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Association of *677 C>T (rs1801133)* and *1298 A>C (rs1801131)* Polymorphisms in the *MTHFR* Gene and Breast Cancer Susceptibility: A Meta-Analysis Based on 57 Individual Studies

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Abstract

Objective: The *677 C>T* and *1298 A>C* polymorphisms of methylenetetrahydrofolate reductase (*MTHFR*) gene have been widely reported and considered to have a significant effect on breast cancer risk, but the results are inconsistent. A meta-analysis based on 57 eligible studies was carried out to clarify the role of *MTHFR* gene polymorphisms in breast cancer.

Methods and Results: Eligible articles were identified by searching databases including PubMed, Web of Science, EMBASE, CNKI and CBM for the period up to August 2012. Finally, a total of 57 studies were included in this meta-analysis. Crude ORs with 95% CIs were used to assess the association between the *MTHFR* polymorphisms and breast cancer risk. The pooled ORs were performed with additive model, dominant model and recessive model, respectively. Subgroup analysis was also performed by ethnicity. The statistical heterogeneity across studies was examined with χ^2-based Q-test. A meta-analysis was performed using the Stata 12.0 software. Overall, the *677 C* allele was significantly associated with breast cancer risk (OR = 0.942, 95%CI = 0.898 to 0.988) when compared with the *677 T* allele in the additive model, and the same results were also revealed under other genetic models. Simultaneously, the *1298 A* allele was not associated with the breast cancer susceptibility when compared with the *1298 C* allele (OR = 0.993, 95%CI = 0.978 to 1.009). Furthermore, analyses under the dominant, recessive and the allele contrast model yielded similar results.

Conclusions: The results of this meta-analysis suggest that *677 C>T* polymorphism in the *MTHFR* gene may contribute to breast cancer development. However, the *1298 A>C* polymorphism is not significantly associated with increased risks of breast cancer.

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Introduction

Breast cancer is currently the most common cancer among women and one of the leading causes of cancer-related death in the world [1]. The etiology of the disease is still not fully understood. Some risk factors such as familial history, age of menarche and of menopause, diet, reproductive history, high estrogen exposure as well as genetic factors may contribute to its development [2,3]. Low-penetrance susceptibility genes combining with environmental factors have been considered as one of the important factors in the progression of cancer [4]. Recently, several common low-penetrant genes have been identified as potential breast cancer susceptibility genes, one of which is 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene. The *MTHFR* gene produces a key 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 circulating form of folate in plasma and provides the methyl group for de novo methionine synthesis through homocysteine remethylation [5].

Folates play an integral role in maintaining DNA stability by regulating DNA biosynthesis, DNA repair and DNA methylation. Low intake of folate may increase the risk of several cancers, including breast cancer [6,7]. This reaction is essential for both purine nucleotide biosynthesis and remethylation of homocysteine to methionine used in DNA methylation [8]. Reduction of the *MTHFR* enzyme activity may increase the cancer risk through impaired DNA repair synthesis and disruption of DNA methylation. In addition, it has been suggested that breast carcinogenesis could be associated with alteration of oestrogen receptor gene methylation patterns [9] and global DNA methylation [10].

The gene encoding *MTHFR* is polymorphic located at 1p36.3 [11]. The two most common polymorphisms in the *MTHFR* gene, *677 C>T (rs1801133)* and *1298 A>C (rs1801131)*, are both associated with reduced enzyme activity [12]. The *MTHFR 677 TT* (homozygote) genotype results in 30% enzyme activity in vitro compared with the *CC* wild-type, whereas the *MTHFR 1298 CC*

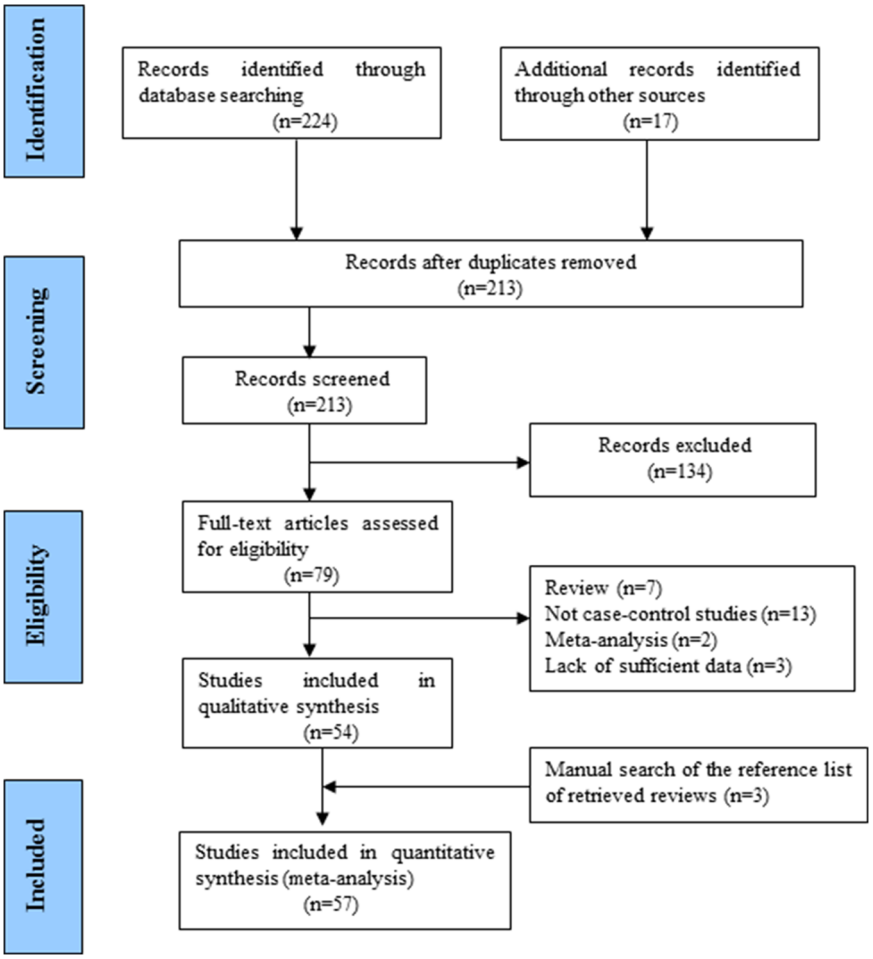

Figure 1. Flow diagram summarizing the search strategy for meta-analysis of MTFHR gene and breast cancer susceptibility.
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genotype has been found to result in 60% enzyme activity in vitro compared with the *AA* wild-type [13,14,15]. A series of studies have investigated the association between the two common polymorphisms of *MTHFR* gene and breast cancer susceptibility, but provided inconclusive results. Some studies found *MTHFR 677 TT* genotype is significantly associated with an increased risk of breast cancer [16,17,18], while no significant association in others [19,20]. For the *1298 A>C* polymorphism, *C* allele was associated with increased risk in the studies of Ergul et al. [18] and Stevens et al. [21], while reduced risk of breast cancer was found for the heterozygous model (*A/C* vs *C/C)* by Chou et al.[22]. Hence, we conducted this systematic meta-analysis of all available studies describing the association between *MTHFR 677 C>T* and *1298 A>C* polymorphisms and the risk of breast cancer.

Materials and Methods

2.1 Literature and Search Strategy

A computerized literature search was conducted for the relevant available studies published in English in PubMed, Web of Science, EMBASE, Chinese National Knowledge Infrastructure database (CNKI) and Chinese Biomedical Literature database (CBM). The literature search was updated on August 1, 2012. The search strategy identified all possible studies using combinations of the following keywords: "methylenetetrahydrofolate reductase", "*MTHFR*", "*MTHFR C677 T*", "*MTHFR Ala222Val*", "*MTHFR A1298 C*", "*MTHFR Glu222Val*", "folate", "one-carbon metabolism", "*rs1801133*", "*rs1801131*", "polymorphism", "genotype", "variant", "breast cancer", and "breast neoplasm". We did not define any minimum number of subjects for the studies included in this meta-analysis. No language restrictions were imposed. The reference lists of reviewed articles, clinical trials, and meta-analyses, were also hand-searched for collecting other relevant studies. Two authors conducted all searches independently. When the same patient population was included in several publications, only the most recent or complete study was used in this meta-analysis.

2.2 Eligibility Criteria

The studies included in this meta-analysis had to meet the following criteria: (1) utilized platinum-based regimens for patients with pathologically proven breast cancer; (2) controls were matched with normal persons; (3) only cohort studies and case-control studies were included in this meta-analysis; (4) evaluation of the *MTHFR 677 C>T* and *1298 A>C* polymorphisms and beast cancer risk; (5) clearly described the source of cases and controls; (6) provided sample sizes, and sufficient genotyping data to calculate odds ratios (ORs) and their 95% confidence intervals (CIs).

Accordingly, the exclusion criteria were: (1) not designed as case-control or cohort studies; (2) reviews; (3) not offering the

Table 1. Main results of pooled odds ratios (ORs) with confidence interval (CI) in the meta-analysis.

Variables	No. of studies	*CC* vs *TT*			*CC* vs *CT*			*CT* vs *TT*		
		OR (95% CI)	Ph	P	OR (95% CI)	Ph	P	OR (95% CI)	Ph	P
Total	57	0.983(0.969 0.997)	0.000	**0.019**	0.984(0.967 1.001)	0.222	0.070	0.983(0.967 1.000)	0.002	**0.048**
Asian	20	0.770(0.633 0.938)	0.015	**0.009**	0.940(0.867 1.018)	0.244	0.130	0.845(0.730 0.977)	0.230	**0.023**
Caucasian	28	0.946(0.818 1.094)	0.006	0.456	1.010(0.952 1.072)	0.191	0.733	0.919(0.792 1.067)	0.004	0.269
Mixed	9	0.894(0.792 1.009)	0.114	0.071	0.940(0.887 0.996)	0.795	**0.036**	0.956(0.839 1.090)	0.061	0.502
Variables	**No. of studies**	***CC+CT* vs *TT* (dominant)**			***CC* vs *CT+TT* (recessive)**			***C* allele vs *T* allele**		
		OR (95% CI)	Ph	P	OR (95% CI)	Ph	P	OR (95% CI)	Ph	P
Total	57	0.990(0.982 0.999)	0.000	**0.020**	0.956(0.923 0.990)	0.052	**0.011**	0.942(0.898 0.988)	0.000	**0.013**
Asian	20	0.808(0.687 0.952)	0.057	**0.011**	0.911(0.845 0.983)	0.057	**0.017**	0.877(0.801 0.960)	0.003	**0.005**
Caucasian	28	0.931(0.807 1.074)	0.002	0.329	1.004(0.950 1.062)	0.151	0.883	0.983(0.924 1.045)	0.018	0.582
Mixed	9	0.921(0.814 1.043)	0.057	0.196	0.935(0.885 0.987)	0.752	**0.016**	0.953(0.857 1.059)	0.000	0.368

P_h: P value of Q-test for heterogeneity test.

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source of cases and controls and other essential information; (4) control population including malignant tumor patients; (5) duplicated publications.

2.3 Quality Assessment

Quality of the studies was assessed using the Newcastle–Ottawa Quality Assessment Scale for cohort studies [23,24]. This scale is composed of eight items to assess patient selection, study comparability and outcome. The scale was recommended by the Cochrane Non-Randomized Studies Methods Working Group [25]. Two investigators performed quality assessment independently. Disagreement was resolved by consensus.

2.4 Data Extraction

Information was independently extracted from all eligible publications by two authors according to the inclusion and exclusion criteria listed above. Disagreement was resolved by discussion between the two authors. The following data were collected from each study: first author's surname, year of publication, ethnicity, the numbers of cases and controls with the frequencies of *CC*, *CT* and *TT* genotypes, and the *AA*, *AC* and *CC* genotypes, respectively. Different ethnicity descents were categorized as Caucasian, Asian and Mixed population. When studies included subjects of more than one ethnicity, data were extracted separately for each ethnic group.

2.5 Statistical Analysis

Crude ORs with 95% CIs were used to assess the strength of association between the *MTHFR 677 C>T* and *1298 A>C* polymorphisms and breast cancer risk. For the two polymorphisms, the meta-analysis examined their associations for the allele model *C* vs *T*; homozygote (*CC* vs *TT*), recessive model (*CC* vs *CT*+*TT*) and dominant model (*CC*+*CT* vs *TT*). Subgroup analyses were stratified by ethnicity. Both fixed-effects model using the Mantel–Haenszel method and random-effects model using the DerSimonian and Laird method were used to pool the results.

Heterogeneity assumption was checked by the Chi-square-based Q-test [26]. 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 included studies was calculated by the fixed-effects model. Otherwise, the random-effects model was used. The significance of the pooled OR was determined by the Z-test, and $P<0.05$ was considered as statistically significant. One-way sensitivity analyses were performed to assess the stability of the results, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual dataset on the pooled OR. 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 by the t-test suggested by Egger ($P<0.05$ was considered representative of statistically significant publication bias) [27]. Hardy–Weinberg equilibrium in the control group was tested by the Chi-square test for goodness of fit, and a *P*-value $<$ 0.05 was considered significant. All of the calculations were performed using STATA (version 12.0; Stata Corporation, College Station, TX), using two-sided *P*-values.

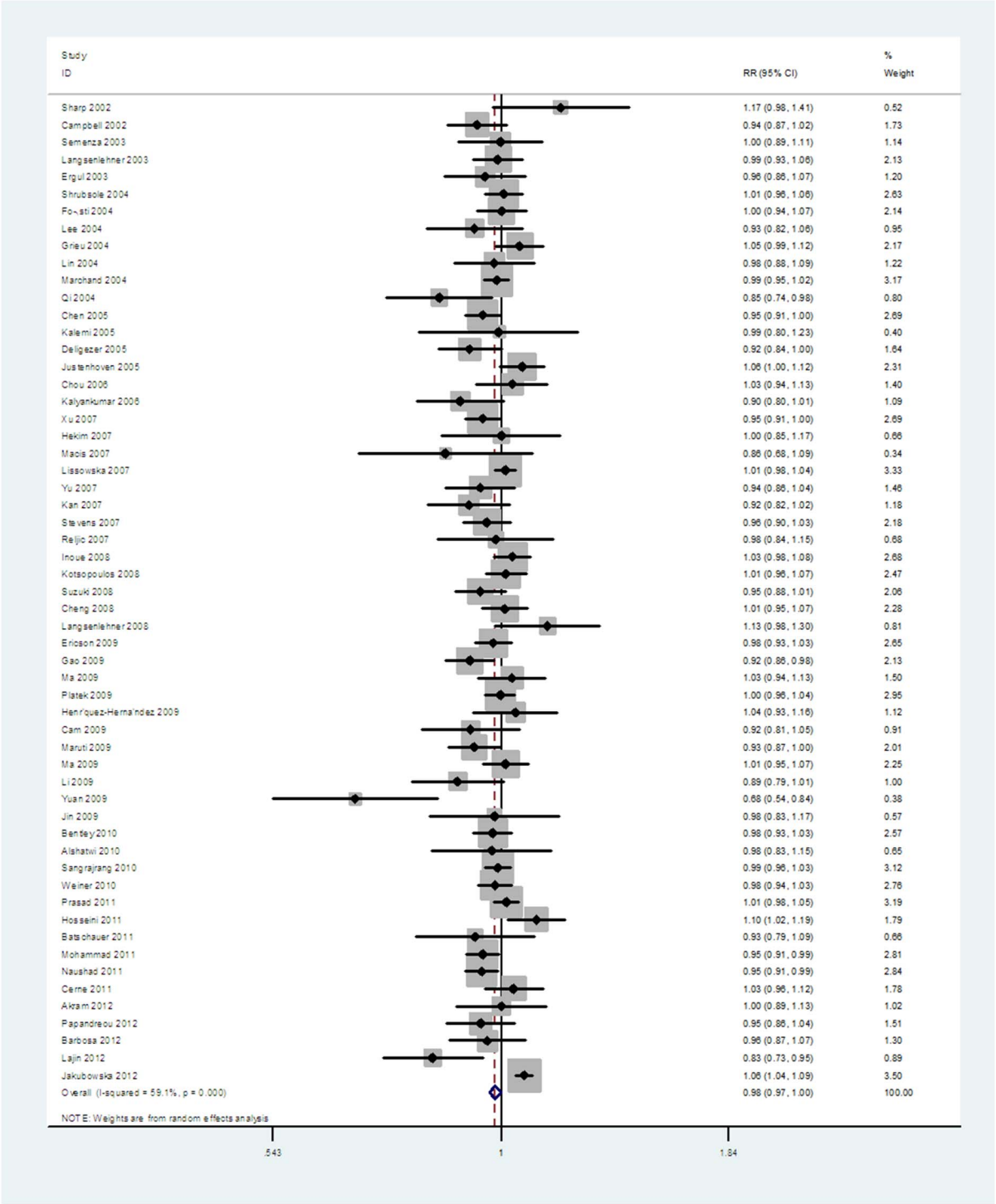

Figure 2. Forest plot of breast cancer susceptibility associated with *MTHFR 677 C>T* polymorphism at additive model (*C* allele vs *T* allele).
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Results

3.1 Study Characteristics

Studies relevant to the searching words were retrieved originally. 57 eligible publications addressing the association between *MTHFR 677 C>T* and *1298 A>C* polymorphisms (25,877 breast cancer cases and 29,781 controls) and breast cancer risk were ultimately analyzed (**Figure 1**). All the cases were histologically confirmed. Controls were mainly healthy populations. A total of 241 articles regarding the association between *MTHFR 677 C>T* (rs1801133) and *1298 A>C* (rs1801131) polymorphisms and breast cancer were identified. After screening the duplicated articles, 28 publications were excluded. And then titles and abstracts were screened, 134 articles were excluded and

Table 2. Main results of pooled odds ratios (ORs) with confidence interval (CI) in the meta-analysis.

Variables	No. of studies	*AA vs CC*			*AA vs AC*			*AC vs CC*		
		OR (95% CI)	Ph	P	OR (95% CI)	Ph	P	OR (95% CI)	Ph	P
Total	29	0.993(0.979 1.008)	0.001	0.367	1.001(0.982 1.021)	0.144	0.888	0.983(0.962 1.006)	0.000	0.143
Asian	9	0.918(0.732 1.152)	0.554	0.461	1.093(0.995 1.201)	0.367	0.065	0.846(0.670 1.068)	0.552	0.159
Caucasian	13	0.781(0.629 0.972)	0.004	**0.026**	0.921(0.854 0.993)	0.634	**0.033**	0.793(0.613 1.025)	0.000	0.077
Mixed	7	1.028(0.835 1.266)	0.058	0.795	1.034(0.959 1.115)	0.232	0.379	1.012(0.843 1.215)	0.157	0.898
Variables	**No. of studies**	***AA+AC vs CC* (dominant)**			***AA vs AC+CC*(recessive)**			***A* allele vs *C* allele**		
		OR (95% CI)	Ph	P	OR (95% CI)	Ph	P	OR (95% CI)	Ph	P
Total	29	0.994(0.985 1.004)	0.000	0.242	0.999(0.972 1.027)	0.021	0.955	0.993(0.978 1.009)	0.000	0.407
Asian	9	0.893(0.714 1.118)	0.554	0.324	1.074(0.981 1.176)	0.359	0.123	1.040(0.958 1.130)	0.348	0.348
Caucasian	13	0.794(0.630 1.001)	0.000	0.051	0.907(0.845 0.974)	0.221	**0.007**	0.876(0.789 0.972)	0.001	**0.013**
Mixed	7	1.025(0.848 1.238)	0.094	0.802	1.038(0.966 1.115)	0.135	0.312	1.019(0.938 1.107)	0.074	0.658

P_h: P value of Q-test for heterogeneity test.

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79 full-test studies were left for further evaluating. Furthermore, 25 publications were excluded because they were 7 review articles, 17 non-case control studies, 2 other meta-analysis, and 3 studies lack of sufficient data. Last, 3 studies were included in this work through manual search of the reference list of retrieved reviews. Hence, 57 publications including 57 studies for *MTHFR 677 C>T (rs1801133)* and 29 studies for *MTHFR 1298 A>C (rs1801131)* were included in this meta-analysis [28–73]. Simultaneously, of 57 studies for *MTHFR 677 C>T (rs1801133)* polymorphism and breast cancer susceptibility, included 28 groups of Caucasians, 20 groups of Asians, and 9 Mixed populations. While 29 studies for *MTHFR 1298 A>C (rs1801131)* polymorphism and breast cancer susceptibility, included 13 groups of Caucasians, 9 groups of Asians, and 7 Mixed populations. The distribution of genotypes in the controls of all studies was in agreement with Hardy–Weinberg equilibrium.

3.2 Association of the *MTHFR* Gene *677C>T* Genotype with Breast Cancer Risk

The main characteristics of these studies were listed in **Table S1**. The association between the *677 C>T* polymorphism and breast cancer risk was investigated under the additive model (allele *C* vs allele *T*). Substantial heterogeneity among the studies ($I^2 = 59.1\%$, $P = 0.0014$) was found. The overall OR under a random-effects model was 0.942 (95%CI = 0.898 to 0.988), suggesting a significant association (**Figure 2**). An overall analysis under other genetic models was then performed. It also revealed a significant association under the dominant model (OR = 0.990, 95%CI = 0.982 to 0.999) and the recessive model (OR = 0.956, 95%CI = 0.923 to 0.990). Furthermore, a significant association was found under other pair-wise comparisons. The results were shown in **Table 1**. In order to analyze characteristic-homogeneous groups, subgroup analysis was carried out by ethnicity. Significant association was found under the additive genetic model.

3.3 Association of the *MTHFR* Gene *1298 a>C* Genotype with Breast Cancer Risk

29 studies were included in the meta-analysis to describe the association between *1298A>C* polymorphism and breast cancer risk. The main characteristics of these studies were listed in **Table S2**. Analysis of *1298 A>C* polymorphism in the *MTHFR* gene with breast cancer risk under the additive model was performed and the random model was used to assess the overall OR value. Compared with the carrier of the *C* allele, the overall OR of the *A* allele was 0.993 (95%CI = 0.978 to 1.009) (**Figure 3**). Under the recessive and the dominant models, the overall OR was 0.999 (95%CI = 0.972 to 1.027) and 0.994 (95%CI = 0.985 to 1.004), respectively. When pair-wise comparisons were made, comparing with the *C/C* genotype, the overall OR of the *A/A* genotype with breast cancer risk was 0.993 (95%CI = 0.979 to 1.008). However, compared with homozygotes of the *CC* allele, significant association of the *AC* genotype with breast cancer risk was not found and the overall OR was 0.983 (95%CI = 0.962 to 1.006). Analyses under different genetic models were shown in **Table 2**.

According to study characteristics, subgroup analysis and sensitivity analysis were performed. The results showed that the *1298A* allele in Caucasian population had significant effect on the risk of breast cancer (OR = 0.876; 95%CI = 0.789 to 0.972), whereas this effect was reversed in Asian (OR = 1.040; 95%CI = 0.958 to 1.130) and Mixed population (OR = 1.019; 95%CI = 0.938 to 1.107) (**Table 2**).

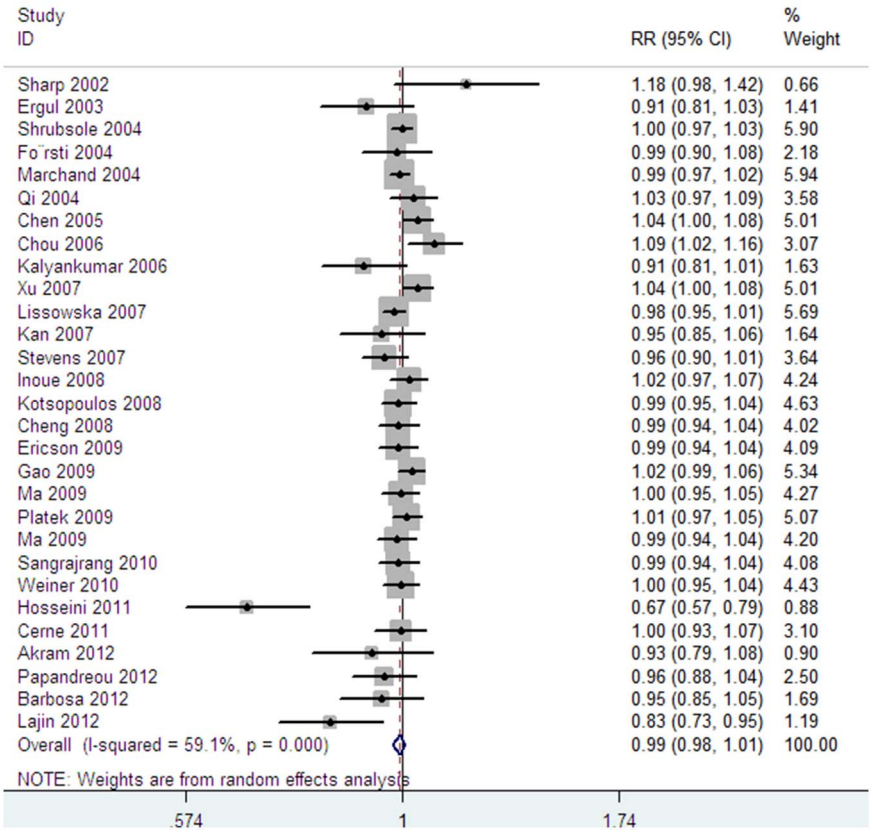

Figure 3. Forest plot of breast cancer susceptibility associated with *MTHFR 1298 A>C* polymorphism at additive model (*A* allele vs *C* allele).
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3.4 Sensitivity Analysis

In order to compare the difference and evaluate the sensitivity of the meta-analyses, we conducted one-way sensitivity analysis to evaluate the stability of the results. The statistical significance of the results was not altered when any single study was omitted (data not shown), confirming the stability of the results.

3.5 Publication Bias

Begg's funnel plot and Egger's test were performed to assess the publication bias. The shapes of the funnel plots did not reveal significant evidence of obvious asymmetry in all comparison models (Figures not show). Furthermore, Egger's test was used to provide statistical evidence for funnel plot symmetry. The results still did not suggest any evidence of publication bias ($P = 0.322$ for

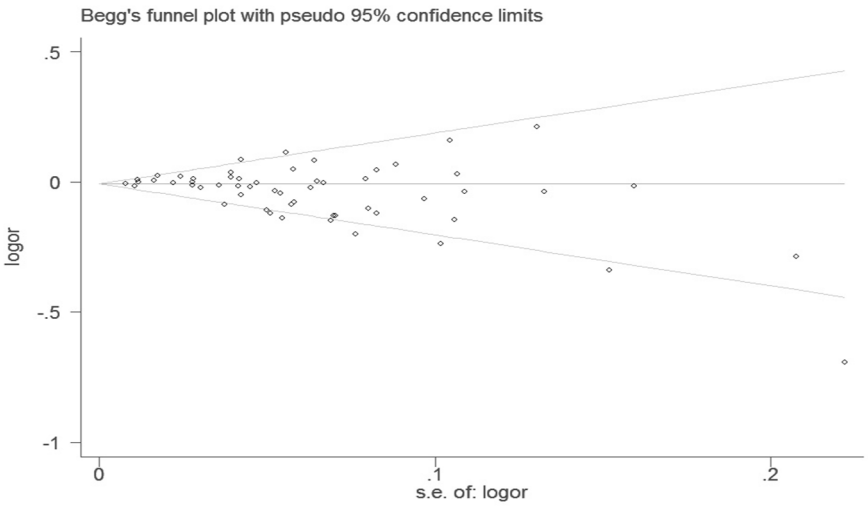

Figure 4. Begg's funnel plot with pseudo 95% confidence limit under the additive genetic model of *677 C>T* genotype.
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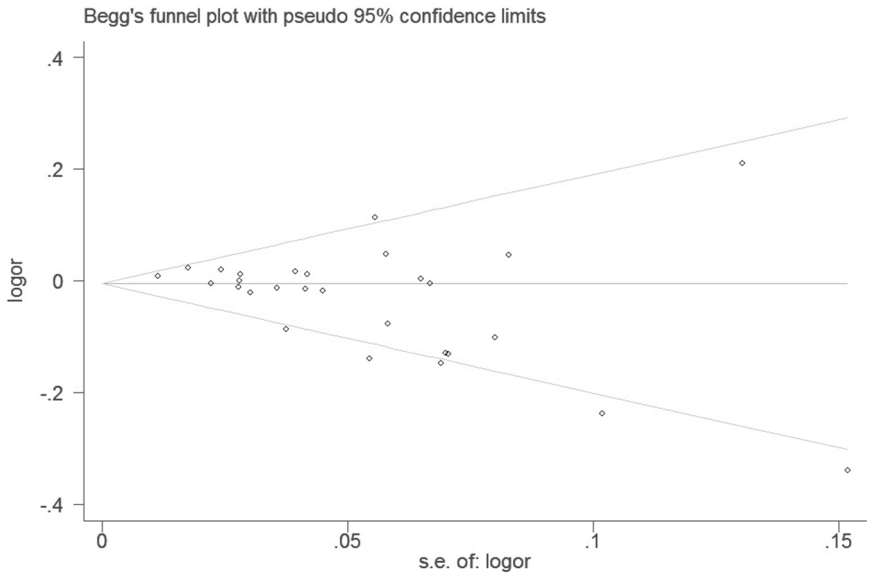

Figure 5. Begg's funnel plot with pseudo 95% confidence limit under the additive genetic model of *1298 A>C* genotype.
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C allele vs *T* allele, **Figure 4**; $P = 0.066$ for *A* allele vs *C* allele, **Figure 5**).

Discussion

It is well recognized that there is individual susceptibility to the same kind of cancer even with the same environmental exposure. Environmental factors and gene genotypes involved in carcinogenesis may account for this difference. Therefore, genetic susceptibility to cancer has been a research focus in scientific community. Genetic epidemiologic studies of single nucleotide polymorphism can provide the relationships between candidate genes and cancer risk. However, individual studies on the relationship between *MTHFR 677 C>T* and *1298 A>C* polymorphisms and cancer risk generated inconsistent results partly because of the small sample size. Meta-analysis is a method that can solve the problem caused by low statistical power in single study to draw a more robust conclusion. Our present study, including 57 published cohort and/or case-control studies, estimated the potential role of *MTHFR 677 C>T* and *1298 A>C* polymorphisms in breast cancer development.

In the meta-analysis of the *677 C>T* polymorphism, a total of 57 studies involving 55,658 subjects, significant association with breast cancer risk was detected in overall comparisons under all genetic models. Results from studies with small sample size or deviating HWE are inconsistent. One explanation may be that small sample size and deviation form HWE may have biased the results. Study characteristics, such as mean age of cases, status of premenopausal and postmenopausal, genotyping method, study design, source of controls and ethnicity, showed some differences in the included studies. But most of them were not responsible for heterogeneity by subgroup analysis and sensitivity analysis.

Another variant of the *MTHFR* gene, the *1298 A>C* polymorphism, which is present in 27,141 subjects and 29 studies, showed that the *A* allele was not significantly associated with increased breast cancer risk when compared with the *C* allele under additive, recessive model and the *AA* vs *CC* genetic model. In the subgroup analysis, *A* allele was not associated the risk of breast cancer in Caucasian populations.

Several potential limitations of this meta-analysis should be considered. First, this meta-analysis mostly focused on papers published in English and Chinese. Second, not all the control subjects were age and sex matched to cases, which may introduce heterogeneity in this meta-analysis. Third, subgroup analysis was only performed according to ethnicity, due to the unavailability of the data on the status of menopausal and conditions on folate intake. Forth, the insufficient information in the included studies did not allow further analysis of the joint effects of the two polymorphisms. However, the advantages of this meta-analysis were also obvious. The large sample size of this study confirmed the reliability of our results. Second, the potential sources of heterogeneity in the meta-analysis were assessed. Third, the associations between the *MTHFR 677 C>T* and *1298 A>C* polymorphisms and breast cancer risk were evaluated under different genetic models.

In conclusion, although the meta-analysis provides evidence that the *MTHFR 1298 A>C* polymorphism is not significantly associated with increased risk of breast cancer, a significant association was found between the *MTHFR 677 C>T* polymorphism and breast cancer risk, especially in Asian populations. Well-designed and large studies are needed to further investigate the association of these polymorphisms with breast cancer susceptibility.

Supporting Information

Table S1 The main characteristics of these studies included in this meta-analysis and the distribution of MTHFR gene 677C>T genotypes and alleles among cases and controls.
(DOCX)

Table S2 The main characteristics of these studies included in this meta-analysis and the distribution of MTHFR gene 1298A>C genotypes and alleles among cases and controls.
(DOCX)

Checklist S1 PRISMA Checklist.
(DOC)

Author Contributions

Conceived and designed the experiments: KL. Performed the experiments: KL. Analyzed the data: KL WL XD. Contributed reagents/materials/analysis tools: WL XD. Wrote the paper: KL.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics 2002. CA Cancer J Clin 55: 74–108.
2. Hankinson SE, Colditz GA, Willett WC (2004) Towards an integrated mod breast cancer etiology. The lifelong interplay of genes, lifestyle, and hormones. Breast Cancer Res 6: 213–218.
3. Dumitrescu RG, Cotarla I (2005) Understanding breast cancer risk–where do we stand in 2005. J Cell Mol Med 9: 208–221.
4. Lichtenstein P, Holm NV, Verkasalo PK (2000) Environmental and heritable factors in the causation of cancer. N Engl J Med 343: 78–85.
5. Bailey LB, Gregory JR (1999) Polymorphisms of methylenete-trahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J Nutr 129: 919–922.
6. Kim YI (1999) Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem 10: 66–88.
7. Mason JB, Choi SW (2000) Folate and carcinogenesis: developing a unifying hypothesis. Adv Enzyme Regul 40: 127–41.
8. Choi SW, Mason JB (2002) Folate status: effects on pathways of colorectal carcinogenesis. J Nutr 132: 2413S–2418S.
9. Nass SJ, Herman JG, Gabrielson E, Iversen PW, Parl FF, et al (2000) Aberrant methylation of the estrogen receptor and E-cadherin 5′ CpG islands increases with malignant progression in human breast cancer. Cancer Res 60: 4346–4348.
10. Soares J, Pinto AE, Cunha CV, André S, Baraõ I, et al (1999) Global DNA hypomethylation in breast carcinoma: correlation with prognostic factors and tumor progression. Cancer 85: 112–118.
11. Rosenberg N, Murata M, Ikeda Y, Opare-Sem O, Zivelin A, et al(2002)The frequent 5,10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in Whites, Japanese, and Africans. Am J Hum Genet 70: 758–762.
12. Rama Devi AR, Govindaiah V, Ramakrishna G, Naushad SM (2004) Prevalence of methylene tetrahydrofolate reductase polymorphism in South Indian population. Curr Sci 86: 440–443.
13. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, et al (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10: 111–113.
14. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R (1998) A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 64: 169–172.
15. Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, et al (2001) The 1298A>C polymorphism in methylenetetrahy-drofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 156: 409–415.
16. Deligezer U, Akisik EE, Dalay N (2005) Homozygosity at the C677T of the MTHFR gene is associated with increased breast cancer risk in the Turkish population. In Vivo 19: 889–893.
17. Ergul E, Sazci A, Utkan Z, Canturk NZ (2003) Polymorphisms in the MTHFR gene are associated with breast cancer. Tumour Biol 24: 286–290.
18. Chen J, Gammon MD, Chan W, Palomeque C, Wetmur JG, et al (2005) One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res 65: 1606–1614.
19. Justenhoven C, Hamann U, Pierl CB, Rabstein S, Pesch B, et al (2005) One-carbon metabolism and breast cancer risk: no association of MTHFR, MTR, and TYMS polymorphisms in the GENICA study from Germany. Cancer Epidemiol Biomark Prev 14: 3015–3018.
20. Platek ME, Shields PG, Marian C, McCann SE, Bonner MR, et al (2009) Alcohol consumption and genetic variation in Methylenetetrahydrofolate reductase and 5-methyltetrahydrofolate homocysteine methyltransferase in relation to breast cancer risk. Cancer Epidemiol Biomark Prev 18: 2453–2459.
21. Stevens VL, McCullough ML, Pavluck AL, Talbot JT, Feigelson HS, et al (2007) Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. Cancer Epidemiol Biomark Prev 16: 1140–1147.
22. Chou YC, Wu MH, Yu JC, Lee MS, Yang T, et al (2006) Genetic polymorphisms of the methylenete-trahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: a case–control study in Taiwan. Carcinogenesis 27: 2295–2300.
23. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, et al. (2003) The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Available: http://www.ohri.ca/programs/clinicalepidemiology/oxford.htm. Accessed September 25, 2009.
24. Schoenleber SJ, Kurtz DM, Talwalkar JA, Roberts LR, Gores GJ (2009). Prognostic role of vascular endothelial growth factor in hepatocellular carcinoma: systematic review and meta-analysis. Br J Cancer 100: 1385–92.
25. The Cochrane Collaborative Review Group on HIV Infection and AIDS (2009) Editorial Policy: Inclusion and Appraisal of Experimental and Non-experimental (Obser-vational) Studies. Available: http://www.igh.org/Cochrane. Accessed September 29, 2009.
26. Cochran WG (1954) The combination of estimates from different experiments. Biometrics 10: 101–29.
27. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–34.
28. Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, et al (2002) Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett 181: 65–71.
29. Campbell IG, Baxter SW, Eccles DM, Choong DY (2002) Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. Breast Cancer Res 4(6):R14.
30. Semenza JC, Delfino RJ, Ziogas A, Anton-Culver H (2003) Breast cancer risk and methylenetetrahydrofolate reductase polymorphism. Breast Cancer Res Treat 77: 217–223.
31. Langsenlehner U, Krippl P, Renner W, Yazdani-Biuki B, Wolf G, et al (2003) The common 677C>T gene polymorphism of methylenetetrahy- drofolate reductase gene is not associated with breast cancer risk. Breast Cancer Res Treat 81: 169–172.
32. Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, et al (2004) MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 13: 190–196.
33. Försti A, Angelini S, Festa F, Sanyal S, Zhang Z, et al (2004) Single nucleotide polymorphisms in breast cancer. Oncol Rep 11: 917–922.
34. Lee SA, Kang D, Nishio H, Lee MJ, Kim DH, et al (2004) Methylenetetrahydrofolate reductase polymorphism, diet, and breast cancer in Korean women. Exp Mol Med 36: 116–121.
35. Grieu F, Powell B, Beilby J, Iacopetta B (2004) Methylenete-Trahydrofolate reductase and thymidylate synthase polymorphisms are not associated with breast cancer risk or phenotype. Anticancer Res 24: 3215–3219.
36. Lin WY, Chou YC, Wu MH, Huang HB, Jeng YL, et al (2004) The MTHFR C677T polymorphism, estrogen exposure and breast cancer risk: a nested case-control study in Taiwan. Anticancer Res 24: 3863–3868.
37. Le Marchand L, Haiman CA, Wilkens LR, Kolonel LN, Henderson BE (2004) MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 13: 2071–2077.
38. Qi J, Miao XP, Tan W, Yu CY, Liang G, et al (2004) Association between genetic polymorphisms in methylenetetrahydrofolate reductase and risk of breast cancer. Chin J Oncol 26: 287–289.
39. Kalemi TG, Lambropoulos AF, Gueorguiev M, Chrisafi S, Papazisis KT, et al (2005) The association of p53 mutations and p53 codon 72, Her 2 codon 655 and MTHFR C677T polymorphisms with breast cancer in Northern Greece. Cancer Lett 222: 57–65.
40. Kalyankumar CH, Jamil K (2006) Methylene tetrahydofolate reductase (MTHFR) C677T and A1298C polymorphisms and breast cancer in South Indian population. Int J Cancer Res 2: 143–151.
41. Lissowska J, Gaudet MM, Brinton LA, Chanock SJ, Peplonska B, et al (2007) Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: a population-based case-control study and meta-analyses. Int J Cancer 120: 2696–2703.
42. Xu X, Gammon MD, Zhang H, Wetmur JG, Rao M, et al (2007) Polymorphisms of one-carbon-metabolizing genes and risk of breast cancer in a population-based study. Carcinogenesis 28: 1504–1509.
43. Hekim N, Ergen A, Yaylim I, Yilmaz H, Zeybek U, et al (2007) No association between methylenetetrahydrofolate reductase C677T polymorphism and breast cancer. Cell Biochem Funct 25: 115–117.
44. Macis D, Maisonneuve P, Johansson H, Bonanni B, Botteri E, et al (2007) Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. Breast Cancer Res Treat 106: 263–271.
45. Yu CP, Wu MH, Chou YC, Yang T, You SL, et al (2007) Breast cancer risk associated with multigenotypic poly-Morphisms in folate-metabolizing genes: a nested case-control study in Taiwan. Anticancer Res 27: 1727–1732.
46. Kan XX, Zou TN, Wu XY, Wang X (2007) Association between mTHFR genotype polymorphism and breast cancer susceptibility in human population from Yunnan. Cancer Res Prev Treat 34: 716–718.
47. Reljic A, Simundic AM, Topic E, Nikolac N, Justinic D, et al (2007) The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and cancer risk: the Croatian case-control study. Clin Biochem 40: 981–985.
48. Inoue M, Robien K, Wang R, Van Den Berg DJ, Koh WP, et al (2008) Green tea intake, MTHFR/TYMS genotype and breast cancer risk: the Singapore Chinese Health Study. Carcinogenesis 29: 1967–1972.

49. Kotsopoulos J, Zhang WW, Zhang S, McCready D, Trudeau M, et al (2008) Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. Breast Cancer Res Treat 112: 585–593.
50. Suzuki T, Matsuo K, Hirose K, Hiraki A, Kawase T, et al (2008) One-carbon metab-olism-related gene polymorphisms and risk of breast cancer. Carcinogenesis 2: 356–362. doi:10.1093/carcin/bgm295.
51. Cheng CW, Yu JC, Huang CS, Shieh JC, Fu YP, et al (2008) Polymorphism of cytosolic serine hydroxymethyltransferase, estrogen and breast cancer risk among Chinese women in Taiwan. Breast Cancer Res Treat 111: 145–155.
52. Langsenlehner T, Renner W, Yazdani-Biuki B, Langsenlehner U (2008) Methylenetetrahydrofolatereductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. Breast Cancer Res Treat 107: 459–460.
53. Ericson U, Sonestedt E, Ivarsson MI, Gullberg B, Carlson J, et al (2009) Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmö Diet and Cancer cohort. Cancer Epidemiol Biomarkers Prev 18: 1101–1110.
54. Gao CM, Tang JH, Cao HX, Ding JH, Wu JZ, et al (2009) MTHFR polymorphisms, dietary folate intake and breast cancer risk in Chinese women. J Hum Genet 54: 414–418.
55. Ma E, Iwasaki M, Kobayashi M, Kasuga Y, Yokoyama S, et al (2009) Dietary intake of folate, vitamin B2, vitamin B6, vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Japan. Nutr Cancer 61: 447–456.
56. Henríquez-Hernández LA, Murias-Rosales A, Hernández González A, Cabrera De León A, Díaz-Chico BN, et al (2009) Gene polymorphisms in TYMS, MTHFR, p53 and MDR1 as risk factors for breast cancer: a case-control study. Oncol Rep 22: 1425–1433.
57. Cam R, Eroglu A, Egin Y, Akar N (2009) Dihydrofolate reduc-Tase (DHRF) 19-bp intron-1 deletion and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms in breast cancer. Breast Cancer Res Treat 115: 431–432.
58. Maruti SS, Ulrich CM, Jupe ER, White E (2009) MTHFR C677T and postmenopausal breast cancer risk by intakes of one-carbon metabolism nutrients: a nested case-control study. Breast Cancer Res 11:R91.
59. Ma E, Iwasaki M, Junko I, Hamada GS, Nishimoto IN, et al (2009) Dietary intake of folate, vitamin B6, and vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Brazilian women. BMC Cancer 9: 122.
60. Alshatwi AA (2010) Breast cancer risk, dietary intake, and methylenetetrahydrofolate reductase (MTHFR) single nucleotide polymorphisms. Food and Chemical Toxicology 48;881–1885.
61. Sangrajrang S, Sato Y, Sakamoto H, Ohnami S, Khuhaprema T, et al (2010) Genetic polymorphisms in folate and alcohol metabolism and breast cancer risk: a case–control study in Thai women. Breast Cancer Res Treat 123: 885–893.
62. Weiner AS, Boyarskih UA, Voronina EN, Selezneva IA, Sinkina TV, et al (2010) Polymorphic Variants of Folate Metabolizing Genes (C677T and A1298C MTHFR and C1420T SHMT1 and G1958A MTHFD) are Not Associated with the Risk of Breast Cancer in the West Siberian Region of Russia. Molecular Biology 44: 720–727.
63. Prasad VV, Wilkhoo H (2011) Association of the Functional Polymorphism C677T in the Methylenetetrahydrofolate Reductase Gene with Colorectal, Thyroid, Breast, Ovarian, and Cervical Cancers. Onkologie 34: 422–426.
64. Hosseini M, Houshmand M, Ebrahimi A (2011) MTHFR polymorphisms and breast cancer risk. Arch Med Sci 7(1):134–7.
65. Batschauer AP, Cruz NG, Oliveira VC, Coelho FF, Santos IR, et al (2011) HFE, MTHFR, and FGFR4 genes polymorphisms and breast cancer in Brazilian women. Mol Cell Biochem 357: 247–253.
66. Mohammad NS, Yedluri R, Addepalli P, Gottumukkala SR, Digumarti RR, et al (2011) Aberrations in one-carbon metabolism induce oxidative DNA damage in sporadic breast cancer. Mol Cell Biochem 349: 159–167.
67. Naushad SM, Pavani A, Digumarti RR, Gottumukkala SR, Kutala VK (2011) Epistatic interactions between loci of one-carbon metabolism modulate susceptibility to breast cancer. Mol Biol Rep 38: 4893–4901.
68. Ziva Cerne J, Stegel V, Gersak K, Novakovic S (2011) Lack of association between methylenetetrahydrofolate reductase genetic polymorphisms and postmenopausal breast cancer risk. Molecular Medicine Reports 4: 175–179.
69. Akram M, Malik FA, Kayani MA (2012) Mutational Analysis of the MTHFR Gene in Breast Cancer Patients of Pakistani Population. Asian Pacific J Cancer Pre 13(4):1599–603.
70. Papandreou CN, Doxani C, Zdoukopoulos N, Vlachostergios PJ, Hatzidaki E, et al (2012) 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 31(2):193–8.
71. Carvalho Barbosa Rde C, Menezes DC, Freire TF, Sales DC, Alencar VH, et al. (2012) Associations of polymorphisms of folate cycle enzymes and risk of breast cancer in a Brazilian population are age dependent. Mol Biol Rep 39: 4899–4907.
72. Lajin B, Sakur AA, Ghabreau L, Alachkar A (2012) Association of polymorphisms in one-carbon metabolizing genes with breast cancer risk in Syrian women. Tumor Biol 33: 1133–1139.
73. Jakubowska A, Rozkrut D, Antoniou A, Hamann U, Scott RJ, et al. (2012) Association of PHB 1630 C4T and MTHFR 677 C>T polymorphisms with breast and ovarian cancer risk in BRCA1/2 mutation carriers: results from a multicenter study. British Journal of Cancer 106, 2016–2024.