. Individuals with the MTHFR 677TT genotype have been shown to have only 30% of in vitro MTHFR enzyme activity compared with the wild type, whereas those with the heterozygous CT genotype have 60% of wild-type MTHFR enzyme activity [5]. Reduction of the MTHFR enzyme activity increases the pool of 5,10-methylene-THF at the expense of the pool of 5-methyl-THF, which impairs the DNA methylation. DNA methylation plays a critical role in regulation of gene expression and maintenance of genomic stability [52, 53] and aberrations in normal methylation patterns have been associated with the development of cancer [53, 54]. More importantly, the homozygous variant genotype MTHFR 677TT has been associated with risk for many different types of cancer, including colorectal [55], gastric [56], endometrial [57], lung cancer [58], and acute leukemia [59].

In the subgroup analysis based on ethnicities, significant associations were found in Asians but not for Caucasians under TT vs. CC and with recessive genetic models, suggesting a possible role of ethnic differences in genetic backgrounds and the environment they live in [60].

Our results showed that significantly increased breast cancer risk in MTHFR T genotype carriers were found in the hospital-based studies but not in population-based studies. The hospital-based studies usually have some biases because such controls may just represent a sample of ill-defined reference population, and may not be representative of the general population very well, particularly when the genotypes under investigation were associated with the disease conditions that the hospital-based controls may have. If considering this kind of selection bias, our results should be interpreted with caution.

When stratified by menopausal status, a more pronounced increased breast cancer risk was observed in postmenopausal women under CT vs. CC and with dominant genetic models. Though results from available case–control studies [7–46] investigating MTHFR C677T and breast cancer risk have been inconsistent, our results are consistent with three of them reporting significant positive associations for postmenopausal women. Ericson et al. observed a significant 34% increase in breast cancer risk among postmenopausal women in Sweden with CT and TT genotypes compared to wild type in a nested case–control study of the Malmo Diet and Cancer cohort [15]. Suzuki reported a significant 83% increased breast cancer risk among postmenopausal Japanese women with the TT genotype compared to wild type [43]. Maruti et al. observed a 62% increased risk of breast cancer among postmenopausal women with the TT genotype [34]. But other investigations have not reported this kind of significant associations. Differences in results may be due to variation between populations with regards to prevalence of polymorphisms in genes related to one-carbon metabolism, intakes of nutrients, and/or other risk factors for breast cancer. Data from future in-depth research on these gene–gene or gene–environment interactions may further elucidate this issue.

Some limitations of this meta-analysis should be acknowledged. Firstly, the controls were not uniformly defined. Although most of the controls were selected mainly from healthy populations, some had benign disease. Therefore, non-differential misclassification bias was possible because these studies may have included the control groups who have different risks of developing breast cancer. Secondly, some studies with small sample size may not have enough statistical power to explore the real association. Thirdly, our results were based on unadjusted estimates, while a more precise analysis should be conducted if all individual data were available, which would allow for the adjustment by other co-variants including age, ethnicity, smoking status, drinking status, obesity, environmental factors, and other lifestyle. In spite of these limitations, our meta-analysis had several strengths. First, substantial number of cases and controls were pooled from different studies, which significantly increased the statistical power of the analysis. Second, no publication biases were detected, indicating that the whole pooled results may be unbiased.

In conclusion, this meta-analysis suggests that the MTHFR T allele is a low-penetrant risk factor for developing breast cancer. However, it is necessary to conduct large sample studies using standardized unbiased genotyping methods, homogeneous breast cancer patients and well matched controls. Moreover, gene–gene and gene–environment interactions should also be considered in the analysis. Such studies taking these factors into account may eventually lead to our better, comprehensive understanding of the association between the MTHFR C677T polymorphism and breast cancer risk.

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