



Association of dietary intake of folate and MTHFR genotype with breast cancer risk

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Genet. Mol. Res. 13 (3): 5446-5451 (2014)

Received June 24, 2013

Accepted December 3, 2013

Published July 24, 2014

DOI <http://dx.doi.org/10.4238/2014.July.24.24>

ABSTRACT. We conducted a hospital-based case-control study to investigate the associations of dietary intake of folate and MTHFR C677T and A1298C polymorphisms with breast cancer in a Chinese population. A 1:1-matched case-control study was conducted. Two hundred and thirty patients who were newly diagnosed and histologically confirmed breast cancer and 230 controls were enrolled from Xinxiang Central Hospital. Folate intake was calculated by standard portion size and relative size for each food item in the questionnaire. Genotyping of MTHFR C677T and A1298C was performed by PCR-RFLP. MTHFR 677TT (OR = 2.26, 95%CI = 1.09-4.87, P = 0.02) and T allele (OR = 1.40, 95%CI = 1.03-1.90, P = 0.03) had an increased risk of laryngeal cancer when compared with the CC genotype. We found any interaction between MTHFR C677T and folate intake (P for interaction = 0.02). In conclusion, our study demonstrated that MTHFR C677T polymorphism and folate are associated with risk of breast cancer.

Key words: Folate; MTHFR; Polymorphism; Breast cancer

INTRODUCTION

Dietary folate and vitamins have an important role in one-carbon metabolism, which is essential for DNA methylation, synthesis and repair (Ulrich, 2005). Low dietary folate intake has been associated with risk of various cancers, such as colorectal cancer, pancreatic cancer, prostate cancer and gastric cancer (Bassett et al., 2013; Lin et al., 2013; Collin, 2013; Gao et al., 2013). Recently, several studies reported a protective effect of folate intake on breast cancer risk (Liu et al., 2013; Yang et al., 2013; Islam et al., 2013). However, evidence from two recent studies have indicated an inverse association (Ericson et al., 2007; Maruti et al., 2009a), and some other studies have shown that the reduced risk only exists in certain populations, such as women with high levels of alcohol intake or premenopausal Chinese women (Levi et al., 2001; Shrubsole et al., 2011).

Previous studies have suggested an association between altered diet and tumorigenesis (Ulrich, 2005), and thus, inherited genetic variation in genes involved with nutrient-metabolizing enzymes could influence the development of cancer. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and it catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate, where it is converted to 5-methyl-THFR.

Two common MTHFR gene polymorphisms, C677T and A1298C, have been widely discussed (de Cássia et al., 2012; Wu et al., 2012). Many studies have examined the association between MTHFR gene polymorphisms and risk of breast cancer, and suggested that MTHFR C677T and A1298C polymorphisms are associated with an increased or decreased risk of breast cancer (Le Marchand et al., 2004; Rossi et al., 2006; Yu and Chen, 2012; Jiao and Li, 2013). Previous studies reported the association between the MTHFR genotype polymorphisms and folate intake and risk of breast cancer (Alshatwi, 2010; Sangrajang et al., 2010; Lajin et al., 2012), but the results are inconsistent. In our study, we conducted a hospital-based case-control study to investigate the associations of dietary intake of folate and MTHFR C677T and A1298C polymorphisms with breast cancer in a Chinese population.

MATERIAL AND METHODS

Subjects

Our study recruited 230 patients who were newly diagnosed and histologically confirmed breast cancer between March 2009 and October 2010 at Xinxiang Central Hospital. Patients with a history of cancer were excluded from our study. Control subjects were selected from subjects for regular health check-up at the same hospital during the same period. All the control subjects matched cases by age (within 5 years). All cases and controls signed informed consent, and the study protocol was approved by the Ethics Committee of Xinxiang Central Hospital.

The demographic characteristics of cases and control subjects were collected by a face to face interview using a structured questionnaire. The medical history, family history of cancer and reproductive factors were collected from medical records. The folate intake was calculated from a food-frequency questionnaire with 80 food terms.

Intake of folate were computed by multiplying frequency by standard portion size and relative size for each food item in the questionnaire, and then the sum of all folate intake from various foods/food groups was calculated as the total folate intake.

Genotype of polymorphisms

The participants were asked to provide 5 mL peripheral blood samples, which were stored at -20°C . Genomic DNA was extracted from peripheral blood samples using QIAGEN FlexiGene DNA kits according to the manufacturer protocol. Genotyping of MTHFR C677T and A1298C genetic polymorphisms was determined using polymerase chain reaction (PCR)-restriction fragment length polymorphism. Primers and probes of MTHFR C677T and A1298C for PCR amplification were designed by the Sequenom[®] Assay Design 3.1 software (Sequenom[®]). PCR for C677T was performed with the primers forward 5'-CGTGGCTCCTGCGTTTCC-3' and reverse 5'-GAGCCGGCCACAGGCAT-3'; PCR for A1298C was performed using the primers forward 5'-CAAATCTGAGGGAGCTGAGT-3' and reverse 5'-CAGATAAGTGGCAGTACAGA-3'. The PCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 2 min, annealing at 64°C for 30 s, extension at 72°C for 90 s, and a final extension at 72°C for 10 min. Cases and controls (10%) were randomly selected to repeat analysis to confirm consistency, and the consistency rate was 100%.

Statistical analysis

All statistical analyses were performed using Stata version 8 (Stata, College Station, TX, USA). Continuous variables are reported as means \pm standard deviation (SD), and categorical variables are reported as frequencies (N) and percentages. The Student *t*-test and the χ^2 test were used to assess differences between cases and controls with regard to demographic characteristics. A goodness-of-fit χ^2 test was used to evaluate the Hardy-Weinberg equilibrium (HWE) in controls. Conditional logistic regression was performed to analyze the association between dietary intake of folate and MTHFR C677T and A1298C polymorphisms with breast cancer and breast cancer risk, with the estimated ORs and their 95% confidence interval (CI) after adjusting for potential confounding variables. Stratified analyses were used to evaluate the potential modifying effect of folate on breast cancer risk with MTHFR genotypes. *P* less than 0.05 was considered to be significance.

RESULTS

The demographic and clinical characteristics of breast cancer cases and controls are shown in Table 1. The mean age of patients and controls were 48.6 ± 9.7 and 48.2 ± 8.8 years, respectively. The breast cancer cases were more likely to be younger at menarche, older at first live birth, more first-degree relatives, and higher folate intake. However, we did not find that live birth and menopausal status were associated with risk of breast cancer.

For the MTHFR gene, there was significant difference between genotype distributions of MTHFR C677T between cases and controls. Subjects carrying MTHFR 677TT (OR = 2.26, 95%CI = 1.09-4.87, *P* = 0.02) and T allele (OR = 1.40, 95%CI = 1.03-1.90, *P* = 0.03) had an increased risk of laryngeal cancer when compared with the CC genotype. However, we did not find significant difference between the MTHFR A1298C polymorphism and risk of breast cancer.

Table 1. Characteristics of breast cancer and control subjects.

	Cases	%	Controls	%	<i>t</i> or χ^2	P value
Age (years, mean \pm SD)	47.5 \pm 8.5		47.3 \pm 8.6		0.35	0.37
Age at menarche (years)	12.6 \pm 1.7		12.9 \pm 1.8		2.5	0.005
Age at first live birth (years)	26.2 \pm 6.7		23.6 \pm 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 relatives						
No	406	93.3	433	99.5	24.4	<0.001
Yes	29	6.7	2	0.5		
Folate intake (μ g/day)	511.5 \pm 94.3		516.5 \pm 104.2		0.74	0.23
Vitamin B ₆ (mg/day)	0.89 \pm 0.26		0.89 \pm 0.28		1.09	0.14
Vitamin B ₁₂ (μ g/day)	7.1 \pm 3.5		7.7 \pm 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		

The associations of MTHFR C677T genotypes and folate intake with breast cancer risk are presented in Table 2, after stratifying by the potential risk factors. In terms of low intake of folate, MTHFR 677TT genotype (adjusted OR = 2.73, 95%CI = 1.31-5.68, P = 0.01) and T allele (adjusted OR = 1.65, 95%CI = 1.14-2.28, P = 0.03) were associated with higher risk of breast cancer, and the association appeared lower among subjects with moderate intake of folate and MTHFR 677TT genotype (adjusted OR = 1.92, 95%CI = 1.01-2.35, P = 0.04). However, no significant association was found in subjects with high intake of folate. Moreover, we found any interaction between MTHFR C677T and folate intake (P for interaction = 0.02).

Table 2. Associations of MTHFR C665T and vitamin B₆ 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

DISCUSSION

Reports regarding the association of folate metabolism and changes in MTHFR activity with breast cancer are conflicting (Alshatwi, 2010; Sangrajang et al., 2010; Lajin et al.,

2012). The present study indicated that there was significant association between MTHFR C677T polymorphism and folate intake and risk of breast cancer, and we found a significant interaction between folate intake and MTHFR C677T polymorphism. We found no association between MTHFR A1298C polymorphism and breast cancer risk. Our findings are consistent with previous studies on the association between variants of MTHFR gene and folate and risk of breast cancer (Gao et al., 2009; Alshatwi, 2010; Hosseini et al., 2011; de Cássia Carvalho et al., 2012; Diakite et al., 2012; Lajin et al., 2012; Wu et al., 2012).

MTHFR is a critical gene for one-carbon and folate metabolism, and plays an important role in DNA synthesis or to homocysteine remethylation (Baylin et al., 2005; Kim, 2006; Koppen et al., 2010; Nikbakht et al., 2012). Reduced activity of MTHFR can interfere with the 5-methyltetrahydrofolate pathway, which leads to elevated levels of the MTHFR substrate 5,10-methylenetetrahydrofolate and reduced levels of 5-methyltetrahydrofolate (Baylin, 2005; Koppen et al., 2010). Therefore, the polymorphisms of MTHFR could alter the folate metabolism, and change the methionine synthesis toward DNA synthesis and repair. Previous several studies have reported that the MTHFR C677T is associated with increased risk of breast cancer (Gershoni-Baruch et al., 2000; Ergul et al., 2003; Maruti et al., 2009b), but some others indicated no association between MTHFR C677T and breast cancer risk (Vařner et al., 2010; de Cássia Carvalho et al., 2012). The inconsistency of findings on that association may be explained by differences in population background, source of control subjects, and sample size.

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

There were several limitations to our study. Firstly, cases were selected in one hospital. Therefore, the selection bias could not be avoided. Secondly, some factors could have influence on the risk of breast cancer, such as clinical status and other genetic factors as well as ethnicities. However, these factors were not included in our analysis. Therefore, large multi-center studies with different ethnicities are warranted to further investigate the impact of folate intake and MTHFR polymorphism on the risk of breast cancer.

In conclusion, our study demonstrated that MTHFR C677T polymorphism and folate are associated with risk of breast cancer, indicating that this nutrient has a role in the development of breast cancer. Further large sample studies are greatly needed to confirm this association.

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