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TYMS Gene Polymorphisms in Breast Cancer Patients Receiving 5—Fluorouracil-Based Chemotherapy

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Breast cancer; Capecitabine; *TYMS*

Introduction

5-Fluorouracil (5-FU) and its oral prodrug capecitabine are chemotherapy drugs that are commonly used to treat advanced gastrointestinal and colorectal cancers. In breast cancer, there are multiple indications for the use of 5-FU in single and multidrug combinations across the breast cancer subtypes.¹ Capecitabine is indicated for single-agent use in HER2-negative advanced breast cancer and in combination with the HER2-targeted agents lapatinib or trastuzumab in HER2-positive metastatic breast cancer.²⁻⁴ The use of capecitabine in patients with residual disease after neoadjuvant chemotherapy is still under active clinical investigation. Data from the CREATE-X (Capecitabine for Residual Cancer as Adjuvant Therapy) trial showed that among HER2-negative breast cancer patients who did not achieve a pathologic complete response (pCR) after neoadjuvant chemotherapy, the use of adjuvant capecitabine was associated with higher rates of 5-year disease-free and overall survival.⁵ Furthermore, numerous other clinical trials evaluating capecitabine in combination with other investigational drugs are in process.

Common side effects of 5-FU and capecitabine include leukopenia, diarrhea, stomatitis, nausea, vomiting, and alopecia. The dose-limiting toxicities include neutropenia, diarrhea, hand-foot syndrome, and hyperbilirubinemia. Patients who experience severe toxicity from 5—FU-based therapy are often genotyped for mutations in the dihydropyrimidine dehydrogenase (*DPYD*) and thymidylate (*TYMS*) genes that have been associated with increased risk of toxicity to fluoropyrimidines. Mutations in the *DPYD* gene that lead to decreased expression of the 5-FU metabolizer dihydropyrimidine dehydrogenase (DPD) have been identified as the cause of decreased 5-FU clearance and increased toxicity.

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Disclosure

The authors declare no conflicts of interest.

Mutations in the *TYMS* gene lead to alterations in the enzyme thymidylate synthase (TS) in the 5-FU metabolic pathway; however, the clinical implications of *TYMS* gene polymorphisms are not as well defined and our understanding of expected toxicities is less clear. Herein, we present 3 cases of breast cancer patients who developed severe toxicity to capecitabine and were found to have *TYMS* gene polymorphisms.

Cases

Case 1

A 39-year-old woman was diagnosed with stage IIIA invasive ductal carcinoma after presenting with a self-detected mass in her right breast that was confirmed using mammography. Biopsy of the primary breast mass was negative for estrogen receptor (ER), progesterone receptor (PR), and HER2 (1+ using immunohistochemistry [IHC]). She underwent staging sentinel lymph node biopsy that was negative for carcinoma. She was treated with neoadjuvant paclitaxel and carboplatin followed by dose-dense adriamycin and cyclophosphamide (per Cancer And Leukemia Group B 40603).⁶ Carboplatin was dose-reduced by 20% during cycles 3 and 4 for neutropenia. She also developed Grade 1 peripheral neuropathy from paclitaxel that resolved when therapy was completed.

After neoadjuvant chemotherapy, she underwent right total mastectomy. Final pathology was downstaged to pathologic stage IA (T1bN0M0). Because of the detection of a positron emission tomography (PET)-avid right intramammary lymph node, the patient received adjuvant radiation therapy to the chest wall and regional lymph nodes. Because she did not achieve a pCR, adjuvant capecitabine treatment was started (per the CREATE-X trial).⁵ 5-FU sensitivity genotyping (Mayo Medical Laboratories) was sent before initiating capecitabine, which revealed heterozygosity in a *DPYD**9A and a *TYMS*3RC allele. With no definitive data in the literature showing that capecitabine should be dose-reduced in the setting of these gene variants, capecitabine 2000 mg/m²/d was started while she was still receiving radiation. However, because she developed Grade 2 hand-foot syndrome and neutropenia, the dose of capecitabine was not escalated upon completion of radiation to the recommended 2500 mg/m²/d dosing. She completed 8 cycles of adjuvant capecitabine and is now being monitored using active surveillance.

Case 2

A 53-year-old Caucasian woman was diagnosed with stage IV invasive ductal carcinoma of the breast after she presented with headaches and magnetic resonance imaging (MRI) of the brain revealed a C2 lytic lesion. She underwent posterior C1 to C2 fusion with biopsy of the lytic lesion. Pathology returned metastatic breast carcinoma that was positive for HER2 (3+ using IHC) and negative for ER and PR. She was also found to have liver metastases in addition to metastases to several other vertebrae. She was treated initially with docetaxel, carboplatin, and trastuzumab. Follow-up MRI showed stabilization of 3 target liver metastases that were thought to be amenable to surgical intervention. PET/computed tomography (CT) scan otherwise showed no evidence of residual hypermetabolic disease. She then proceeded to left mastectomy with axillary node dissection and elective right

mastectomy. Final pathology revealed TON0 disease. Bilateral mastectomies were followed by percutaneous ablation of the liver metastases.

Six months later, she presented with shoulder pain, for which MRI of the cervical spine incidentally revealed a right cerebellar lesion that was associated with significant edema and effacement of the fourth ventricle. She underwent surgical excision of the cerebellar tumor followed by partial brain radiation. She continued single-agent trastuzumab until she developed headaches and difficulties with writing. MRI of the brain revealed a recurrent right cerebellar mass, which was subsequently resected via suboccipital craniotomy. Pathology was notable for now weakly ER-positive (30%), PR-negative, and HER2-positive (3+ using IHC) status. She received postoperative radiation to the surgical bed in the right cerebellum. PET/CT showed no evidence of extracranial disease; she was then treated with the aromatase inhibitor letrozole in addition to trastuzumab and pertuzumab. She continued this regimen for almost a year, until repeat MRI of the brain showed disease progression. She was then enrolled in a clinical trial (TBCRC022) with the HER2-targeted agent neratinib (240 mg) and capecitabine (1500 mg/m²/d). Seven days after starting the trial, she experienced Grade 3 nausea, vomiting, and diarrhea. Per protocol, the study drugs were held for 5 days and then resumed at full dose. However, the next day her symptoms returned in full severity and she withdrew from the clinical trial shortly thereafter.

Treatment was then switched to ado-trastuzumab emtansine, with recent imaging showing a decrease in size of intracranial lesions and no evidence of extracranial disease. Because of the severity of her symptoms during capecitabine treatment, 5-FU sensitivity genotyping was sent, which revealed heterozygosity in the *DPYD**5 allele as well as heterozygosity in the *TYMS* 3RC allele.

Case 3

A 54-year-old woman presented with a self-detected mass in her right breast that was confirmed using mammography. Biopsy of the primary breast mass revealed invasive ductal carcinoma with lobular features that was ER-positive, PR-positive, and HER2-positive (3+ using IHC). She was treated with neoadjuvant dose-dense adriamycin and cyclophosphamide followed by 12 weeks of paclitaxel and trastuzumab. Chemotherapy was complicated by Grade 1 peripheral neuropathy and nausea. She then underwent lumpectomy of the right breast (final pathologic staging ypT2N0) followed by adjuvant radiation. After 12 weeks of single-agent trastuzumab treatment, an echocardiogram showed decreased ejection fraction. Trastuzumab was held briefly, but then resumed after a repeat echocardiogram showed stable cardiac function. Single-agent trastuzumab was ultimately discontinued early after 46 weeks when her ejection fraction declined again. The patient then continued on to endocrine therapy with anastrozole.

Less than 1 year later, the patient presented with repeated episodes of erythema of the right breast that persisted despite multiple courses of antibiotics. Skin biopsy revealed an inflammatory breast cancer recurrence that was ER-negative, PR-negative, and HER2 equivocal (2+ using IHC, fluorescent in situ hybridization [FISH] 4.3 HER2 copies per cell). Systemic therapy was restarted with docetaxel and carboplatin. Because of only moderate response to chemotherapy, trastuzumab and pertuzumab were added after 4 cycles of

docetaxel and carboplatin. After completing 6 cycles of docetaxel/carboplatin and 2 cycles of trastuzumab/pertuzumab, she developed new nodularity of the breast, for which biopsies were positive for dermal recurrence. She underwent bilateral mastectomy and pathology showed residual disease (stage ypT4dypN3) that was ER-negative, PR-negative, and HER2-negative (2+ using IHC, FISH 3.4 HER2 copies per cell). After multidisciplinary discussion, she was treated with radiation and single-agent trastuzumab. Pertuzumab and anastrozole were added to the treatment regimen after she completed radiation, because of the HER2 and hormone receptor status of her initial tumor.

One month later, she presented with another skin recurrence that was ER-negative, PR-negative, and HER2-negative (2+ using IHC, FISH 3.9 HER2 copies per cell). Systemic therapy was switched to single-agent capecitabine 2000 mg/m²/d. During the first cycle she developed Grade 2 diarrhea. She continued the same dose of capecitabine but switched to a 7 day on, 7 day off cycle. Cycle 2 was complicated again by Grade 2 vomiting and diarrhea, so she was dose-reduced to 1800 mg/m²/d on a 7 day on, 7 day off schedule. During the third cycle she developed severe hypovolemic shock from vomiting and diarrhea, requiring intensive care unit admission. 5-FU sensitivity genotyping was sent, which revealed a heterozygous rs67376798 mutation in *DPYD* as well as a heterozygous *9A mutation. Genotyping was also significant for *TYMS* 2R and 3RC polymorphisms. Imaging during the hospitalization showed progression of disease so systemic therapy was switched to single-agent carboplatin. Unfortunately, after 2 cycles of carboplatin she had rapid progression of disease and associated clinical decline and she was transitioned to hospice care.

Discussion

Capecitabine is metabolized to 5-FU, which irreversibly inhibits TS, an enzyme that converts deoxyuridine monophosphate to deoxythymidine monophosphate, a necessary step for DNA synthesis. Most of 5-FU is metabolized by DPD (encoded by the *DPYD* gene), and several studies have highlighted the role of DPD deficiency in the development of severe 5-FU-related toxicity.^{7,8} Gene polymorphisms of the *TYMS* gene, which encodes for TS, have been associated with increased toxicity to fluoropyrimidines when used to treat gastrointestinal malignancies.⁹⁻¹² When certain alleles and variants in the *DPYD* gene known to be associated with high risk for toxicity are identified, the recommendations are to consider either dose reductions or even alternate therapy. However, when polymorphisms in *TYMS* are discovered, the clinical applications are less clear. In this report we describe 3 patients who developed varying degrees of toxicity to capecitabine and were determined to have gene polymorphisms in *TYMS*.

Fluoropyrimidine drug sensitivity genotyping, performed by Mayo Clinic Laboratories (Rochester, MN), is a pharmacogenomics test that detects polymorphisms in the *DPYD* and *TYMS* genes on the basis of polymerase chain reaction sequencing. The variants in the *TYMS* gene that have been reported to be associated with increased 5-FU toxicity typically involve tandem repeats of a 28-base pair sequence in the promoter region or a 6-base pair variation in the 3'-untranslated region of *TYMS*. Wild type (consisting of 3 tandem repeats and designated 3RG) expresses more translational activity compared with promoters with only 2 tandem repeats (designated 2R).¹³ The 3RC genotype is a variant that consists of a

single nucleotide polymorphism G>C at the 12th nucleotide of the second repeat of the 3R. All 3 patients described in this report were found to have this *3RC* allele, yet each patient showed different degrees of toxicity to capecitabine (Table 1).

In a retrospective study of 90 colorectal cancer patients who received 5—FU-based chemotherapy, 18% of patients were found to have a *2R/2R* *TYMS* promoter genotype, 32% *2R/3RC*, 16% *2R/3RG*, 15% *3RC/3RG*, 5% *3RC/3RC*, and 14% *3RG/3RG*. Patients with *2R/3RC* and *2R/3RG* genotypes exhibited increased toxicity compared with patients with *3R/3R* genotypes, which included patients with *3RC/3RG* and *3RG/3RG* genotypes. Despite recent data that showed decreased transcriptional activity of *3RC* alleles, the retrospective study did not show increased toxicity for patients harboring *3RC* alleles.^{9,14} With regard to the concurrent *DPYD* gene variations identified in the patients in cases 1 and 2, these are variants that exhibit normal DPD enzyme activity. Notably, the patient in case 3 exhibited the *2R/3RC* genotype as well as a clinically relevant variation in *DPYD*. The *DPYD* allele rs67376798 detected in the patient in case 3 is a nonfunctional allele and manifests as partial DPD deficiency. This *DPYD* allele in combination with the *TYMS 2R/3RC* genotype likely explains the Grade 4 toxicity to capecitabine.

With the push toward personalized medicine, pharmacogenetics is becoming increasingly important in cancer therapeutics. Identification of clinically significant gene polymorphisms has the potential to individualize treatment decisions on the basis of predictions of drug efficacy and toxicity. Clinical trials are currently under way in colorectal cancer patients to evaluate chemotherapy dose escalation on the basis of pharmacogenetics, yet similar clinical applicability in breast cancer is still nascent. In breast cancer patients, certain polymorphisms in the *TYMS* 5′-untranslated region (UTR) and 3′-UTR have been shown to be associated with TS expression but whether the polymorphisms have an effect on response to 5—FU-based chemotherapy is unknown.¹⁵ Another study failed to find any association between *TYMS* gene polymorphisms and toxicity in breast cancer patients treated with neoadjuvant 5-FU.¹⁶ Although theoretically, data related to 5—FU-based chemotherapy in colon cancer patients can be extrapolated to breast cancer patients, the conflicting results suggest that variations in toxicity and efficacy of 5-FU treatment might not only be patient-specific because of variable penetrance or yet-to-be discovered gene polymorphisms, but also might be tumor type-specific. This case report ultimately highlights the need for further inquiry into the clinical relevance of *TYMS* gene polymorphisms in breast cancer patients.

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Clinical Practice Points

- Although not as extensively used as in gastrointestinal malignancies, the 5-fluoropyrimidine (5-FU) oral prodrug capecitabine is often used to treat patients with metastatic breast cancer. Clinical indications for capecitabine use in breast cancer patients are increasing.
- We describe 3 cases of breast cancer patients who developed severe toxicity to capecitabine and were found to have thymidylate synthase (*TYMS*) gene polymorphisms.
- Tests such as 5-FU drug sensitivity genotyping are available to assess risk of toxicity on the basis of the presence of certain alleles and variants of the dihydropyrimidine dehydrogenase and *TYMS* genes. Although dihydropyrimidine dehydrogenase enzyme deficiency is well described, less is known about the clinical relevance of *TYMS* gene polymorphisms.
- Our case series illustrates how *TYMS* genotype variations might affect clinical decision-making regarding the use of capecitabine in breast cancer patients.

Table 1Summary of Capecitabine Toxicities and Associated *DPYD* and *TYMS* Mutations

Case	<i>DPYD</i> Mutations Identified	<i>TYMS</i> Mutations Identified	Toxicities
1	*9A heterozygous	3RC heterozygous	Grade 2 hand-foot syndrome Grade 2 neutropenia
2	*5 Heterozygous	3RC heterozygous	Grade 3 nausea, vomiting, and diarrhea
3	rs67376798 heterozygous *9A heterozygous	2R heterozygous 3RC heterozygous	Grade 4 nausea, vomiting, and diarrhea