Polymorphisms of MTHFR and TYMS predict capecitabine-induced hand-foot syndrome in patients with metastatic breast cancer

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Abstract

Background: Breast cancer is a global problem, and a large number of new cases are diagnosed every year. Capecitabine is effective in patients with metastatic breast cancer (MBC). Hand-foot syndrome (HFS) is a common adverse effect of capecitabine. In this study, we investigated the association between single nucleotide polymorphisms (SNPs) in genes involved in capecitabine metabolism pathways and capecitabine-induced HFS in Chinese patients with MBC to identify some predictive genetic biomarkers.

Methods: We selected 3 genes involved in capecitabine metabolism and screened genetic variants in these target genes. We genotyped a total of 22 SNPs in the thymidylate synthase gene (TYMS), the methylene tetrahydrofolate reductase gene (MTHFR), and the ribonucleotide reductase M1 gene (RRM1) in 342 MBC patients treated with capecitabine-based chemotherapy. The genotype distributions of each SNP in patients with and without HFS were assessed using Pearson’s χ2 test, and the relationship between HFS and genotypes of SNPs was determined using logistic regression analysis. The association between SNPs and their corresponding gene expression was analyzed using the Blood expression quantitative trait loci (eQTL) browser online tools.

Results: We found 4 positive sites for HFS in the TYMS and MTHFR genes: TYMS rs2606241 (P = 0.022), TYMS rs2853741 (P = 0.019), MTHFR rs3737964 (P = 0.029), and MTHFR rs4846048 (P = 0.030). Logistic regression analyses showed that the genotype AG of MTHFR rs3737964 [odds ratio (OR) = 0.54, 95% confidence interval (CI) 0.31–0.97, P = 0.038] and MTHFR rs4846048 (OR = 0.54, 95% CI 0.30–0.98, P = 0.042) were protective factors of HFS, whereas the genotype CT of TYMS rs2853741 (OR = 2.25, 95% CI 1.31–3.87, P = 0.012) increased the risk of HFS. The association between the genotype GT of TYMS rs2606241 (OR = 1.27, 95% CI 0.73–2.23, P = 0.012) and HFS was uncertain. Further eQTL analyses confirmed that the alleles of rs3737964 and rs4846048 affected the gene expression levels of MTHFR in cis.

Conclusions: We have identified four potentially useful pharmacogenetic markers, TYMS rs2606241, TYMS rs2853741, MTHFR rs3737964, and MTHFR rs4846048 to predict capecitabine-induced HFS in MBC patients.

Keywords: Capecitabine, Polymorphism, Metastatic breast cancer, Hand-foot syndrome, TYMS, MTHFR

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Background

Breast cancer is a global problem, and 1.7 million new cases are diagnosed per year [1]. Approximately 6%–10% of breast cancer patients present metastatic disease when initially diagnosed, and over 30% of patients with non-metastatic disease will relapse [2]. Capecitabine (N4-pentyloxy carbonyl-5'-deoxy-5-fluorocytidine) has been widely used for the treatments of breast [3, 4], colon [5], and gastric cancers [6], and has also been considered as an option for hepatocellular carcinoma [7] and rectal cancer [8]. Capecitabine is often administered as second-line monotherapy for metastatic breast cancer (MBC) patients whose disease is resistant to anthracycline, taxane, or both [9]. The common capecitabine-induced adverse events include hand-foot syndrome (HFS), increased bilirubin, diarrhea, stomatitis, nausea, neutropenia, and cardiotoxicity [10, 11]. Despite not life-threatening, HFS, characterized by tenderness, redness, and swelling of palms and soles, can be very debilitating and impair the quality of life. Although HFS is manageable, if it is not handled well, it can deteriorate rapidly and lead to treatment interruptions which may affect the treatment efficacy [12].

Capecitabine, a novel oral fluoropyrimidine carbamate, may be converted to 5-flourouracil (5-FU) selectively in tumors through a cascade of different enzymes [13]. Thymidylate synthase (TYMS), methylene tetrahydrofolate reductase (MTHFR), and ribonucleotide reductase M1 (RRM1) are involved in capecitabine metabolism pathways. The metabolic pathways by which 5-FU and the prodrug capecitabine are converted to active nucleotide analogues are described in details [14]. TYMS catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxothymidine monophosphate (dTMP), MTHFR converts 5-10 methylentetrahydrofolate (5-10 MTHF) into 5-methyltetrahydrofolate (5-MeTHF) [14], and RRM1 is involved in ribonucleotide reductase reaction converting fluorouridine diphosphate (FUDP) to fluoro deosyuridine diphosphate (FdUDP) [15]. Single nucleotide polymorphisms (SNPs) are used in researches comparing genotype frequencies and copy number variations between cases and controls to identify new cancer-susceptible genes and potential markers predicting therapy response and drug resistance. Any failure in the metabolism system, with a special distinction of SNP genotypes present in the drug-metabolic genes, might result in drug resistance or therapy toxicity.

The availability of tools for predicting toxicity would allow physicians to choose proper treatment regimens to elicit a positive response while keeping adverse effects under acceptable levels. There have been several studies about the biomarkers predicting toxicity of capecitabine, which have been mainly focused on a limited number of known candidates, such as dihydropyrimidine dehydrogenase gene (DPYD) [16, 17] and cytidine deaminase gene (CDD) [18], based on white patient population. However, until recently, only a limited number of SNPs in MTHFR [19], TYMS [17], and no SNPs in RRM1 genes associated with HFS have been identified. Accordingly, we genotyped 22 SNPs in MTHFR, TYMS, and RRM1 genes involved in capecitabine metabolism pathways and combined the medical data with the experimental results in hope of seeking potential genetic markers, which may help identify candidate patients suitable for capecitabine treatment, thus promoting the benefit from capecitabine and avoiding severe adverse effects.

Patients and methods

Patient selection

The study was conducted on female MBC patients admitted to the National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College (Beijing, China) between January 2010 and September 2012. All patients received capecitabine-based therapy (capecitabine: 1000 mg/m² orally twice daily, on days 1–14), mostly in combination with docetaxel (75 mg/m², 1-h intravenous infusion on day 1) and vinorelbine (25 mg/m² for 20-min intravenous infusion or 60 mg/m² orally on days 1 and 8) every 3 weeks as one cycle. The inclusion criteria were as follows: female patients with MBC confirmed by pathological or cytological techniques; patients without other malignant cancers; patients eligible for capecitabine-based therapy; complete medical records including age at diagnosis, tumor size, lymph node status, stage, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, menstrual situation, and previous locoregional or systemic therapy; and complete follow-up data. Regular outpatient or telephone follow-ups were carried out, and the last follow-up was March 1, 2014. Follow-up examinations included computed tomography (CT) of metastases, breast ultrasounds, and tumor marker examination. Treatment responses were evaluated every two cycles of treatment (21 days per cycle) according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.0.

SNP selection

We selected genes possibly related to toxicity of capecitabine-based chemotherapy in MBC patients, according to the metabolism pathway of capecitabine. Three candidate genes were selected: TYMS, MTHFR, and RRM1. A total of 22 SNPs from the public database (http://hapmap.ncbi.nlm.nih.gov/) and the 1000 Genomes Project database (http://www.1000genomes.org) [20] in 3 key genes
in the Chinese Han population were genotyped. All loci were in the balance of Hardy–Weinberg ($P > 0.05$), with minor allele frequency greater than 0.05.

**DNA extraction**

We collected 2 mL heparin-anticoagulated blood samples from all participants, and extracted DNA from peripheral blood leucocytes by the phenol–chloroform method using a blood DNA kit (BioTeke Corporation, Beijing, China). The blood samples were stored at $-80 \degree C$, then added to a centrifuge tube after melting at room temperature. The tube was tightly capped and centrifuged for 15 min at $5000 \times g$. The supernatant was discarded, and the pellet was suspended with lysis buffer containing 10 mmol/L Tris–HCl (pH 8.0), 0.1 mol/L ethylene diamine tetraacetic acid, 20 μg/mL RNA enzyme, and 0.5% sodium-dodecyl sulphate for 1 h at 37 °C. The cellular lysates were digested overnight at 37 °C with proteinase K (100 μg/mL). After digestion, samples were subsequently blended with a same volume of Tris–HCl-satured phenol (pH 7.4) and centrifuged for 15 min at 8000×g.

The aqueous phase was added with a same volume of phenol–chloroform (1:1) in a new tube and centrifuged for 15 min at 8000×g. The aqueous phase was collected again in a new tube and precipitated with a 10% volume of ammonium acetate (10 mol/L) and a two times volume of absolute ethanol at $-20 \degree C$. The pellet was rinsed with 75% ethanol twice and resuspended with appropriate TE buffer. The concentration of extracted DNA was assessed using a spectrophotometer. According to Sequenom, the concentration should be higher than 10 ng/μL, and the $A_{260}/A_{280}$ ratio between 1.8 and 2.0. The extracted DNA samples were stored in 1.5-mL EP tubes at $-80 \degree C$.

**SNP genotyping**

Polymerase chain reaction (PCR) and extension primers were designed according to Assay Design 3.1 software (Sequenom Inc., San Diego, CA, USA) and synthesized by the Beijing Genomics Institute (Beijing, China). High-throughput MassARRAY spectrometry platform (Sequenom Inc.) was used for SNP genotyping. The homogeneous Mass EXTEND (hME) reaction is shown in Fig. 1. Following PCR amplification of a locus of interest, a primer extension was performed using an hME primer that was designed to anneal next to the SNP. The key feature of the scheme was the use of a terminator mixture that yielded allele-specific extension products differing in length and mass by at least one nucleotide. The genotyping was completed by Bomiao Biological Technology (Beijing, China). The polymorphisms genotyped are shown in Table 1. For quality control, we

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**Fig. 1** The homogeneous Mass EXTEND (hME) reaction. A deoxyguanosine triphosphate (dGTP) was used along with terminators dideoxadenosine 5′-triphosphate (ddATP), dideoxyctidine 5′-triphosphate (ddCTP), and dideoxymethylidine 5′-triphosphate (ddTTP). For the T allele (T), ddATP was incorporated, extending the primer to a 24-mer. For the C allele (C), dGTP was incorporated prior to the termination of the extension by incorporation of a ddATP, yielding a 25-mer. U primer marked unextended primer
| Gene | Localization | SNP | PCR primer (5′ → 3′) | hME primer (5′ → 3′) |
|------|--------------|-----|----------------------|---------------------|
| **TYMS** | 18p11.32 | 2790 | First: ACGTTGGATGGGATGCGAGGTAAGAGTTC  
Second: ACGTTGGATGAGGGGAGATCGACGACAG  | GATTTTGACCTAGTCTCC |
| | | 2,853,741 | First: ACGTTGGATGGGAAAGAGATCGACGACAG  
Second: ACGTTGGATGAGGGGAGATCGACGACAG  | GGACCAGCTTTCTGGTCCTGGT |
| | | 2,606,241 | First: ACGTTGGATGCGAGAGAAGAGGTCTAGT  
Second: ACGTTGGATGCGAGAGAAGAGGTCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 3,786,362 | First: ACGTTGGATGGGACACGACAGTCTAGT  
Second: ACGTTGGATGCGAGAGAAGAGGTCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,004,474 | First: ACGTTGGATGAAAGAGAGAGATTGCTAGT  
Second: ACGTTGGATGAAAGAGAGAGATTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 9,947,507 | First: ACGTTGGATGACGAGAGAGATTGCTAGT  
Second: ACGTTGGATGACGAGAGAGATTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 699,517 | First: ACGTTGGATGGGACACGACAGTCTAGT  
Second: ACGTTGGATGCGAGAGAAGAGGTCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 9,967,368 | First: ACGTTGGATGGGACACGACAGTCTAGT  
Second: ACGTTGGATGCGAGAGAAGAGGTCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,587 | First: ACGTTGGATGACGAGAGAGATTGCTAGT  
Second: ACGTTGGATGACGAGAGAGATTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| **MTHFR** | 1p36.3 | 1,801,133 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,801,131 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 3,737,964 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 4,846,049 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 2,274,976 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 3,753,582 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 4,846,048 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 725,519 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 720,106 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,042,858 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,042,927 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,980,412 | First: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  
Second: ACGTTGGATGCTTAGGGAAGAGTGCTAGT  | GGACCAGCTTTCTGGTCCTGGT |
| **RRM1** | 11p15.5 | 725,519 | First: ACGTTGGATGCTTACTAGGAAAGATGGT  
Second: ACGTTGGATGCTTACTAGGAAAGATGGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 720,106 | First: ACGTTGGATGCTTACTAGGAAAGATGGT  
Second: ACGTTGGATGCTTACTAGGAAAGATGGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,042,858 | First: ACGTTGGATGCTTACTAGGAAAGATGGT  
Second: ACGTTGGATGCTTACTAGGAAAGATGGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,042,927 | First: ACGTTGGATGCTTACTAGGAAAGATGGT  
Second: ACGTTGGATGCTTACTAGGAAAGATGGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 11,030,918 | First: ACGTTGGATGCTTACTAGGAAAGATGGT  
Second: ACGTTGGATGCTTACTAGGAAAGATGGT  | GGACCAGCTTTCTGGTCCTGGT |
| | | 1,980,412 | First: ACGTTGGATGCTTACTAGGAAAGATGGT  
Second: ACGTTGGATGCTTACTAGGAAAGATGGT  | GGACCAGCTTTCTGGTCCTGGT |

TYMS thymidylate synthase, MTHFR methylene tetrahydrofolate reductase, RRM1 ribonucleotide reductase M1
designed parallel group and blank group (DNA-free), and the reappear rate of the parallel group was 100%. Besides, all trial and analysis personnel were blinded to disease condition of all samples.

**Statistical analysis**

Statistical analysis was performed using SPSS 19.0 and SNPStats softwares (IBM Inc., Chicago, IL, USA). The PLINK software was used to calculate genotype frequency, P value, and 95% confidence interval (CI). Hardy–Weinberg Equilibrium (HWE) was checked using the χ² test or Fisher’s test. The loci deviated from HWE were excluded. The associations between polymorphisms and toxicity were estimated by unconditional logistic regression, and odds ratios (OR) and 95% CI were calculated. The ORs were adjusted for potential confounders, such as age and menstruation. All statistical tests were two-sided and considered significant when P was less than 0.05. Furthermore, we also examined the potential expression quantitative trait loci (eQTL) effects of the significant SNPs by extracting data from the Blood eQTL browser (https://www.genenetwork.nl/bloodeqtlbrowser/) [21].

**Results**

**Patient characteristics**

A total of 342 female patients with MBC were enrolled in our study, aging from 26 to 81 years (median, 51 years). All patients received capecitabine-based treatment, mostly in combination with docetaxel (59.9%) and vinorelbine (28.7%). The patient characteristics are summarized in Table 2. Of the 342 patients, 7 (2.0%) had complete response (CR), 194 (56.7%) had partial response (PR), 90 (26.3%) had stable disease (SD), 39 (11.4%) had progressive disease (PD), and 12 (3.5%) had unknown disease status; 141 (41.2%) experienced leukopenia, 109 (31.9%) had neutropenia, 101 (29.5%) had increased aspartate transaminase, 193 (56.4%) had HFS, 204 (59.6%) had nausea and vomiting, and 72 (21.1%) had increased bilirubin. HFS and increased bilirubin were relatively specific capecitabine-induced adverse events.

**Associations between gene polymorphisms and risks of increased bilirubin and HFS**

No association between polymorphisms of the 3 candidate genes and the risk of increased bilirubin was found (data not shown). There was no significant association between polymorphisms of RRM1 and the risk of HFS (Table 3). The most investigated polymorphisms of MTHFR, rs1801133 and rs1801131, were not related with 5-FU toxicities in our study. We found that TYMS rs2606241 (P=0.022), TYMS rs2853741 (P=0.019), MTHFR rs3737964 (P=0.029), and MTHFR rs4846048 (P=0.030) were associated with the risk of HFS (Table 3).

We performed unconditional logistic regression analysis using the SNPStats software. The results indicated that the genotype AG of MTHFR rs3737964 (OR=0.54, 95% CI 0.31–0.97, P=0.038) and MTHFR rs4846048 (OR=0.54, 95% CI 0.30–0.98, P=0.042) were protective factors for HFS, whereas the genotype CT of TYMS rs2853741 (OR=2.25, 95% CI 1.31–3.87, P=0.012) was a risk factor for HFS (Table 4). However, the association between the genotype GT of TYMS rs2606241 (OR=1.27, 95% CI 0.73–2.23, P=0.012) and HFS was still uncertain.

**Associations between the SNPs and corresponding gene expression**

TYMS rs2606241 and rs2853741 were not associated with TYMS expression, whereas MTHFR rs3737964 and rs4846048 were significantly associated with MTHFR expression (both P<0.001).

**Discussion**

The present study demonstrated that genetic polymorphisms in MTHFR and TYMS were associated with capecitabine-induced HFS in MBC patients. We identified 4 SNPs significantly associated with HFS. Further analysis showed that the genotype AG of MTHFR rs3737964 and the genotype AG of MTHFR rs4846048 were protective factors for HFS, whereas the genotype CT of TYMS rs2853741 was a risk factor.

Until recently, few researches have explored the biomarkers for toxicity of capecitabine in patients with MBC. Two retrospective studies [22, 23] and an exploratory analysis [24] have shown that HFS occurrence in capecitabine-treated patients might be associated with improved efficacy and suggested that early dose adjustment according to the severity of HFS might improve its efficacy. However, there is still little information available about predictive biomarkers for HFS. Rs9936750, a polymorphism in an intergenic region of different genes, was shown to be associated with an increased risk of capecitabine-induced HFS [25], but the sample size is too small to be convincing and the result needs further confirmation. Another variant rs3215400 in the cytidine deaminase promoter has been demonstrated to be plausibly associated with severe capecitabine-induced HFS [18], but such association was not observed in another study [26]. In the present study, we identified 4 potential predictive biomarkers for HFS in Chinese female MBC patients treated with capecitabine, so that capecitabine therapy might be further tailored to patient response.

Optimal efficacy of 5-FU requires elevated intratumoral concentration of 5-10 MTHF, which is mainly...
Table 2  Clinicopathological characteristics of 342 metastatic breast cancer patients (MBC) with or without hand-foot syndrome (HFS)

| Characteristic                                      | Total [cases (%)] | Patients with HFS [cases (%)] | Patients without HFS [cases (%)] |
|----------------------------------------------------|-------------------|-------------------------------|----------------------------------|
| **Age (years)**                                    |                   |                               |                                  |
| ≤ 40                                               | 57 (16.7)         | 30 (8.8)                      | 27 (7.9)                         |
| > 40                                               | 285 (83.3)        | 163 (47.7)                    | 122 (35.7)                       |
| **Family history of cancer**                       |                   |                               |                                  |
| None                                               | 275 (80.4)        | 152 (44.4)                    | 123 (36.0)                       |
| Breast cancer or ovarian cancer                    | 19 (5.6)          | 8 (2.3)                       | 11 (3.2)                         |
| Other malignancies                                 | 48 (14.0)         | 33 (9.6)                      | 15 (4.4)                         |
| **Menstrual station**                              |                   |                               |                                  |
| Premenopause                                       | 218 (63.7)        | 120 (35.1)                    | 98 (28.7)                        |
| Postmenopause                                      | 118 (34.5)        | 71 (20.8)                     | 47 (13.7)                        |
| Unclear                                            | 6 (1.8)           | 2 (0.6)                       | 4 (1.2)                          |
| **Clinical stage**                                 |                   |                               |                                  |
| I+II                                               | 154 (45.0)        | 89 (26.0)                     | 65 (19.0)                        |
| III                                                | 143 (41.8)        | 85 (24.9)                     | 58 (17.0)                        |
| IV                                                 | 14 (4.1)          | 5 (1.5)                       | 9 (2.6)                          |
| Unclear                                            | 31 (9.1)          | 14 (4.1)                      | 17 (5.0)                         |
| **Pathological type**                              |                   |                               |                                  |
| Intraductal carcinoma                              | 4 (1.2)           | 2 (0.6)                       | 2 (0.6)                          |
| Infiltrating ductal carcinoma                      | 306 (89.5)        | 172 (50.3)                    | 134 (39.2)                       |
| Invasive lobular carcinoma                         | 17 (5.0)          | 11 (3.2)                      | 6 (1.8)                          |
| Others                                             | 11 (3.2)          | 7 (2.0)                       | 4 (1.2)                          |
| Unclear                                            | 4 (1.2)           | 1 (0.3)                       | 3 (0.9)                          |
| **Pathological grade**                             |                   |                               |                                  |
| Grade 1                                            | 10 (2.9)          | 8 (2.3)                       | 2 (0.6)                          |
| Grade 2–3                                          | 170 (49.7)        | 96 (28.1)                     | 74 (21.6)                        |
| Unclear                                            | 162 (47.4)        | 89 (26.0)                     | 73 (21.3)                        |
| **Vascular invasion**                              |                   |                               |                                  |
| No                                                 | 308 (90.1)        | 174 (50.9)                    | 134 (39.2)                       |
| Yes                                                | 34 (9.9)          | 19 (5.6)                      | 15 (4.4)                         |
| **Axillary lymph node metastasis at initial diagnosis** |                 |                               |                                  |
| No                                                 | 106 (31.0)        | 54 (15.8)                     | 52 (15.2)                        |
| Yes                                                | 225 (65.8)        | 136 (39.8)                    | 89 (26.0)                        |
| Unclear                                            | 11 (3.2)          | 3 (0.9)                       | 8 (2.3)                          |
| **Distant metastasis at initial diagnosis**        |                   |                               |                                  |
| No                                                 | 324 (94.7)        | 186 (54.4)                    | 138 (40.4)                       |
| Yes                                                | 14 (4.1)          | 5 (1.5)                       | 9 (2.6)                          |
| Unclear                                            | 4 (1.2)           | 2 (0.6)                       | 2 (0.6)                          |
| **ER status**                                      |                   |                               |                                  |
| Positive                                           | 215 (62.9)        | 123 (36.0)                    | 92 (26.9)                        |
| Negative                                           | 117 (34.2)        | 65 (19.0)                     | 52 (15.2)                        |
| Unclear                                            | 10 (2.9)          | 5 (1.5)                       | 5 (1.5)                          |
| **PR status**                                      |                   |                               |                                  |
| Positive                                           | 213 (62.3)        | 117 (34.2)                    | 96 (28.1)                        |
| Negative                                           | 118 (34.5)        | 70 (20.5)                     | 48 (14.0)                        |
| Unclear                                            | 11 (3.2)          | 6 (1.8)                       | 5 (1.5)                          |
| **HER2 status**                                    |                   |                               |                                  |
| Positive                                           | 83 (24.3)         | 47 (13.7)                     | 36 (10.5)                        |
controlled by MTHFR [27]. As 5-10 MTHF inhibits TYMS activity in conjunction with 5-fluorodeoxyuridine 5′-monophosphate (5-FdUMP), reduced MTHFR activity, which is associated with increased levels of 5-10 MTHF, theoretically leads to more effective TYMS inhibition. The metabolic pathway of capecitabine is shown in Fig. 2. Previous clinical studies have suggested that MTHFR gene polymorphisms might have impact on fluoropyrimidine responsiveness [28, 29]. Two of the most investigated polymorphisms of MTHFR, rs1801133 (Ala-222Val, 677C>T) and rs1801131 (Glu429Ala, 1298A>C), have been proved to have close association with the onset of some cancers, including colorectal cancer [30] and breast cancer [31]. Several meta-analyses have demonstrated that MTHFR C677T polymorphism may be a risk factor for thyroid [32], breast and ovarian cancers [33]. Accordingly, we investigated influence of MTHFR rs1801133 and rs1801131 on toxicities of capecitabine therapy in patients with MBC, but found no such associations. This result was in agreement with those of some [34–36], whereas other studies have demonstrated significant associations between MTHFR rs1801133 and 5-FU-induced toxicity [37, 38]. The reason for this discrepancy is not clear. It may be due to different sample sizes or patient selection. Large-scale studies are needed to determine whether testing for these two variants is clinically useful. Our results showed that the frequencies of the genotype AG of MTHFR rs4846048 (P = 0.030) and MTHFR rs3737964 (P = 0.029) were significantly lower in patients with capecitabine-induced HFS than in those without HFS. Further multivariate unconditional logistic regression analysis indicated that the genotype AG of MTHFR rs3737964 (OR = 0.54, 95% CI 0.31–0.97, P = 0.038) and MTHFR rs4846048 (OR = 0.54, 95% CI

| Characteristic                        | Total [cases (%)] | Patients with HFS [cases (%)] | Patients without HFS [cases (%)] |
|---------------------------------------|-------------------|--------------------------------|----------------------------------|
| Negative                              | 230 (67.3)        | 130 (38.0)                     | 100 (29.2)                       |
| Unclearb                              | 29 (8.5)          | 16 (4.7)                       | 13 (3.8)                         |
| Therapy                               |                   |                                |                                 |
| Capecitabine                          | 20 (5.8)          | 10 (2.9)                       | 10 (2.9)                         |
| Docetaxel plus capecitabine           | 205 (59.9)        | 123 (36.0)                     | 82 (24.0)                        |
| Vinorelbine plus capecitabine         | 98 (28.7)         | 48 (14.0)                      | 50 (14.6)                        |
| Othersc                               | 19 (5.6)          | 12 (3.3)                       | 7 (2.0)                          |
| Therapy line                          |                   |                                |                                 |
| First-line                            | 211 (61.7)        | 121 (35.4)                     | 90 (26.3)                        |
| Multi-line                            | 131 (38.3)        | 72 (21.1)                      | 59 (17.3)                        |
| Metastatic site                       |                   |                                |                                 |
| No visceral metastasis (including local recurrence) | 104 (30.4) | 60 (17.5) | 44 (12.9) |
| Visceral metastasis                   | 238 (69.6)        | 133 (38.9)                     | 105 (30.7)                       |
| Maintenance therapy                   |                   |                                |                                 |
| Yes                                   | 137 (40.1)        | 94 (27.5)                      | 43 (12.6)                        |
| No                                    | 205 (59.9)        | 99 (28.9)                      | 106 (31.0)                       |
| Response evaluation                   |                   |                                |                                 |
| CR                                    | 7 (2.0)           | 5 (1.5)                        | 2 (0.6)                          |
| PR                                    | 194 (56.7)        | 107 (31.3)                     | 87 (25.4)                        |
| SD                                    | 90 (26.3)         | 56 (16.4)                      | 34 (9.9)                         |
| PD                                    | 39 (11.4)         | 20 (5.8)                       | 19 (5.6)                         |
| Unclear                               | 12 (3.5)          | 5 (1.5)                        | 7 (2.0)                          |
| Survival conditiond                   |                   |                                |                                 |
| Alive                                 | 218 (63.7)        | 124 (36.3)                     | 94 (27.5)                        |
| Dead                                  | 124 (36.3)        | 69 (20.2)                      | 55 (16.1)                        |

ER estrogen receptor, PR progesterone receptor, HER2 human epidermal growth factor receptor 2, CR complete response, PR partial response, SD stable disease, PD progressive disease

a Breast cancer or ovarian cancer or other malignances of first- or second-degree relatives

b Equivocal results (HER2 ++ ) without fluorescence in situ hybridization testing
c Other capecitabine-based therapies
d Patients’ survival conditions by last follow-up
Table 3 Frequencies of 22 SNPs of 3 genes in metastatic breast cancer patients with and without capecitabine-induced HFS

| Gene | SNP   | Genotype | Patients with HFS [cases (%)] | Patients without HFS [cases (%)] | P value |
|------|-------|----------|-------------------------------|-----------------------------------|---------|
|      | Total |          |                               |                                   |         |
|      | TYMS  | rs2790   | AA                            | 78 (40.4)                         | 0.316   |
|      |       |          | GA                            | 87 (45.1)                         |         |
|      |       |          | GG                            | 26 (13.5)                         |         |
|      |       |          | NA                            | 2 (1.0)                           |         |
|      |       | rs15872  | TT                            | 73 (37.8)                         | 0.265   |
|      |       |          | GC                            | 96 (49.7)                         |         |
|      |       |          | CC                            | 22 (11.4)                         |         |
|      |       |          | NA                            | 2 (1.0)                           |         |
|      |       | rs699517 | TT                            | 73 (37.8)                         | 0.195   |
|      |       |          | GC                            | 97 (50.3)                         |         |
|      |       |          | CC                            | 20 (10.4)                         |         |
|      |       |          | NA                            | 3 (1.6)                           |         |
|      |       | rs2606241| TT                            | 44 (22.8)                         | 0.022   |
|      |       |          | GC                            | 113 (58.5)                        |         |
|      |       |          | CC                            | 34 (21.6)                         |         |
|      |       |          | NA                            | 3 (1.6)                           |         |
|      |       | rs2853741| TT                            | 38 (19.7)                         | 0.019   |
|      |       |          | GC                            | 113 (58.5)                        |         |
|      |       |          | CC                            | 39 (20.2)                         |         |
|      |       |          | NA                            | 3 (1.6)                           |         |
|      |       | rs376362 | TT                            | 135 (69.9)                        | 0.055   |
|      |       |          | CT                            | 48 (24.9)                         |         |
|      |       |          | CC                            | 7 (3.6)                           |         |
|      |       |          | NA                            | 3 (1.6)                           |         |
|      |       | rs9947507| TT                            | 193 (100.0)                       | NA      |
|      |       | rs9967368| CC                            | 55 (28.5)                         | 0.231   |
|      |       |          | CG                            | 97 (50.3)                         |         |
|      |       |          | GG                            | 38 (19.7)                         |         |
|      |       |          | NA                            | 3 (1.6)                           |         |
|      | MTHFR  | rs1801133| TT                            | 60 (31.1)                         | 0.662   |
|      |       |          | GC                            | 99 (51.3)                         |         |
|      |       |          | CC                            | 31 (16.1)                         |         |
|      |       |          | NA                            | 3 (1.6)                           |         |
|      |       | rs2274976| GT                            | 163 (84.5)                        | 0.558   |
|      |       |          | GT                            | 26 (13.5)                         |         |
|      |       |          | AA                            | 1 (0.5)                           |         |
|      |       |          | NA                            | 3 (1.6)                           |         |

Table 3 (continued)

| Gene | SNP   | Genotype | Patients with HFS [cases (%)] | Patients without HFS [cases (%)] | P value |
|------|-------|----------|-------------------------------|-----------------------------------|---------|
|      | rs9967368 | CC | 61 (31.6) | 58 (38.9) | 0.359 |
|      | rs9947507 | TT | 193 (100.0) | 149 (100.0) | NA |
|      | rs3737964 | GG | 165 (85.5) | 113 (75.8) | 0.029 |
|      | rs3753582 | TT | 160 (82.9) | 131 (87.9) | 0.389 |
|      | rs4846048 | AA | 165 (85.5) | 115 (77.2) | 0.030 |
|      | rs4846049 | GT | 49 (25.4) | 49 (32.9) | 0.338 |
|      | rs720106 | TT | 142 (73.6) | 98 (65.8) | 0.054 |
|      | rs1042858 | TT | 116 (60.1) | 78 (52.3) | 0.491 |
|      | rs1042927 | AA | 118 (61.1) | 81 (54.4) | 0.415 |
|      | rs1980412 | CC | 61 (31.6) | 58 (38.9) | 0.359 |
|      | rs11030918 | TT | 103 (53.4) | 74 (49.7) | 0.378 |
|      | rs1801133 | TT | 60 (31.1) | 52 (34.9) | 0.662 |
|      | rs1801133 | GC | 99 (51.3) | 69 (46.3) |         |
|      | rs1801133 | CC | 31 (16.1) | 25 (16.8) |         |
|      | rs1801133 | NA | 3 (1.6) | 3 (2.0) |         |
|      | rs2274976 | GG | 163 (84.5) | 131 (87.9) | 0.558 |
|      | rs2274976 | GA | 26 (13.5) | 17 (11.4) |         |
|      | rs2274976 | AA | 1 (0.5) | 0 (0.0) |         |
|      | rs2274976 | NA | 3 (1.6) | 1 (0.7) |         |

P value < 0.05 was considered statistically significant (in italics)

SNP, single nucleotide polymorphism; HFS, hand-foot syndrome; TYMS, thymidylate synthase; MTHFR, methylene tetrahydrofolate reductase; RRM1, ribonucleotide reductase M1; NA, not applicable

0.30–0.98, P = 0.042) were protective factors for HFS. Due to the catalytic deficit of MTHFR, subsequent to its polymorphic variants, the increased 5-10 MTHF concentration enhanced the formation and stability of the inhibitory complex consisting of 5-10 MTHF, TYMS,
and 5-FdUMP, thereby increasing the potential toxicity of fluoropyrimidines [39]. Lying in the promoter region of \(MTHFR\), rs3737964 did not result in coding amino acid polymorphisms, but possibly led to transcription factor binding difference. rs4846048, located on the \(3'\)-untranslated region (\(3'\)-UTR) of \(MTHFR\), might influence the microRNA (miRNA) binding regulation, thus enhancing the message RNA (mRNA) expression. Further eQTL analyses based on the Blood eQTL browser confirmed that the alleles of rs3737964 and rs4846048 could affect the gene expression levels of \(MTHFR\) in cis. Therefore, it was inferred that the \(MTHFR\) enzymatic activity in subjects with the genotype AG of rs3737964 and rs4846048 might be improved through increasing mRNA transcription, and hence to protect patients from HFS.

\(TYMS\) is an enzyme that catalyzes the conversion of dUMP to dTMP, and is the main intracellular target of the active 5-FU metabolite, 5-FdUMP, which forms a ternary complex with \(TYMS\) and 5-10 MTHF [40]. Elevated \(TYMS\) expression or activity is a well-known mechanism of resistance to 5-FU [41]. Ooyama et al. [42] found that the copy number of \(TYMS\) (18p11.32) showed a strong association with drug resistance, which may lead to the use of \(TYMS\) copy number as a predictive marker for drug sensitivity of fluoropyrimidines. The rs2612091 and rs2741171 variants, lying downstream of \(TYMS\) within an intron of enolase superfamily member 1 (\(ENOSF1\)), were significant associated with HFS, irrespective of the two \(TYMS\) polymorphisms \(5'\)-variable number of tandem repeat (\(5'\)-VNTR) 2R/3R [36] and \(3'\)-UTR 6 bp ins-del [43]) that have previously been reported to affect 5-FU toxicity [16]. Therefore, \(TYMS\) rs2612091 and rs2741171 were not included in the present study. We included 9 less-investigated polymorphisms of \(TYMS\) and found differences in frequencies of \(TYMS\) rs2606241 and rs2853741 between patients with and without HFS. Our results showed that the frequencies of the genotype CT of \(TYMS\) rs2606241 \((P=0.022)\) and the genotype CT of \(TYMS\) rs2853741 \((P=0.019)\) were significantly higher in patients with capecitabine-induced HFS than in those without HFS. Logistic regression reflected that the genotype CT of \(TYMS\) rs2853741 \((OR=2.25, 95\%)\)

### Table 4 Unconditional logistic regression analyses assessing associations of gene polymorphisms with HFS

| Gene     | SNP  | Genetic model | Genotype | Patients without HFS [cases (%)] | Patients with HFS [cases (%)] | OR (95% CI) | \(P\) value | AIC    | BIC    |
|----------|------|---------------|----------|---------------------------------|--------------------------------|-------------|------------|--------|--------|
| \(TYMS\) | rs2606241 | Codominant   | GG       | 34 (22.8)                        | 44 (22.8)                     | 1.00        | 0.012      | 449.7  | 476.4  |
|          |      |               | GT       | 69 (46.3)                        | 113 (58.5)                    | 1.27 (0.73–2.23) |            |        |        |
|          |      |               | TT       | 44 (29.5)                        | 33 (17.1)                     | 0.55 (0.28–1.06) |            |        |        |
|          |      | Dominant      | GG       | 34 (22.8)                        | 44 (22.8)                     | 1.00        | 0.960      | 456.5  | 479.4  |
|          |      |               | GT + TT  | 113 (75.8)                       | 146 (75.6)                    | 0.99 (0.58–1.68) |            |        |        |
|          |      | Recessive     | GG + GT  | 103 (69.1)                       | 157 (81.3)                    | 1.00        | 0.004      | 448.4  | 471.3  |
|          |      |               | TT       | 44 (29.5)                        | 33 (17.1)                     | 0.46 (0.27–0.79) |            |        |        |
|          |      | Overdominant  | GG + TT  | 78 (52.3)                        | 77 (39.9)                     | 1.00        | 0.018      | 450.9  | 473.8  |
|          |      |               | GT       | 69 (46.3)                        | 113 (58.5)                    | 1.72 (1.10–2.70) |            |        |        |
|          |      | Log-additive  | NA       | NA                              | NA                            | 0.74 (0.53–1.03) | 0.071      | 453.3  | 476.2  |
|          | rs2853741 | Codominant   | TT       | 48 (32.2)                        | 38 (19.9)                     | 1.00        | 0.012      | 449.6  | 476.4  |
|          |      |               | CT       | 68 (45.6)                        | 113 (58.5)                    | 2.25 (1.31–3.87) |            |        |        |
|          |      |               | CC       | 31 (20.8)                        | 39 (20.2)                     | 1.71 (0.81–3.31) |            |        |        |
|          |      | Dominant      | TT       | 48 (32.2)                        | 38 (19.9)                     | 1.00        | 0.005      | 448.5  | 471.4  |
|          |      |               | TC + CC  | 99 (66.4)                        | 152 (78.8)                    | 2.09 (1.25–3.49) |            |        |        |
|          |      | Recessive     | TT + TC  | 116 (77.9)                       | 151 (78.2)                    | 1.00        | 0.950      | 456.4  | 479.4  |
|          |      |               | CC       | 31 (20.8)                        | 39 (20.2)                     | 0.98 (0.57–1.70) |            |        |        |
|          |      | Overdominant  | TT + CC  | 79 (53.0)                        | 77 (38.9)                     | 1.00        | 0.012      | 450.1  | 473.1  |
|          |      |               | TC       | 68 (45.6)                        | 113 (58.5)                    | 1.77 (1.13–2.77) |            |        |        |
|          |      | Log-additive  | NA       | NA                              | NA                            | 1.35 (0.97–1.88) | 0.075      | 453.3  | 476.2  |
| \(MTHFR\) | rs4846048 | NA           | AA       | 115 (77.2)                       | 165 (85.5)                    | 1.00        | 0.042      | 453.7  | 476.7  |
|          |      |               | AG       | 33 (22.1)                        | 25 (13.0)                     | 0.54 (0.30–0.98) |            |        |        |
|          | rs3737964 | NA           | GG       | 113 (75.8)                       | 165 (85.5)                    | 1.00        | 0.038      | 458.8  | 470.3  |
|          |      |               | AG       | 33 (22.1)                        | 25 (13.0)                     | 0.54 (0.31–0.97) |            |        |        |

The lower the AIC and BIC values, the more accurate the model. HFS, hand-food syndrome; OR, odds ratio; CI, confidence interval; AIC, Akaike’s information criterion; BIC, Bayes information criterion; NA, not applicable.
CI 1.31–3.87, $P=0.012$) seemed to raise the susceptibility to HFS. Although the association was uncertain, the genotype GT of $TYMS$ rs2606241 (OR=1.27, 95% CI 0.73–2.23, $P=0.012$) tended to increase the risk of HFS. Lecomte et al. [43] suggested that the low $TYMS$ mRNA expression level in patients with 2R/2R genotype was associated with a higher risk of 5-FU-induced adverse events. Falling both in the promoter region of $TYMS$, the genotype GT of rs2606241 and the genotype CT of rs2853741 did not change the coding amino acid sequence. However, the mRNA expression might be reduced due to the impact on the transcription binding sites. Therefore, we hypothesized that the decreasing $TYMS$ mRNA expression in patients with the genotype GT of rs2606241 and the genotype CT of rs2853741 might increase the risk of 5-FU-induced HFS because of the high efficacy of $TYMS$ inhibition. However, further eQTL data did not afford much information about the association between rs2606241 and rs2853741 and gene expression level of $TYMS$. Whether these two polymorphisms are implicated in gene regulation at a post-transcriptional level through decreased mRNA stability needs further validation.

The specific mechanisms underlying the effect of the above mentioned genotypes on HFS are still not quite valid. We also cannot neglect that these results must be view as preliminary and need additional confirmation. Further prospective studies on a larger number of
patients are desirable to confirm and quantify these associations in additional datasets and to understand the mechanistic origins of capecitabine toxicity. Besides, further efforts to identify additional polymorphisms and rare variants associated with capecitabine toxicity remain valid.

Conclusions

In summary, we have identified a panel of potentially useful pharmacogenetic markers predicting capecitabine-induced HFS in MBC patients. Our findings may help clinicians identify patients who have a low risk of capecitabine-induced HFS and improve treatment decision for MBC patients.

Abbreviations

MBC: metastatic breast cancer; HFS: hand-foot syndrome; 5-FU: 5-fluorouracil; TYMS: thymidylate synthase; MTHFR: methylene tetrahydrofolate reductase; RRM1: ribonucleotide reductase M1; dUMP: deoxyuridine monophosphate; dTMP: deoxymethylcytidine monophosphate; MTHF: 5-MTHF: 5-methylenetetrahydrofolate; TYMS: thymidylate synthase; MTHFR: methylene tetrahydrofolate reductase; MBC: metastatic breast cancer; HFS: hand-foot syndrome; 5-FU: 5-fluorouracil; 

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Authors' contributions

All authors have contributed significantly, and all authors were in agreement with the content of the manuscript. BX and FM directed the study, obtained financial support and were responsible for study design. SL drafted the initial manuscript. YL, YF, RC, QL, SC, PZ and QL collected clinical data. All authors read and approved the final manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

The study was approved by the ethics committee of National Cancer Center/ National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College. All patients voluntarily signed an informed consent form.

Consent for publication

We have obtained consent from the participants to publish and report individual patient data.

Competing interests

The authors declare that they have no competing interests.

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