Association between *MTHFR* 677C/T and 1298A/C gene polymorphisms and breast cancer risk

X.F. Zhang[1], T. Liu[2], Y. Li[1] and S. Li[2]

[1]Department of Breast, Liao Ning Cancer Hospital and Institute, Shenyang, China
[2]Department of Radiology, The First Affiliated Hospital of China Medical University, Shenyang, China

Corresponding author: T. Liu
E-mail: liuting255@163.com

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ABSTRACT. We performed a case-control study to investigate the association between single nucleotide polymorphisms in the *MTHFR* gene (677C/T and 1298A/C) and risk of breast cancer. This case-control study included 216 breast cancer cases and 216 controls. The *MTHFR* 677C/T and 1298A/C gene polymorphisms were assessed by polymerase chain reaction restriction fragment length polymorphism. We observed an increased likelihood of breast cancer patients having a higher age at menarche and first live birth, and a greater family history of breast cancer, especially among first-degree relatives. In addition, individuals with the TT genotype of *MTHFR* 677C/T were associated with increased risk of breast cancer by logistic regression analysis; the adjusted odds ratio (95%CI) was 3.05 (1.17-8.87). In conclusion, the results of our study indicated that the *MTHFR* C677T gene polymorphism could play a role in the development of breast cancer.

Key words: MTHFR; Polymorphism; Breast cancer; Risk

INTRODUCTION

Breast cancer is the most frequently diagnosed type of cancer in females, with ~1.38 million new cases estimated to have occurred in 2008, making it the second most common malignancy among females worldwide (10.9% of all cancers). The etiology of breast cancer is not well understood. Many previous studies have shown that genetic factors, such as *BRCA1*, *BRCA2*, *RAD51*, *XRCC2*, and *ERCC5*, may play an important role in the development of breast cancer (Hirotsu et al., 2015; Michalska et al., 2015; Wang et al., 2015).

Previous studies have also reported that low-penetrance susceptibility genes may play an important role in the development of cancer (Lichtenstein et al., 2000). Several low-penetrant genes have been reported to affect the susceptibility of breast cancer, including the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene. The gene encoding MTHFR is located at lp36.3; the *MTHFR* gene has two common polymorphisms, 677C/T and 1298A/C, that affect the enzyme activity (Rosenberg et al., 2002; Rama Devi et al., 2004). Previous studies have reported that the TT genotype of *MTHFR* 677C/T could reduce the enzyme activity by 70% *in vitro*, compared to the CC wild-type; in addition, the CC genotype of *MTHFR* 1298A/C has been found to reduce the *in vitro* enzyme activity compared to the AA wild-type (Frosst et al., 1995; Weisberg et al., 1998; Weisberg et al., 2001).

Previous studies have also reported an association between the *MTHFR* 677C/T and 1298A/C gene polymorphisms and susceptibility to breast cancer (He et al., 2014; Rai, 2014; Wang et al., 2014; López-Cortés et al., 2015); however, these results were inconclusive. Therefore, we conducted a case-control study to investigate the association between *MTHFR* 677C/T and 1298A/C gene polymorphisms and the risk of breast cancer.

MATERIAL AND METHODS

Subjects

This case-control study included 216 breast cancer cases and 216 controls. All cases were newly diagnosed via histopathological confirmation with primary breast cancer at the Tumor Hospital of Liaoning Province between January 2013 and December 2014. Patients with primary tumors other than breast cancer, tumors of an unknown origin, or with any histopathological diagnosis other than breast cancer were excluded from the study. The controls were randomly selected from among individuals who underwent a regular health examination in the hospital. The control subjects were frequency matched to the cases by sex and age (±5 years). All control subjects were confirmed to have no history of cancer or other breast diseases.

The demographic and clinical characteristics of breast cancer patients and control subjects were collected from medical records. A written informed consent was obtained from each participant prior to the commencement of the study. The study was approved by the ethics committee of the Tumor Hospital of Liaoning Province.

Genotype polymorphisms

Five milliliters of blood was obtained from each participant; this was used for genomic DNA extraction. The collected supernatant was refrigerated until further use. The *MTHFR* 677C/T and

1298A/C gene polymorphisms were analyzed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. The primers used for the detection of *MTHFR* 677C/T were 5'-CGTGGCTCCTGCGTTTCC-3' (forward) and 5'-GAGCCGGCCACAGGCAT-3' (reverse), and those used to detect the 1298A/C polymorphism were 5'-CAAATCTGAGGGAGCTGAGT-3' (forward) and 5'-CAGATAAGTGGCAGTACAGA-3' (reverse). The reaction conditions were set as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 45 s, and extension at 72°C for 30 s, and a final extension at 72°C for 5 min.

Statistical analysis

Statistically significant differences in the demographic characteristics of the cases and controls were assessed by the χ^2 test. The Hardy-Weinberg equilibrium (HWE) was determined and tested by Fisher's exact test for the *MTHFR* 677C/T and 1298A/C polymorphisms in the controls. The association between genetic factors and risk of breast cancer was analyzed using logistic regression models adjusted for potential confounding factors; the results of these analyses were expressed as the odds ratio (OR) with 95%CI. A P value < 0.05 was considered to be statistically significant. All data was analyzed using the statistical software SPSS Statistics (v.16.0; SPSS Inc., Chicago, IL, USA).

RESULTS

The demographic characteristics of breast cancer patients and controls are summarized in Table 1. The mean ages of breast cancer patients and controls were 51.4 ± 7.6 and 50.6 ± 8.1 years, respectively. A comparison of the demographic characteristics between breast cancer patients and control subjects revealed the likelihood of breast cancer patients having a higher age at menarche and first live birth, and a greater chance of a family history of breast cancer (among first-degree relatives). However, we observed no significant differences between the patients and controls in terms of the menopausal status, folate intake, and vitamin B_6 and B_{12} intake.

Table 1. Characteristics of breast cancer patients and control subjects.

Variables	Patients	%	Controls	%	*t* or χ^2	P value
Age, years	51.4 ± 7.6		50.6 ± 8.1		1.06	0.15
Age at menarche, years	12.6 ± 2.1		13.1 ± 1.9		2.59	<0.05
Age at first live birth, years	27.7 ± 5.9		24.2 ± 6.4		5.91	<0.05
Menopausal status						
Premenopausal	103	47.69	92	42.59		
Postmenopausal	113	52.31	124	57.41	1.13	0.29
Breast cancer in first-degree relatives						
No	105	48.61	215	99.54		
Yes	11	5.09	1	0.46	17.62	<0.05
Folate intake		514.3 ± 87.5		522.7 ± 92.4	0.97	0.17
Vitamin B_6		0.82 ± 0.18		0.85 ± 0.21	1.59	0.06
Vitamin B_{12}		7.3 ± 3.3		7.6 ± 3.8	0.88	0.19

An analysis of the Hardy-Weinberg equilibrium revealed that the *MTHFR* 677C/T and 1298A/C polymorphisms were in line with the Hardy-Weinberg equilibrium in the control group (Table 2). Logistic regression analysis revealed that the TT genotype of *MTHFR* 677C/T was associated with an increased risk of breast cancer; the adjusted OR (95%CI) for this analysis was

3.05 (1.17-8.87). However, we observed no significant associations between the *MTHFR* 1298A/C gene polymorphism and risk of breast cancer.

Table 2. Association between the *MTHFR* 677C/T and 1298A/C gene polymorphisms and risk of breast cancer.

Genotypes	Cases	%	Controls	%	P value for Hardy-Weinberg equilibrium	OR (95%CI)[1]	P value
MTHFR 677C/T							
CC	114	52.78	128	59.26		1.0 (Ref.)	-
CT	83	38.43	81	37.50		1.15 (0.76-1.74)	0.49
TT	19	8.80	7	3.24	0.17	3.05 (1.17-8.87)	0.01
CT + TT	102	47.22	88	40.74		1.30 (0.87-1.94)	0.17
MTHFR 1298A/C							
AA	98	45.37	105	48.61		1.0 (Ref.)	-
AC	87	40.28	84	38.89		1.11 (0.72-1.70)	0.62
CC	31	14.35	27	12.50	0.12	1.23 (0.66-2.31)	0.49
AC + CC	118	54.63	111	51.39		1.14 (0.77-1.69)	0.5

[1]Adjusted for age, age of menarche and age of first live birth. OR = odds ratio; CI = confidence interval.

DISCUSSION

It is well known that individuals may not develop the same type of cancer despite being exposed to similar environmental conditions. Therefore, genetic variations may play an important role in the development of cancers. Previous epidemiological studies have reported that single nucleotide polymorphisms in the candidate genes could contribute to cancer risk. Many studies have reported an association between the *MTHFR* 677C/T and 1298A/C gene polymorphisms and susceptibility to breast cancer (He et al., 2014; Rai, 2014; Wang et al., 2014; López-Cortés et al., 2015); however, these results are inconsistent because of the small sample size used. In our study, the TT genotype of *MTHFR* 677C/T was found to be associated with an increased risk of breast cancer.

MTHFR is an important gene involved in one-carbon and folate metabolism; this gene also plays a role in DNA synthesis and homocysteine re-methylation (Koppen et al., 2010; Nikbakht et al., 2012). Polymorphisms in the *MTHFR* gene could alter the enzyme activity and influence the capacity of the MTHFR enzyme, thereby altering the folate metabolism, and DNA synthesis and repair. Previous studies have reported an association between the polymorphisms in the *MTHFR* gene and increased risk of several types of cancers, such as head and neck cancer, childhood leukemia, lung cancer, colon cancer, and skin cancer (Deng et al., 2014; Fang et al., 2014; Niu et al., 2015; Pei et al., 2015; Wang et al., 2015). However, the results of these studies have been inconsistent, with the differences generally being attributed to the different types of cancers, sample size, and study populations.

Several studies have also reported an association between polymorphisms in the *MTHFR* gene and breast cancer risk (He et al., 2014; Rai, 2014; Wang et al., 2014). Wang et al. (2014) discovered an association between *MTHFR* C677T polymorphisms and increased risk of breast cancer in a case-control study with 230 patients and control subjects each. On the other hand, He et al. (2014) reported that the TT genotype of *MTHFR* C677T was associated with a higher risk of breast cancer, compared to the CC genotype. A recent meta-analysis also reported an association between the *MTHFR* C677T gene polymorphism and increased risk of breast cancer (Zhong et al., 2014). Another meta-analysis of 57 studies reported that the *MTHFR* C677T gene polymorphism contributed to the development of breast cancer (Li et al., 2014). Therefore, the results of our study are in line with those of previous studies.

In conclusion, the results of our study indicated that the *MTHFR* C677T gene polymorphism could contribute to the development of breast cancer, which suggests that this polymorphism could influence the etiology of breast cancer. However, studies with larger sample sizes are required to confirm these findings.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Deng F, Gao Y, L V JH and Gao JM (2014). Methylenetetrahydrofolate reductase gene polymorphisms and skin cancer risk: a meta-analysis. *Cancer Genet.* 207: 299-305.

Fang XY, Xu WD, Huang Q, Yang XK, et al. (2014). 5,10-Methylenetetrahydrofolate reductase polymorphisms and colon cancer risk: a meta-analysis. *Asian Pac. J. Cancer Prev.* 15: 8245-8250.

Frosst P, Blom HJ, Milos R, Goyette P, et al. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.* 10: 111-113.

He JM, Pu YD, Wu YJ, Qin R, et al. (2014). Association between dietary intake of folate and MTHFR and MTR genotype with risk of breast cancer. *Genet. Mol. Res.* 13: 8925-8931.

Hirotsu Y, Nakagomi H, Sakamoto I, Amemiya K, (2015). Detection of BRCA1 and BRCA2 germline mutations in Japanese population using next-generation sequencing. *Mol. Genet. Genomic Med.* 3: 121-129.

Koppen IJ, Hermans FJ and Kaspers GJ (2010). Folate related gene polymorphisms and susceptibility to develop childhood acute lymphoblastic leukaemia. *Br. J. Haematol.* 148: 3-14.

Li K, Li W and Dong X (2014). Association of 677 C>T (rs1801133) and 1298 A>C (rs1801131) polymorphisms in the MTHFR gene and breast cancer susceptibility: a meta-analysis based on 57 individual studies. *PLoS One* 9: e71290.

Lichtenstein P, Holm NV and Verkasalo PK (2000). Environmental and heritable factors in the causation of cancer. *N. Engl. J. Med.* 343: 78-85.

López-Cortés A, Echeverría C, Oña-Cisneros F, Sánchez ME, et al. (2015). Breast cancer risk associated with gene expression and genotype polymorphisms of the folate-metabolizing MTHFR gene: a case-control study in a high altitude Ecuadorian mestizo population. *Tumour Biol.* [Epub ahead of print].

Michalska MM, Samulak D, Romanowicz H and Smolarz B (2015). Single nucleotide polymorphisms (SNPs) of RAD51-G172T and XRCC2-41657C/T homologous recombination repair genes and the risk of triple-negative breast cancer in Polish women. *Pathol. Oncol. Res*. [Epub ahead of print].

Nikbakht M, MalekZadeh K, Kumar JA, Askari M, et al. (2012). Polymorphisms of *MTHFR* and *MTR* genes are not related to susceptibility to childhood ALL in North India. *Exp. Oncol.* 34: 43-48.

Niu YM, Deng MH, Chen W, Zeng XT, et al. (2015). MTHFR C677T gene polymorphism and head and neck cancer risk: a meta-analysis based on 23 publications. *Dis. Markers.* 2015: 681313.

Pei JS, Hsu CM, Tsai CW, Chang WS, et al. (2015). The association of methylenetetrahydrofolate reductase genotypes with the risk of childhood leukemia in Taiwan. *PLoS One* 10: e0119776.

Rai V (2014). The methylenetetrahydrofolate reductase C677T polymorphism and breast cancer risk in Asian populations. *Asian Pac. J. Cancer Prev.* 15: 5853-5860.

Rama Devi AR, Govindaiah V, Ramakrishna G and Naushad SM (2004). Prevalence of methylene tetrahydrofolate reductase polymorphism in South Indian population. *Curr. Sci.* 86: 440-443.

Rosenberg N, Murata M, Ikeda Y, Opare-Sem O, et al. (2002). The frequent 5,10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in Whites, Japanese, and Africans. *Am. J. Hum. Genet.* 70: 758-762.

Wang H, Wang T, Guo H, Zhu G, et al. (2015). Association analysis of ERCC5 gene polymorphisms with risk of breast cancer in Han women of northwest China. *Breast Cancer* [Epub ahead of print].

Wang X, Yue K and Hao L (2015). Meta-analysis of methylenetetrahydrofolate reductase polymorphism and lung cancer risk in Chinese. *Int. J. Clin. Exp. Med.* 8: 1521-1525.

Wang ZG, Cui W, Yang LF, Zhu YQ, et al. (2014). Association of dietary intake of folate and MTHFR genotype with breast cancer risk. *Genet. Mol. Res.* 13: 5446-5451.

Weisberg I, Tran P, Christensen B, Sibani S, et al. (1998). A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol. Genet. Metab.* 64: 169-172.

Weisberg IS, Jacques PF, Selhub J, Bostom AG, et al. (2001). The 1298A.C polymorphism in methylene tetrahydrofolate reductase (MTHFR): *in vitro* expression and association with homocysteine. *Atherosclerosis* 156: 409-415.

Zhong S, Chen Z, Yu X, Li W, et al. (2014). A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in breast cancer. *Mol. Biol. Rep.* 41: 5775-5785.