RESEARCH ARTICLE

Methionine Synthase Reductase A66G Polymorphism is not Associated with Breast Cancer Susceptibility - a Meta-analysis

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Abstract

Background: Several studies have investigated the association between methionine synthase reductase (MTRR) A66G polymorphism and breast cancer risk, but controversial results were yielded. Therefore, we performed a meta-analysis to provide a more robust estimate of the effect of this polymorphism on susceptibility to breast cancer. Materials and Methods:Case-control studies investigating the relationship between MTRR A66G polymorphism and breast cancer risk were included by searching PubMed, EMBASE, China National Knowledge Infrastructure and Wanfang Database. Either fixed-effects or random-effects models were applied to calculate odds ratios(ORs) and 95% confidence intervals (CIs) by RevMan5.2 software. Results: A total of 9 studies bearing 7,097 cases and 7,710 controls were included in the meta-analysis. The results were that the combined ORs and 95%CIs of MTRR 66AG, GG, (AG+GG) genotypes were 0.98(0.91-1.05), 1.06(0.97-1.16) and 1.02(0.94-1.10), respectively with *p*=0.52, 0.19 and 0.65. We also performed subgroup analysis by specific ethnicity. The results of the combined analysis of MTRR 66AG, GG, (AG+GG) genotypes and breast cancer in Asian descent were Z=0.50, 0.53 and 0.21, with *p* all>0.05; for breast cancer in Caucasian descent, the results were Z=1.14, 1.65 and 0.43, with *p* all>0.05. Conclusions: Our findings suggested that MTRR A66G polymorphism was not associated with breast cancer susceptibility.

Keywords: Breast cancer - methionine synthase reductase - polymorphism - susceptibility - meta-analysis

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Introduction

Breast cancer is the most common cancer among women around the world, with an estimated 232, 340 new cases in 2013 in the U.S. alone, which represents close to 30% of all estimated new cancer cases in women (Siegel et al., 2013). Almost 70% of the patients develop metastasis and die of the disease and about 10% of the patients have metastases when diagnosed with breast cancer. Despite positive reductions in mortality, due largely to improvements in the early detection, treatment, surgery and radiation support, preventing breast cancer prior to its development remains the most effective way to reduce mortality (Marmot et al., 2013). Therefore, it would be meaningful to identify biomarkers associated with the risk of breast cancer. However, the biologic mechanisms of breast cancer have not been fully clarified. It is generally considered that the interaction between genetic susceptibility and environmental exposure plays an important role in the etiology of breast cancer (Xu and Chen, 2009). It has been suggested that an inverse relationship exists between dietary intake of folate and breast cancer risk and that host polymorphisms may modify the individual's ability to maintain an intact genome in face of genotoxic stress (Baylin et al., 2001; Widschwendter and Jones, 2002; Maruti et al., 2009). Thus, functional polymorphisms in genes encoding folate-metabolizing enzymes may have determined susceptibility to breast cancer.

One-carbon metabolism is a network of interrelated biological reactions in which a one-carbon unit is transferred among a series of folate-derived compounds. It provides essential cofactors in the production of primary methyl donor for DNA methylation. It also supplies the methyl group for DNA synthesis. Therefore, it has an impact on both genetic and epigenetic processes (Stern et al., 2000). Genes involved in the folate metabolic pathway include methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), thymidylate synthase (TS), serine hydroxymethyltransferase (SHMT), cystathionine -β-synthase (CBS) etc. and most of them are polymorphic (Xu and Chen, 2009; Naushad et al., 2011). Perturbations of the one-carbon metabolism may play an important role in tumorigenesis and development due to its effects on gene expression through DNA methylation and on genome integrity through DNA synthesis and repair, particularly, tumor suppressor genes inactivation, proto-oncogenes activation and chromosome segregation abnormality. All of the above processes may be involved in the development of cancers, including breast cancer (Choi and Mason, 2000; Duthie, 2011; Naushad et al., 2011). MTR is responsible for synthesis of methionine through irreversible transfer of a methyl group from

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5-methyltetrahydrofolate. MTR is maintained its active form by MTRR, an enzyme that regenerates functional MTR by reductive methylation. The A66G polymorphism at codon 22 is the most common functional polymorphism in MTRR and the variant enzyme has a lower affinity for MTR (Wilson et al., 1999). Changes in MTRR activity may significantly influence DNA methylation and synthesis. Several case -control studies have investigated the association between this polymorphism and breast cancer risk, however, these studies yielded inconclusive or controversial results. To clarify this issue, we did a meta-analysis to provide a more robust estimate of the effect of MTRR A66G polymorphism on the susceptibility to breast cancer.

Materials and Methods

Studies identification

A literature search was conducted to identify studies investigating the relationship between MTRR A66G polymorphism and breast cancer risk. The PubMed, EMBASE, China National Knowledge Infrastructure platform (CNKI) and Wanfang database were searched (prior to Sep. 20, 2013) with the following subject terms and keywords: "MTRR" or "methionine synthase reductase" and "polymorphism" or "variant" in combination with "breast cancer" or "breast carcinoma", without any restriction on language. All the references cited in the eligible studies were reviewed to identify additional publications. Two authors reviewed the retrieved literatures independently and any disagreement was resolved by discussion between them.

Inclusion criteria

The inclusion criteria were defined as follows: (1) raw materials were published literatures in public; (2) case-control study; (3) the breast cancer group had confirmed pathologically diagnosis; (4) genotype frequencies for both cases and controls were available; (5) genotypes distribution in the control group was in agreement to Hardy-Weinberg equilibrium (HWE).

Data extraction

The following data were collected from each eligibility study: first author's name, year of publication, country, source of control group and genotypes distribution of the MTRR A66G polymorphism in cases and controls. Two investigators extracted the information from each study independently and disagreements were resolved by discussion.

Statistical analysis

We used odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of association between MTRR A66G polymorphism and breast cancer risk. HWE in the control group was checked using the χ^2-test. The χ^2-based Q-statistic test was used to assess heterogeneity. Heterogeneity was deem to be present when $p<0.05$, in which case the random-effects model (DerSimonian-Laird) was selected, otherwise, the fixed-effects model (Mantel-Haenszel) was selected to calculate the combined OR. The significance of the combined OR was determined by the Z-test. Publication bias was evaluated visually through funnel plot and sensitivity analysis was performed by sequential removal of individual studies. All the p values were for a two-sided test and $p<0.05$ was considered as statistically significant. The data analyses were performed using the software Statistical Analysis System v9.0 (SAS Institute, Cary, NC) and Review Manager v5.2 (The Cochrane Collaboration, Oxford, UK).

Results

Study characteristics

A total of 21 potentially relevant publications were identified based on the literature search criteria. 8 articles were unrelated and excluded firstly; during further screening, one study was excluded as case group were restricted to BRCA mutation carriers (Beetstra et al., 2008); The genotype frequencies of MTRR A66G polymorphism in cases and controls provided were not sufficient in three studies. Overall, 9 case-control studies bearing 7097 cases and 7710 controls about the association between MTRR A66G polymorphism and breast cancer risk were included in this meta-analysis (Shrubsole et al., 2006; Xu et al., 2007; Lissowska et al., 2007; Kotsopoulos et al., 2008; Suzuki et al., 2008; Burcos et al., 2010; Sangrajrang et al., 2010; Lajin et al., 2012; Weiner et al., 2012). The countries in which these studies had been carried out include China, India, Japan, Canada, Syria, Poland, Thailand, Russia, Romania and USA. Among these publications, there were three studies of Asian descent (Shrubsole et al., 2006; Suzuki et al., 2008; Sangrajrang et al., 2010) and six of Caucasian descent including one mixed population (about 93% were Caucasians) (Lissowska et al., 2007; Xu et al., 2007; Kotsopoulos et al., 2008; Burcos et al., 2010; Lajin et al., 2012; Weiner et al., 2012). All the cases had histologically confirmed diagnosis of breast cancer and the controls were mainly matched for age and sex. Genotypes distribution among the controls of all included studies were in agreement with HWE, except one study (Kotsopoulos et al., 2008). The detailed characteristics of included studies are available in Table 1.

Meta-analysis results

Evaluation of the association between MTRR 66AG genotype and breast cancer risk: The heterogeneity test showed $\chi^2=11.69$, $p=0.11$, indicating that there was no significant heterogeneity among these studies. The fixed-effects model was used to calculate the combined OR and 95%CI, which were 0.98 (0.91-1.05), Z=0.64, $p=0.52$, which suggested that there was no significant association between 66AG genotype and breast cancer risk (Figure 1).

Evaluation of the association between MTRR 66GG genotype and breast cancer risk: The heterogeneity test showed $\chi^2=7.04$, $p=0.43$, indicating that there was no significant heterogeneity between these studies. The fixed-effects model was used to calculate the combined OR and 95%CI, which were 1.06 (0.97-1.16), Z=1.30, $p=0.19$, which suggested that there was no significant association

Table 1. Characteristics of the Included Studies of MTRR A66G Polymorphism and Breast Cancer Risk

First author	Year	Country	Ethnicity	Control source	Case			Control			HWE
					AA	AG	GG	AA	AG	GG	
Burcos	2010	Romania	Caucasian	Hospital	0	37	23	3	32	25	YES
Kotsopoulos	2008	Canada	Caucasian	Hospital	222	448	270	179	360	243	NO
Lajin	2012	Syria	Caucasian	Population	40	59	20	43	58	25	YES
Lissowska	2007	Poland	Caucasian	Population	358	970	663	430	1110	753	YES
Sangrajrang	2010	Thailand	Asian	Hospital	295	218	46	229	210	46	YES
Shrubsole	2006	China	Asian	Population	621	393	70	687	422	76	YES
Suzuki	2008	Japan	Asian	Hospital	205	205	42	456	366	90	YES
Weiner	2012	Russia	Caucasian	Unknown	162	387	285	158	394	216	YES
Xu	2007	USA	Mixed	Population	279	549	230	276	600	223	YES

HWE, Hardy-Weinberg equilibrium

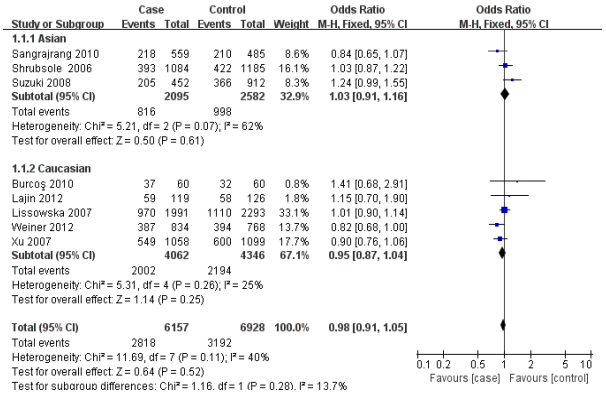

Figure 1. Meta-Analysis of *MTRR* 66AG Genotype and Breast Cancer Risk

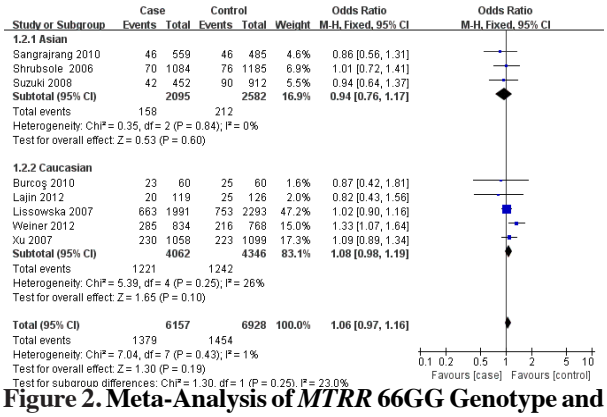

Figure 2. Meta-Analysis of *MTRR* 66GG Genotype and Breast Cancer Risk

between 66GG genotype and breast cancer risk (Figure 2).

Evaluation of the association between MTRR 66 (AG+GG) genotypes and breast cancer risk: The heterogeneity test showed χ^2=8.66, p=0.28, indicating that there was no significant heterogeneity among these studies. The fixed-effects model was used to calculate the combined OR and 95%CI, which were 1.02 (0.94-1.10), Z=0.46, p=0.65, which suggested that there was no significant association between 66 (AG+GG) genotypes and breast cancer risk (Figure 3).

A subgroup analysis was also performed by specific ethnicity. Combined analysis of MTRR 66AG, GG, (AG+GG) genotypes and breast cancer in Asian descent: the results showed Z=0.50, 0.53, 0.21, with p all>0.05 (Figure 1-3), indicating that there were no significant association between MTRR A66G polymorphism and

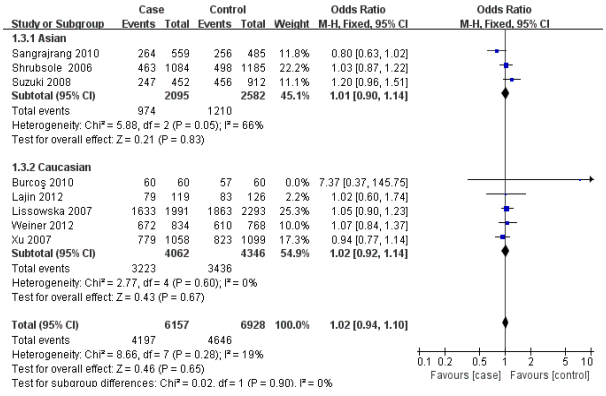

Figure 3. Meta-Analysis of *MTRR* 66(AG+GG) Genotypes and Breast Cancer Risk

the risk of breast cancer in Asian population; Regarding Caucasian descent, the results showed Z=1.14, 1.65, 0.43, with p all>0.05 (Figure 1-3), indicating that there were also no significant association between A66G polymorphism and the development of breast cancer in Caucasian population.

Publication Bias and Sensitivity Analysis

Funnel plots were applied to assess the publication bias and the results showed all points in the funnel plots were symmetrically distributed, suggesting that there was no significant bias (funnel plots not shown). Sensitivity analysis was performed by sequential removal of individual studies and results suggested that no individual study significantly affected the combined ORs. Finally, we carried out an additional sensitivity analysis by including the study by Kotsopoulos et al. (2008), in which the distribution of genotypes was not in agreement with HWE. However, no significant changes on the combined ORs were observed (data not shown).

Discussion

Breast cancer is a manifestation of abnormal genetic and epigenetic changes, and the interaction between environmental exposure and genetic susceptibility has been a key cause of breast cancer (Russo et al., 1998; Xu and Chen, 2009). Functional polymorphisms in the genes encoding folate-metabolism enzymes may play a role in breast cancer development. Human MTRR is a housekeeping gene and locates at 5p15.2-p15.3 (Leclerc et al., 1998). MTRR plays a key role in folate-dependent

homocysteine metabolism and is responsible for MTR activity regulation by reductive methylation. The most common polymorphism in MTRR is A66G substitution, leading to a change of isoleucine to methionine at amino acid 22. The variant enzyme has a 3- to 4-fold lower affinity for MTR (Wilson et al., 1999). Few studies have investigated the association between MTRR A66G polymorphism and cancer susceptibility and found this polymorphism has been associated with a reduced risk for acute lymphoblastic leukemia and an increased risk for hepatocellular carcinoma (Gast et al., 2007; Kwak et al., 2008). Meanwhile, there are controversial findings about the role of MTRR A66G polymorphism on breast cancer susceptibility.

To investigate the effect of MTRR A66G polymorphism on the breast cancer risk through a more robust analysis, we did a meta-analysis of 9 case-control studies that examined the association between MTRR A66G polymorphism and risk of breast cancer. In the present meta-analysis, the results we acquired were that the combined ORs and 95%CIs of MTRR 66AG, GG, (AG+GG) genotypes were 0.98 (0.91-1.05), 1.06 (0.97-1.16) and 1.02 (0.94-1.10), respectively with p=0.52, 0.19 and 0.65. We found no significant impact of MTRR A66G polymorphism on breast cancer susceptibility. We also performed subgroup analysis by specific ethnicity. The results of the combined analysis of MTRR 66AG, GG, (AG+GG) genotypes and breast cancer in Asian descent were Z=0.50, 0.53 and 0.21, with p all>0.05; for breast cancer in Caucasian descent, the results were Z=1.14, 1.65 and 0.43, with p all>0.05, indicating that there were no significant association between MTRR A66G polymorphism and the risk of breast cancer in both Asian and Caucasian population.

However, some limitations need to be addressed in interpreting the results of present meta-analysis. Selection bias could have occurred because studies without sufficient data were excluded. Our analysis largely used unadjusted estimates, because not all included studies had adjusted by the same potential confounders, such as age, menopause status and exposures, which may influence the combined results. As we know, folate intake status has an important impact on DNA methylation and synthesis, which may influence cancer risk by potential gene-nutrition interactions (Suzuki et al., 2008; Christensen et al., 2010).

In summary, our meta-analysis found no association between MTRR A66G polymorphism and breast cancer susceptibility. Since limited sample cases were included in this study, larger sample sizes and case-control studies are required in future to verify biomarkers associated with the risk of breast cancer.

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