Drug-related genetic polymorphisms affecting severe chemotherapy-induced neutropenia in breast cancer patients

A hospital-based observational study

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Abstract

Chemotherapy-induced neutropenia (CIN) is one of the major adverse events that necessitate chemotherapy dose reduction. This study aimed to evaluate the association between grade 4 neutropenia and genetic polymorphisms in breast cancer patients. In this genetic polymorphism association study, peripheral blood samples from 100 consecutive breast cancer outpatients, between August 2012 and September 2014, treated with doxorubicin and cyclophosphamide (AC) combination chemotherapy were genotyped for polymorphisms in adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1), cytochrome P450 (CYP) enzyme-coding genes (CYP2B6 and CYP3A5), glutathione S-transferase (GST), and excision repair complementing 1 (ERCC1). Associations between grade 4 neutropenia and genotypes as well as risk factors were examined using multivariate logistic regression. From 100 patients, 32.0% had grade 4 neutropenia. Multivariate logistic regression analysis revealed that ERCC1 118C > T (odds ratio [OR], 3.43; 95% confidence interval [CI], 1.22–9.69; P = 0.020), CYP2B6*6 (OR, 4.51; 95% CI, 1.21–16.95; P = 0.026), body mass index (BMI) (OR, 6.94; 95% CI, 1.15–41.67; P = 0.035), and baseline white blood cell (WBC) count (OR, 2.99; 95% CI, 1.06–8.40; P = 0.030) were significant predictors of grade 4 neutropenia. ERCC1 and CYP2B6 gene polymorphisms were associated with the extent of grade 4 neutropenia in patients receiving AC chemotherapy. In addition to previously known risk factors, BMI and WBC counts, ERCC1 and CYP2B6 gene polymorphisms were also identified as independent strong predictors of grade 4 neutropenia.

Abbreviations: ABCB1 = adenosine triphosphate-binding cassette subfamily B member 1, AC = doxorubicin and cyclophosphamide, ALT = alanine aminotransferase, ANC = absolute neutrophil count, AST = aspartate aminotransferase, BMI = body mass index, CI = confidence interval, CIN = chemotherapy-induced neutropenia, CYP = cytochrome P450, ERCC1 = excision repair cross-complementing 1, G-CSF = granulocyte colony-stimulating factor, GSTs = glutathione S-transferases, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, WBC = white blood cell.

Keywords: breast cancer, cyclophosphamide, doxorubicin, genetic polymorphism, neutropenia

1. Introduction

Chemotherapy-induced neutropenia (CIN) is a major dose-limiting toxicity of systemic cancer chemotherapy and frequently necessitates the reduction of the initial chemotherapeutic dose. The degree and duration of neutropenia determine the risk of life-threatening infections. CIN is directly associated with concomitant morbidity and mortality rates and health care costs. Therefore, it is critical to identify risk factors for CIN, and an adequate management of CIN in cancer patients is essential. Several risk factors, such as female gender, older age, and lower pretreatment blood cell counts, for CIN are well known and were previously reported. In addition, pharmacogenetic factors, as potential risk factors for CIN, garnered increased interest in recent years.

Anthracycline-based chemotherapy containing doxorubicin and cyclophosphamide (AC) is a standard regimen commonly used worldwide to treat early-stage breast cancer. Doxorubicin is subject to transport by organic anion transporters belonging to the amphiphilic solute transporter family 22 and is mainly exported by adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) transporter. ABCB1 polymorphisms significantly impact the levels of doxorubicin in breast cancer patients, resulting in significantly increased serum levels and reduced clearance in patients with ABCB1 variant alleles. Conversely, cyclophosphamide is administered as a prodrug that undergoes Phase I activation metabolism by the cytochrome P450 (CYP) enzymes CYP2B6, CYP3A4, and
CYP3A5 and phase II inactivation primarily through conjugation with a thiol or sulfate via glutathione S-transferases (GSTs).[9–12] The active metabolite, 4-hydroxy-cyclophosphamide diffuses into cancer cells and is responsible for the alkylating ability of cyclophosphamide. Functional genes related to drug transport and metabolism and DNA repair impact the cytotoxic effects associated with chemotherapy. Nevertheless, little is known regarding pharmacogenomics in breast cancer treatment.

Importantly, 1 study found that severe neutropenia during breast cancer treatment with standard-dose adjuvant AC combination chemotherapy was observed more frequently in Asian patients than in Caucasian patients.[13] Interethnic differences and pharmaecoethnicity are increasingly recognized as underlying interindividual variations in drug responsiveness and anticancer drug responsiveness, respectively. One potential reason for this effect is genetic differences in the functions of drug metabolizing enzymes and/or transporters. Therefore, it is critical to determine the role of pharmacogenetic polymorphisms in each ethnic group to improve treatment outcomes and reduce adverse events.

In this study, we assessed the association between grade 4 neutropenia and genetic polymorphisms in breast cancer patients receiving AC combination chemotherapy to examine potential effects of gene polymorphisms on severe CIN. In addition, we assessed potential risk factors predisposing patients receiving adjuvant AC combination chemotherapy to severe neutropenia.

2. Patients and methods

2.1. Study design and patients

In this genetic polymorphism association study, a total of 100 consecutive Japanese breast cancer outpatients receiving 4 cycles of AC combination therapy between August 2012 and September 2014 at the Department of Medical Oncology at Seirei Hamamatsu General Hospital were analyzed. Patients were eligible for this pharmacogenetic study if they had histologically confirmed breast cancer and planned to receive 4 cycles of the standard AC regimen (60-mg/m² doxorubicin and 600-mg/m² cyclophosphamide on day 1 of a 21-day cycle) as neoadjuvant or adjuvant chemotherapy, were ≥20 years of age at diagnosis, had an Eastern Cooperative Oncology Group performance status of 0 to 1, and adequate baseline hematologic, hepatic, and renal functions (white blood cell [WBC] count ≥3 × 10³/L; platelet count ≥100 × 10³/L; alanine aminotransferase [ALT] <100 IU/L; total bilirubin <2 mg/dL; and serum creatinine <1.5 mg/dL).

Written informed consent was obtained from all patients. This study was approved by the Institutional Review Board of the Seirei Hamamatsu General Hospital and complied with the provisions of the Declaration of Helsinki. The STROBE checklist is available as Supplementary Material (see Checklist 1, Supplementary Material, http://links.lww.com/MD/B370).

2.2. Assessment

Hematologic toxicities were graded according to the Common Terminology Criteria for Adverse Events, version 4.0. As absolute neutrophil count (ANC) is the most important indicator and most readily quantifiable parameter of myelotoxicity, clinically significant and severe myelotoxicity induced by chemotherapy was defined as the presence of grade 4 neutropenia (ANC < 0.5 × 10⁹/L). The main evaluation parameter was the percentage of patients with grade 4 neutropenia. The neutropenia grade was based on the lowest recorded neutrophil count during the first cycle of AC chemotherapy.

Several baseline variables, including age, body mass index (BMI), clinical stage, and baseline laboratory data of all patients that could potentially influence severe CIN were included in this study. The cutoff values for laboratory data were based on the medians in this study. Patients with BMI > 25 kg/m² were considered overweight; this cutoff was chosen in accordance with the universal BMI criteria developed by the World Health Organization.[14]

2.3. Pharmacogenetic analysis

Five milliliters of whole venous blood samples from all patients were collected into EDTA-2Na Venoject II tubes (Terumo, Tokyo, Japan) and stored at −25°C before DNA extraction. Leukocyte genomic DNA was extracted directly from the blood specimen using a QIAamp DNA Blood Maxi Kit (Qiagen N.V., Limburg, the Netherlands). We selected the following polymorphisms in 5 genes that were associated with functional effects on gene expression, level, or activity: ABCB1 1236C>T, ABCB1 2677G>T/A, ABCB1 3435C>T, CYP2B6*4 (785A>G), CYP2B6*5 (1459C>T), CYP2B6*6 (516G>T and 785A>G), CYP2B6*9 (516G>T), CYP3A5 6986A>G, GSTM1/T1 null, GSTP1 313A>G, excision repair cross-complementing 1 (ERCC1) 118C>T, and ERCC1 8092G>T. Genotyping for all gene polymorphisms was performed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. PCR conditions, primer, and restriction enzymes used for all reactions are shown in Table 1. All patients were classified into 2 groups: homozygous wild-type or carriers of 1 or more variant alleles.

2.4. Statistical analysis

The optimal sample size was not estimated a priori because this is an exploratory study. Continuous variables were summarized as means ± standard deviation, and categorical variables were presented as frequencies and proportions. Categorical variables were compared using the chi-square test. If assumptions of the chi-square test were not met, that is, >20% of the cells in the contingency table had expected values of <5 or the minimum expected frequency was <1, Fisher’s exact test was used. The genotypic distribution of each polymorphism was also examined for deviation from Hardy–Weinberg equilibrium using the chi-square test. Risk factors associated with grade 4 neutropenia were examined using the chi-square test or Fisher’s exact test in univariate analysis and multivariate logistic regression analysis. Risk factors with P values of < 0.15 by univariate analysis were included in multivariate analysis. Results of multivariate analysis were reported as odds ratios (ORs) with 95% confidence intervals (CIs) and P values. All statistical tests were 2-sided, and P values of <0.05 were considered statistically significant. All statistical analyses were performed using PASW Statistics for Windows, version 22.0 (SPSS, Chicago, IL).

3. Results

3.1. Patient characteristics

In this genetic polymorphism association study, a total of 100 patients with breast cancer were included. The baseline
Table 1
Primer sequences, annealing temperatures, restriction enzymes, and digestion temperatures used for genetic polymorphisms analysis in this study.

| Gene | Polymorphism | rs number | Primer | Annealing temperature | Restriction enzyme | Digestion temperature |
|------|--------------|-----------|--------|-----------------------|--------------------|-----------------------|
| ABCB1 | 1236C>T | rs1128503 | F: 5'-TTCAGTTGATGCCAGTCTC-3' | 52°C | HaeII | 37°C |
|       | 2677G>T/A | rs2032582 | R: 5'-CTACACATCCAGCTACGTC-3' | 52°C | NheI | 37°C |
|       | 2677G>T/A | rs2032582 | R: 5'-CTACACATCCAGCTACGTC-3' | 52°C | NheI | 37°C |
| CYP2B6 | 516G>T | rs374274 | F: 5'-CTGAGGCTCTTCTCAAGG-3' | 56°C | BstI | 37°C |
|       | 785A>G | rs2279343 | R: 5'-GACGAGGAGGAGGAGGAGG-3' | 60°C | StyI | – |
|       | 1459C>T | rs3211371 | R: 5'-GTAAGATGATGAGGGATGAGG-3' | 60°C | BglII | 37°C |
| CYP3A5 | 6986A>G | rs776746 | F: 5'-GACGAGGAGGAGGAGGAGG-3' | 56°C | SspI | 37°C |
| GST | M1 | – | F: 5'-GAACTCTCTGAAAGTTAAGGCT-3' | 56°C | – | – |
| T1 | – | – | F: 5'-TCCTTCTGAATGACACATGCG-3' | 60°C | MboII | 55°C |
| P1 313A>G | rs1695 | – | F: 5'-ACCCGAAGGTCTTGGAGAAG-3' | 64°C | BsmAI | 55°C |
| ERCC1 | 118C>T | rs16165 | R: 5'-GTACGCAGAGGACGAGGACG-3' | 63°C | BstDII | 65°C |
| 8092G>T | rs3212986 | – | F: 5'-GGGCACCTCAGTCTTTTTCT-3' | 60°C | MboII | 37°C |

ABCB1 = adenosine triphosphate-binding cassette subfamily B member 1; CYP = cytochrome P450; ERCC1 = excision repair cross-complementing 1; GSTs = glutathione S-transferases.

The mean age of study participants was 52 years. All patients were female and received a combination of 60-mg/m² doxorubicin and 600-mg/m² cyclophosphamide as adjuvant or neo-adjuvant treatment.

Gene polymorphisms were evaluated using genomic DNA extracted from peripheral leukocytes in all patients included in this study. The observed genotype distributions were in line with Hardy–Weinberg equilibrium (P > 0.05 for all variants).

The genotype frequencies of gene polymorphisms included in this study were as follows: for ABCB1 1236C>T, the genotype frequency for CC was 14.0% and that for CT and TT was 86.0%; for ABCB1 2677G>T/A, the genotype frequency for GG was 22.0% and that for GT, GA, TT, TA, and AA was 78.0%; for ABCB1 3435C>T, the genotype frequency for CC was 35.0% and that for CT and TT was 65.0%; for CYP2B6*,4, the frequencies of genotypes with and without *4 mutations were 78.0% and 22.0%, respectively; for CYP2B6*,5, the frequencies of genotypes with and without *5 mutations were 95.0% and 5.0%, respectively; for CYP2B6*,6, the frequencies of genotypes with and without *6 mutations were 72.0% and 28.0%, respectively; for CYP2B6*,9, the frequencies of genotypes with and without *9 mutations were 99.0% and 1.0%, respectively; for CYP3A5 6986A>G, the genotype frequency for AA was 2.0% and that for AG and GG was 98.0%; for GSTM1, the frequencies of null/null and other genotypes were 24.0% and 76.0%, respectively; for GSTP1 313A>G, the genotype frequency for AA was 68.0% and that for AG and GG was 32.0%; for ERCC1 118C>T, the genotype frequency for CC was 58.0% and that for CT and TT was 42.0%; and for ERCC1 8092G>T, the genotype frequency for GG was 56.0% and that for GT and TT was 44.0%. The observed genotype frequencies were in line with an expected Hardy–Weinberg equilibrium (P > 0.05 for all variants).

3.2. Association between grade 4 neutropenia incidence and genotypes

Of a total of 100 patients, the proportion of patients with grade 4 neutropenia was 32.0% (n = 32); the genotypes of these patients were classified into 2 groups (Table 3). Patients with the ERCC1 118 CT and TT genotypes had a significantly higher rate of grade 4 neutropenia than those with the CC genotype (45.2% vs 22.4%, P = 0.017). The rate of grade 4 neutropenia was higher in patients not harboring CYP2B6*6 allele than in those harboring CYP2B6*6 allele, which trended toward significance (37.5% vs 17.9%, P = 0.063). Similarly, the rate of grade 4 neutropenia was higher in patients harboring ABCB1 1236 CT and TT genotypes than in those harboring the CC genotype, which trended toward significance (36.0% vs 7.1%, P = 0.061).

3.3. Association between grade 4 neutropenia incidence and patient characteristics

We performed univariate analysis to examine the relationship of patient characteristics with grade 4 neutropenia (Table 4). The following factors exhibited either a significant correlation or a trend toward significant correlation in univariate analysis:...
baseline WBC count ($P=0.012$), baseline AST ($P=0.017$), baseline hemoglobin level ($P=0.049$), and BMI ($P=0.110$). No association was found between clinical stage and grade 4 neutropenia. The cutoff values for all parameters were determined based on the median value for continuous variables.

### Table 2
Baseline demographics and clinical characteristics of the study patients.

| Gender | Male | Female |
|--------|------|--------|
| Age, y | 52.1±10.2 | 52.1±10.2 |
| Height, cm | 156.4±5.6 | 156.4±5.6 |
| Weight, kg | 54.2±7.9 | 54.2±7.9 |
| BMI, kg/m² | 22.1±5.1 | 22.1±5.1 |
| Doxorubicin dose, mg/m² | 600 | 600 |
| Cyclophosphamide dose, mg/m² | 600 | 600 |
| WBC, mm³ | 5.69±1.4 | 5.69±1.4 |
| Hb, g/dL | 12.7±1.2 | 12.7±1.2 |
| PLT, x10⁹/L | 240.7±54.9 | 240.7±54.9 |
| AST, IU/L | 19.3±7.6 | 19.3±7.6 |
| ALT, IU/L | 17.4±12.2 | 17.4±12.2 |
| ER | Positive | 65 (65.0%) |
| | Negative | 35 (35.0%) |
| PgR | Positive | 45 (45.0%) |
| | Negative | 55 (55.0%) |
| Type of chemotherapy | Adjuvant | 78 (78.0%) |
| | Neoadjuvant | 22 (22.0%) |
| Stage | I | 23 (23.0%) |
| | II | 36 (36.0%) |
| | IIIa | 13 (13.0%) |
| | IIIb | 2 (2.0%) |
| | IIIc | 1 (1.0%) |
| | IV | 1 (1.0%) |
| Type of chemotherapy | Adjuvant | 78 (78.0%) |
| | Neoadjuvant | 22 (22.0%) |
| Stage | I | 23 (23.0%) |
| | II | 36 (36.0%) |
| | IIIa | 13 (13.0%) |
| | IIIb | 2 (2.0%) |
| | IIIc | 1 (1.0%) |
| | IV | 1 (1.0%) |

3.4. Risk factors for grade 4 neutropenia

To explore genetic polymorphisms as potential predictors of grade 4 neutropenia, we performed univariate analysis. The following 7 factors exhibited either a significant correlation or a trend toward a significant correlation in univariate analysis and were included in the multivariate logistic regression analysis: baseline WBC count, baseline AST, baseline hemoglobin level, baseline BMI, and genotypes ERCC1 118C>T, CYP2B6 *6, and ABCB1 1236C>T.

The multivariate analysis adjusted for these risk factors showed that ERCC1 118C>T: non-CC (OR, 3.432; 95% CI, 1.16–9.687; $P=0.020$), non-*6 CYP2B6 allele (OR, 4.505; 95% CI, 1.212–16.949; $P=0.025$), body mass index (BMI) < 25 kg/m² (OR, 6.944; 95% CI, 1.151–41.667; $P=0.035$), and baseline WBC count <5.68 x 10⁹/L (OR, 2.985; 95% CI, 1.060–8.403; $P=0.038$) were significant determinants of grade 4 neutropenia in patients receiving AC combination chemotherapy (Table 5).

3.5. Association between the number of identified risk factors and grade 4 neutropenia development

Based on multivariate analysis results, 4 patient-related risk factors, including BMI, baseline WBC, ERCC1 118C>T, and CYP2B6*6, were determined as significantly associated with grade 4 neutropenia development. The number of risk factors was significantly associated with grade 4 neutropenia development and their associations are summarized in Table 6. All patients but 1 had more than 1 risk factor. The frequency of grade 4 neutropenia was observed in 7.1% of patients with 1 risk factor, 11.8% with 2 risk factors, 51.4% with 3 risk factors, and 57.1% with all 4 risk factors. Compared with those carrying 1 risk factor, OR for grade 4 neutropenia development in patients carrying 2, 3, and 4 risk factors were 1.733 (95% CI, 0.176–17.047; $P=1.000$), 13.722 (95% CI, 1.625–115.895; $P=0.0129$), and 17.333 (95% CI, 1.750–171.662; $P=0.039$), respectively.

### Table 3
Association between grade 4 neutropenia incidence and genotypes by univariate analysis.

| Polymorphism | N | Grade 4 neutropenia (%) | N (%) | P |
|--------------|---|-------------------------|-------|---|
| ABCB1 1236C>T |   |                         |       |   |
| CC           | 14 | 1 (7.1)                 | 0.061 |   |
| CT and TT    | 86 | 31 (36.0)               |       |   |
| ABCB1 2677G>T/A | |                         |       |   |
| GG           | 22 | 6 (27.3)                | 0.591 |   |
| GT, GA, TT, TA, AA | 78 | 25 (33.3)              |       |   |
| ABCB1 3435C>T |   |                         |       |   |
| CC           | 35 | 9 (25.7)                | 0.325 |   |
| CT and TT    | 64 | 23 (35.4)              |       |   |
| CYP2B6       |   |                         |       |   |
| Not harboring *4 allele | 78 | 23 (29.5)         | 0.313 |   |
| Harboring *4 allele | 22 | 9 (40.9)            |       |   |
| Not harboring *5 allele | 95 | 30 (31.6)          | 0.695 |   |
| Harboring *5 allele | 5  | 2 (40.0)             |       |   |
| Not harboring *6 allele | 72 | 27 (37.5)         | 0.065 |   |
| Harboring *6 allele | 28 | 5 (17.9)             |       |   |

ABCB1 = adenosine triphosphate-binding cassette subfamily B member 1, CYP = cytochrome P450, N = number of patients.
In this study, we evaluated the effect of genetic polymorphisms on grade 4 neutropenia induced by AC combination chemotherapy in breast cancer patients. The multivariate logistic regression analysis revealed 4 factors, CYP2B6, ERCC1, BMI, and baseline WBC count, to be the independent and significant risk factors. Our findings showing low BMI and low baseline WBC count as risk factors for grade 4 neutropenia was consistent with those of previous reports. We showed a strong association between CYP2B6 and ERCC1 genotypes and grade 4 neutropenia development. OR for patients with grade 4 neutropenia and possessing 1 or more CYP2B6*6 and ERCC1 118C>T variant alleles were 4.505 (95% CI, 1.212–16.949) and 3.432 (95%CI, 1.216–9.687), respectively. Therefore, in addition to previously reported risk factors, we found that CYP2B6 and ERCC1 polymorphisms were good predictors of grade 4 neutropenia.

In this analysis, the frequency of grade 4 neutropenia was lower in patients harboring CYP2B6*6 allele than in those not harboring CYP2B6*6 allele. CYP2B6 is the primary enzyme that substantially metabolizes cyclophosphamide to its active metabolite, 4-hydroxycyclophosphamide. Several studies showed that CYP2B6 polymorphisms altered the pharmacokinetics of cyclophosphamide and 4-hydroxycyclophosphamide.[32–35] As CYP2B6*6 allele is associated with its reduced protein and mRNA expression,[31] it is likely that the lower levels of 4-hydroxycyclophosphamide led to the lower frequency of grade 4 neutropenia in patients with CYP2B6*6 allele.

Increased ERCC1 mRNA levels are directly associated with platinum resistance in various cancers. Furthermore, ERCC1 gene polymorphisms were already reported to associate with the efficacy and cytotoxicity of platinum agents.[32–33] Given that ERCC1 118C>T polymorphisms were associated with differential mRNA levels and lower enzyme levels[36] and that the T

### Table 4

| Patient characteristics | Grade 4 neutropenia | Patient characteristics | Grade 4 neutropenia |
|-------------------------|---------------------|-------------------------|---------------------|
| Age, y                  | N                   | N (%)                   | P                   |
| ≥52                     | 50                  | 18 (36.0)               | 0.392               |
| <52                     | 50                  | 14 (28.0)               |                     |
| BMI, kg/m²              |                     |                         |                     |
| ≥25                     | 15                  | 2 (13.3)                | 0.110               |
| <25                     | 85                  | 30 (35.3)               |                     |
| Baseline WBC, x10⁹/L   | ≥5.68               | 50                      | 10 (20.0)           | 0.012               |
|                         | <5.68               | 50                      | 22 (44.0)           |                     |
| Baseline Hb, g/dL      | ≥12.9               | 52                      | 12 (23.1)           | 0.049               |
|                         | <12.9               | 48                      | 20 (41.7)           |                     |

### Table 5

| Risk factor               | OR      | 95% CI      | P       |
|---------------------------|---------|-------------|---------|
| ABCB1 1236C>T: CT or TT   | 10.406  | 0.939–115.350| 0.056   |
| CYP2B6 not harboring *6   | 4.505   | 1.212–16.949 | 0.025   |
| ERCC1 118C>T: CT or TT    | 3.432   | 1.216–9.687  | 0.020   |
| BMI, kg/m²: <25           | 6.944   | 1.511–41.667 | 0.035   |
| Baseline WBC, x10⁹/L: <5.68 | 2.985     | 1.068–8.403  | 0.038   |
| Baseline Hb, g/dL: <12.9  | 2.801   | 0.956–8.197  | 0.060   |
| Baseline AST, IU/L: ≤17   | 2.227   | 0.702–6.250  | 0.129   |

### Table 6

| Number of risk factors | Grade 4 neutropenia | OR      | 95% CI | Unadjusted P | Adjusted P |
|------------------------|---------------------|---------|--------|--------------|------------|
| 1                      | 14                  | 1 (7.1) | 1.000 (ref.) | –           | –          |
| 2                      | 34                  | 4 (11.8) | 1.733 | 0.176–17.047 | 1.000      |
| 3                      | 37                  | 19 (51.4) | 13.722 | 1.625–115.895 | 0.004      |
| 4                      | 14                  | 8 (57.1) | 17.333 | 1.750–171.662 | 0.013      |

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, Hb = hemoglobin, N = number of patients, PLT = platelet count, WBC = white blood cells.

### 4. Discussion

This study aimed to determine whether doxorubicin/cyclophosphamide-related genetic polymorphisms were associated with grade 4 neutropenia development and to assess risk factors for grade 4 neutropenia including genetic polymorphisms in Japanese patients receiving AC combination chemotherapy. Several factors, such as female gender,[15–17] younger age,[16–20] lower BMI,[21,23] and lower pretreatment blood cell counts,[17,23,24] and hemoglobin levels,[19,23–27] were identified as risk factors for severe CIN and/or febrile neutropenia. However, a pharmacogenetic approach based solely on polymorphisms involved in antitumor agent activity appears to be insufficient. A multifaceted approach, including genetic factors, is required to address CIN. Thus, germline polymorphisms are attractive candidates that can be easily assessed in available samples.
allele was associated with reduced translation and presumably reduced DNA repair capability, lower DNA repair capacity resulting from non-CC ERCC1 118C>T alleles might lead to decreased ability to repair the damage done by platinum agents in normal tissue to precipitate increased toxicity. The findings of this study showed that possessing at least 1 ERCC1 118C>T variant allele was associated with a 3.4-fold increase in the risk for developing grade 4 neutropenia. One study reported that ERCC1 gene products were crucial for the repair of 4-hydroxy-cyclophosphamide-induced DNA damage and that ERCC1 mutants might exhibit hypersensitivity to DNA crosslinking agents.13,14 Therefore, our findings suggested that ERCC1 118C>T polymorphisms might affect DNA repair of normal cells damaged by cyclophosphamide.

In this study, the incidence of grade 4 neutropenia was 32.0%. Furthermore, all patients but one developed grade 4 neutropenia during the first treatment cycle. In a study among breast cancer patients treated with standard-dose adjuvant AC combination chemotherapy, Ma et al13 reported that the incidence of grade 4 neutropenia was higher in Chinese patients (23%) than in Caucasian patients (0.3%), demonstrating significant interethnic differences. This study might have also encountered patients with different pharmacoeffectiveness. Granulocyte colony-stimulating factor (G-CSF) was proven to effectively reduce the incidence of febrile neutropenia when prophylactically given 24 to 72 hours after chemotherapy. However, due to high cost, G-CSF is not routinely prescribed to all patients. Because Asian patients have higher frequency of severe neutropenia, those with 1 or more risk factors might be considered for prophylactic G-CSF administration.

To the best of our knowledge, this is the first clinical report showing an association between CYP2B6 and ERCC1 polymorphisms and severe neutropenia in patients treated with AC combination chemotherapy. However, the study has 2 major limitations. First, pharmacokinetic data for doxorubicin and cyclophosphamide were not available in this study as blood samples were collected before the first cycle of chemotherapy. Therefore, we could not elucidate the mechanisms underlying CYP2B6 polymorphisms and cyclophosphamide toxicity. The second limitation was the relatively small sample size used to evaluate the association.

In conclusion, we observed a significant association of grade 4 neutropenia with genetic polymorphisms in CYP2B6 and ERCC1. Our analysis also demonstrated its association with BMI and WBC, 2 previously identified risk factors for CIN. Our findings from clinical data might contribute to future clinical practice. However, our study included a relatively small sample size; thus, future, large-scale studies are critical to evaluate the utility of CYP2B6 and ERCC1 polymorphisms as predictors of grade 4 neutropenia.

Acknowledgments
The authors wish to thank all the patients and medical staff who participated in this study.

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