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Association of the *ABCB1* 3435C>T polymorphism and treatment outcomes in advanced gastric cancer patients treated with paclitaxel-based chemotherapy

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Abstract. We evaluated the frequency of *ABCB1* polymorphisms (2677G/T>A and 3435C>T) and studied the association between the polymorphisms and clinical outcomes of paclitaxel-based chemotherapy in advanced gastric cancer patients. This study was performed in 43 gastric cancer patients and a control group consisting of 118 healthy volunteers. Patients were treated with paclitaxel combined with an infusional 5-fluorouracil and low-dose leucovorin. Genomic DNA from peripheral blood mononuclear cells was used to determine *ABCB1* polymorphisms by direct sequencing. Genotypes were investigated for their association with survival and toxicity. The *ABCB1* 3435 C allele was more frequent in gastric cancer patients than healthy volunteers ($p<0.001$). The 2677G>T/A and 3435C>T polymorphisms were independent factors associated with shorter progression-free survival (PFS) ($p=0.024$, $p=0.001$, respectively). In combined analysis of 2677 and 3435 polymorphisms, the 3435C>T polymorphism was an independent factor for poor PFS ($p=0.01$). The 3435CT and TT genotypes were associated with mucositis ($p=0.04$), and the variant genotypes at 2677 loci were associated with diarrhea ($p=0.034$). Our data suggest that the *ABCB1* polymorphism at 3435 is associated with clinical outcomes after paclitaxel-based combined chemotherapy in advanced gastric cancer patients.

Introduction

Gastric cancer is the fourth most common type of cancer and the second leading cause of cancer-related death in the world (1). Various agents and their combinations have been shown to be effective in tumor response. Taxanes have also demonstrated promising activity in treating gastric cancer. Response rates of single agent taxanes range between 10-25% and 20-50% when used in combination with other agents (2). We reported that paclitaxel or doxetaxel combined with an infusional 5-fluorouracil and low-dose leucovorin (FLT) regimen was effective and had tolerable toxicity when used to treat advanced gastric cancer patients (3,4).

Despite the clinical activity of paclitaxel, the variability of toxicity and response remains an important consideration for patients treated with paclitaxel (5). Such inter-patient variation may be associated with polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters, and drug targets (6). However, there is a paucity of *in vivo* pharmacogenetic studies focusing on paclitaxel-based therapy for gastric cancer.

ABCB1 (P-glycoprotein, multidrug resistance 1) is a transmembrane protein that acts as an energy-dependent drug efflux pump for chemotherapeutic drugs, including taxanes (7). ABCB1 eliminates the parent drug through hepatobiliary and intestinal secretion (8). In addition, ABCB1 has been suggested to have a role in the proliferation and survival of epithelial cells and malignant cells during tumorigenesis (9). ABCB1 has been associated with intestinal tumorigenesis in $ApcM^{in/+}$ mice. Yamada *et al* reported that *Mdr1*-deficient Min ($Apc^{Min/+}Mdr1a/b^{-/-}$) mice developed fewer intestinal polyps than did $Apc^{Min/+}Mdr1a/b^{+/+}$ mice (10).

More than 50 single-nucleotide polymorphisms (SNP) and 3 insertion/deletion polymorphisms have been reported in the *ABCB1* gene, and some of these variants affect the expression and function of P-glycoprotein (P-gp) (11,12). Hoffmeyer *et al* showed that a synonymous polymorphism in exon 26, 3435C>T, correlated with the level of P-gp expression in the intestine (13). Individuals homozygous for the 3435C>T polymorphism had lower P-gp expression and showed higher plasma levels of the P-gp substrate digoxin. Other study also showed a weak association between placental P-gp expression and polymorphisms at position 2677 (GG>G/mut>mut/mut) (14). Polymorphisms of *ABCB1* may increase the efflux of chemotherapeutic agents

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from tumor cells or increase their elimination from the body, resulting in lower plasma concentrations (and vice versa), thereby influencing their therapeutic efficacy. Recent studies reported that an association between *ABCB1* 2677G>T/A and response to paclitaxel in ovarian cancer patients as well as paclitaxel-induced neuropathy and neutropenia were related with homozygous variants at the 2677 and 3435 loci of *ABCB1* (8,15). We previously showed that *ABCB1* 2677 and 3435 polymorphisms of metastatic breast cancer patients are related to clinical outcomes including disease control rates, overall survival, and chemo-resistance (16). However, no previous studies have implicated these variants in association with toxicity or survival differences after paclitaxel therapy in advanced gastric cancer patients.

In the present study, we evaluated the frequency of *ABCB1* polymorphisms in gastric cancer patients and studied whether the variant *ABCB1* genotype is associated with toxicity or clinical outcomes of paclitaxel-based chemotherapy in gastric cancer patients.

Materials and methods

Patient eligibility. From August 2003 to April 2005, a total of 60 patients with metastatic and/or relapsed gastric adenocarcinoma were enrolled for paclitaxel plus infusional 5-fluorouracil (5-FU) and low leucovorin chemotherapy (3). Among these 60 patients, we enrolled 43 patients who had available genotype information. The study was approved by the appropriate ethics review board. Informed consent was obtained from all participants.

Eligibility criteria included the following: patient age 18 years or older, an Eastern Cooperative Oncology Group (ECOG) performance scale ≤2, evaluable disease with or without measurable lesions, and adequate hematological, renal, and hepatic functions. Patients were either chemotherapy-naïve or treated with only one prior chemotherapy regimen. Patients were excluded from the study if they had concurrent cancer, peripheral neuropathy with a grade ≥2 (as defined by the National Cancer Institute Common Toxicity Criteria (NCI-CTC), brain metastasis, or uncontrolled comorbidity conditions. Healthy volunteers consisted of subjects of the same ethnic and geographical origin as the patients. One hundred and eighteen healthy volunteers were included after a physical check-up and health status screening.

Treatment schedule. Paclitaxel was infused over a period of 3 h at a dose of 175 mg/m^2. Within 2 h after completion of paclitaxel, 20 mg/m^2 leucovorin was intravenously injected and followed by 1000 mg/m^2/day 5-FU as a 24-h continuous infusion for 3 days. The treatment was repeated every 21 days. Chemotherapy was given until the disease progressed, the patient experienced unacceptable toxicity, or the patient withdrew from treatment. The treatment continued for a maximum of 12 cycles.

Response and toxicity assessment. Tumor response measurements were recorded according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.0).

Table I. Patient characteristics.

Characteristics	No. of patients (%)
No. of enrolled patients	43
Age (years) median (range)	47 (23-68)
Sex	
Male	26 (60.5)
Female	17 (39.5)
ECOG	
0-1	13 (30.2)
2	30 (69.8)
Tumor pathology	
Moderate differentiated	11 (25.6)
Poorly differentiated	18 (41.9)
Signet ring cell	10 (23.3)
Mucinous	1 (2.3)
Undetermined	3 (6.9)
Chemotherapy status	
1st	24 (55.8)
2nd	19 (44.2)

ECOG, Eastern Cooperative Oncology Group performance scale.

Every two cycles and all the responses had to be confirmed by a second measurement after an additional 4 weeks. Complete blood cell counts and toxicity assessments were performed weekly, and performance status and serum chemistry were assessed before each cycle. Toxicity was evaluated according to NCI-CTC (version 2.0).

Genotyping. Peripheral blood mononuclear cells (PBMCs) were isolated from blood using Ficoll-Paque (Pharmacia, Uppsala, Sweden) following the manufacturer's instructions. Genomic DNA (gDNA) from lymphocytes was extracted using the LaboPass™ Blood kit (COSMO Genotech Co., Ltd., Seoul, Korea). Extracted gDNA was amplified by PCR using an Eppendorf Mastercycler Gradient (Eppendorf North America, Inc., Westbury, NY, USA). PCR cycling was performed with the initial denaturation at 95˚C for 2 min followed by 30 cycles of denaturation at 94˚C for 30 sec, annealing at 57˚C for 20 sec, and extension at 72˚C for 30 sec. Forward (F) and reverse (R) primers were designed on the basis of target gene sequences obtained from GenBank: *ABCB1* 2677G>T/A: 5'-ATTGCAATAGCAGGAGTTGT-3' (F), 5'-CTGGCTTTGCTACTTTCTGT-3' (R); and 3435C>T: 5'-ACAATTATGACCTTGTTGGG-3' (F), 5'-TTCTCTTCA CTTCTGGGAGA-3' (R). Sequencing of PCR products was performed according to the manufacturer's instructions using the CEQ 8000 Dye Terminator Kit (CEQ™ 8000 Beckman Coulter, Inc., Fullerton, CA, USA). Sequence data were analyzed and compared using the GeneDoc system (www.psc.edu/biomed/genedoc) and FinchTV software (http://www.geospiza.com) (Geospiza Inc., Seattle, WA,

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Adjusted odds ratios for frequency of *ABCB1* 2677 and 3435 between gastric cancer patients (n=43) and healthy s (n=118).

	Gastric cancer patients (%)	Healthy volunteers (%)	Odds ratio[a] (95% CI)	P-value[a]
2677 Allele[b]				
G	46 (53.5)	130 (55.1)		
T/A	40 (46.5)	106 (44.9)		0.704
2677 Genotype[c]				
GG	14 (32.6)	38 (32.2)		
GT and GA	18 (41.9)	54 (45.8)		
TT, TA, and AA	11 (25.5)	26 (22.0)		0.869
3435 Allele[b]				
C	70 (81.4)	120 (50.8)	4.79 (2.40-9.54)	<0.001
T	16 (18.6)	116 (49.2)	Reference	
3435 Genotype[c]				
CC	30 (69.8)	53 (44.9)	10.3 (2.70-39.9)	0.001
CT	10 (23.3)	14 (11.9)	13.5 (2.81-64.8)	0.001
TT	3 (6.9)	51 (43.2)	Reference	
Haplotype[d]				
11	44 (51.2)	73 (30.9)	Reference	
12	2 (2.3)	55 (23.3)	0.05 (0.01-0.25)	<0.001
21	26 (30.2)	48 (20.4)	1.01 (0.48-2.10)	0.983
22	14 (16.3)	60 (25.4)	0.39 (0.17-0.89)	0.026

CI, confidence interval. The haplotypes were named as follows: 11 for alleles without mutations (2677G-3435C), 12 for alleles with (2677G-3435T), 21 for alleles with (2677T/A-3435C), and 22 for alleles carrying both mutations (2677T/A-3435T). [a]Odds ratio (95% CI) and p-value were calculated in the logistic regression model with adjustment for sex and age. OR ratio is shown when p-value was significant. [b]Number represents number of alleles (percentage). [c]Number represents number of patients (percentage). [d]Number represents number of haplotypes (percentage).

USA). The *ABCB1* haplotype frequency was estimated by the expectation-maximization algorithm PHASE version 2.1 (http://www.depts.washington.edu/ventures/clickthru/releaseAgreement.php/raf=PHASEV2). The haplotype nomenclature was used as previously described (17). The haplotypes were named as follows: 11 for alleles without mutations (2677G-3435C), 12 for alleles with (2677G-3435T), 21 for alleles with (2677T/A-3435C), and 22 for alleles carrying both mutations (2677T/A-3435T). The diplotype nomenclature was used as described previously (18).

Statistical analysis. Differences in allele or genotype frequencies between healthy volunteers and patients were determined using logistic regression analysis, with adjustment for age and sex. Adherence of genotype frequencies to the Hardy-Weinberg equilibrium was assessed by a goodness-of-fit χ^2 test. The univariate association between genotypes and tumor response or disease control was calculated using a Fisher's exact test. Logistic regression analysis was also performed to find the relation between genotype and tumor response/disease control rate with adjustment for sex, age, performance status, and line of chemotherapy. Chi-squared trend analysis was used to analyze the significance of trends for genotypes and grade of toxicity.

Progression-free survival (PFS) was defined as the time interval between the date of first paclitaxel administration to the date of disease progression or death from any cause. Overall survival (OS) was calculated as the date of the first paclitaxel administration to the date of death or the date of last contact (censored observation). The Kaplan-Meier method was used to estimate the distribution of time to events. Univariate associations between genotype and OS and between genotype and PFS were assessed using the log-rank test. Cox regression analysis was applied to determine the contribution of the genotypes and other factors to OS and PFS. All tests were two-sided and differences were considered significant when p-values were <0.05. All analyses were performed using SPSS for Windows (version 12.0, SPSS, Chicago, IL, USA).

Results

Frequencies of genotypes, diplotypes and haplotypes. The characteristics of the 43 treated patients are listed in Table I. The healthy volunteer group consisted of 50 males and 68 females, and the median age was 29 (range, 20-65) years.

The *ABCB1* 2677G>T/A, 3435C>T genotypes were examined in all patients and healthy volunteers (Tables II and III).

Table III. Comparison of *ABCB1* 2677 and 3435 diplotype frequencies between gastric cancer patients (n=43) and healthy volunteers (n=118).

	Chromosome 1		Chromosome 2		Number (%)[b]	
Diplotype[a]	2677G>T/A	3435C>T	2677G>T/A	3435C>T	Patients	Volunteers
1	G	C	G	C	14 (32.6)	21 (17.8)
2	G	C	◆	◆	11 (25.6)	26 (22.0)
3	G	C	T/A	T/A	5 (11.6)	5 (4.2)
4	T/A	T/A	◆	◆	7 (16.3)	26 (22.0)
5	T/A	T/A	T/A	T/A	1 (2.3)	13 (11.0)
6	◆	◆	◆	◆	5 (11.6)	27 (22.9)

◆Any combination of alleles that is not mutually exclusive with another diplotype consisting of the 2677 and 3435 SNPs. [a]The diplotype nomenclature was used as described previously (18). [b]Number represents number of diplotypes. *ABCB1* diplotypes 1-3 showed a higher frequency in gastric cancer patients than in healthy volunteers (adjusted OR 2.48, 95% CI: 1.05-5.81, p=0.037).

Table IV. Associations between *ABCB1* polymorphisms and chemotherapy-induced diarrhea and mucositis.

	Diarrhea grade					Mucositis grade				
	0	1	2	3	4	0	1	2	3	4
2677 Genotype[a]										
GG	13	1	0	0	0	14	0	0	0	0
Other	18	6	5	0	0	27	1	1	0	0
P-value[b]				0.034					0.347	
3435 Genotype[a]										
CC	24	3	3	0	0	30	0	0	0	0
Other	7	4	2	0	0	11	1	1	0	0
P-value[b]				0.172					0.040	
Haplotype[c]										
11	35	5	4	0	0	43	0	1	0	0
12	1	0	1	0	0	2	0	0	0	0
21	18	5	3	0	0	25	1	0	0	0
22	8	4	2	0	0	12	1	1	0	0
P-value[b]				0.193					0.251	
Diplotype[d]										
3-5	7	4	2	0	0	11	1	1	0	0
Other	24	3	3	0	0	30	0	0	0	0
P-value[b]				0.172					0.040	

[a]Number of patients. [b]P-value was calculated by chi-squared trend analysis; [c]number of haplotypes; [d]number of diplotypes.

There was no difference in the *ABCB1* 2677 genotype between healthy volunteers and cancer patients. The frequency of the 3435 C allele carrier in cancer patients was higher than the frequency in healthy volunteers (adjusted OR 4.79, 95% CI: 2.40-9.54, p<0.001). Therefore, frequency of the *ABCB1* TT genotype was lower in gastric cancer patients than in healthy volunteers (CC genotype, adjusted OR 10.3, 95% CI: 2.70-39.9, p=0.001; CT genotype, adjusted OR 13.5, 95% CI: 2.81-64.8, p=0.001). The haplotype 11 showed higher frequencies in gastric cancer patients (other haplotypes, adjusted OR 0.42, 95% CI: 0.23-0.78, p=0.006) ABCB1 diplotypes 1-3 also showed a higher frequency in gastric cancer patients than in healthy volunteers (adjusted OR 2.48, 95% CI: 1.05-5.81, p=0.037) (Tables II and III).

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Comparison of progression-free survival according to *ABCB1* genotypes, haplotypes, and diplotypes in gastric cancer treated with paclitaxel-based chemotherapy.

	No.[a]	PFS (median, month)	HR (95% CI)[b]	P-value
2677 Genotype				
GG	14	4.4	1	
Others	29	3.0	2.64 (1.13-6.15)	0.024
3435 Genotype				
CC	30	3.1	1	
Others	13	3.0	4.63 (1.90-11.3)	0.001
Haplotype[c]				
11	44	4.4	1	
12	2	1.3	2.51 (0.56-11.2)	0.228
21	26	3.0	1.62 (0.93-2.82)	0.086
22	14	3.0	3.52 (1.71-7.25)	0.001
Diplotype[d]				
1	14	4.4	1	
2	11	3.1	1.43 (0.51-4.02)	0.495
3	5	3.0	8.09 (2.05-31.8)	0.003
4	7	2.0	5.66 (1.81-17.6)	0.003
5	1	6.3	6.36 (0.62-65.1)	0.119
6	5	3.0	2.87 (0.75-11.0)	0.123

[a]Number of patients with the 2677 and 3435 genotype, the number of haplotypes with haplotypes 11, 12, 21, 22 and, the number of diplotypes with diplotypes 1-6, respectively. [b]HR was calculated for *ABCB1* genotypes, haplotypes, and diplotypes, respectively, by Cox regression analysis (adjusted for sex, age, performance status, tumor response, and line of chemotherapy). [c]The haplotypes were named as follows: 11 for alleles without mutations (2677G-3435C), 12 for alleles with (2677G-3435T), 21 for alleles with (2677T/A-3435C), and 22 for alleles carrying both mutations (2677T/A-3435T). [d]The diplotype nomenclature is described in Table III.

None of the patient characteristics, except the number of previous regimens, were associated with any type of *ABCB1* polymorphism. Patients carrying the *ABCB1* 3435 CC genotype had received prior chemotherapy more often than patients with the 3435 CT genotype (% of second line chemotherapy: 53% vs. 10%, p=0.026).

Association between polymorphisms and chemotherapy-induced toxicities. We evaluated the association between polymorphisms and toxicities from all 43 patients. The frequency of major gastrointestinal (GI) toxicities from the FLT regimen such as diarrhea and mucositis were compared between different genotypes, haplotypes, and diplotypes of *ABCB1*. Generally, the toxicities were mild (grade 1-2). Patients with 2677 T or 2677 A alleles were more likely to experience diarrhea compared to the 2677 wild-type allele (p=0.034). *ABCB1* genotypes carrying the 3435T allele and diplotypes 3, 4, and 5 (those carrying at least one TT haplotype) were associated with mucositis compared with other types (p=0.040 and p=0.040, respectively) (Table IV). None of the *ABCB1* genotypes, haplotypes, or diplotypes were associated with hematologic toxicities.

Association between polymorphisms and tumor response or progression. Of the 43 patients enrolled, 42 were fully eligible for response evaluation. One patient was unevaluable because of the absence of measurable lesions. Forty-two patients had disease progression or died within the median follow-up duration of 36 weeks (range, 1-215 weeks). We could not find any difference in either response rate or disease control rate between the different genotypes, haplotypes, or diplotypes.

Patients with *ABCB1* 2677 T or 2677 A alleles showed a higher risk of poor PFS when compared with individuals who had the 2677 GG genotype (PFS; 3.0 months vs. 4.4 months, p=0.024). Patients with *ABCB1* 3435 variants also had a higher probability of shorter PFS than individuals carrying 3435 CC genotype when adjusted for other factors on multiple regression analysis (p=0.001) (Table V).

Patients harboring haplotype 12 had shorter PFS than patients harboring haplotypes 11 and 21 (p=0.039, p=0.039, respectively) (Fig. 1). The hazard ratios of haplotypes 22 and 21 for PFS compared to haplotype 11 were 3.52 (95% CI: 1.71-7.25, p=0.001) and 1.62 (95% CI: 0.93-2.82, p=0.086), respectively. *ABCB1* diplotypes 3 and 4 were associated with a higher risk of poor PFS compared to diplotype 1 (diplotype 1 vs. 3, HR=8.09; 95% CI: 2.05-31.8, p=0.003; diplotype 1 vs. 4, HR=5.66; 95% CI: 1.81-17.6, p=0.003).

When both the 2677 and 3435 polymorphisms were included in the Cox analysis model, we found that the

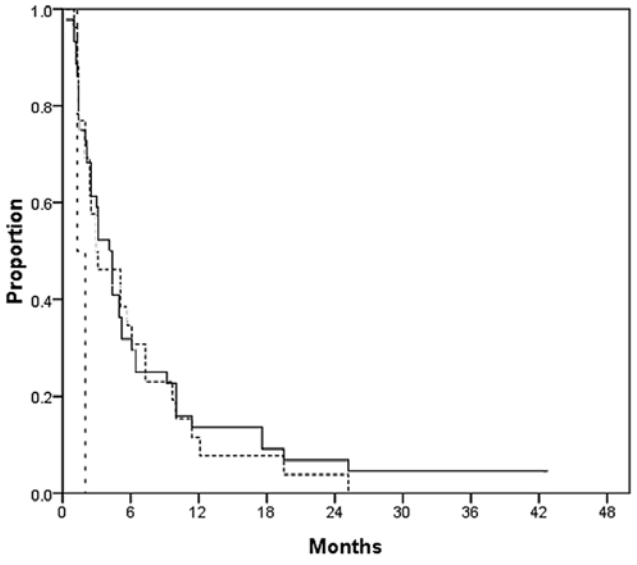

Figure 1. Comparison of progression-free survival between haplotype 11 (–), 12 (- -) and 21 (- - -). (11 vs. 12, p=0.039; 21 vs. 12, p=0.039; log-rank test).

Table VI. Association between *ABCB1* polymorphisms and clinical outcomes by combined analysis of 2677 and 3435 genotypes.

	Toxicity/ Diarrhea[a]	Toxicity/ Mucositis[a]	PFS
ABCB1 2677[b]	0.034	0.347	0.245
ABCB1 3435[b]	0.172	0.040	0.010
Haplotype[c]	0.193	0.251	0.001
Diplotype[c]	0.172	0.040	0.003

PFS, progression-free survival. [a]P-value for toxicity was calculated using chi-squared trend analysis. Patients with 2677 T or 2677 A alleles were more likely to experience diarrhea compared to the 2677 wild-type allele (p=0.034). *ABCB1* genotypes carrying the 3435T allele and diplotype grouping of diplotypes 3, 4, and 5 were more strongly associated with mucositis compared to other types (p=0.040 and p=0.040, respectively). [b]P-value for survival was adjusted for sex, age, performance status, tumor response, and line of chemotherapy, *ABCB1* 2677, 3435 SNPs using Cox regression analysis. 3435C>T polymorphism was an independent factor that was associated with poor PFS (HR of 3.60 for 3435 variants, 95% CI: 1.35-9.56, p=0.010). [c]P-value for survival was adjusted for sex, age, performance status, tumor response, and line of chemotherapy using Cox regression analysis. The hazard ratio of the haplotype 22 for PFS compared with haplotype 11 was 3.52 (95% CI: 1.71-7.25, p=0.001). *ABCB1* diplotype 3 and diplotype 4 were associated with a higher risk of poor PFS compared with diplotype 1 (diplotype 1 vs. 3, HR=8.09; 95% CI: 2.05-31.8, p=0.003; diplotype 1 vs. 4, HR=5.66; 95% CI: 1.81-17.6, p=0.003).

3435C>T polymorphism was an independent factor that was associated with poor PFS (HR of 3.60 for 3435 variants, 95% CI: 1.35-9.56, p=0.010) (Table VI). For overall survival, we found no associated ABCB1 polymorphisms when a combined analysis was done with both the ABCB1 2677 and 3435 polymorphisms.

Discussion

In this study, 3435 C allele was seen at a higher frequency in cancer patients than healthy volunteers, consistent with the results of Tahara *et al* showing that 3435 TT genotype was associated with a reduced risk of gastric cancer in a Japanese population (9). P-gp, a *ABCB1* gene product, is part of the gastrointestinal barrier that protects cells against xenobiotics from diet, bacterial toxins, drugs, and other biologically active carcinogens (19,20). It has been reported that genetic polymorphism of *ABCB1* gene may affect the expression and function of the ABCB1 efflux pump (11,12). Nakamura *et al* showed that decreased mRNA levels in healthy Japanese subjects carrying the 3435 C alleles as compared with subjects with the 3435 T allele (21). Based on these reports, it is possible that the 3435 C allele may alter the function of ABCB1 in normal gastric mucosa and, be more susceptible to neoplastic transformation by carcinogens.

In the current study, we found that SNP in *ABCB1* was related with the chemotherapy-induced toxicity and progression-free survival. The *ABCB1* polymorphisms may affect the substantial inter-individual difference in drug clearance and influence the sensitivity or resistance of tumor cells, which might contribute to the variability in toxicity as well as efficacy and survival in gastric cancer.

Notably, PFS decreased while toxicity increased in patients with variant alleles in our study. Individuals with 3435 CC had a longer PFS and a lower frequency of GI toxicity, although patients harboring 3435 CC had been treated much more with prior palliative chemotherapy than patients with 3435 CT. In cancers that already overexpress ABCB1 compared with normal tissue, polymorphisms might be more deterministic in efflux phenotypes than an expression difference itself. In contrast, in normal tissues where ABCB1 is expressed at low basal levels, it is likely that polymor-phisms influence drug penetration by altering expression (18).

Another explanation could be that the mechanism of regulation of ABCB1 expression differs between normal and neoplastic tissues, at least in gastric cancer. In addition, the drug efflux function of ABCB1 could possibly be related with toxicity to normal tissues, but not related to drug efficacy in tumor cells. *ABCB1* polymorphism could be rather associated with the mutation of apoptosis-related genes (22). However, none of the previous studies examined whether there is a difference in the functional effect of *ABCB1* polymorphisms in gastric cancer tissue and normal tissue. Further *in vivo* and *ex vivo* studies are required to resolve this issue.

This study included patients who had been treated with prior palliative chemotherapy and chemo-naïve patients. We previously reported that the risk of tumor progression was higher in chemo-naïve patients than previously treated patients with advanced gastric cancer (3). ABCB1 could affect inherent and acquired resistance against chemo-therapeutic agents in gastric cancer (23). *In vitro* studies

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shown that acquired drug-resistant cell lines have duplication of the ABCB1 chromosome region (24). Therefore, prior palliative chemotherapy history could affect the expression of ABCB1, resulting in the development of drug resistance. In our study, despite the fact that more patients with the 3435 CC allele had been treated with prior chemotherapy than patients with other variants, these former patients had a lower probability of progression. It is possible that previous treatment may not be enough to induce treatment-resistant ABCB1 induction in our patients. It is also possible that *ABCB1* genotypes are related to patient prognosis independently of treatment resistance induction, especially when considering that *ABCB1* is known to play a significant survival role in normal and dysplastic cells during tumorigenesis, progression, and metastasis (9).

Our study has some limitations. Firstly, *ABCB1* genotype of both healthy volunteers and cancer patients was not shown to be in Hardy-Weinberg equilibrium ($p<0.001$). Lee *et al* considered that this deviation in their Vietnamese population study could be explained in part by the numerous races involved in the study (25). In our study, however, all healthy volunteers were Koreans and thus of the same ethnic background. A large and well-controlled study for gene-environmental interactions is therefore needed to further investigate the influence of the 3435 polymorphisms in gastric cancer carcinogenesis. Secondly, the number of patients was not enough to perform a sufficiently powered study for the association between genotype and toxicity. A well-designed large study is needed to confirm the association between *ABCB1* SNP and chemotherapy-induced toxicities. Thirdly, a combination chemotherapy regimen with paclitaxel and 5-fluorouracil (5-FU) was used. 5-FU is metabolized principally by dihydropyrimidine dehydrogenase, which is responsible for the degradation of 80-90% of 5-FU to its inactive metabolite. 5-FU is not considered to be a substrate of ABCB1 (26). Furthermore, diarrhea and oral mucositis are frequently reported as gastrointestinal side effects caused by 5-FU (27). 5-FU has established activity against gastric cancer and high ABCB1 expression was observed in several tumors (e.g., colon, liver, and pancreas) after 5-FU chemotherapy (28,29). For these reasons we could not assume that the efficacy and toxicity of our regimen were related solely to the pharmacogenetic effect of *ABCB1* on paclitaxel.

In conclusion, *ABCB1* 3435 polymorphisms could be associated with the risk of a short PFS and increased toxicity after paclitaxel and 5-FU combined chemotherapy. Further, larger studies are needed to determine the relative roles of various *ABCB1* genotypes in gastric cancer patient on the response and toxicity of paclitaxel combined with other drugs.

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