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Original Article

Association of MTHFR, MTRR and MTR polymorphisms with breast cancer risk: a study in Chinese females

Zhaofeng Niu[1], Haidong Zhao[2], Xiaoke Hou[1]

[1]Department of Galactophore, Yuncheng Central Hospital, Yuncheng, Shanxi Province, China; [2]Breast Disease Center, The Second Affiliated Hospital of Dalian Medical University, Dalian, Liaoning Province, China

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Abstract: Literature revealed that folate metabolism imbalance may be involved in predisposition to cancer. MTHFR, MTRR and MTR are essential enzymes for maintenance of folate hemostasis and homocysteine (Hcy) in the blood. We aimed to investigate the association between homocysteine metabolism gene polymorphism (MTHFR rs1801133, MTR 1805087 and MTRR rs1801394) and risk of breast cancer in West China. A total of 466 patients with breast cancer and 470 normal control subjects were enrolled into our study. The MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 polymorphism was analyzed by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). By logistic regression analysis, we observed that the CT (adjusted OR=2.67, 95% CI=1.60-4.45) and TT (adjusted OR=2.67, 95% CI=1.60-4.45) genotypes of MTHFR rs1801133 were significantly associated with higher risk of breast cancer, compared with the CC genotype. In addition, the CT+TT of MTHFR rs1801133 was significantly associated with risk of breast cancer in comparison to the CC genotype (adjusted OR=1.43, 95% CI=1.09-1.88). The CC genotype of MTRR rs1801394 showed higher association with an increased risk of breast cancer as compared with the AA genotype (adjusted OR=1.96, 95% CI=1.14-3.37). The results of our study indicate that the MTHFR rs1801133 and MTRR rs1801394 polymorphisms are potential risk factors for the development of breast cancer in the Chinese population.

Keywords: MTHFR, MTRR, MTR, polymorphism, breast cancer

Introduction

In female, breast is particularly prominent as the hallmark of pubertal development. Breast cancer begins in any part of breast, caused by abnormal cells growth and division. It is one of the oldest known forms of malignancies. Unfortunately globally, it remains a major public health issue in China as well as world. Breast cancer is the leading cause of death of women in worldwide due to it spread to other organ [1, 2]. Development of human breast cancers is a multistep process, arising from genetic alterations that drive the transformation of normal mammary epithelial cells into highly malignant derivatives [3]. Literature revealed that folate metabolism imbalance may be involved in predisposition to cancer. Folate metabolism pathway regulates the intracellular folate pool for synthesis and methylation of DNA [4, 5].

The serum folate enters into tissue cells through folate receptors, and then it was turned into tetrahydrofolate via dihydrofolate reductase. The tetrahydrofolate is converted into 5,10-methylenetetrahydrofolate [6]. Methylenetetrahydrofolate reductase (MTHFR) then transformed the 5,10-methylenetetrahydrofolateinto 5-methyltetrahydrofolate [7, 8], which supplied a methyl group for transformation of homocysteine to methionine in a reaction catalyzed by methionine synthase (MTR) [9, 10]. MTR provides cobalamin to be a coenzyme. The cobalamin (I) cofactor is oxidized to form cobalamin (II), which causes inactivation of MTR. The methionine synthase reductase (MTRR) is required for inverse conversion of oxidized cobalamin (II) to CH3-cobalamin (III) to keep the activity of MTR [11]. Therefore, the MTHFR, MTRR and MTR are essential enzymes for maintenance of folate hemostasis and homocysteine (Hcy) in the

Table 1. Primer sequences and restriction enzyme and product size of MTHFR rs1801133, MTR 1805087 and MTRR rs1801394

Genes	Primer sequence	Product size	Restriction enzyme	Restriction enzyme digestion
MTHFR rs1801133	F: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'	198 bp	*Hinf* I	CC: 198 bp; TT: 175 and 23 bp; CT: 198, 175 and 23 bp
	R: 5'-AGGACGGTGCGGTGAGAGTG-3'			
MTR 1805087	F: 5'-GAACTAGAAGACAGAAATTCTCTA-3'	189 bp	*Mae* III	AA: 189 bp; CC: 159 and 30 bp; AC: 189, 159 and 30 bp
	R: 5'-CATGGAAGAATATCAAGATATTAGA-3'			
MTRR rs1801394	F: 5'-GCAGCCATCGCAGAAGACAT-3'	151 bp	*Nde* I	AA: 44 and 22 bp; GG: 66 bp; AG: 66, 44 and 22 bp
	R: 5'-GTGAAGATCTGCAGAAAATCCATGTA-3'			

blood. Several genetic polymorphisms in MTHFR, MTRR and MTR have been reported to affect the level of serum folate [12], including MTHFR rs1801133 and rs1801131, MTR 1805087 and MTRR rs1801394. However, the association between MTRR and MTR polymorphisms and plasma folate level is controversial, and no study reported the MTHFR, MTRR and MTR genetic polymorphisms and risk of breast cancer in north China. Therefore, we performed a case-control study to investigate the association between homocysteine metabolism gene polymorphism MTHFR rs1801133, MTR 1805087 and MTRR rs1801394) and risk of breast cancer in West China.

Material and methods

Subjects

A case-control study design was taken in this study. During January 2014 and July 2016, a total of 466 patients with breast cancer and 470 normal control subjects were enrolled into our study. All the patients with breast cancer were collected from the outpatient clinics and inpatient departments of Galactophore of Yuncheng Central Hospital. All the patients were confirmed without any other malignant tumors, metastatic tumors, recurrent tumors and malnutrition. In addition, patients who receive any form of anti-cancer treatment prior to enrollment were also excluded from this study.

Controls who were recruited from the Yuncheng Central Hospital's outpatient clinics and health examination centers. All the control subjects are free of any malignant tumors and metabolic diseases. Controls were matched to patients with regard to age.

All cases were divided into two subgroups according to breast status: a) patients with early cancer stage (including stages I and II) and b) patients with advanced cancer stage (including stages III and IV), according to the American Joint Committee for Cancer Staging and End-Results reporting in 1992 [13]. Demographic information of all the participants was collected from medical records, including sex, age, family history of cancer, smoking and drinking habits.

The subjects were sub-divided into non-smokers and smokers; smokers were defined as those who smoked at least one cigarette per day for a period of six months. Furthermore, individuals were also categorized as non-drinkers and drinkers; drinkers were defined as those who drank at least 50 mL white wine or a bottle of beer at least once a week for six consecutive months. Written informed consents were obtained from all subjects prior to enrollment, and the study protocols were approved by the ethics committee of Yuncheng Central Hospital.

DNA extraction

Peripheral blood was collected from all the subjects in 0.5 M EDTA tubes. Genomic DNA was isolated from whole blood using the standard phenol-chloroform extraction method [14].

MTHFR, MTRR and MTR polymorphisms

The MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 polymorphisms were analyzed by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). Genomic DNA was amplified (Applied Biosystems, Veriti, Singapore) using the following PCR conditions: for MTHFR rs1801133, 94°C for 4 min; 35 cycles at 94°C for 45 s, 58°C for 35 s, 72°C for 45 s; final extension at 72°C for 7 min; for MTR 1805087, 94°C for 4 min; 35 cycles at 94°C for 30 s, 53°C for 36 s, 72°C for 45 s; final extension at 72°C for

Table 2. Demographic characteristics of included patients and controls

Variables	Patients N=466	%	Controls N=470	%	t or χ^2 value	*P* value
Age, years		49.78±11.06		49.49±10.90	0.48	0.482
Socioeconomic status						
Lower	119	25.54	127	27.02		
Middle	214	45.92	192	40.85		
Upper	133	28.54	151	32.13	2.58	0.276
Physical activity						
Never	208	44.64	282	60.00		
Seldom	68	14.59	105	22.34		
Often	90	19.31	83	17.66	9.33	0.009
Menopausal status						
Premenopausal	325	69.74	299	63.62		
Postmenopausal	141	30.26	171	36.38	3.95	0.047
Smoking						
No	456	97.85	464	98.72		
Yes	10	2.15	6	1.28	1.05	0.305
Passive smoking from husband						
No	226	48.50	240	51.06		
Yes	274	58.80	196	41.70	9.03	0.003
Drinking						
No	387	83.05	411	87.45		
Yes	79	16.95	59	12.55	3.60	0.058
Body mass index (BMI), kg/m^2		23.04±4.14		22.40±3.63	2.55	0.023
Nulliparous						
No	437	93.78	448	95.32		
Yes	29	6.22	22	4.68		
Age at first live birth, year		26.63±3.23		25.82±3.21	3.84	0.898
Breastfeeding						
No	92		71			
Yes	374		399			
Months of breastfeeding, months		7.43±2.88		8.22±2.74	-4.303	0.17
Benign breast disease						
No	276	59.23	383	81.49		
Yes	190	40.77	87	18.51	9.03	0.003
First-degree relative with cancer						
No	386	82.83	441	93.83		
Yes	80	17.17	29	6.17	27.50	<0.001
Intake of folic acid, μg/d		192.35±44.22		222.06±46.32	-10.034	0.21

7 min; for MTRR rs1801394, 94°C for 4 min; 35 cycles at 94°C for 45 s, 60°C for 35 s, 72°C for 45 s; final extension at 72°C for 7 min. The primers used for amplification of the MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 gene polymorphisms were shown in **Table 1**. The 50 μL PCR reaction mixture contained 5 μL 10× PCR buffer solution, 4 μL dNTP (2.5 mM), 2 μL forward and reverse primers (10 mM), 2.5 U TaqDNA polymerase, 2 μL DNA template, and hydrogen peroxide. Amplification success of samples was monitored on 2% agarose gel by Gel electrophoresis.

Statistical analysis

The categorical variables were showed as frequencies and percentages of total number. Pearson's chi-squared and Fisher's exact tests were adopted to analyze the inter-group differ-

Table 3. Genotype distributions of MTHFR rs1801133, MTR 1805087 and MTRR rs1801394

Genotypes	Patients		Controls		χ^2 value	*P* value	χ^2 for HWE in controls	*P* value
MTHFR rs1801133								
CC	235	50.43	274	58.30				
CT	172	36.91	168	35.74				
TT	59	12.66	28	5.96	14.06	0.001	0.11	0.739
MTR 1805087								
AA	362	77.68	358	76.17				
AG	73	15.67	79	16.81				
GG	31	6.65	33	7.02	0.30	0.859	59.50	<0.001
MTRR rs1801394								
AA	259	55.58	251	53.40				
AG	160	34.33	189	40.21				
GG	47	10.09	30	6.38	6.27	0.043	0.50	0.480

ences. Whether the genotype frequencies of MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 were departure from Hardy-Weinberg equilibrium (HWE) was analyzed by chi-square test. The association between MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 and risk of breast cancer was analyzed using the method of multiple logistic regression analysis. Results were expressed using odds ratios (ORs) and 95% confidence intervals (CIs). The wild-type genotype of MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 was considered as reference group. Chi-square or student t tests analysis were taken to analyze the interaction between MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 polymorphisms and environmental factors in the risk of breast cancer. All the analysis was adopted using SPSS version17.0 for Windows (SPSS, Inc., Chicago, IL, USA), and *P* value less than 0.05 was considered as significant difference.

Results

The social demographic variables of included patients and controls were shown in **Table 2**. According to chi-squared and student t analysis, we observed that patients were more likely to have less physical activity (χ^2=9.33, P=0.009) and higher BMI (t=2.55, P=0.023), be premenopausal status (χ^2=3.95, P=0.047), have a habit of passive smoking from husband (χ^2=9.03, P=0.003) and a history of benign breast disease (χ^2=9.03, P=0.003) as well as a history of first-degree relative with cancer (χ^2=27.50, P<0.001).

The genotype distributions of MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 are list in **Table 3**. The genotypic distributions of CC, CT and TT in the MTHFR rs180113 (χ^2=14.06, P=0.001) and AA, AG and GG in MTRR rs1801394 (χ^2=6.27, P=0.043) showed significant different between patients with breast cancer and controls. However, no significant difference was observed in the genotypic distributions of MTR 1805087. The MTHFR rs1801133 (χ^2=0.11, P=0.739) and MTRR rs1801394 (χ^2=0.50, P=0.480) were in line with the Hardy-Weinberg equilibrium (HWE), while MTR 1805087 (χ^2=59.50, P<0.001) was not.

By logistic regression analysis, we observed that the CT (adjusted OR=2.67, 95% CI=1.60-4.45) and TT (adjusted OR=2.67, 95% CI=1.60-4.45) genotypes of MTHFR rs1801133 were significantly associated with higher risk of breast cancer, compared with the CC genotype (**Table 4**). In addition, the CT+TT of MTHFR rs1801133 was significantly associated with risk of breast cancer in comparison to the CC genotype (adjusted OR=1.43, 95% CI=1.09-1.88). The CC genotype of MTRR rs1801394 showed higher association with increased risk of breast cancer as compared with the AA genotype (adjusted OR=1.96, 95% CI=1.14-3.37). However, MTR 1805087 polymorphism was not seemed to affect the breast cancer risk.

We observed a significant interaction between MTRR rs1801394 polymorphism and physical activity (χ^2=6.39, P=0.041), and a significant interaction between intake of folic acid and

Table 4. Association between MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 genetic polymorphisms and risk of breast cancer

Genotypes	Patients	%	Controls	%	OR (95% CI)	*P* value	Adjusted OR (95% CI)[1]	*P* value
MTHFR rs1801133								
CC	235	50.43	274	58.30	1.0 (Ref.)		1.0 (Ref.)	
CT	172	36.91	168	35.74	2.06 (1.25-3.39)	0.004	2.17 (1.28-3.67)	0.004
TT	59	12.66	28	5.96	2.46 (1.52-3.98)	<0.001	2.67 (1.60-4.45)	<0.001
CT+TT	231	49.57	196	41.70	1.37 (1.06-1.78)	0.016	1.43 (1.09-1.88)	0.011
MTR 1805087								
AA	362	77.68	358	76.17	1.0 (Ref.)		1.0 (Ref.)	
AG	73	15.67	79	16.81	0.93 (0.56-1.55)	0.778	1.10 (0.64-1.90)	0.737
GG	31	6.65	33	7.02	1.02 (0.57-1.82)	0.956	1.17 (0.63-2.20)	0.618
AG+GG	104	22.32	112	23.83	0.92 (0.68-1.25)	0.583	1.02 (0.74-1.40)	0.922
MTRR rs1801394								
AA	259	55.58	251	53.40	1.0 (Ref.)		1.0 (Ref.)	
AG	160	34.33	189	40.21	1.52 (0.93-2.48)	0.095	1.62 (0.96-2.74)	0.072
GG	47	10.09	30	6.38	1.85 (1.12-3.06)	0.017	1.96 (1.14-3.37)	0.015
AG+GG	207	44.42	219	46.60	1.09 (0.84-1.41)	0.504	1.08 (0.82-1.43)	0.57

[1]Adjusted for age, physical activity, menopausal, passive smoking, BMI, benign breast disease and first-degree relative with cancer.

Table 5. Interaction between MTHFR rs1801133 and MTRR rs1801394 and environmental factors in the risk of breast cancer

Variables	MTHFR rs1801133				t or χ^2 value	*P* value	MTRR rs1801394				t or χ^2 value	*P* value
	CC	%	CT+TT	%			AA	%	AG+GG	%		
Physical activity												
Never	330	64.83	260	60.89			304	59.61	286	67.14		
Seldom	88	17.29	85	19.91			99	19.41	74	17.37		
Often	91	17.88	82	19.20	1.65	0.437	107	20.98	66	15.49	6.39	0.041
Menopausal												
Premenopausal	344	67.58	280	65.57			339	66.47	285	66.90		
Postmenopausal	165	32.42	147	34.43	0.42	0.516	171	33.53	141	33.10	0.02	0.889
Passive smoking												
No	282	55.40	218	51.05			268	52.55	232	54.46		
Yes	227	44.60	209	48.95	1.77	0.184	242	47.45	194	45.54	0.34	0.559
BMI												
<24	277	54.42	250	58.55			291	57.06	236	55.40		
≥24	232	45.58	177	41.45	1.61	0.205	219	42.94	190	44.60	0.26	0.610
Benign breast disease												
No	361	70.92	298	69.79			360	70.59	299	70.19		
Yes	148	29.08	129	30.21	0.14	0.705	150	29.41	127	29.81	0.02	0.894
First-degree relative with cancer												
No	447	87.82	380	88.99			448	87.84	379	88.97		
Yes	62	12.18	47	11.01	0.31	0.577	62	12.16	47	11.03	0.29	0.593
Intake of folic acid, μg/d	214.07±245.16		203.53±48.49		23.44	0.001	212.09±44.70		205.87±49.41		2.02	0.044

MTHFR rs1801133 (t=23.44, P=0.001) and MTRR rs1801394 (t=2.02, P=0.044) polymorphisms (**Table 5**).

Discussion

In the present study, we performed a case-control study to examine the association of MTHFR rs1801133, MTR 1805087 and MTRR rs1801394 with susceptibility to breast cancer. In our study, we observed that the MTHFR rs1801133 and MTRR rs1801394 polymorphisms were associated with risk of breast cancer in this Chinese population.

In our study, we observed that those carrying the inactive T allele of MTHFR rs1801133 were associated with higher risk of breast cancer

when compared with the active genotype (CC genotype). MTHFR is an essential enzyme for maintenance of folate hemostasis and homocysteine (Hcy) in the blood [15, 16]. Alteration of the C to T allele in the non-synonymous rs1801133 due to an original mutation changes alanine to valine at amino acid 222 in the N-terminal catalytic domain of the MTHFR protein, leading to a more thermolabile enzyme with a lower enzymatic activity which reduces 5-methyltetrahyrofolate and plasma folate levels, but increases the 5,10-methylenetetrahydrofolate and plasma Hcy levels [17]. Folate plays a key role in nucleotide synthesis of methylation. Decreased of enzyme activity undergoes diminished folate deficiency and aberrant DNA methylation, and is associated with development of cancer [18]. Prevalence of the T allele varies amongst the geographical populations of them Chinese has the highest ratio of this allele [19]. This variety has been highly influenced by the environmental factors such as migration, sexual reproduction, and natural selection together with the epigenetic mechanism [20, 21]. Therefore, MTHFR rs1801133 polymorphism is suggested as a specific susceptibility marker of breast cancer.

We observed that the GG genotype of MTRR rs1801394 genetic polymorphism exposed significantly associated with risk of breast cancer when compared with the AA genotype. The rs1801394 of MTRR is the replacement of A for G at nucleotide 66, and this mutation causes the substitution of isoleucine by methionine. This genetic mutation is lied on the putative flavin mononucleotide-binding domain of the MTRR enzyme, and it has correlation with MTR [11]. Therefore, the broken of binding MTRR to the MTR-cobalamin-complex could reduce the process of homocysteine remethylation [22]. Our study reported that the GG genotype was associated with risk of breast cancer. Previous studies have reported similar results [23, 24]. Wu et al. conducted a study with 96 breast cancer patients and 85 controls, and reported that the MTRR rs1801394 was positively correlated with risk of breast cancer [24]. However, several studies did not find significant association between MTRR rs1801394 and risk of developing breast cancer [25, 26]. Therefore, further sufficiently larger studies are suggested to examine our findings. Moreover, our study found the MTHFR rs1801133 and MTRR rs1801394 polymorphisms were associated with the level of folic acid. Several previous studies have indicated that MTHFR rs1801133 and MTRR rs1801394 polymorphisms have interaction with the serum folate concentration [27-29].

Two limitations of the present study should be acknowledged. First, the breast cancer patients and control subjects were selected from only one hospital, and they may not be sufficiently representative of other populations. Second, our study had limited statistical power due to a small sample size. Therefore, further large-scale studies are needed to confirm our results.

In summary, the results of our study indicate that the MTHFR rs1801133 and MTRR rs1801394 polymorphisms are potential risk factors for the development of breast cancer in the Chinese population, suggesting that these polymorphisms could contribute to the risk of breast cancer.

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Disclosure of conflict of interest

None.

Address correspondence to: Zhaofeng Niu, Department of Galactophore, Yuncheng Central Hospital, No. 173 West Hongqi Sreet, Yuncheng 044-000, Shanxi Province, China. E-mail:niuzhaofeng6@163.com

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