Relationship between genetic polymorphisms of methylenetetrahydrofolate reductase and breast cancer chemotherapy response

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ABSTRACT. Activity of methylenetetrahydrofolate reductase (MTHFR), an enzyme involved in folate metabolism, is influenced by mutations in the corresponding gene, contributing to a decrease in 5,10-MTHF. Due to such polymorphisms, individuals differ in MTHFR enzyme activity and plasma folate levels. We investigated the relationship between two common *MTHFR* polymorphisms (C677T and A1298C) and breast cancer (BC) chemotherapy response. From February 2013 to January 2016, 148 advanced BC patients at the Center Hospital of Cangzhou were enrolled and treated with six different chemotherapy regimens. Subjects were genotyped using polymerase chain reaction-restriction fragment length polymorphism. Forty-one (27.7%), 70 (47.3%), and 37 (25.0%) patients carried the C/C, C/T, and

T/T C677T genotypes, respectively; 101 (68.2%), 42 (28.4%), and 5 (3.4%) had the A/A, A/C, and C/C genotypes of A1298C, respectively. Total chemotherapy efficacy was 66.9% (99/148), with 7 (4.7%), 92 (62.2%), 36 (24.3%), and 13 (8.8%) cases showing complete response, partial response, no change, and progressive disease, respectively. Chemotherapy regimens did not differ in effectiveness ($P > 0.05$). Efficacy rates associated with C677T C/C, C/T, and T/T genotypes were 58.5, 58.6, and 91.9%, respectively, with T/T carriers exhibiting significantly better responses than the C/C ($P < 0.05$) and C/T groups ($P < 0.05$). Effectiveness among A1298C A/A, A/C, and C/C carriers was 70.6, 64.3, and 0.0%, respectively, but no difference was established between these genotypes in this regard ($P > 0.05$). The *MTHFR* C677T genotype may be associated with BC chemotherapy response, and could be of great value in guiding individualized treatment for this disease.

INTRODUCTION

DNA methylation can affect gene expression, and is closely associated with tumor incidence and development. Changes in this process, in which the provision of active methyl by folic acid metabolism is crucial, play an important role in carcinogenesis and contribute to the deactivation of tumor suppressor genes (Kim et al., 1997; Jacob et al., 1998). Methylenetetrahydrofolate reductase (MTHFR), an enzyme involved in folate metabolism (Bailey and Gregory, 1999), reduces 5,10-MTHF to 5-methyltetrahydrofolate, the predominant circulating folate form and substrate for the remethylation of homocysteine to methionine, which leads to the subsequent production of *S*-adenosylmethionine, the universal methyl group donor required for DNA methylation (Kim, 2000).

The C677T MTHFR polymorphism is common in breast cancer, and its frequency differs dramatically between countries, areas, and nations. For example, some studies estimate the frequency of the 677T allele to be 34% among Asians, including Han Chinese people, and approximately 22 to 44% in European populations (Botto and Yang, 2000; Song et al., 2001). In addition, this variant has been shown to influence enzyme activity (Frosst et al., 1995), possibly contributing to folic acid metabolism disorder, and the interaction between this polymorphism and low folate intake has been identified as a potential breast cancer risk factor (Shrubsole et al., 2004). Moreover, a number of reports have suggested that *MTHFR* variants are associated with methotrexate chemotherapy toxicity and 5-fluorouracil (5-FU) efficiency in patients with breast cancer, gastrointestinal carcinoma, and leukemia (Toffoli et al., 2000, 2003; Ruzzo et al., 2006; Shitara et al., 2010).

The *MTHFR* gene, comprising 11 exons and 10 introns, is located on chromosome 1p36.3. To date, 20 polymorphic sites have been identified in this sequence, some of which cause a decrease in enzyme activity (Gao et al., 2009; Ma et al., 2009). The C677T and A1298C variants are a particular focus of study, and have been reported to have a close link to several human diseases. It has been found that the enzyme activity of C677T heterozygotes is greater than that of individuals homozygous for the variant allele (Frosst et al., 1995). Furthermore,

the 677TT genotype is closely connected with higher plasma homocysteine and lower folate levels (Deloughery et al., 1996). Another common *MTHFR* polymorphism, A1298T, might also affect the encoded enzyme's specific activity, homocysteine levels, and plasma folate concentration, although to a lesser extent than the C677T variation (van der Put et al., 1998).

Breast cancer chemotherapy has developed remarkably as the drugs used to treat this disease have improved. To date, cyclophosphamide (CTX), anthracycline, 5-FU, and paclitaxel (PTX) combination therapy has been the standard treatment for breast cancer. Chemotherapy has many advantages, not only ameliorating the symptoms of advanced breast cancer, but also prolonging survival time, although individuals can exhibit different responses to the same regimen. Thus, some researchers have focused on variations in drug sensitivity between cases in terms of pharmacogenetics and pharmacogenomics to establish a reliable prediction index and guide individualized treatment. This approach will be of significance to the enhancement of chemotherapy drug efficacy and safety. Therefore, we aimed to analyze the relationship between *MTHFR* and the sensitivity of breast cancer to chemotherapy.

MATERIAL AND METHODS

Study subjects

Between February 2013 and September 2015, 148 patients were recruited from the Cangzhou Central Hospital and all were confirmed as having breast cancer by pathology and cytology. This group comprised Han Chinese women, 29 to 71 years old, with a median age of 50 years. Thirty-nine had previously received chemotherapy, and 58 neoadjuvant chemotherapy. Measurable lesions were observed in the primary foci (62 cases), lymph nodes (57), liver (9), lung (21), and other regions (11). Chemotherapy regimens were as follows: CAF [CTX + epirubicin (EADM) + 5-FU; N = 41], AT [EADM + PTX; N = 39], NP [vinorelbine (NVB) + cisplatin (DDP); N = 19], NA [NVB + EADM; N = 17], CAP [CTX + EADM + DDP; N = 16], and TP [PTX + DDP; N = 16]. All subjects were confirmed as having measurable lesions by computed tomography. Routine blood examination results and liver and kidney function of the study subjects were within normal range. Moreover, electrocardiogram tests of patients revealed no significant abnormalities, and performance status scores were all over 60 points. All participants signed a consent form.

MTHFR genotyping

Blood (2 mL) was obtained from all subjects for genomic DNA extraction using a QIAGEN DNA Blood Mini Kit (QIAGEN, Inc., Valencia, CA, USA) following the manufacturer protocol. DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm.

MTHFR variants were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. Genomic DNA was amplified by PCR using primers specific for the C677T and A1298C regions, and polymorphisms were detected by restriction enzyme digestion (Table 1). The cycling conditions employed for each sequence variant are shown in Table 2. The resulting fragments were analyzed on 2-2.5% agarose gels.

Table 1. Primer sequences and methods used for detection of *MTHFR* gene polymorphisms.		
C677T	Forward: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'; Reverse: 5'-AGGACGGTGCGGTGAGAGTG-3'	*Hin*fI-based RFLP
A1298C	Forward: 5'-CTTTGGGGAGCTGAAGGACTACTAC-3'; Reverse: 5'-CACTTTGTGACCATTCCGGTTTG-3'	*Mbo*II-based RFLP

RFLP = restriction fragment length polymorphism.

Table 2. Polymerase chain reaction (PCR) conditions for amplification of the *MTHFR* gene.	
Locus	PCR conditions
C677T	94°C (2 min); 40 cycles: 94°C (30 s), 62°C (30 s), 72°C (30 s); 72°C (7 min)
A1298C	94°C (5 min); 35 cycles: 95°C (30 s), 58°C (30 s), 72°C (30 s); 72°C (7 min)

Chemotherapy regimens

The above mentioned chemotherapy regimens were administered in a 3-week period and curative effect was evaluated after two cycles of treatment.

Evaluation of treatment

According to the World Health Organization efficacy standards for solid tumors, treatment outcome was categorized as complete response (CR), partial response (PR), no change (NC), or progressive disease (PD). Therapy resulting in CR or PR was considered effective.

Statistical analysis

Polymorphism frequency data were evaluated for deviation from Hardy-Weinberg equilibrium. Data are reported as medians and ranges or means and standard deviations. All tests were two-tailed, and P values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

MTHFR genotype frequencies and total chemotherapy efficacy

Among the 148 patients, the distribution of *MTHFR* C677T genotypes was as follows: C/C, 41 cases (27.7%); C/T, 70 (47.3%); and T/T, 37 (25.0%). For the A1298C variant, the following genotype frequencies were observed: A/A, 101 cases (68.2%); A/C, 42 (28.4%); and C/C, 5 (3.4%). Total chemotherapy efficacy was 66.9% (99/148), with CR being observed in 7 patients (4.7%), PR in 92 (62.2%), NC in 36 (24.3%), and PD in 13 (8.8%) (Table 3).

Relationship between *MTHFR* genotype and chemotherapy response

There were no statistically significant differences between the six chemotherapy regimens in terms of efficacy (P = 0.41). The effectiveness rates associated with *MTHFR* C677T C/C, C/T, and T/T genotypes were 58.5, 58.6, and 91.9%, respectively, with T/T carriers showing significantly better responses than individuals with C/C (P < 0.05) and C/T

genotypes (P < 0.05). The effectiveness of chemotherapy for patients with A/A, A/C, and C/C *MTHFR* A1298C genotypes was 70.6, 64.3, and 0.0%, respectively, although no statistically significant difference was detected between these groups (P > 0.05; Table 3).

Table 3. Chemotherapy efficiency rate in patients with different *MTHFR* genotypes.

	C677T (%)			A1298C (%)		
	C/C	C/T	T/T	A/A	A/C	C/C
CR	2 (4.9)	5 (7.1)	0 (0)	2 (2.0)	5 (11.9)	0 (0)
PR	22 (53.6)	36 (51.4)	34 (91.9)	70 (69.4)	22 (52.4)	0 (0)
NC	15 (36.6)	19 (27.1)	2 (5.4)	24 (23.8)	10 (23.8)	3 (60.0)
PD	2 (4.9)	10 (14.4)	1 (2.7)	5 (5.0)	5 (11.9)	2 (40.0)
Total efficiency rate	24 (58.5)	41 (58.6)	34 (91.9)	72 (70.6)	27 (64.3)	0 (0)

CR = complete response; PR = partial response; NC = no change; PD = progressive disease.

Relationship between *MTHFR* genotype and efficacy of different chemotherapy regimens

The effectiveness rates of the CAF, AT, NP, NA, CAP, and TP regimens were 58.5 (24/41), 87.2 (34/39), 63.2 (12/19), 52.9 (9/17), 43.8 (7/16), and 81.3% (13/16), respectively, but no significant difference was established (P > 0.05). As shown in Table 4, the efficacy of all regimens except that of CAP was 100% in the T/T genotype group; however, owing to the small number of patients for each treatment, no statistically significant difference was observed. Moreover, no patient undergoing AT, NA, CAP, or TP chemotherapy carried the A1298C C/C genotype, and none in the CAP group had the A/C genotype. In each chemotherapy regimen subgroup, treatment effectiveness did not significantly differ according to the *MTHFR* A1298C genotype.

Table 4. Relationship between the *MTHFR* genotype and chemotherapy efficacy.

Regimen	N	C677T (%)				A1298C (%)			
		C/C	C/T	T/T	P	A/A	A/C	C/C	P
CAF	41	7 (63.6)	10 (57.1)	7 (100.0)	0.37	17 (70.0)	7 (50.0)	0 (0)	0.45
AT	39	7 (100.0)	12 (72.1)	15 (100.0)	0.68	24 (88.9)	10 (80.0)	-	-
NP	19	4 (100.0)	5 (40.0)	2 (100.0)	0.65	10 (100.0)	2 (33.3)	0 (0)	0.07
NA	17	2 (28.6)	2 (50.0)	5 (100.0)	0.68	7 (50.0)	2 (100.0)	-	-
CAP	16	0 (0)	5 (71.4)	3 (42.9)	-	7 (50.0)	-	-	-
TP	16	2 (100.0)	7 (60.0)	2 (100.0)	-	7 (60.0)	6 (100.0)	-	-

CAF = cyclophosphamide (CTX) + epirubicin (EADM) + 5-fluorouracil (5-FU); AT = EADM + paclitaxel (PTX); NP = vinorelbine (NVB) + cisplatin (DDP); NA = NVB + EADM; CAP = CTX + EADM + DDP; TP = PTX + DDP.

DISCUSSION

The *MTHFR* C677T and A1298C polymorphisms are of clinical significance, and a considerable number of studies have focused on the former. The C677T variant consists of a C-to-T substitution at position 677, causing the replacement of alanine with valine in the translated protein, leading to decreased enzyme stability and activity. With the development of chemotherapy drugs, chemotherapy currently employed has had remarkable achievements in breast cancer treatment. However, the effects of the same chemotherapy may differ between patients. Researchers have therefore proposed that pharmacogenetics and pharmacogenomics might contribute to such drug sensitivity differences between individuals, and could be used to establish a reliable prediction index to guide personalized chemotherapy.

Some studies have found that *MTFHR* polymorphisms correlate with chemotherapy toxicity and the clinical efficacy of 5-FU (Marsh et al., 2000; Lu et al., 2004; Ma et al., 2009). In addition, the C677T variation in this gene has been reported to be associated with the survival of patients with advanced breast cancer, although the A1298C polymorphism was not found to be significantly related to the mortality rate of this disease (Marsh et al., 2000). Martin et al. (2006) concluded that these *MTHFR* sequence variants do influence patient survival in breast cancer cases, with different genotypes exerting divergent effects on disease outcome, and that this relationship demonstrates race specificity. Shrubsole et al. (2005) reported that the A1298C genotype does not affect risk of death, but the C677T polymorphism may modify breast cancer survival.

In this study, we found that the effectiveness of chemotherapy for patients with the *MTHFR* C677T T/T genotype was significantly higher than for those carrying the C/C or C/T genotype. An analysis of various regimens revealed that the A1298C polymorphism of this gene had no significant relationship with breast cancer chemotherapy efficacy, although some differences were noted in this respect. This variant might therefore be a potential marker to predict chemotherapy response of breast cancer patients, albeit with lower sensitivity than the *MTHFR* C677T polymorphism. van der Put et al. (1998) reported that the effects of the A1298C variation on the cellular level of folic acid are significantly lower than those of the C677T polymorphism, thus the former's impact on chemotherapy efficacy is also weaker than the latter's, consistent with our results.

In conclusion, the *MTHFR* C677T polymorphism demonstrates greater utility for the development of individualized breast cancer treatment. Future studies using larger sample sizes are needed to confirm this result and improve the applied value of this sequence variation.

Conflicts of interest

The authors declare no conflict of interest.

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