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
**INTRODUCTION**

Breast cancer (BC) was the most commonly diagnosed malignancy worldwide in 2020. 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. 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. 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. 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. Moreover, gemcitabine can be used continuously until disease progression because of its feasible toxicity profile.

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. In pancreatic cancer, CMPK1 polymorphism or a combination model of CDA/DCK/DCTD/SLC28A3/SLC29A1 or CDA/RRM1/SLC29A1 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. 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. 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. 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. 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 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 ≥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.

**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. 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. 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
polygenetic analysis. In this study, 39 (42.9%) 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 CDCSL, 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     | 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 |
|-------|---------|-----|---------|---------|-----|---------|-------------|---------|-------------|---------|-------------|---------|
| CDC5L rs992160 | 59 (72.8) | 5 (55.6) | 0 (0) | 3.265 (1.479-7.208) | 0.003 |
| CMPK1 rs1044457 | 45 (78.9) | 15 (53.6) | 4 (66.7) | 4.444 (1.905-10.363) | <0.001 |
| CMPK1 rs35687416 | 53 (73.6) | 11 (61.1) | 0 (0) | 29.784 (2.258-392.837) | 0.010 |
| CMPK1 rs9436883 | 41 (63.1) | 21 (87.5) | 2 (100) | 0.271 (0.080-0.912) | 0.035 |
| DCK rs4694362 | 50 (78.1) | 13 (54.2) | 1 (33.3) | 4.051 (1.749-9.387) | 0.001 |
| DCK rs7684954 | 62 (72.9) | 2 (33.3) | 0 (0) | 6.042 (1.866-19.562) | 0.003 |
| SLC29A1 rs693955 | 28 (73.7) | 33 (73.3) | 3 (37.5) | 5.078 (1.776-14.519) | 0.002 |
| CMPK1 rs9436883 | 43 (64.2) | 19 (86.4) | 2 (100) | 0.281 (0.085-0.922) | 0.036 |
| DCTD rs12507552 | 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 |
| DCTD rs13137332 | 11.6 (8.9-14.3) | 8.2 (6.1-12.6) | 7.1 (5.4-15.1) | 3.704 (1.615-8.497) | 0.002 |
| NTSC3A rs12668520 | 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 |
| SLC29A1 rs760370 | 10.7 (7.7-14.3) | 7.9 (5.3-12.0) | 14.5 (—) | 1.786 (1.021-3.124) | 0.042 |
| TLE4 rs10125657 | 9.5 (6.5-11.6) | 13.5 (7.1-18.0) | 17.0 (—) | 0.438 (0.174-1.105) | 0.081 |
| TLE4 rs2807312 | 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.

### 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 |
| CMPK1 rs9436883 | 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 |
| DCTD rs13137332 | 9.6 (7.1-12.2) | 13.8 (6.5-18.3) | — | 0.281 (0.085-0.922) | 0.036 |
| NTSC3A rs12668520 | 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 |
| SLC29A1 rs760370 | 10.2 (7.4-14.3) | 10.5 (5.8-13.8) | 5.6 (4.0-8.2) | — | — |
| TLE4 rs10125657 | 10.7 (7.7-14.3) | 7.9 (5.3-12.0) | 14.5 (—) | 1.786 (1.021-3.124) | 0.042 |
| TLE4 rs2807312 | 9.5 (6.5-11.6) | 13.5 (7.1-18.0) | 17.0 (—) | 0.438 (0.174-1.105) | 0.081 |

CI, confidence interval; Het, heterozygote; HR, hazard ratio; Maj hom, major allele homozygote; Min hom, minor allele homozygote.
**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 rs2807321 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. 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. 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 rs104457 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. Another previous study reported, however, that TLE4 expression was not associated with favorable prognosis in pancreatic cancer patients. 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. 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. 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, 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). 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.
TENT4A, formerly known as POLS, encodes DNA polymerase kappa (Pol κ), which is involved in replication of damaged DNA in a process called translesion synthesis (TLS). DNA polymerases involved in TLS include pol h, pol i, pol k, pol σ, pol ξ, 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. 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. 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. 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

| Table 3. Associations between genetic polymorphisms and overall survival |
|-----------------|-------------|-----------------|
|                  | Median, months (95% CI) | Additive Dominant Recessive |
|                  | HR (95% CI) | P-value | HR (95% CI) | P-value | HR (95% CI) | P-value |
| FKBP5 rs1360780  | 17.3 (15.0-19.3) | 14.5 (12.9-16.9) | 13.0 (11.3-19.2) | 2.373 (1.218-4.621) | 0.011 |
| NME7 rs1320094   | 17.8 (15.7-19.3) | 16.7 (15.4-18.6) | 15.2 (13.3-18.9) | 3.156 (0.909-10.954) | 0.070 |
| SLC29A1 rs760370 | 15.4 (14.5-18.0) | 18.5 (15.8-20.0) | 13.0 (11.3-19.2) | 5.537 (1.154-25.563) | 0.032 |
| TENT4A rs2279655 | 18.0 (15.2-18.4) | 16.1 (13.8-18.5) | 14.5 (12.9-17.8) | 2.946 (1.214-6.994) | 0.014 |

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

FUNDING
This work was supported by a grant from the South Korean Ministry of Health and Welfare [grant number HA17C0055] and by the South Korean National R&D Program for Cancer Control, Ministry of Health and Welfare [grant number 1720150].

DISCLOSURE
The authors have declared no conflicts of interest.

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