RESEARCH ARTICLE

# Association of Dietary Intake of Folate, Vitamin $B_6$ and $B_{12}$ and MTHFR Genotype with Breast Cancer Risk

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## Abstract

**Aim: We aimed to investigate the associations of dietary intake of folate, vitamin $B_6$ and $B_{12}$ and MTHFR genotype with breast cancer in a Chinese population. Methods: A matched case-control study was conducted, and 435 patients with newly diagnosed and histologically confirmed breast cancer and 435 controls were collected. The folate intake, vitamin $B_6$ and vitamin $B_{12}$ were calculated, and MTHFR C665T, C677T and A1298C were analyzed by PCR-RFLP. Results: We found vitamin $B_{12}$ was likely to reduce the risk of breast cancer, and MTHFR 665TT was associated with increased risk of breast cancer. Folate intake, vitamin $B_{12}$ intake and variants of MTHFR C677T and MTHFR A1298C demonstrated no association with risk of breast cancer. However, we found patients with low intake of vitamin $B_6$ and MTHFR 665TT genotype had a higher risk of breast cancer (OR=1.87, 95% CI=1.29-2.77), the association being less pronounced among subjects with a moderate intake of vitamin $B_6$ and MTHFR 665TT genotype (OR=1.58, 95% CI=1.03-2.49, *P*=0.03). Conclusion: Our study indicated that the MTHFR C665T polymorphism and vitamin $B_6$ are associated with risk of breast cancer, which indicated roles for nutrients in developing breast cancer.**

**Keywords:** Folate - vitamin - MTHFR - polymorphism - breast cancer

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## Introduction

Breast cancer is a major health problem worldwide, and it is the leading type of cancer in Chinese females (IARC, 2008). It is known that breast cancer is caused by a complex combination of genetic and environmental factors (Szakacs et al., 2006), and the genes and environment share the stage for most cancers. Exposure to genotoxic agents during breast development, null parity, exposure to ionizing radiation, age at the first child's birth and a family history are well-established risk factors for breast cancer, but majority of the causes is still obscure. However, the DNA repair deficiencies in breast cancer development has been reported to be associated with breast cancer risk due to deficient in the repair of DNA damage (Roberti et al., 2006).

Previous studies have suggested an association between altered diet levels and tumorigenesis (Ulrich et al., 2005), and thus inherited genetic variation in the gene involved in the diet metabolizing enzymes could influence the development of cancer. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and its catalysis creates an irreversible reduction in 5,10-methlenetetrahydrofolate (THF), which is irreversible reduction in 5,10-methlenetetrahydrofolate (THF), and converted into 5-methyl-THFR during process. Previous epidemiologic study indicated the variant of MTHFR C665T (Ala222Val) was associated with risk of breast cancer (Semenza et al., 2003; Rossi et al., 2006; Yu et al., 2012; Jiao et al., 2013). Another two common MTHFR gene polymorphisms, C677T and A1298C, were widely discussed with the risk of breast cancer (de Cássia et al., 2012; Wu et al., 2012). Therefore, we aimed to investigate the associations of dietary intake of folate, vitamin $B_6$ and $B_{12}$ and MTHFR genotype with breast cancer in Chinese population.

## Materials and Methods

*Subjects*

Eligible cases were a consecutive series of female patients with newly diagnosed and histologically confirmed breast cancer. Cases were recruited between January 2009 and December 2010 at The Second Affiliated Hospital of Guangzhou Medical University. Eligible controls confirmed not to have any cancer were selected from the same hospitals during the same period, and one control matched to each case by age (within 5 years). All the cases in our study were collected from patients for health examination and treatment for gynecological. Of all eligible cases, 476 were collected, and 435 patients agreed to participate into our study, with a participation rate of 91.4%.

Face to face interviews were conducted by trained interviewers using a structured questionnaire, and the questionnaire included demographic characteristics,

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**Table 1. Characteristics of Breast Cancer and Control Subjects**

| | Cases | % | Controls | % | *t* or $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|
| Age (mean±SD), years | 47.5±8.5 | | 47.3±8.6 | | 0.35 | 0.37 |
| Age at menarche, years | 12.6±1.7 | | 12.9±1.8 | | 2.5 | 0.005 |
| Age at first live birth, years | 26.2±6.7 | | 23.6±7.1 | | 5.6 | <0.001 |
| Menopausal status | | | | | | |
| Premenopausal | 198 | 45.5 | 183 | 42.1 | 1.05 | 0.31 |
| Postmenopausal | 237 | 54.5 | 252 | 57.9 | | |
| Breast cancer in first-degree relative | | | | | | |
| No | 406 | 93.3 | 433 | 99.5 | 24.4 | <0.001 |
| Yes | 29 | 6.7 | 2 | 0.5 | | |
| Folate intake, ug/day | 511.5±94.3 | | 516.5±104.2 | | 0.74 | 0.23 |
| Vitamin B6, mg/day | 0.89±0.26 | | 0.89±0.28 | | 1.09 | 0.14 |
| Vitamin B12, ug/day | 7.1±3.5 | | 7.7±4.6 | | 2.17 | 0.02 |
| MTHFR C665T | | | | | | |
| CC | 139 | 31.9 | 168 | 38.6 | 6.36 | 0.04 |
| CT | 216 | 49.7 | 209 | 48.1 | | |
| TT | 80 | 17.4 | 58 | 13.3 | | |
| MTHFR C677T | | | | | | |
| CC | 250 | 57.5 | 255 | 58.6 | 0.14 | 0.93 |
| CT | 153 | 35.2 | 150 | 34.5 | | |
| TT | 32 | 7.3 | 30 | 6.9 | | |
| MTHFR A1298C | | | | | | |
| AA | 206 | 47.4 | 214 | 49.3 | 0.36 | 0.84 |
| AC | 176 | 40.5 | 172 | 39.5 | | |
| CC | 53 | 12.1 | 49 | 11.2 | | |

medical history, family history of cancer and reproductive factors as well as a food-frequency questionnaire with 86 food terms.

The folate intake, vitamin $B_6$ and vitamin $B_{12}$ were computed by multiplying frequency by standard portion size and relative size for each food item in questionnaire, and then the sum of all folate intake from various foods/ food groups was calculated as the total folate intake.

*Genotype of polymorphisms*

Peripheral blood samples were obtained from each case and control, and stored at -20°C until use. Genomic DNA samples were extracted from the blood using QIAGEN FlexiGene DNA kits according to the manufacture's protocol. Genotyping of MTHFR C665T, C677T and A1298C genetic polymorphism was determined using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The condition of PCR was as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, annealing at 60°C for 65 s, extension at 72°C for 90 s, and final extension at 72°C for 5 minutes. A random sample of 5% of cases and controls was genotyped again by different researchers. The reproducibility was 100%.

*Statistical analysis*

Odds ratios (ORs) were used to measure the association between MTHFR genotype, dietary intake and breast cancer risk. Conditional logistical regression models were used to obtain the estimated ORs and their 95% confidence intervals (Cls) after adjusting for potential confounding variables, and statistical significance was examined by the Wald chi-square test. Stratified analyses were used to evaluate the potential modifying effect of modifying effect of folate intake, vitamin $B_6$ and vitamin $B_{12}$ on breast cancer risk with MTHFR genotypes. Statistical analyses were performed by using Stata version 8 (Stata, College Station, TX). P value less than 0.05 was considered to be significant.

**Table 2. Associations of MTHFR C665T and Vitamin $B_6$ intake with breast cancer risk**

| Dietary intake | MTHFR C665T | OR(95% CI) | *P*-value |
|---|---|---|---|
| Vitamin $B_6$ | | | |
| Low (<0.6 mg/day) | CC | - | |
| | CT | 1.35(0.97-1.74) | 0.15 |
| | TT | 1.87(1.29-2.77) | 0.01 |
| Moderate(0.6-1.0 mg/day) | CC | - | |
| | CT | 1.26(0.91-1.62) | 0.26 |
| | TT | 1.58(1.03-2.49) | 0.03 |
| High (>1.0 mg/day) | CC | - | |
| | CT | 1.11(0.82-1.47) | 0.38 |
| | TT | 1.42(0.93-2.36) | 0.17 |

## Results

The characteristics of cases and controls at baseline are shown in Table 1. The breast cancer cases tended to be older at first live birth, younger at menarche and more first-degree relatives. For dietary habit, we found vitamin $B_{12}$ were more likely to reduce the risk of breast cancer. However, we did not found intake of vitamin $B_6$ was associated with risk of breast cancer. For MTHFR gene, MTHFR 665TT was associated with increased risk of breast cancer, and no significant association was found between variants of MTHFR C677T and MTHFR A1298C and risk of breast cancer.

The associations of MTHFR C665T genotypes and Vitamin $B_6$ intake with breast cancer risk are presented in Table 2, after stratifying by the levels of Vitamin $B_6$. In terms of low intake of Vitamin $B_6$, MTHFR 665TT genotype was associated with higher risk of breast cancer (OR=1.87, 95% CI=1.29-2.77, *P*=0.01), and the association appeared lower among subjects with moderate intake of Vitamin $B_6$ and MTHFR 665TT genotype (OR=1.58, 95% CI=1.03-2.49, *P*=0.03). However, no significant association was found in subjects with high intake of high Vitamin $B_6$ (*P*=0.17). Moreover, we did not find interaction between MTHFR C665T and Vitamin $B_6$ (*P*=0.24).

## Discussion

In our study, we found no association between MTHFR C665T polymorphism and Vitamin $B_6$ and breast cancer risk. However, we did not find a significant association of intake of folate and Vitamin $B_{12}$ with risk of breast cancer. Our findings are consistent with previous studies on variants of MTHFR gene, folate, vitamin $B_6$ and vitamin $B_{12}$ (Semenza et al., 2003; Chen et al., 2005; Cho et al., 2007; Larsson et al., 2007; Gao et al., 2009; Alshatwi et al., 2010; de Cássia et al., 2012; Lajin et al., 2012).

MTHFR is a critical gene in the one-carbon metabolism pathway. Three non-synonymous missnese SNPs, C665T, C667T and A1298C, in the coding region were extensively studies. However, the previous association studies on the MTHFR polymorphism and breast cancer risk showed inconsistent results. Several studies have reported that the MTHFR C677T was associated with increased risk of breast cancer in pre-menopausal women or in those with ovarian cancer (Gershoni-Baruch et al., 2000; Ergul

et al., 2003; Maruti et al., 2009), but some others showed no association between MTHFR C677T and breast cancer risk (Vaĭner et al., 2010; de Cássia et al., 2012). Previous studies reported that MTHFR A1298C was not associated with breast cancer risk in various populations (Vaĭner et al., 2010; de Cássia et al., 2012; Qiu et al., 2011), while two studies indicated the MTHFR A1298C polymorphism was associated with risk of breast cancer (Hosseini et al., 2011; Akram et al., 2012). Our study did not find significant association between polymorphisms in MTHFR C667T and A1298C and breast cancer risk. The inconsistent of the results might be induced by different populations, sample size, study design and by chance. For MTHFR C665T, various studies reported that variant of MTHFR C665T was associated with risk of breast cancer (Semenza et al., 2003; Rossi et al., 2006; Yu et al., 2012; Jiao et al., 2013). In our study, we found MTHFR C665T polymorphisms was significantly associated with increased risk of breast cancer, which was in line with previous studies.

Generally, folate is able to prevent the development of tumors before established preneoplastic lesions, but it would improve tumorigenesis once lesions have been established (Kim, 2006; Lin et al., 2008; Xu et al., 2008). Although increased folate intake may be good for population deficient in this nutrient, increased intake in women with already-sufficient levels of folate may provide no further benefit or actually harmful. Previous several studies did not report a significant association with breast cancer risk (Vaĭner et al., 2010; Islam et al., 2013), which is in line with our results. The results indicated folate intake could not play a protective role in breast cancer for women who already have deficient in nutrition.

Our study found that Vitamin $B_6$ has a protective effect on breast cancer risk. There is a substantial body of data supporting the biological plausibility. Vitamin $B_6$ acts as a critical coenzyme in two different steps, one is in the synthesis of 5,10- methylenetetrahydrofolate, which is critical for synthesis, repair and methylation of DNA, and another is catabolism of homocysteine to glutathione, which is involved in the detoxification of many carcinogenic compounds and preventing cells from oxidative DNA damage (Selhub et al., 2002; Matsubara et al., 2003). Experimental study suggested that Vitamin $B_6$ exerts other anticarcinogenic effects by reducing cell proliferation, nitric oxide production and angiogenesis (Matsubara et al., 2003), and clinical studies provide evidence that Vitamin $B_6$ play a role in protection of breast cancer (Yang et al., 2013). Our study also indicated Vitamin $B_6$ has an important role in preventing of breast cancer.

In conclusion, our study indicated that MTHFR C665T polymorphism and Vitamin $B_6$ are associated with risk of breast cancer, which indicated these nutrients have a role in developing breast cancer. Further large sample study are greatly needed to confirm their association.

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