

ORIGINAL RESEARCH

# Potential role of *CMPK1*, *SLC29A1*, and *TLE4* polymorphisms in gemcitabine-based chemotherapy in HER2-negative metastatic breast cancer patients: pharmacogenetic study results from the prospective randomized phase II study of eribulin plus gemcitabine versus paclitaxel plus gemcitabine (KCSG-BR-13-11)

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**Background:** In this study, we evaluated the association between genetic polymorphisms of 23 genes associated with gemcitabine metabolism and the clinical efficacy of gemcitabine in breast cancer patients.

**Patients and methods:** This prospective, pharmacogenetic study was conducted in cooperation with a phase II clinical trial. A total of 103 genetic polymorphisms of the 23 genes involved in gemcitabine transport and metabolism were selected for genotyping. The associations of genetic polymorphisms with overall survival, progression-free survival (PFS), and 6-month PFS were analyzed.

**Results:** A total of 91 breast cancer patients were enrolled in this study. In terms of 6-month PFS, rs1044457 in *CMPK1* was the most significant genetic polymorphism [55.9% for CT and TT and 78.9% for CC,  $P < 0.001$ , hazard ratio (HR): 4.444, 95% confidence interval (CI): 1.905-10.363]. For the rs693955 in *SLC29A1*, the median duration of PFS was 5.4 months for AA and 10.5 months for CA and CC ( $P = 0.002$ , HR: 3.704, 95% CI: 1.615-8.497). For the rs2807312 in *TLE4*, the median duration of PFS was 5.7 months for TT and 10.4 months for CT and CC ( $P = 0.005$ , HR: 4.948, 95% CI: 1.612-15.190). In survival analysis with a multi-gene model, the TT genotype of rs2807312 had the worst PFS regardless of other genetic polymorphisms, whereas the CA genotype of rs693955 or the CT genotype of rs2807312 without the AA genotype of rs693955 had the best PFS compared with those of other genetic groups ( $P < 0.001$ ).

**Conclusions:** Genetic polymorphisms of rs1044457 in *CMPK1*, rs693955 in *SLC29A1*, and rs2807312 in *TLE4* were significantly associated with the 6-month PFS rate and/or the duration of PFS. Further studies with a larger sample size and expression study would be helpful to validate the association of genetic polymorphisms and clinical efficacy of gemcitabine.

**Key words:** gemcitabine, breast cancer, genetic polymorphism, pharmacogenetics

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## INTRODUCTION

Breast cancer (BC) was the most commonly diagnosed malignancy worldwide in 2020.<sup>1</sup> In women, BC was the most common and most fatal malignancy. With recent advances in treatment strategies, BC mortality has decreased steadily. Metastatic BC (MBC), however, still has dismal prognosis.<sup>2</sup> Gemcitabine (2',2'-difluorodeoxycytidine; dFdC), a novel S-phase-specific cytidine nucleoside analogue of deoxycytidine, has broad antitumor activity with significant monotherapy activity in BC, with response rates ranging from 22% to 42% depending on the patients' pretreatment characteristics.<sup>3,4</sup> Recently, gemcitabine has been reported to have high activity with a feasible toxicity profile as a combination chemotherapeutic agent for patients with MBC with taxane and eribulin.<sup>5,6</sup> The National Comprehensive Cancer Network (NCCN) guideline for BC recommends gemcitabine as a preferred regimen for human epidermal growth factor receptor 2 (HER2)-negative MBC. In addition, gemcitabine with carboplatin or paclitaxel combination therapy is suggested for patients with high tumor burden, rapidly progressing disease, or visceral crisis.<sup>7</sup> Moreover, gemcitabine can be used continuously until disease progression because of its feasible toxicity profile.<sup>5,7</sup>

Genetic polymorphisms are an important factor affecting the activity of transporters and enzymes involved in gemcitabine metabolism and can explain the interindividual difference in gemcitabine efficacy. The association between genetic polymorphism and the clinical efficacy of gemcitabine has been studied in lung, pancreas, and BC patients.<sup>8-13</sup> In pancreatic cancer, *CMPK1* polymorphism<sup>10</sup> or a combination model of *CDA/DCK/DCTD/SLC28A3/SLC29A1*<sup>9</sup> or *CDA/RRM1/SLC29A1*<sup>12</sup> were associated with survival of pancreatic cancer patients following gemcitabine treatment. Moreover, *CDA*, *NT5C2*, *RRM1*, and *SLC29A1* polymorphisms affected the survival of lung cancer patients.<sup>8,13</sup> Our previous study of genetic polymorphisms in MBC suggested that *SLC28A3*, *SLC29A1*, and *RRM1* predicted clinical outcome in patients with MBC receiving paclitaxel plus gemcitabine (PG) chemotherapy.<sup>11</sup> These previous studies, however, have different results in terms of the affected genes in cancer patients following gemcitabine treatment, and there were only a few studies focusing on BC patients.<sup>11,14-17</sup> In addition, most of the studies focused on a small number of genetic polymorphisms, which might not be enough to evaluate the association between genetic polymorphisms and the clinical efficacy of gemcitabine. Because several genes are involved in gemcitabine transport and metabolism, it is necessary to analyze as many genes as possible simultaneously. Therefore, this study is aimed to focus on a large number of genetic polymorphisms in genes found to be involved in gemcitabine transport and metabolism.

We previously conducted a phase II clinical trial of gemcitabine-based chemotherapy as the first line of treatment in patients with HER2-negative MBC.<sup>6</sup> In patients who participated in the clinical trial, we evaluated the association between 103 genetic polymorphisms in 23 genes and the clinical efficacy of gemcitabine.

## PATIENTS AND METHODS

### Patients

This prospective pharmacogenetic study was conducted in cooperation with a phase II clinical trial of eribulin plus gemcitabine (EG) versus PG for treatment of HER2-negative MBC (Korean Cancer Study Group Trial: KCSG BR13-11; [ClinicalTrials.gov](https://clinicaltrials.gov) number: NCT02263495). Pathologically confirmed HER2-negative metastatic or recurrent BC patients without previous cytotoxic chemotherapy for metastatic disease were eligible for the study. Patients aged  $\geq 19$  years with adequate bone marrow, renal, and liver function were included. Patients with previous gemcitabine chemotherapy regardless of treatment setting, parenchymal or leptomeningeal brain metastases, and persistent peripheral neuropathy grade 2 or more caused by previous treatment were excluded. The details of inclusion and exclusion criteria are described in our previous clinical trial.<sup>6</sup>

### Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, Madison, WI). The 103 genetic polymorphisms of the 23 genes involved in gemcitabine transport and metabolism were selected for genotyping based on a previous study.<sup>10</sup> The candidate genetic polymorphisms were genotyped using the MassARRAY system (Sequenom, Inc., San Diego, CA) and Birdseed calling algorithm in SpectroTYPER software (Sequenom, Inc.). The 28-bp tandem repeat in the *TYMS* 5'-untranslated enhanced region (*TSER*) and a 6-bp deletion/insertion in the *TYMS* 3'-untranslated region (*TS* 3'-UTR) was detected using a protocol previously described.<sup>18</sup> Sequencing was carried out for genetic polymorphisms that failed genotyping. After excluding two genetic polymorphisms with a minor allele frequency (MAF)  $< 0.01$ , call rate  $< 90\%$ , or Hardy-Weinberg equilibrium  $P$  value  $< 0.001$ , 101 polymorphisms from 23 genes were finally selected for analysis (Supplementary Table S1, available at <https://doi.org/10.1016/j.esmoop.2021.100236>).

### Statistical analysis

We presented the categorical variables as numbers (percentage) and continuous variables as median (range). The associations of genetic polymorphisms with overall survival (OS), progression-free survival (PFS), and 6-month PFS were evaluated with hazard ratios (HRs) using Cox regression analysis with stepwise selection. Chemotherapy arm and cancer subtype were adjusted for the multivariable analyses, and multicollinearity was checked by variance inflation factor values in the models. Three genetic models (additive, dominant, and recessive) were constructed, respectively. A  $P$  value  $< 0.05$  was considered significant, and variables with a  $P$  value  $< 0.1$  according to a univariable analysis were included in the multivariable analysis. All statistical analyses were carried out using the R package (version 4.0.5). A schematic diagram of genetic

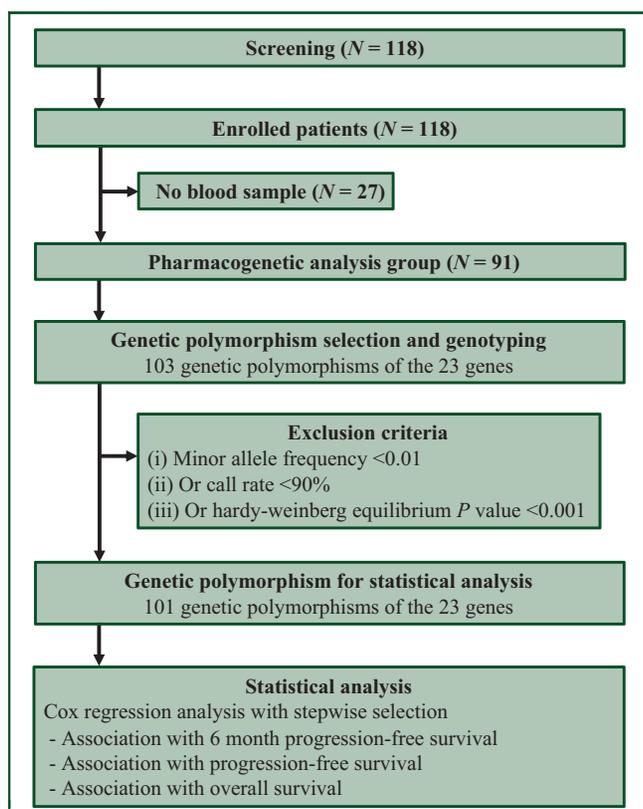


Figure 1. Schematic diagram for genetic polymorphism selection and statistical analysis.

polymorphism selection and statistical analysis are summarized in Figure 1.

## RESULTS

### Clinical characteristics

Of the 118 patients enrolled in the clinical trial, 91 patients were included in this study (Figure 1). Forty-five patients were treated with EG chemotherapy, and 46 were treated with PG chemotherapy. Supplementary Table S2, available at <https://doi.org/10.1016/j.esmoop.2021.100236> describes the clinical characteristics of the patients included in pharmacogenetic analysis. In this study, 39 (42.9%) patients were premenopausal. Hormone receptor-positive BC was found in 80 cases (87.9%) and triple-negative BC in 11 cases (12.1%). Recurred stage IV BC after curative resection was found in 67 cases (73.6%), and *de novo* MBC was found in 24 (26.4%) cases.

### Associations between genetic polymorphisms and 6-month PFS rate

Nine genetic polymorphisms were found to be associated with 6-month PFS. Specifically, rs992160 in *CDC5L*, rs1044457 and rs35687416 in *CMPK1*, rs4694362 and rs7684954 in *DCK*, rs693955 in *SLC29A1*, and rs7039267 in *TLE4* were associated with a lower 6-month PFS rate. rs9436883 in *CMPK1* and rs28363340 in *TENT4A* were associated, however, with a higher 6-month PFS rate. Among them, rs1044457 in *CMPK1* and rs4694362 in *DCK*

were the most significant genetic polymorphisms. For the rs1044457 in *CMPK1*, the 6-month PFS rate was 55.9% for CT and TT genotypes and 78.9% for the CC genotype ( $P < 0.001$  for the dominant genetic model, HR: 4.444, 95% CI: 1.905-10.363). For the rs4694362 in *DCK*, the 6-month PFS rate was 51.9% for TC and CC genotypes and 78.1% for the TT genotype ( $P = 0.001$  for the dominant genetic model, HR: 4.051, 95% CI: 1.749-9.387). The associations between genetic polymorphisms and 6-month PFS rates are summarized in Table 1.

### Associations between genetic polymorphisms and PFS

Two genetic polymorphisms were found to be associated with prolonged PFS, rs9436883 in *CMPK1* and rs13137332 in *DCTD*, and five genetic polymorphisms were determined to be associated with shorter PFS, rs12507552 in *DCTD*, rs693955 and rs760370 in *SLC29A1*, rs2279655 in *TENT4A*, and rs2807312 in *TLE4*. Among them, rs693955 and rs760370 in *SLC29A1* and rs2807312 in *TLE4* were the most significant genetic polymorphisms. For the rs693955 in *SLC29A1*, the median PFS duration was 5.4 months for the AA genotype and 10.5 months for CA and CC genotypes ( $P = 0.002$  for the recessive genetic model, HR: 3.704, 95% CI: 1.615-8.497). For the rs760370 in *SLC29A1*, the median duration of PFS was 5.6 months for the GG genotype and 10.4 months for AA and AG genotypes ( $P = 0.002$  for the recessive genetic model, HR: 5.535, 95% CI: 1.839-16.656). For the rs2807312 in *TLE4*, median duration of PFS was 5.7 months for the TT genotype and 10.4 months for CT and CC genotypes ( $P = 0.005$  in the recessive genetic model, HR: 4.948, 95% CI: 1.612-15.190). The associations between genetic polymorphisms and PFS are summarized in Table 2.

Further survival analysis was carried out using rs2807312 in *TLE4*, and rs693955 in *SLC29A1*, two genetic polymorphisms which mostly affected PFS in the recessive model. In this analysis, the TT genotype of rs2807312 had a shorter PFS compared with that of CC and CT genotypes ( $P = 0.022$ ), and the AA genotype of rs693955 showed a shorter PFS ( $P = 0.044$ ) (Figure 2A and B). We carried out survival analysis with a two-gene combination model (Figure 2C). In this analysis, the TT genotype of rs2807312 had the worst PFS regardless of other genetic polymorphisms, whereas the CA genotype of rs693955 or the CT genotype of rs2807312 without the AA genotype of rs693955 had the best PFS compared with those of other genetic groups ( $P < 0.001$ ).

### Associations between genetic polymorphisms and OS

Three genetic polymorphisms were found to be associated with OS; rs1360780 in *FKBP5* was associated with shorter OS in additive and recessive genetic models ( $P = 0.011$  and 0.046, respectively), and rs760370 in *SLC29A1* was associated with shorter OS in the recessive genetic model ( $P = 0.032$ ). In additive and dominant genetic models, rs2279655 in *TENT4A* was associated with shorter OS ( $P = 0.014$  and 0.013, respectively). The associations between genetic polymorphisms and OS are summarized in Table 3.

**Table 1. Associations between genetic polymorphisms and 6-month progression-free survival**

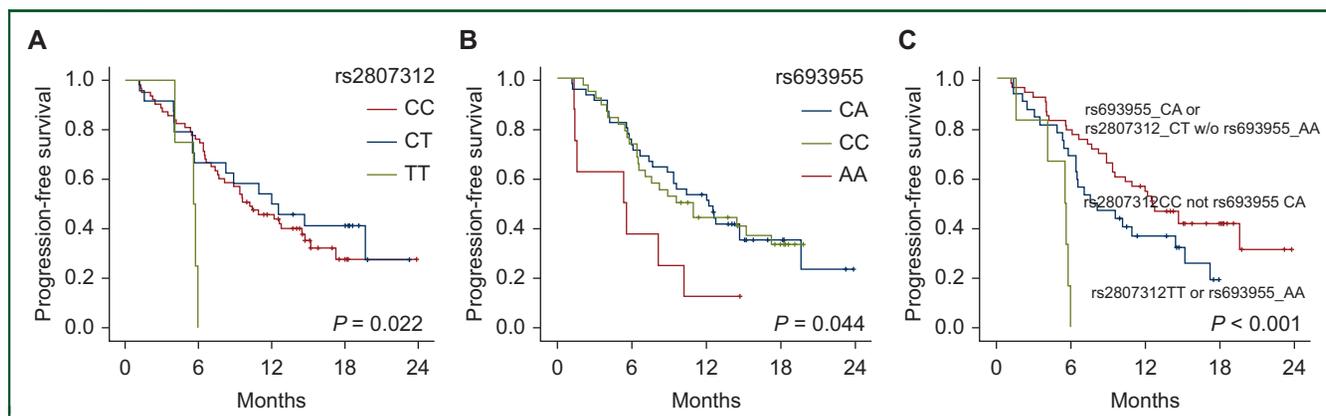
n	Nonprogressive, n (%)			Additive		Dominant		Recessive				
	Maj hom	Het	Min hom	Maj hom	Het	Min hom	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
<i>CDC5L</i> rs992160	81	9	1	59 (72.8)	5 (55.6)	0 (0)	3.265 (1.479-7.208)	0.003	—	—	—	—
<i>CMPK1</i> rs1044457	57	28	6	45 (78.9)	15 (53.6)	4 (66.7)	—	—	4.444 (1.905-10.363)	<0.001	—	—
<i>CMPK1</i> rs35687416	72	18	1	53 (73.6)	11 (61.1)	0 (0)	—	—	—	—	29.784 (2.258-392.837)	0.010
<i>CMPK1</i> rs9436883	65	24	2	41 (63.1)	21 (87.5)	2 (100)	—	—	0.271 (0.080-0.912)	0.035	—	—
<i>DCK</i> rs4694362	64	24	3	50 (78.1)	13 (54.2)	1 (33.3)	—	—	4.051 (1.749- 9.387)	0.001	—	—
<i>DCK</i> rs7684954	85	6	0	62 (72.9)	2 (33.3)	0 (0)	6.042 (1.866-19.562)	0.003	—	—	—	—
<i>SLC29A1</i> rs693955	38	45	8	28 (73.7)	33 (73.3)	3 (37.5)	—	—	—	—	5.078 (1.776-14.519)	0.002
<i>TENT4A</i> rs28363340	67	22	2	43 (64.2)	19 (86.4)	2 (100)	0.299 (0.093-0.960)	0.043	0.188 (0.053-0.666)	0.010	—	—
<i>TLE4</i> rs7039267	37	38	16	29 (78.4)	28 (73.7)	7 (43.8)	2.204 (1.285-3.779)	0.004	—	—	3.806 (1.604-9.031)	0.002

CI, confidence interval; Het, heterozygote; HR, hazard ratio; Maj hom, major allele homozygote; Min hom, minor allele homozygote.

**Table 2. Associations between genetic polymorphisms and progression-free survival**

n	Median, months (95% CI)			Additive		Dominant		Recessive				
	Maj hom	Het	Min hom	Maj hom	Het	Min hom	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
<i>CMPK1</i> rs9436883	65	24	2	8.9 (6.1-10.9)	14.2 (8.9-17.0)	10.8 (6.4-15.1)	0.501 (0.280-0.894)	0.019	0.444 (0.234-0.843)	0.013	—	—
<i>DCTD</i> rs12507552	65	23	3	9.6 (7.1-12.5)	10.9 (6.5-14.5)	6.4 (3.5-7.7)	—	—	—	—	4.368 (1.308-14.589)	0.017
<i>DCTD</i> rs13137332	82	9	0	9.6 (7.1-12.2)	13.8 (6.5-18.3)	—	0.281 (0.085-0.922)	0.036	0.359 (0.111-1.165)	0.088	—	—
<i>NT5C3A</i> rs12668520	51	31	9	11.6 (8.9-14.3)	8.2 (6.1-12.6)	7.1 (5.4-15.1)	1.406 (0.973-2.029)	0.069	—	—	—	—
<i>SLC29A1</i> rs693955	38	45	8	9.8 (6.5-14.5)	12.0 (7.7-14.1)	5.4 (1.3-14.7)	—	—	—	—	3.704 (1.615-8.497)	0.002
<i>SLC29A1</i> rs760370	53	34	4	10.2 (7.4-14.3)	10.5 (5.8-13.8)	5.6 (4.0-8.2)	—	—	—	—	5.535 (1.839-16.656)	0.002
<i>TENT4A</i> rs2279655	66	24	1	10.7 (7.7-14.3)	7.9 (5.3-12.0)	14.5 (—)	—	—	1.786 (1.021-3.124)	0.042	—	—
<i>TLE4</i> rs10125657	80	10	1	9.5 (6.5-11.6)	13.5 (7.1-18.0)	17.0 (—)	0.438 (0.174-1.105)	0.081	—	—	—	—
<i>TLE4</i> rs2807312	63	24	4	10.0 (7.6-12.6)	12.3 (5.3-12.0)	5.7 (4.1-6.0)	—	—	—	—	4.948 (1.612-15.190)	0.005

CI, confidence interval; Het, heterozygote; HR, hazard ratio; Maj hom, major allele homozygote; Min hom, minor allele homozygote.



**Figure 2.** Progression-free survival of the breast cancer patients treated with gemcitabine-based chemotherapy according to genetic polymorphisms in (A) rs2807312, (B) rs693955, and (C) combination of two genetic polymorphisms.

## DISCUSSION

We evaluated genetic polymorphisms of 23 gemcitabine metabolism-associated genes in BC patients treated with gemcitabine-based chemotherapy. In terms of 6-month PFS, rs1044457 in *CMPK1* was the most significant genetic polymorphism. The genetic polymorphisms of rs693955 in *SLC29A1* and rs2807312 in *TLE4* were significantly associated with the duration of PFS. In detail, the AA genotype of rs693955 in BC with gemcitabine chemotherapy had poor clinical outcomes compared with those of CA and CC genotypes, and the TT genotype of rs2807312 had poor outcomes compared with those of the CT and CC genotypes. In survival analysis with a multi-gene model, the TT genotype of rs2807312 had the worst PFS regardless of other genetic polymorphisms, whereas the CA genotype of rs693955 or the CT genotype of rs2807312 without the AA genotype of rs693955 had the best. In terms of OS, rs1360780 in *FKBP5*, rs2279655 in *TENT4A*, and rs760370 in *SLC29A1* were associated.

Gemcitabine requires intracellular phosphorylation for metabolite activation by deoxycytidine kinase (dCK) and cytidine monophosphate kinase 1 (CMPK1). Specifically, dCK phosphorylates gemcitabine to gemcitabine monophosphate, and then CMPK phosphorylates gemcitabine monophosphate to gemcitabine diphosphate.<sup>4</sup> In this study, genetic polymorphisms in *DCK* and *CMPK1*, which encode dCK and CMPK1, respectively, were associated with the clinical efficacy of gemcitabine. *CMPK1* was the most important gene, as three of its genetic polymorphisms were associated with the clinical efficacy of gemcitabine. Especially, rs1044457 was the most significant genetic polymorphism in the dominant genetic model. CT and TT genotypes showed lower 6-month PFS than that of the CC genotype. According to a previous study, nuclear expression of *CMPK1* was associated with poor prognosis in triple-negative BC.<sup>19</sup> Because rs1044457 is located in the 3'-UTR, it can affect the expression of *CMPK1*. Therefore, rs1044457 would likely affect the 6-month PFS rate. Further studies to examine the expression of rs1044457 would be helpful to understand the mechanisms that support the association of rs1044457 with the clinical efficacy of gemcitabine. In this study, rs35687416 was associated with 6-

month PFS in the recessive genetic model (GG and GT genotypes versus TT genotype). However, statistical power was weak because there was only one patient with the TT genotype.

*TLE4* encodes transducin-like enhancer of split 4 (TLE4), a transcriptional corepressor. One previous study reported the association of *TLE4* with gemcitabine toxicity.<sup>20</sup> Another previous study reported, however, that *TLE4* expression was not associated with favorable prognosis in pancreatic cancer patients.<sup>21</sup> In this study, two genetic polymorphisms in *TLE4* were associated with clinical efficacy: rs2807312 and rs7039267. Further studies are required to explore the association of *TLE4* with the clinical efficacy of gemcitabine.

*SLC28A1*, *SLC28A3*, and *SLC29A1* encode nucleoside transporters that allow gemcitabine to enter the cell. *SLC28A1* and *SLC28A3* encode human concentrative nucleoside transporter (hCNT) 1 and 3, respectively. *SLC29A1* encodes human equilibrative nucleoside transporter (hENT) 1. According to previous studies, hCNT and hENT levels were associated with gemcitabine sensitivity and improved clinical efficacy of gemcitabine.<sup>22-26</sup> In addition, one previous study reported that genetic polymorphisms in these genes were associated with gemcitabine clearance, which can affect the clinical efficacy of gemcitabine.<sup>27</sup> Consistent with previous studies, genetic polymorphisms in *SLC29A1*, rs693955 and rs760370, were associated with OS, PFS, and 6-month PFS in this study. No genetic polymorphisms in *SLC28A1* or *SLC28A3*, however, were associated with the clinical efficacy of gemcitabine.

Phosphorylation by dCK is a rate-limiting step and is essential for gemcitabine activation. Previous studies showed that decreased expression of dCK was associated with gemcitabine resistance,<sup>28-31</sup> and that dCK expression also was associated with the clinical efficacy of gemcitabine in various types of cancer patients (OS in pancreatic cancer patients and recurrence-free survival rate in biliary tract cancer patients).<sup>32-34</sup> In this study, rs4694362 and rs7684954 in *DCK* were associated with 6-month PFS. Expression studies of rs7684954 would be helpful to understand the mechanisms that support the association of genetic polymorphisms in *DCK* with clinical efficacy.

**Table 3. Associations between genetic polymorphisms and overall survival**

n	Median, months (95% CI)				Additive		Dominant		Recessive	
	Maj hom	Het	Min hom	Maj hom	Het	Min hom	HR (95% CI)	P value	HR (95% CI)	P value
FKBP5 rs1360780	28	7	17.3 (15.0-18.3)	17.3 (14.7-19.8)	14.3 (7.3-21.1)	2.373 (1.218-4.621)	0.011	—	3.640 (1.023-12.957)	0.046
NME7 rs1320964	39	19	17.8 (14.7-19.3)	16.7 (14.5-18.6)	15.2 (13.3-18.9)	—	—	3.156 (0.909-10.954)	—	0.070
SLC29A1 rs760370	34	4	15.4 (14.5-18.0)	18.5 (15.8-20.0)	13.0 (7.8-19.4)	—	—	—	5.537 (1.154-25.563)	0.032
TENT4A rs2279655	24	1	18.0 (15.2-18.4)	16.1 (12.8-18.5)	14.5 (-)	2.946 (1.241-6.994)	0.014	3.269 (1.282-8.336)	—	0.013

CI, confidence interval; Het, heterozygote; HR, hazard ratio; Maj hom, major allele homozygote; Min hom, minor allele homozygote.

*TENT4A*, formerly known as *POLS*, encodes DNA polymerase kappa (Pol  $\kappa$ ), which is involved in replication of damaged DNA in a process called translesion synthesis (TLS). DNA polymerases involved in TLS include pol  $\eta$ , pol  $\iota$ , pol  $\kappa$ , pol  $\theta$ , pol  $\psi$ , pol  $\sigma$ , pol  $\xi$ , and Rev1. Several previous studies reported that levels of these special DNA polymerases were associated with drug resistance as well as with the clinical efficacy of DNA damaging agents.<sup>35-38</sup> In this study, two genetic polymorphisms in *TENT4A* were associated with OS, PFS, and 6-month PFS; rs2279655 was associated with OS and PFS, and rs28363340 was associated with 6-month PFS. According to the previous studies, rs274713, rs274717, and rs2279653 were associated with the clinical efficacy of gemcitabine.<sup>10,39</sup> This association, however, was not reproduced in the present study.

Screening for genetic polymorphisms before gemcitabine treatment would be helpful for optimizing clinical efficacy. Depending on the number of genetic polymorphisms, customized chip or allele-specific polymerase chain reaction can be selected as a genotyping method. According to the results of the present study, rs1044457 in *CMPK1*, rs693955 in *SLC29A1*, and rs2807312 in *TLE4* were significantly associated with clinical efficacy of gemcitabine. rs1044457, rs693955, and rs2807312 are common variants in general populations, with MAF of 0.4036, 0.1444, and 0.08988, respectively.<sup>40</sup> Therefore, selective screening of these genetic polymorphisms can be cost-effective and useful in patients subject to gemcitabine treatment. After further studies to validate the results of the present study, cost-effective analysis can be considered to apply genotyping to clinical practice.

There have been only a few pharmacogenetic studies of gemcitabine focused on BC patients (Supplementary Table S3, available at <https://doi.org/10.1016/j.esmoop.2021.100236>). These studies evaluated the associations between genetic polymorphisms and clinical efficacy, such as survival and toxicity. Compared with previous studies, the present study has some strong points. This is the first study to analyze a large number of genetic polymorphisms simultaneously in BC patients treated with gemcitabine-based chemotherapy. In addition, the numbers of genetic polymorphisms/genes and patients included in this study are the largest to date. An extensive analysis of the genes associated with gemcitabine transport and metabolism in BC patients provide tangible insight into the use of gemcitabine in BC treatment.

This study has some limitations. First, statistical significance was not maintained when multiple testing correction was conducted. Some genetic polymorphisms, however, showed statistical significance in previous studies as well as in the present study: rs1044457 and rs35687416 in *CMPK1* and rs992160 in *CDC5L*. This could improve the reliability of the results in this study. Second, sample size was small, and we did not have a validation cohort. This study, however, had the largest sample size to date of such studies of BC patients. Further large-scale studies are required to validate our results. Third, further studies, such as expression studies that could support the associations we identified, were not

conducted. Several previous studies identified the association between gene expression and the clinical efficacy of gemcitabine. Expression studies of the genetic polymorphisms identified in this study would be helpful to demonstrate the results of this study.

In conclusion, we identified several genetic polymorphisms associated with the clinical efficacy of gemcitabine in BC patients. The genetic polymorphisms of rs1044457 in *CMPK1*, rs693955 in *SLC29A1*, and rs2807312 in *TLE4* were significantly associated with the 6-month PFS rate and/or the duration of PFS. This is the largest pharmacogenetic study of gemcitabine-based BC treatment in a prospective clinical trial. The results of this study may contribute to the personalized treatment of BC. Further studies with a larger sample size and expression studies would be helpful to validate the associations between genetic polymorphisms and the clinical efficacy of gemcitabine.

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## DISCLOSURE

The authors have declared no conflicts of interest.

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