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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 cross-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; $P=0.025$), 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; $P=0.038$) 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.[1] CIN is directly associated with concomitant morbidity and mortality rates and health care costs.[2,3] 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.[4,5] 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.[6,7] *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.[8] Conversely, cyclophosphamide is administered as a prodrug that undergoes phase I activation metabolism by the cytochrome P450 (CYP) enzymes CYP2B6, CYP3A4, and

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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 pharmacoethnicity 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/m^2 doxorubicin and 600-mg/m^2 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 $\geq 3 \times 10^9$/L; platelet count $\geq 100 \times 10^9$/L; aspartate aminotransferase [AST] < 100 IU/L; alanine aminotransferase [ALT] < 100 IU/L; total bilirubin <2 mg/dL; 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 $< 0.5 \times 10^9$/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/m^2 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′-TTCACTTCAGTTACCCATC-3′ R: 5′-CATAGAGCCTCTGCATCA-3′	52°C	HaeIII	37°C
	2677G > T/A 2677G	rs2032582	F: 5′-TACCCATCATTGCAATAGCAG-3′ R: 5′-TTTAGTTTGACTCACCTTGCTAG-3′	52°C	NheI	37°C
	2677G > T/A 2677T		F: 5′-GCACTGAAAGATAAGAAAGAACTAGAAGCT-3′ R: 5′-GAGCATAGTAAGCAGTAGGGAG-3′	56°C	HindIII	37°C
	2677G > T/A 2677A		F: 5′-TACCCATCATTGCAATAGCAGGAG-3′ R: 5′-TTTAGTTTGACTCACCTTCCCAG-3′	52°C	BsrI	37°C
	3435C > T	rs1045462	F: 5′-TGTTTTCAGCTGCTTGATGG-3′ R: 5′-AAGGCATGTATGTTGGCCTC-3′	52°C	Sau3AI	37°C
CYP2B6	516G > T	rs3745274	F: 5′-GGTCTGCCCATCTATAAAC-3′ R: 5′-CTGATTCTTCACATGTCTGCG-3′	56°C	BsrI	
	785A > G	rs2279343	F: 5′-GACAGAAGGATGAGGGAGGAA-3′ R: 5′-CTCCCTCTGTCTTTCATTCTGT-3′	60°C	StyI	
	1459C > T	rs3211371	F: 5′-TGAGAATCAGTGGAAGCCATAGA-3′ R: 5′-TAATTTTCGATAATCTCACTCCTGC-3′	60°C	BglII	
CYP3A5	6986A > G	rs776746	F: 5′-CATGACTTAGTAGACAGATGA-3′ R: 5′-GGTCCAAACAGGGAAGAAATA-3′	56°C	SspI	37°C
GST	M1		F: 5′-GAACTCCCTGAAAAGCTAAAGC-3′ R: 5′-GTTGGGCTCAAATATACGGTGG-3′	56°C	–	–
	T1		F: 5′-TTCCTTACTGGTCCTCACATCTC-3′ R: 5′-TCACCGGATCATGGCCAGCA-3′			
	P1 313A > G	rs1695	F: 5′-ACCCCAGGGCTCTATGGGAA-3′ R: 5′-TGAGGGCACAAGAAGCCCCT-3′	64°C	BsmAI	55°C
ERCC1	118C > T	rs11615	F: 5′-TCATCCCTATTGATGGCTTCTGCCC-3′ R: 5′-GACCATGCCCAGAGGCTTCTCATAG-3′	63°C	BsrDI	65°C
	8092G > T	rs3212986	F: 5′-GGGCACCTTCAGCTTTCTTT-3′ R: 5′-CAGAGACAGTGCCCCAAGAG-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.

demographic and clinical characteristics are shown in Table 2. The mean age of study participants was 52 years. All patients were female and received a combination of 60-mg/m^2 doxorubicin and 600-mg/m^2 cyclophosphamide as adjuvant or neoadjuvant 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 *GSTM/T1*, 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%, $P = 0.065$). Similarly, the rate of grade 4 neutropenia was higher in patients harboring *ABCB1* 1236 CT and TT genotyes 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:

Table 2

Baseline demographics and clinical characteristics of the study patients.

		All patients (N = 100)
Gender	Female	100 (100%)
	Male	0 (0%)
Age, y		52.1 ± 10.2
Height, cm		156.4 ± 5.6
Weight, kg		54.2 ± 7.9
BMI, kg/m^2		22.1 ± 3.1
Doxorubicin dose, mg/m^2		60
Cyclophosphamide dose, mg/m^2		600
WBC, mm^3		5.69 ± 1.4
Hb, g/dL		12.7 ± 1.2
PLT, $\times 10^9$/L		240.7 ± 54.9
ASL, IU/L		19.3 ± 7.6
ALT, IU/L		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%)
	IIa	36 (36.0%)
	IIb	19 (19.0%)
	IIIa	13 (13.0%)
	IIIb	2 (2.0%)
	IIIc	7 (7.0%)

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, ER = estrogen receptor, Hb = Hemoglobin, N = number of patients. PgR = progesterone receptor, PLT = Platelet Count, WBC = white blood cells.

Data of age, BMI, height, weight, WBC, Hb, PLT, AST, and ALT are shown as median values and ranges.

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.

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 BIM, 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) $< 25\,kg/m^2$ (OR, 6.944; 95% CI, 1.151–41.667; $P = 0.035$), and baseline WBC count $< 5.68 \times 10^9$/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.

		Grade 4 neutropenia				Grade 4 neutropenia	
Polymorphism	***N***	***N* (%)**	***P***	**Polymorphism**	***N***	***N* (%)**	***P***
ABCB1 1236C > T				*CYP2B6*			
CC	14	1 (7.1)	0.061	Not harboring *9 allele	99	32 (32.3)	1.000
CT and TT	86	31 (36.0)		Harboring *9 allele	1	0 (0.0)	
ABCB1 2677G > T/A				*CYP3A5* 6986A > G			
GG	22	6 (27.3)	0.591	AA	2	1 (50.0)	0.590
GT, GA, TT, TA, AA	78	25 (33.3)		AG and GG	98	31 (31.6)	
ABCB1 3435C > T				*GSTM1, T1* null			
CC	35	9 (25.7)	0.325	P/P	24	6 (25.0)	0.401
CT and TT	64	23 (35.4)		P/N or N/P, N/N	76	26 (34.2)	
CYP2B6				*GSTP1* 313A > G			
Not harboring *4 allele	78	23 (29.5)	0.313	AA	68	21 (30.9)	0.727
Harboring *4 allele	22	9 (40.9)		AG and GG	32	11 (34.4)	
CYP2B6				*ERCC1* 118C > T			
Not harboring *5 allele	95	30 (31.6)	0.695	CC	58	13 (22.4)	0.017
Harboring *5 allele	5	2 (40.0)		CT and TT	42	19 (45.2)	
CYP2B6				*ERCC1* 8092G > T			
Not harboring *6 allele	72	27 (37.5)	0.065	GG	56	20 (35.7)	0.370
Harboring *6 allele	28	5 (17.9)		GT and TT	44	12 (27.3)	

ABCB1 = adenosine triphosphate-binding cassette subfamily B member 1, CYP = cytochrome P450, *N* = number of patients.

Table 4

Association between grade 4 neutropenia incidence and patient characteristics by univariate analysis.

Patient characteristics	N	Grade 4 neutropenia N (%)	P	Patient characteristics	N	Grade 4 neutropenia N (%)	P
Age, y				Baseline PLT ($\times 10^9$/L)			0.321
≥52	50	18 (36.0)	0.392	≥232	51	14 (27.5)	
<52	50	14 (28.0)		<232	49	18 (36.7)	
BMI, kg/m^2				Baseline AST (IU/L)			0.017
≥25	15	2 (13.3)	0.110	≥17	61	14 (23.0)	
<25	85	30 (35.3)		<17	39	18 (46.2)	
Baseline WBC, $\times 10^9$/L				Baseline ALT (IU/L)			0.328
≥5.68	50	10 (20.0)	0.012	≥14	54	15 (27.8)	
<5.68	50	22 (44.0)		<14	46	17 (37.0)	
Baseline Hb, 9/dL							
≥12.9	52	12 (23.1)	0.049				
<12.9	48	20 (41.7)					

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

Table 5

Multivariate analysis of associations between grade 4 neutropenia and clinical variables.

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/m^2: <25	6.944	1.151–41.667	0.035
Baseline WBC, $\times 10^9$/L: <5.68	2.985	1.060–8.403	0.038
Baseline Hb, g/dL: <12.9	2.801	0.958–8.197	0.060
Baseline AST, IU/L: <17	2.227	0.792–6.250	0.129

AST = aspartate aminotransferase, BMI = body mass index, CI = confidence interval, Hb = hemoglobin, OR = odds ratio, 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,[18–20] lower BMI,[21,22] and lower pretreatment blood cell counts,[17,23,24] and hemoglobin levels,[19,25–27] 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.

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.[28–30] 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.[32–35] Given that *ERCC1* 118C > T polymorphisms were associated with differential mRNA levels and lower enzyme levels [36] and that the T

Table 6

Number of risk factors and grade 4 neutropenia development.

Number of risk factor	Grade 4 neutropenia N	(%)	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	1.000
3	37	19 (51.4)	13.722	1.625–115.895	0.004	0.012
4	14	8 (57.1)	17.333	1.750–171.662	0.013	0.039

CI = confidence interval, *N* = number of patients, OR = odds ratio.

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-hydroxycyclophosphamide-induced DNA damage and that *ERCC1* mutants might exhibit hypersensitivity to DNA crosslinking agents.[37] 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 al[13] reported that the incidence of grade 4 neutropenia was higher in Chinese patients (25%) than in Caucasian patients (0.3%), demonstrating significant interethnic differences. This study might have also encountered patients with different pharmacoethnicity. 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.

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