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MTRR A66G polymorphism and breast cancer risk: a meta-analysis

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Abstract Methionine synthase reductase (MTRR) is one of the important enzymes involved in the folate metabolic pathway and its functional genetic polymorphisms may be associated with breast cancer risk. However, this relationship remains inconclusive. For better understanding the effect of *MTRR A66G* polymorphism on breast cancer risk, a meta-analysis was performed. By searching PubMed and EMBASE, a total of six case–control studies, containing 6,084 cases and 6,756 controls, were included. The strength of association between *MTRR A66G* polymorphism and breast cancer risk was assessed by odds ratio (OR) with the corresponding 95% confidence interval (95% CI). The results strongly suggested that there was no significant association between *MTRR A66G* polymorphism and breast cancer susceptibility in overall comparisons in all genetic models (additive model: OR 1.00, 95% CI 0.89–1.11, $P = 0.943$; dominant model: OR 1.00, 95% CI 0.91–1.10, $P = 0.989$; recessive model: OR 1.00, 95% CI 0.91–1.09, $P = 0.926$). Similarly, in subgroup analyses for ethnicity (Caucasian, Asian and mixed population) and folate intake status (high and low folate intake), the results were negative. Sensitivity analysis demonstrated that omitting any study did not perturb the results. In conclusion, this meta-analysis strongly suggests that *MTRR A66G* polymorphism is not associated with breast cancer risk, especially in Caucasians and Asians.

Keywords Methionine synthase reductase · Gene polymorphism · Breast cancer · Meta-analysis

Introduction

Breast cancer, a manifestation of abnormal genetic as well as epigenetic changes, is a major challenge to women's health. Though the exact etiology remains unknown, an accumulating body of evidence has suggested that an inverse relationship exists between folate intake and breast cancer risk [1–3]. Based on this finding, it is proposed that individual variation in the genes that assist in folate metabolism may have an effect on this risk.

One-carbon metabolism, also referred as folate-mediated one-carbon metabolism, is a network of biological reactions that plays a critical role in DNA methylation and synthesis, and therefore has an impact on both genetic and epigenetic pro-carcinogenic processes [4]. Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) are three important enzymes involved in the folate metabolic pathway. Several case–control studies have been conducted to evaluate the association between their genetic polymorphisms and breast cancer risk, with controversial or inconclusive results [5–7]. According to a more precise estimation of this relationship derived from meta-analysis,

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MTHFR T allele was a low-penetrant risk factor for developing breast cancer [8, 9] and *MTR A2756G* polymorphism may contribute to susceptibility to breast cancer among Europeans [10]. But no meta-analysis was performed regarding MTRR and breast cancer risk.

Both MTRR and MTR play a role in regulating the reaction in which methionine is produced through the irreversible transfer of a methyl group from 5-methyltetrahydrofolate. MTR is maintained in its active form by MTRR. A genetic polymorphism at nucleotide 66 (A-to-G) is a functional polymorphism of MTRR and the variant has a lower affinity for MTR [11]. In light of the potential contribution of *MTR A2756G* polymorphism to breast cancer risk, it is reasonable that MTRR genotype may also have an impact on breast cancer risk. To clarify this problem, a meta-analysis was performed.

Methods

Study identification and selection

Before the study, inclusion criteria were defined as follows: (a) articles evaluating the association between *MTRR A66G* polymorphism and breast cancer risk; (b) study designed as case–control; (c) sufficient data available to estimate an odds ratio (OR) with its 95% confidence interval (95% CI).

A literature search of PubMed and EMBASE (updated to 2010/03/28) was conducted using the following terms: 'MTRR' or 'methionine synthase reductase', 'polymorphism(s)', 'breast cancer' or 'breast carcinoma', without restriction on language. The retrieved literatures were then read in their entirety to assess their appropriateness for the inclusion in this meta-analysis by the two authors (Hu and Zhou) independently. The reference lists of reviews and retrieved articles were searched simultaneously to find additional eligible studies. If studies had partly overlapped subjects, only the one with a larger sample size was selected. Any disagreement was resolved by discussion between the two authors.

Data extraction

The following variables were extracted from each study if available: first author's surname, publication year, ethnicity, matching criteria, numbers of cases and controls, and genotype distributions in both cases and controls.

Statistical analysis

The strength of association between *MTRR A66G* polymorphism and breast cancer risk was assessed by OR with the corresponding 95% CI. The pooled OR was calculated by a fixed-effects model (the Mantel–Haenszel method) when between-study heterogeneity was absent [12]. Otherwise, a random-effects model (the DerSimonian and Laird method) [13] was selected. Statistical between-study heterogeneity was checked by the Q test [14] and it was considered statistically significant with $P < 0.10$. The OR and its 95% CI in each comparison was assessed in dominant (*GG/AG* vs. *AA*), additive (*GG* vs. *AA*) and recessive (*GG* vs. *AG/AA*) genetic models. In addition, subgroup analyses for ethnicity (Caucasian, Asian and mixed population) and folate intake status (high and low folate intake) were conducted, and influence analysis was performed by omitting each study to find potential outliers [15]. Sensitivity analysis was also conducted by excluding the Hardy–Weinberg equilibrium (HWE)-violating studies. The potential publication bias was examined visually in a funnel plot of log [OR] against its standard error (SE), and the degree of asymmetry was tested by Egger's test ($P < 0.05$ was considered a significant publication bias) [16]. In the control populations, HWE was tested, but a deviation from HWE was allowed in a mixed control population. This meta-analysis was performed using the software STATA version 10.0.

Results

Study characteristics

A total of seven publications met the inclusion criteria. Of these studies, one [17] was excluded as cases involved were restricted to BRCA mutation carriers. As a result, a total of six publications [7, 18–22] containing 6,084 cases and 6,756 controls were included in this meta-analysis. Table 1 lists the main characteristics of these studies. Among these publications, there were two studies of Caucasian descent [19, 21], three of Asian descent [7, 18, 22] and one of mixed population (about 93% were Caucasians) [20]. In addition, two of these studies [18, 22] presented *MTRR A66G* polymorphism genotype distributions according to the folate intake status (high (Tertile 2 + 3) and low (Tertile 1) folate intake) (Table 2). All of the cases were histologically confirmed as breast cancer. Controls were mainly healthy or hospital-based populations and matched with age and sex. Genotype distributions in the controls of all studies were in agreement with HWE, except one study [21].

Meta-analysis results

As shown in Table 3, no between-study heterogeneity was found in overall comparisons in all genetic models. When all the eligible studies were pooled into the meta-analysis, *MTRR A66G* polymorphism did not reveal any relationship with breast cancer susceptibility (additive model: OR 1.00,

Table 1 Main characteristics of studies included in this meta-analysis

References	Country	Ethnicity	Source of controls	Matching criteria	Sample size (case/control)	Genotype (case/control)			HWE
						AA	AG	GG	
Shrubsole et al. [18]	China	Asian	Population based	Age, sex	1193/1310	621/687	393/422	70/76	Yes
Lissowska et al. [19]	Poland	Caucasian	Population based	Age, sex, study site	1995/2296	358/430	970/1110	663/753	Yes
Xu et al. [20]	USA	Mixed	Population based	Age, sex	1102/1141	279/276	549/600	230/223	Yes
Kotsopoulos et al. [21]	Canada	Caucasian	Hospital based	Sex	952/817	222/179	448/360	270/243	No
Suzuki et al. [22]	Japan	Asian	Hospital based	Age, sex, menopausal status	456/912	205/456	205/366	42/90	Yes
Sangrajrang et al. [7]	Thailand	Asian	Hospital based	Sex	600/642	295/229	218/210	46/46	Yes

HWE Hardy–Weinberg equilibrium

Table 2 Genotype frequencies for cases and controls in different folate intake status

References	High (case/control)			Low (case/control)		
	AA	AG	GG	AA	AG	GG
Shrubsole et al. [18]	371/419	243/265	43/41	196/199	127/130	22/27
Suzuki et al. [22]	124/301	134/244	18/61	80/155	68/121	21/28

High high folate intake (Tertile 2 + 3), *Low* low folate intake (Tertile 1)

95% CI 0.89–1.11, $P = 0.943$; dominant model: OR 1.00, 95% CI 0.91–1.10, $P = 0.989$; recessive model: OR 1.00, 95% CI 0.91–1.09, $P = 0.926$). Next, the effect of *MTRR A66G* was evaluated according to specific ethnicity and different folate intake status (Table 3; Fig. 1). Similarly, no significant association was found.

Sensitivity analysis

Influence analysis was performed to assess the influence of each individual study on the pooled OR by sequential removal of individual studies. The results suggested that no individual study significantly affected the pooled ORs (Fig. 2). Sensitivity analysis by excluding HWE-violating study did not perturb the overall results.

Publication bias

Funnel plot and Egger's test were performed to assess the publication bias. The shape of the funnel plot did not indicate any evidence of obvious asymmetry (Fig. 3) and the Egger's test suggested the absence of publication bias ($P = 0.476$).

Discussion

Similar to MTR, MTRR is also a critical enzyme for the biosynthesis of methionine, the precursor for methylation reactions, and for the regeneration of tetrahydrofolate for nucleotide biosynthesis. Changes in this enzyme may significantly influence DNA methylation, synthesis and repair. The *A66G* SNP at codon 22 is one of the most common polymorphisms in *MTRR* gene and the variant MTRR enzyme proved to have a lower affinity for MTR [11] and be inconsistently associated with elevated blood or plasma homocysteine levels [23, 24]. The elevation of

Table 3 Results of meta-analysis for *MTRR A66G* polymorphism and breast cancer risk

Analysis	Cases/controls	Additive model (GG vs. AA)		Dominant model (AG/GG vs. AA)		Recessive model (GG vs. AA/AG)	
		OR (95% CI)	P/P_h	OR (95% CI)	P/P_h	OR (95% CI)	P/P_h
Overall	6084/6756	1.00 (0.89–1.11)	0.943/0.794	1.00 (0.91–1.10)	0.989/0.227	1.00 (0.91–1.09)	0.926/0.772
Ethnicity							
Caucasian	2391/3075	1.01 (0.87–1.16)	0.947/0.302	1.02 (0.90–1.16)	0.739/0.508	0.98 (0.88–1.10)	0.776/0.284
Asian	2095/2582	0.96 (0.76–1.20)	0.694/0.565	1.00 (0.82–1.24)	0.975/0.053	0.94 (0.76–1.17)	0.598/0.840
Mixed	1058/1099	1.02 (0.80–1.31)	0.874/-	0.94 (0.77–1.14)	0.504/-	1.09 (0.89–1.34)	0.409/–
Folate intake							
High	933/1331	0.97 (0.68–1.37)	0.853/0.173	1.11 (0.93–1.32)	0.238/0.454	0.87 (0.47–1.61)	0.662/0.079
Low	514/660	1.08 (0.70–1.66)	0.738/0.202	1.03 (0.82–1.31)	0.778/0.455	1.07 (0.70–1.62)	0.768/0.222

P_h, P values for heterogeneity

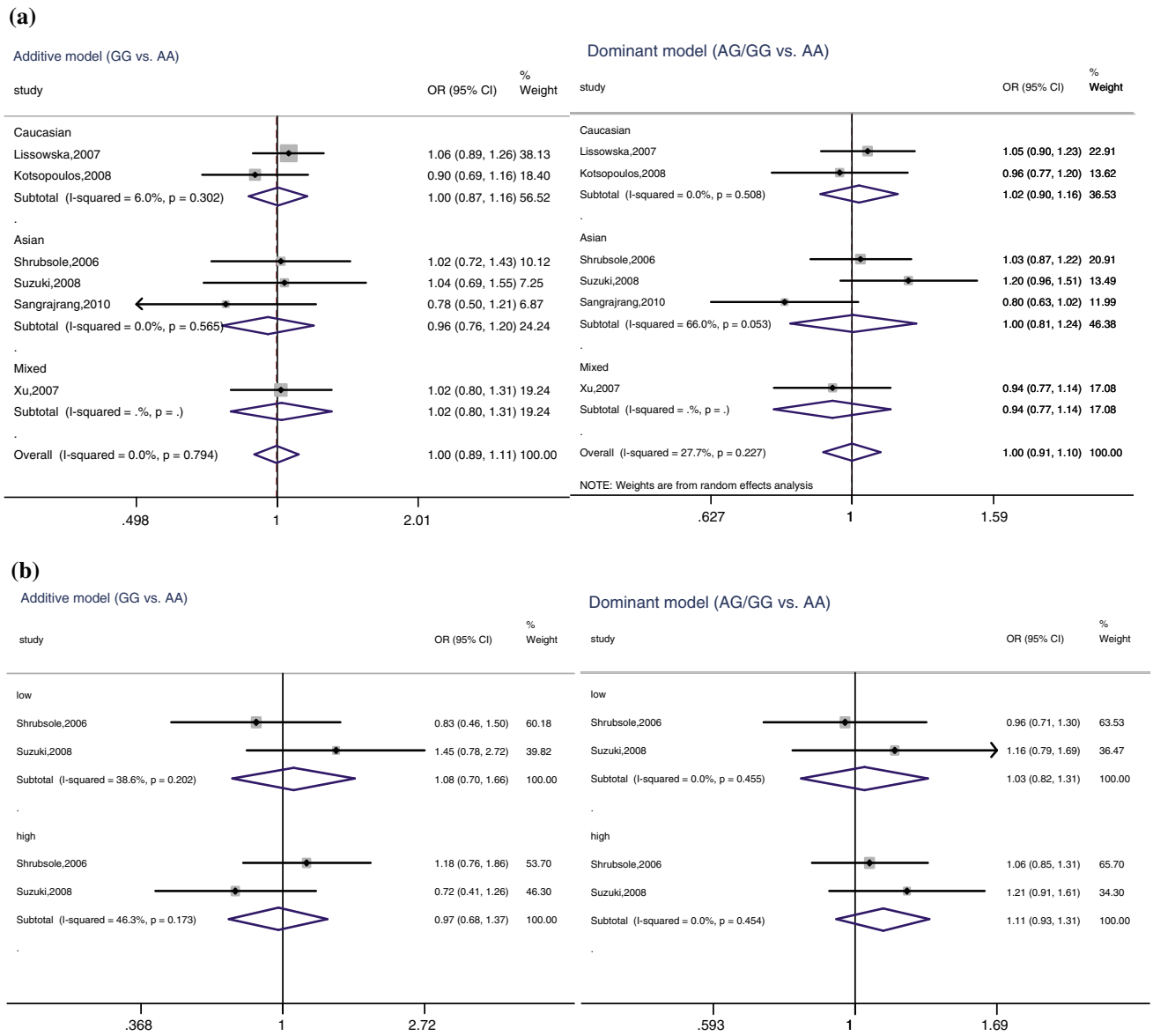

Fig. 1 Meta-analysis of *MTRR A66G* polymorphism in breast cancer. **a** overall meta-analysis and subgroup analysis on ethnicities, **b** subgroup analysis on folate intake status (low and high folate intake)

homocysteine is a marker of DNA hypomethylation, which is an early and consistent event in cancer development [25]. Evidences supported that the variant *G allele-bearing* genotype of *MTRR* was significantly associated with an increased risk of hepatocellular carcinoma [26] and esophageal squamous cell carcinoma [27], and that the *GG* homozygotic genotype was a risk factor for colorectal cancer [28]. Regarding the association between *MTRR A66G* polymorphism and breast cancer susceptibility, six case–control studies were found by searching PubMed and Embase, with inconclusive results owing to the relatively small sample size and different patient population. However, meta-analysis can well resolve these problems. In this study, the results strongly suggested that the *MTRR A66G* polymorphism was not associated with breast cancer risk in all genetic models. Subgroup analysis based on ethnicities and influence analysis did not perturb the results.

Besides the variants of one-carbon metabolism-related genes, folate intake status is another important factor that has an impact on DNA methylation and synthesis. An interactive effect on cancer risk may exist between gene polymorphisms and diet. High folate intake is believed to be associated with decreased breast cancer risk, which might compensate the effect of gene polymorphisms on cancer susceptibility. On the contrary, low folate intake and gene variants may have synergistic effect on cancer risk, because deficiencies of folate may lead to low level of cellular *S*-adenosylmethionine, or DNA hypomethylation,

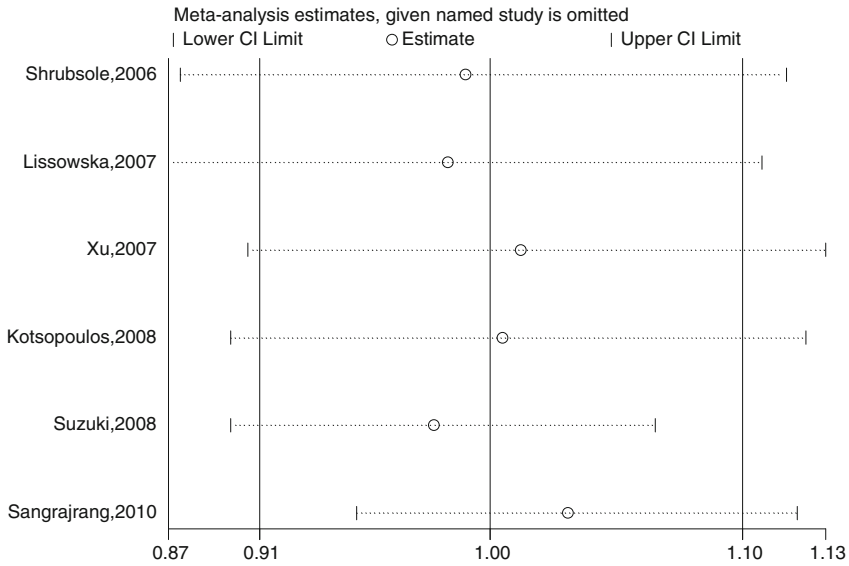

Fig. 2 Influence analysis for AG/GG versus AA in the overall meta-analysis. This figure shows the influence of individual studies on the summary OR. The *middle vertical axis* indicates the overall OR and the *two vertical axes* indicate its 95% CI. *Open circles* indicate the pooled OR when the left study is omitted in this meta-analysis. The two ends of the *dotted lines* represent the 95% CI

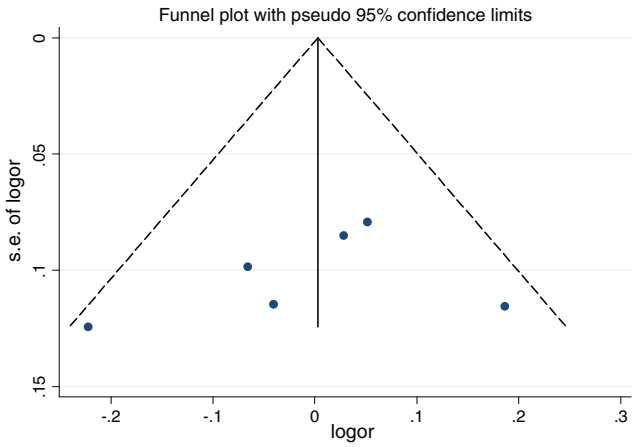

Fig. 3 Funnel plot of *MTRR A66G* polymorphism and breast cancer risk for publication bias

which may increase the susceptibility of genes to mutation or alter gene expression [29]. Suzuki et al. [22] had reported that an interaction existed between *MTRR A66G* polymorphism and low folate intake, and it was significantly associated with high risk of breast cancer among postmenopausal women. However, this meta-analysis did not reveal any significant association either in low folate intake subgroup or high folate intake subgroup, and no interaction effect between *MTRR A66G* polymorphism and folate intake on breast cancer risk was found.

However, some limitations still exist in this meta-analysis. First, this study is a study-level and not an individual patient-level meta-analysis. It is known that study-level analysis can lead to biased assessments and use of aggregated summary values has some limitations in explaining the heterogeneity [30, 31]. Second, OR value was obtained without correction. More accurate OR should be corrected by age, menopause status and other exposure factors that are potentially associated with breast cancer risk or gained through subgroup analysis. However, this meta-analysis failed to explore this association by subgroup analysis based on menopause status, for most studies did not provide related information. Third, of these six studies, most subjects were Caucasians and Asians, and there was no African in any of the studies. Therefore, the conclusion about this association in African populations should be further investigated.

In conclusion, this study strongly suggested that the *MTRR A66G* polymorphism was not associated with breast cancer among Caucasians and Asians

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