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# Association between dietary intake of folate and *MTHFR* and *MTR* genotype with risk of breast cancer

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**ABSTRACT.** We investigated the association between dietary intake of folate, vitamin B6, and the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) genotype with breast cancer. A matched case-control study was conducted, and 413 patients with newly diagnosed and histologically confirmed breast cancer and 436 controls were recruited. Folate intake, vitamin B6, and vitamin B12 levels were calculated, and the *MTHFR* C677T and *A1298C* and *MTR A2756G* polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism. Breast cancer cases were generally older, older at first live birth, and younger at menarche, had a higher body mass index, were smokers, had higher energy intake, and more first-degree relatives with breast cancer as well as more live births compared to controls. With respect to energy intake, we found that higher energy intake were more likely to increase the risk of breast cancer. The *MTHFR* 667TT genotype was associated with a moderately increased risk of breast cancer when compared with the CC genotype, and a significant odds

 

ratio (OR; 95% confidence interval, CI) was found (OR = 1.70, 95%CI = 1.06-2.73). Individuals carrying T allele were associated with higher risk of breast cancer when compared with C allele (OR = 1.34, 95%CI = 1.06-1.70). We did not find a significant effect of the *MTHFR A1298C* and *MTR A2756G* on the risk of breast cancer. We did not find any association between folate intake and MTHFR C677T polymorphisms. In conclusion, we found that the *MTHFR* C667T polymorphism is associated with the risk of breast cancer, indicating that this genotype plays a role in breast cancer development.

**Key words:** Folate; Vitamin; MTHFR; Polymorphism; Breast cancer

## INTRODUCTION

Breast cancer is one of the most commonly cancer in women worldwide (IARC, 2012). By far, it is the most frequent cancer among women with an estimated 1.67 million new cases diagnosed in 2012 (25% of all cancers). It is well known that family or personal history of cancer, nulliparous and history of hormone replacement therapy are the risk factors for breast cancer. Although many women are exposed to these risk factors, only few develop breast cancer, which indicates that some hereditary factors play a role in the susceptibility to breast carcinogenesis (Szakacs et al., 2006).

B vitamins, including folate, vitamin B6, and vitamin B12, play an important role in the metabolism of coenzymes in one-carbon units, and they are also important in DNA synthesis, repair, and methylation (Ulrich, 2005). Previous studies have indicated a potential association between folate intake and other vitamins and risk of several cancers, such as oral and pharyngeal cancer, breast cancer, colorectal cancer, and esophageal cancer (Bravi et al., 2013; Liu et al., 2013; Morita et al., 2013; Sharp et al., 2013). For breast cancer, a recent meta-analysis showed high folate intake did not protect against breast cancer development (Castillo et al., 2012). Another meta-analysis showed that folate intake has no effect on the incidence of breast cancer (Qin et al., 2013). The results are inconsistent.

In addition to folate and vitamins intake, functional polymorphisms in folate-metabolizing genes influence the susceptibility to cancer. The enzyme 5,10-methylenetetrahydrofolate reductase (5,10-methylene THF) irreversibly catalyzes the conversion of 5,10-methylenetetrhydrofolate to 5-methyl THF, the methyl donor in DNA methylation. There are two common genetic polymorphisms in the *MTHFR* gene, including *MTHFR C677T* and *A1298C* polymorphisms (López-Cortés et al., 2013). TT genotype of MTHFR C667T and the CC genotype of A1298C exhibit approximately 35 and 60% of the normal MTHFR activity, respectively. Methionine synthase (*MTR*) catalyzes the remethylation of homocysteine to methionine, and it has one common genetic polymorphism of *MTR A2756G* (López-Cortés et al., 2013). Previous several case-control studies reported the association between *MTHFR C677T* and *A1298C* and *MTR A2756G* polymorphisms and risk of breast cancer, but few studies reported their association in Chinese population (Semenza et al., 2003; Rossi et al., 2006; Suzuki et al., 2008; Ma et al., 2009; de Cássia Carvalho Barbosa et al., 2012a; Wu et al., 2012; Yu and Chen, 2012).

In the current study, therefore, we performed a case-control study and investigated

the association between dietary intakes of folate and *MTHFR* genotype with breast cancer in a Chinese population, and we additionally analyzed their association between gene polymorphisms and dietary intake.

## MATERIAL AND METHODS

### Subjects

This study recruited 342 breast cancer patients who were newly diagnosed and histologically confirmed. They were recruited at Xinxiang Medical College between January 2009 and December 2010. Four hundred and sixteen health controls without any cancer were recruited from the health examination center of our hospital during the same period, and 381 control subjects agreed to participate in this study (participation rate of 91.5%). Of the eligible cases, 310 breast cancer patients agreed to participate in the study, with a participation rate of 91.6%. Informed written consent for participation was gained from all patients and control subjects. Ethical approval was obtained from the ethics committee of Xinxiang Medical College.

A structured questionnaire was designed to collect the clinical and demographic information, including 62 food items, age, history of cancer, and menopausal situation, etc.

### Genotype of polymorphisms

Peripheral blood samples were collected from all breast cancer cases before treatment, and blood samples were obtained from control subjects after enrolling in study. Samples were stored at -40°C until further analysis. The *MTHFR C677T* and *A1298C* and *MTR A2756G* polymorphisms were identified by performing polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. The primer sequences of *MTHFR C677T* and *A1298C* and *MTR A2756G* were designed using the Sequenom® Assay Design, Version 3.1 software (Sequenom; San Diego, CA, USA). Each PCR reaction mix comprised 50 ng genomic DNA, 200 μM dNTP, 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA), and 200 μM primers, in a total volume of 20 μL. The cycling programme of PCR conditions included preliminary denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 30 s, annealing at 62°C for 62 s, extension at 72°C for 30 s, and a final elongation for 10 min at 72°C. To confirm the genotyping results, we randomly selected 10% of cases and controls, and the samples were genotyped again. Quality control analysis confirmed 100% concordance.

### Statistical analysis

Continuous variables are reported as the means ± SD and categorical variables are expressed as frequency and percentage of subjects. The continuous variables and categorical variables are analyzed using the independent-samples Student *t*-test and $\chi^2$ test, respectively. An unconditional logistical regression model was performed to determine the effects of *MTHFR* and *MTR* polymorphisms and dietary intake on breast cancer risk with results expressed as Odds Ratios (ORs) and their 95% confidence intervals (CIs) after adjusting for potential confounding variables. The statistical significance was examined using the Wald chi-square test.

Interaction between folate intake, vitamin B6, and vitamin B12 and *MTHFR* and MTR polymorphisms on breast cancer risk was conducted. The homozygous genotypes for the *MTHFR* and *MTR* polymorphisms were used as references. Statistical significance was considered at $P < 0.05$, and the analysis was conducted using the Stata version 8 software (StataCorp; College Station, TX, USA).

## RESULTS

Table 1 shows the background characteristics of breast cancer cases and control subjects. Compared to controls, the breast cancer case group was generally older, older at menarche, order at first live birth, and had more first-degree relatives with breast cancer. For energy intake, we found that higher energy intake was likely to increase the risk of breast cancer. However, we did not find that intake of vitamin B6 and vitamin B12 was associated with risk of breast cancer.

**Table 1.** Characteristics of breast cancer cases and control subjects.

| | Cases (N = 310) | % | Controls (N = 381) | % | *t* or $\chi^2$ | P value |
|---|---|---|---|---|---|---|
| Age (means ± SD), years | 50.1 ± 8.7 | | 44.3 ± 7.9 | | 9.17 | <0.001 |
| Age at menarche, years | 12.7 ± 1.9 | | 13.0 ± 1.8 | | 2.13 | 0.017 |
| Age at first live birth, years | 27.2 ± 6.7 | | 23.8 ± 7.4 | | 6.27 | <0.001 |
| Menopausal status | | | | | | |
| Premenopausal | 125 | 40.3 | 139 | 36.5 | 1.07 | 0.302 |
| Postmenopausal | 185 | 59.7 | 242 | 63.5 | | |
| Smoking status | | | | | | |
| Never | 259 | 83.5 | 338 | 88.7 | 3.88 | 0.05 |
| Current and ever | 51 | 16.5 | 43 | 11.3 | | |
| Drinking status | | | | | | |
| Never | 267 | 86.1 | 346 | 90.8 | 3.75 | 0.053 |
| Current and ever | 43 | 13.9 | 35 | 9.2 | | |
| Breast cancer in first-degree relative | | | | | | |
| No | 287 | 92.6 | 379 | 99.5 | 15.19 | <0.001 |
| Yes | 23 | 7.4 | 2 | 0.5 | | |
| Live births | | | | | | |
| <1 | 30 | 9.7 | 22 | 5.8 | 4.96 | 0.08 |
| 1- 3 | 192 | 61.9 | 260 | 68.2 | | |
| >3 | 89 | 28.7 | 99 | 26.0 | | |
| Energy, kcal/day | 1713.6 ± 538.5 | | 1637.3 ± 507.5 | | 1.91 | 0.03 |
| Folate intake, μg/day | 504.5 ± 110.3 | | 515.2 ± 108.3 | | 1.28 | 0.1 |
| Vitamin $B_6$, mg/day | 0.75 ± 0.22 | | 0.77 ± 0.23 | | 1.16 | 0.12 |
| Vitamin $B_{12}$, μg/day | 7.2 ± 5.1 | | 6.9 ± 4.6 | | 0.81 | 0.21 |

We found that the *MTHFR* 667TT genotype was associated with a moderately significant increased risk of breast cancer when compared with the CC genotype (OR = 1.70, 95%CI = 1.06-2.73) (Table 2). Similarly, individuals carrying T allele was associated with higher risk of breast cancer when compared with C allele (OR = 1.34, 95%CI = 1.06-1.70). However, we did not find a significant effect of the *MTHFR A1298C* and *MTR A2756G* on the risk of breast cancer.

The associations between the *MTHFR* C667T polymorphism and folate intake with breast cancer risk are shown in Table 3. We did not find any association between folate intake and MTHFR C677T polymorphisms, and there was no significant interaction between them.

**Table 2.** Association between *MTHFR* C677T and A1298C and MTR A2756G genotypes and breast cancer risk.

| Gene | Cases, N | % | Controls, N | % | P value | OR (95%CI)[1] |
|---|---|---|---|---|---|---|
| *MTHFR* C677T | | | | | | |
| CC | 159 | 51.3 | 220 | 57.7 | - | 1.0 (Ref.) |
| CT | 97 | 31.3 | 117 | 30.7 | 0.43 | 1.15 (0.81-1.63) |
| TT | 54 | 17.4 | 44 | 11.5 | 0.02 | 1.70 (1.06-2.73) |
| C allele | 415 | 66.9 | 557 | 73.1 | - | 1.0 (Ref.) |
| T allele | 205 | 33.1 | 205 | 26.9 | 0.01 | 1.34 (1.06-1.70) |
| *MTHFR* A1298C | | | | | | |
| AA | 138 | 44.5 | 173 | 45.4 | - | 1.0 (Ref.) |
| AC | 132 | 42.6 | 155 | 40.7 | 0.69 | 1.07 (0.76-1.49) |
| CC | 40 | 12.9 | 53 | 13.9 | 0.82 | 0.95 (0.58-1.55) |
| C allele | 408 | 65.8 | 501 | 65.7 | - | 1.0 (Ref.) |
| A allele | 212 | 34.2 | 261 | 34.3 | 0.98 | 1.0 (0.79-1.25) |
| MTR A2756G | | | | | | |
| AA | 97 | 31.3 | 127 | 33.3 | - | 1.0 (Ref.) |
| AG | 131 | 42.3 | 167 | 43.8 | 0.88 | 1.03 (0.71-1.48) |
| GG | 82 | 26.5 | 87 | 22.8 | 0.30 | 1.23 (0.81-1.88) |
| A allele | 325 | 52.4 | 421 | 55.2 | - | 1.0 (Ref.) |
| G allele | 295 | 47.6 | 341 | 44.8 | 0.29 | 1.12 (0.90-1.39) |

[1]Adjusted for age, age at menarche, age at first live birth, smoking status, drinking status, breast cancer in first-degree relative, and energy.

**Table 3.** Interaction between dietary intake and the *MTHFR* C667T polymorphism for breast cancer risk.

| Dietary intake | MTHFR C667T | Cases | % | Controls | % | OR (95%CI) | P value |
|---|---|---|---|---|---|---|---|
| Folate intake | | | | | | | |
| Low (<400 µg/day) | C allele | 73 | 67.2 | 88 | 72.4 | - | - |
| | T allele | 36 | 32.8 | 34 | 27.6 | 1.28 (0.70-2.33) | 0.39 |
| Moderate (400-510 mg/day) | C allele | 64 | 69.3 | 91 | 71.6 | - | - |
| | T allele | 29 | 30.7 | 36 | 28.4 | 1.15 (0.61-2.14) | 0.65 |
| High (>510 mg/day) | C allele | 77 | 72.4 | 93 | 70.5 | - | - |
| | T allele | 30 | 27.6 | 39 | 29.5 | 0.93 (0.51-1.69) | 0.80 |

## DISCUSSION

Our study found an association between the *MTHFR* C667T polymorphism and risk of breast cancer. However, we observed no significant association of intake of folate, vitamin B6 and vitamin B12 with risk of breast cancer, and no significant interaction between *MTHFR C677T* polymorphism and folate intake on the risk of breast cancer. Recently, several studies have reported the association between *MTHFR C677T* and *A1298C* and *MTR A2756G* polymorphisms and risk of breast cancer, but the results of our study are consistent with those of previous studies (Cho et al., 2007; Larsson et al., 2007; Gao et al., 2009; Webb et al., 2011; de Cássia Carvalho Barbosa et al., 2012a, b; Lajin et al., 2012; Diakite et al., 2012). Therefore, we investigated the role of *MTHFR C677T* and *A1298C* and *MTR A2756G* polymorphisms in the risk of breast cancer.

It is reported that folate plays a protective role against the development of cancer before the establishment of preneoplastic lesions, but it also may enhance tumorigenesis once lesions have been established (Kim, 2006; Xu et al., 2008; Lin et al., 2008). It has been reported that high intake of folate is beneficial to women who are deficient in nutrient gain. Several previous studies have reported inconsistent results for folate intake and breast cancer risk (Ma et al., 2009; Wu et al., 2012; Tavani et al., 2012; Islam et al., 2013; Qin et al., 2013;

Yang et al., 2013). In our study, we reported that folate intake does not play a protective role in breast cancer risk in Chinese population. The inconsistency of these studies may be attributed to differences in ethnicities, source of control subjects, and sample size, among other reasons. Further confirmation of existing findings is required.

Our study showed that the *MTHFR* C677T polymorphism plays an important role in breast cancer risk, and that *MTHFR* 677TT was correlated with an increased risk of breast cancer. Our findings are consistent with those of several other studies (Prasad and Wilkhoo, 2011; Hosseini et al., 2011; Akram et al., 2012). A few studies have reported a non-significant or reverse association between the *MTHFR* C677T polymorphism and breast cancer risk (Gao et al., 2009; Akram et al., 2012). Because these studies were performed in different ethnicities, direct comparisons of the results are difficult. A recent meta-analysis has indicated that the *MTHFR* 677TT variant genotype is associated with elevated breast cancer risk by pooling 37 studies. The discrepancies among studies may have been caused by differences in variant frequencies between races. The frequency of the *MTHFR* 677TT genotype was 8.4% in the control group, but was 14.1% in a study conducted in the Chinese population (Gao et al., 2009). The source of the control population might contribute to the differences observed among populations. Thus, multi-center studies are greatly needed to confirm their association.

There were two limitations in the current study. First, the breast cancer cases and control subjects were selected from one hospital, which could not well represent populations in other places. Second, the sample size in this study is relative small, which could decrease the statistical power to find difference between breast cancer cases and control subjects. We did not find significant association between *MTHFR A1298C* and *MTR A2756G* polymorphisms and risk of breast cancer, which may be caused by the relative sample size. Therefore, large sample studies are greatly needed to confirm their association in the future.

In summary, this case-control study found that the *MTHFR* C667T polymorphisms were associated with risk of breast cancer. However, no association was found between dietary intake of folate, vitamin B6 and vitamin B12 and risk of breast cancer. Further large sample studies are greatly needed to confirm their association.

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