의학석사 학위논문

ABCB1, *FCGR2A*, and *FCGR3A* polymorphisms in patients with HER2-positive metastatic breast cancer who were treated with first-line taxane plus trastuzumab chemotherapy

탁센과 트라스투주맙 병용 요법으로 치료받은 HER2-양성 전이성 유방암 환자에서 *ABCB1*, *FCGR2A*, *FCGR3A* 유전자 다형성의 임상적 의미

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ABCB1, *FCGR2A*, and *FCGR3A* polymorphisms in patients with HER2-positive metastatic breast cancer who were treated with first-line taxane plus trastuzumab chemotherapy

by

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A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Master of Science in Medicine (Molecular Oncology Major) at Seoul National University College of Medicine

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Abstract

ABCB1, *FCGR2A*, and *FCGR3A* polymorphisms in patients with HER2-positive metastatic breast cancer who were treated with first-line taxane plus trastuzumab chemotherapy

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The aim of this study was to elucidate clinical implications of *ABCB1*, *FCGR2A*, and *FCGR3A* polymorphisms in patients with HER2-positive metastatic breast cancer (MBC) after taxane plus trastuzumab (TH) chemotherapy. Using genomic DNA samples extracted from mononuclear cells of consecutive patients with HER2-positive MBC who received first-line TH, we analyzed five polymorphisms (*ABCB1* 1236C>T, *ABCB1* 2677G>T/A, *ABCB1* 3435C>T, *FCGR2A* 131H/R, and *FCGR3A* 158V/F), and then correlated them with response rate (RR), progression-free survival (PFS), overall survival (OS), and adverse events of patients. A total of 57 women were analyzed. The median age was 46 years (range, 27-72 years). *ABCB1* 2677T carriers had a longer PFS (p=0.037) along with a tendency toward a longer OS (p=0.057). *ABCB1* 3435CC genotype carriers had a shorter PFS (p=0.039) along with a tendency toward a shorter OS (p=0.093). In combined

analysis, PFS was significantly longer in *ABCB1* 1236CC and/or 2677TT carriers compared to the others (p=0.006). *FCGR2A* 131H/R and *FCGR3A* 158V/F polymorphisms were not significantly associated with RR, PFS, and OS. In conclusion, our data support that *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T polymorphisms may predict PFS after first-line TH chemotherapy in patients with HER2-positive MBC. In contrast, *FCGR2A* 131H/R and *FCGR3A* 158V/F polymorphisms were not significantly correlated to clinical outcomes after TH chemotherapy. Further studies with a more statistical power are necessary to validate our findings.

Keywords: *ABCB1*; *FCGR2A*; *FCGR3A*; polymorphism; taxane; trastuzumab

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Tables and Figures

Introduction

The overexpression of human epidermal growth factor receptor 2 (HER2) in primary breast carcinomas is associated with increased risks of relapse and chemotherapy resistance as well as poor prognosis (1-4). Trastuzumab, the humanized anti-HER2 immunoglobulin G (IgG) monoclonal antibody, was introduced to overcome these problems (5-7). Trastuzumab, when used in combination with taxane, could drastically improve the prognosis of patients with HER2-positive metastatic breast cancer (MBC) (5).

P-glycoprotein (P-gp), which is encoded in the *ABCB1* (also known as MDR1) gene, is known to act as an energy-dependent drug efflux pump for various chemotherapeutic drugs such as anthracyclines, vinca alkaloids, and taxanes (8). Recently, single nucleotide polymorphisms (SNPs) of the *ABCB1* gene have been associated with taxane clearance and clinical outcomes of patients treated with taxane (9-12). *ABCB1* 1236C>T polymorphism (rs1128503) in exon 12 was significantly related to docetaxel clearance (9). *ABCB1* 2677G>T/A polymorphism (rs2032582) in exon 21 is associated with treatment response to paclitaxel monotherapy in patients with ovarian cancer (10). In addition, *ABCB1* 3435C>T polymorphism (rs1045642) in exon 26 is also a significant clinical predictor of paclitaxel monotherapy in patients with breast cancer and gastric cancer (11, 12). However, the effects of these polymorphisms on clinical outcomes after first-line trastuzumab plus taxane (TH) chemotherapy in patients with HER2-positive MBC have not yet been

studied.

On the other hand, trastuzumab is known to bind to the juxtamembrane portion of the extracellular domain of HER2, and inhibit the dimerization of HER2, leading to the enhancement of HER2 degradation and inhibition of cell cycle progression via inhibition of the mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase and Akt (PI3K/Akt) pathways (13). In addition, immune mechanisms, including antibody-dependent cellular cytotoxicity (ADCC), may also be involved (14, 15). In ADCC, IgG, which is bound to tumor cells, is engaged by effector cells via their fragment C (Fc) gamma receptors (16).

Polymorphisms of genes encoding Fc gamma receptors have been reported to predict response to monoclonal antibody therapy in patients with malignancies (17-20). The 131 histidine (H)/arginine (R) polymorphism (rs1801274) of the *FCGR2A* gene encoding Fc gamma receptor IIa and the 158 valine (V)/phenylalanine (F) polymorphism (rs396991) of *FCGR3A* gene encoding Fc gamma receptor IIIa were predictive factors for both rituximab therapy in patients with follicular lymphoma and cetuximab therapy in patients with colorectal cancer (17-19). These polymorphisms were also predictive for response to TH chemotherapy in patients with HER2-positive MBC (20). In contrast, these polymorphisms were not significant clinical indicators in patients with B-cell chronic lymphocytic leukemia receiving rituximab (21). In this study, we also aimed to validate the clinical implications of *FCGR2A* and *FCGR3A* polymorphisms in patients with HER2-positive MBC who were treated with first-line TH chemotherapy.

Materials and Methods

Patient population

Patients with MBC who planned to receive chemotherapy were asked to participate in the pharmacogenomic study at the Seoul National University Hospital (IRB Registration No. H-0610-020-186). After informed consent was obtained, patients donated their blood for research purposes and were registered in a database. From this database, we selected patients with HER2-positive MBC who were treated with TH as first-line chemotherapy between February 2004 and July 2009. We conducted this study using genomic deoxyribonucleic acid (DNA) samples extracted from mononuclear cells and the clinical data of included patients (IRB Registration No. H-0907-060-287). Patients were eligible for this study if they were at least 20 years of age and received TH as first-line chemotherapy for histologically confirmed HER2-positive MBC. HER2 positivity was defined as an intensity of 3+ by immunohistochemistry (IHC) or as a HER2/CEP17 (centromeric probe for chromosome 17) ratio of more than 2.0 by fluorescent *in situ* hybridization (FISH) (22). All patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. In addition, patients were also eligible if they had received neoadjuvant or adjuvant chemotherapy including taxanes, anthracyclines, hormonal therapy at least 12 months before TH therapy. Exclusion criteria were previous chemotherapy, radiotherapy, or surgery within three weeks, history of neoadjuvant or adjuvant chemotherapy

including trastuzumab or the other HER2-blocking agents, or previous exposure to excessive dose of anthracyclines. The maximum cumulative dose of anthracycline was 360 mg/m^2 body surface area (BSA) of doxorubicin, 720 mg/m^2 BSA of epirubicin, or equivalent.

TH regimens that were considered eligible were weekly paclitaxel plus trastuzumab, tri-weekly paclitaxel plus trastuzumab, and tri-weekly docetaxel plus trastuzumab. The weekly paclitaxel plus trastuzumab regimen consists of paclitaxel 80 mg/m^2 BSA every week, and trastuzumab at a loading dose of 4 mg/kg on day 1 of cycle 1 and 2 mg/kg/week thereafter. The tri-weekly paclitaxel plus trastuzumab regimen consists of a tri-weekly administration of paclitaxel 175 mg/m^2 BSA and trastuzumab at a loading dose of 8mg/kg, followed by 6 mg/kg. The tri-weekly docetaxel plus trastuzumab regimen consists of docetaxel 75 mg/m^2 BSA every 3 weeks plus trastuzumab at the same dose as that in the tri-weekly paclitaxel plus trastuzumab regimen.

Study objectives

The main objective of this study was to evaluate the effects of *ABCB1* 1236C>T, *ABCB1* 2677G>T/A, *ABCB1* 3435C>T, *FCGR2A* 131H/R, and *FCGR3A* 158V/F polymorphisms on treatment response, progression-free survival (PFS), overall survival (OS), and adverse events in patients with MBC who received TH as first-line treatment.

Acquisition of clinical data

Data regarding patient demographics, pathologic classification, treatment

response, PFS, OS, and adverse events were retrospectively obtained by reviewing medical records. Data collection was completed before blood sample analysis. Treatment response was evaluated using a spiral computed tomography (CT) scan by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (23). PFS was calculated from the initiation of chemotherapy to documented disease progression or death from any cause. OS was calculated from the start of chemotherapy to death from any cause.

Adverse events were assessed in all patients with version 3.0 of the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE). Hematologic toxicity was assessed every 3 weeks. Left ventricular (LV) ejection fraction was assessed by echocardiography at baseline in all patients and when clinically significant cardiac symptoms developed. These echocardiography data were used to evaluate cardiac toxicity.

Analysis of genetic polymorphisms

Genomic DNA was extracted from peripheral blood mononuclear cells using QIAmp DNA extraction kits (Qiagen Inc., Valencia, CA, USA). Then, DNA concentration was measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

ABCB1 1236C>T, *ABCB1* 2677G>T/A, and *ABCB1* 3435C>T polymorphisms were analyzed using previously described polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays (24).

FCGR2A 131H/R and *FCGR3A* 158V/F polymorphisms were analyzed

using the ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA). The genomic DNA flanking the polymorphisms of interest was amplified by PCR with forward and reverse primer pairs and standard PCR reagents in a 10 μL reaction volume, which contained 10 ng of genomic DNA, 0.5 pM of each oligonucleotide primer, 1 μL of 10X PCR Gold buffer, 250 μM dNTP, 3 mM $MgCl_2$, and 0.25 unit i-StarTaq DNA polymerase (iNtRON Biotechnology, Seongnam, Korea). After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (Roche, Basel, Switzerland) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37℃ for 60 minutes and at 72℃ for 15 minutes to purify the amplified products. One microliter of the purified amplification products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmol of genotyping primer for the primer extension reaction. The primer extension reaction was carried out for 25 cycles at 96℃ for 10 seconds, 50℃ for 5 seconds, and 60℃ for 30 seconds. The reaction products were treated with 1 unit of SAP at 37℃ for 1 hour and at 72℃ for 15 minutes to remove excess fluorescent dye terminators. One microliter of the final reaction sample containing the extension products was added to 9 μL of Hi-Di formamide (ABI). The mixture was incubated at 95℃ for 5 minutes and on ice for 5 minutes, and then analyzed by electrophoresis in an ABI Prism 3730xl DNA analyzer. The results were analyzed using GeneScan analysis software (ABI).

Statistical analysis

Statistical analysis of the categorical variables was performed using the Pearson's chi-square test or the Fisher's exact test, as appropriate. The median PFS and OS were calculated using the Kaplan-Meier method. Comparison of the survival data was performed using the log rank test. The Hardy-Weinberg equilibrium analysis was performed to compare the observed and expected genotype frequencies. All statistical tests were two-sided, with significance defined as $p<0.05$. All analyses were performed using PASW Statistics version 18.0 (SPSS Inc., Chicago, IL, USA).

Ethical considerations

Signed informed consents for chemotherapy and blood sample collection for the pharmacogenomic research were obtained from all patients before treatment. This study protocol was reviewed and approved by the Institutional Review Board of the Seoul National University Hospital (IRB Registration No. H-0907-060-287) and conducted in accordance with the precepts established by the Helsinki Declaration.

Results

Patient characteristics

A total of 124 patients were eligible for this study between February 2004 and July 2009. These patients were asked to donate blood for research purpose before TH therapy. Among them, 57 patients agreed to participate and were enrolled in this study. The demographic and clinical characteristics of the patients are summarized in Table 1. The median age of patients was 46 years (range, 27-72 years). Twenty one patients (36.8%) were estrogen receptor (ER)-positive and 10 patients (17.5%) were progesterone receptor (PgR)-positive. Thirty-five patients (61.4%) had recurrent disease after curative treatment, while 22 patients (38.6%) were initially diagnosed with metastatic disease. Thirty-five patients (61.4%) underwent breast-conserving surgery and neoadjuvant or adjuvant chemotherapy, and 14 patients among them received adjuvant radiotherapy. Sites of metastasis were as follows: lymph node in 36 patients (63.2%), lung in 27 patients (47.4%), pleura in 6 patients (10.5%), bone in 25 patients (43.9%), liver in 16 patients (28.1%), brain in 4 patients (7.0%), adrenal gland in 2 patients (3.5%), spleen in 1 patient (1.8%), and thigh in 1 patient (1.8%). Fifty one patients (89.5%) had an ECOG performance status of 0 or 1 at baseline. Treatment regimens were as follows: 22 patients (38.6%) received weekly paclitaxel plus trastuzumab, 26 patients (45.6%) received tri-weekly paclitaxel plus trastuzumab, and 9 patients (15.8%) received tri-weekly docetaxel plus trastuzumab.

Table 1. Baseline characteristics

Characteristics		Values
Age - years	Median (range)	46 (27-72)
HER2 status - No (%)	IHC(3+)	38 (66.7)
	IHC(2+) and FISH(+)	19 (33.3)
Estrogen receptor status - No (%)	Positive	21 (36.8)
	Negative	36 (63.2)
Progesterone receptor status - No (%)	Positive	10 (17.5)
	Negative	47 (82.5)
Disease status at treatment	Relapsed	35 (61.4)
	Initially metastatic	22 (38.6)
Prior treatments - No (%)	Curative surgery	35 (61.4)
	Neoadjuvant/adjuvant chemotherapy	35 (61.4)
	Prior taxane	15 (26.3)
	Prior anthracycline	24 (42.1)
	Adjuvant radiotherapy	14 (24.6)
	Adjuvant hormone therapy	9 (15.8)
No of metastatic sites	1	18 (31.6)
	2	27 (47.4)
	3	3 (5.3)
	≥4	9 (15.8)
Sites of metastasis - No (%)	Lymph node	36 (63.2)
	Lung	27 (47.4)
	Pleura	6 (10.5)
	Bone	25 (43.9)
	Liver	16 (28.1)
	Brain	4 (7.0)
	Others[a]	4 (7.0)
ECOG performance status	0 or 1	51 (89.5)
	2	6 (10.5)
Treatment regimens	Weekly paclitaxel + trastuzumab	22 (38.6)
	Tri-weekly paclitaxel + trastuzumab	26 (45.6)
	Tri-weekly docetaxel + trastuzumab	9 (15.8)

Abbreviations: HER2 = human epidermal growth factor receptor 2; IHC = immunohostochemistry; FISH = fluorescent *in situ* hybridization; ECOG = Eastern Cooperative Oncology Group.

[a] Others include adrenal gland, spleen, and thigh

Treatment results

During a median follow-up of 30.6 months (range, 0.6-75.9 months), 34 patients (59.6%) experienced disease progression, and 16 patients (28.1%) died. Treatment response was evaluated in 50 patients, because 7 patients did not have measurable lesions: 4 patients had bone metastasis only and 3 patients had only small lesions of longest diameter <10 mm with spiral CT scan. Response rate (RR) of first-line TH chemotherapy was 76.0%: 6 patients (12.0%) had complete remission (CR) and 32 patients (64.0%) had partial response (PR). The median PFS was 15.1 months (95% confidence interval (CI), 10.3-19.8 months). The median OS was 47.0 months (95% CI, 34.5-59.4 months).

Treatment-related anemia developed in 45 patients (78.9%) as follows: grade 1 in 29 patients, grade 2 in 12 patients, grade 3 in 2 patients, and grade 4 in 2 patients. Neutropenia developed in 36 patients (63.2%) as follows: grade 1 in 8 patients, grade 2 in 20 patients, grade 3 in 6 patients, and grade 4 in 2 patients. Thrombocytopenia occurred in 4 patients (7.0%) as follows: grade 1 in 2 patients and grade 3 in 2 patients. Grade 2 LV dysfunction was observed in 2 patients (3.5%).

Effects of clinical variables on treatment outcomes are shown in Table 2. Patients who were previously treated with taxane had a significantly shorter OS (p=0.009). Age, hormone receptor status, performance status, and treatment regimen were not significantly associated with clinical outcomes.

Table 2. Risk factors for response rate, progression-free survival, and overall survival

Variables		No of patients (%)	RR (%)	*p*	Median PFS (95% CI)	*p*	Median OS (95% CI)	*p*
Age	<50 years	35 (61.4)	76.7	1.000	14.6 (8.2-21.0)	0.805	47.0 (32.3-61.7)	0.651
	≥50 years	22 (38.6)	75.0		15.1 (9.8-20.3)		Not reached[a]	
Hormone receptor status	ER(+) or PR(+)	21 (36.8)	83.3	0.497	18.5 (13.7-23.2)	0.356	Not reached[a]	0.088
	ER(-) and PR(-)	36 (63.2)	71.9		12.2 (7.8-16.6)		47.0 (35.2-58.8)	
Any prior chemotherapy	Yes	35 (61.4)	71.0	0.332	13.0 (10.0-16.0)	0.360	47.0 (33.1-60.8)	0.157
	No	22 (38.6)	84.2		17.7 (16.3-19.1)		Not reached[a]	
Prior taxane therapy	Yes	15 (26.3)	61.5	0.256	11.5 (10.0-13.0)	0.091	23.3 (5.5-41.1)	0.009
	No	42 (73.7)	81.1		17.6 (13.3-21.9)		47.0 (35.3-58.6)	
ECOG PS	0 or 1	51 (89.5)	77.3	0.621	15.1 (10.5-19.6)	0.447	47.0 (24.7-69.3)	0.848
	2	6 (10.5)	66.7		7.2 (0.0-20.2)		38.9 (0.0-79.7)	
Treatment regimens	Weekly TH	22 (38.6)	75.0	1.000	16.6 (9.3-23.9)	0.697	38.9 (22.6-55.2)	0.260
	Tri-weekly TH	26 (45.6)	76.2		15.1 (9.4-20.7)		Not reached[a]	
	Tri-weekly DH	9 (15.8)	77.8		13.0 (10.5-15.6)		47.0 (34.5-59.4)	

Abbreviations: RR = response rate; PFS = progression-free survival; CI = confidence interval; OS = overall survival; ER = estrogen receptor; PR = progesterone receptor; ECOG PS = Eastern Cooperative Oncology Group performance status; TH = paclitaxel plus trastuzumab; DH = docetaxel plus trastuzumab.

[a] In these groups, OS did not reach the median value during a median follow-up of 30.6 months. Thus, the median OS could not be calculated.

Association between genotypes and treatment results

Genotype frequencies are shown in Table 3. *FCGR3A* 158V/F polymorphism could not be analyzed in 3 patients. All genotypes were in Hardy-Weinberg equilibrium (p>0.05). The association between genotype and treatment results is summarized in Table 4.

ABCB1 2677T allele carriers (GT+TA+TT) had a longer PFS (42.1 months (95% CI, 12.7-71.4 months) vs. 13.0 months (95% CI, 10.6-15.4 months); p=0.037; Figure 1) along with a tendency toward a longer OS (54.7 months (95% CI, 43.0-66.4 months) vs. 38.9 months (95% CI, 18.1-59.7 months); p=0.057; Figure 2). *ABCB1* 2677G or A alleles were not significantly associated with RR, PFS, and OS (data not shown).

PFS was significantly different according to *ABCB1* 3435C>T genotypes (p=0.026; Figure 3). OS also tended to be different according to *ABCB1* 3435C>T genotypes (p=0.199; Figure 4). *ABCB1* 3435CC genotype carriers had a shorter PFS than CT or TT genotype carriers (13.0 months (95% CI, 10.8-15.2 months) vs. 19.1 months (95% CI, 0.0-38.5 months); p=0.039) along with a tendency toward a shorter OS (38.9 months (95% CI, 19.7-58.1 months) vs. 54.7 months (95% CI, 43.0-66.4 months); p=0.093). In combined analysis, *ABCB1* 1236CC and/or 2677TT carriers had significantly longer PFS compared to the others (p=0.006; Figure 5)

On the other hand, *FCGR2A* 131H/R and *FCGR3A* 158V/F were not significantly associated with RR, PFS, and OS.

None of these polymorphisms were associated with any grades of hematologic or

cardiac toxicities (data not shown).

Table 3. Genotype distribution and Hardy-Weinberg equilibrium analysis

Polymorphisms		No of patients	Observed frequency (%)	Expected frequency (%)	*p*
ABCB1	CC	13	22.8	23.3	
1236C>T	CT	29	50.9	49.9	0.887
(rs1128503)	TT	15	26.3	26.8	
ABCB1	GG	13	22.8	21.6	
2677G>T/A	GT+GA	27	47.4	49.8	0.717
(rs2032582)	TT+TA+AA	17	29.8	28.6	
ABCB1	CC	29	50.9	49.2	
3435C>T	CT	22	38.6	41.9	0.556
(rs1045642)	TT	6	10.5	8.9	
FCGR2A	H/H	39	68.4	65.1	
131H/R	H/R	14	24.6	31.2	0.110
(rs1801274)	R/R	4	7.0	3.7	
FCGR3A	V/V	2	3.7	5.8	
158V/F	V/F	22	40.7	36.6	0.400
(rs396991)	F/F	30	55.6	57.6	

Table 4. Association between polymorphisms and treatment outcomes

Polymorphisms		Frequency (%)	RR[a] (%)	*p*	Median PFS (95% CI)	*p*	Median OS (95% CI)	*p*
ABCB1	CC	22.8	81.8		22.2 (8.0-36.4)		Not reached[b]	
1236C>T	CT	50.9	68.0	0.520	13.0 (10.2-15.9)	0.191	47.0 (8.0-85.9)	0.933
(rs1128503)	TT	26.3	85.7		14.7 (6.5-22.9)		33.7 (14.9-52.6)	
ABCB1	T carriers[c]	47.4	86.4	0.470	42.1 (12.7-71.4)	0.037	54.7 (43.0-66.4)	0.057
2677G>T/A	Others[d]	52.6	76.0		13.0 (10.6-15.4)		38.9 (18.1-59.7)	
(rs2032582)								
	CC	50.9	74.1		13.0 (10.8-15.3)		38.9 (19.7-58.1)	
ABCB1	CT	38.6	76.5	1.000	17.6 (8.2-27.1)	0.026	54.7 (43.0-66.5)	0.199
3435C>T	TT	10.5	83.3		Not reached[b]		Not reached[b]	
(rs1045642)	CC	50.9	74.1	0.754	13.0 (10.8-15.2)	0.039	38.9 (19.7-58.1)	0.093
	CT+TT	49.1	78.3		19.1 (0.0-38.5)		54.7 (43.0-66.4)	
ABCB1 combined	1236CC and/or 2677TT	35.1	83.3	0.497	27.4	0.006	54.7	0.496
	Others	64.9	71.9		12.4 (10.3-19.8)		47.0 (23.5-70.4)	
FCGR2A	H/H	68.4	76.5		17.6 (11.0-24.2)		47.0 (24.4-69.5)	
131H/R	H/R	24.6	69.2	0.748	10.6 (0.0-24.4)	0.605	38.9 (16.0-61.8)	0.456
(rs1801274)	R/R	7.0	100		11.5 (4.5-18.5)		Not reached[b]	
FCGR3A	V/V	3.7	100		14.6		Not reached[b]	
158V/F	V/F	40.7	64.7	0.450	19.1 (13.1-25.1)	0.628	47.0 (23.3-70.6)	0.861
(rs396991)	F/F	55.6	82.8		13.0 (10.0-16.1)		54.7	

Abbreviations: RR = response rate; PFS = progression-free survival; CI = confidence interval; OS = overall survival; H = histidine allele; R = arginine allele; V = valine allele; F = phenylalanine allele.

[a] Response rate was evaluated in 47 patients.

[b] In these groups, PFS and OS did not reach the median value during a median follow-up of 30.6 months. Thus, the median PFS and OS could not be calculated.

[c] GT+TA+TT

[d] GG+GA+AA

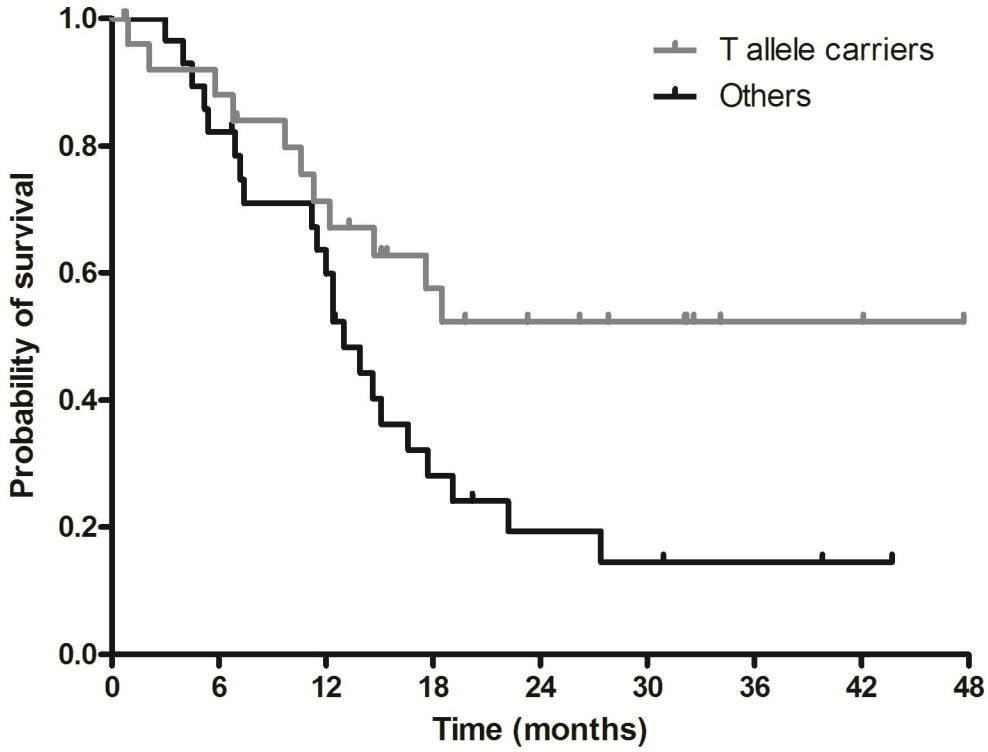

Figure 1. PFS according to *ABCB1* 2677G>T/A polymorphism

ABCB1 2677T allele carriers had longer PFS compared to the others (p=0.037).

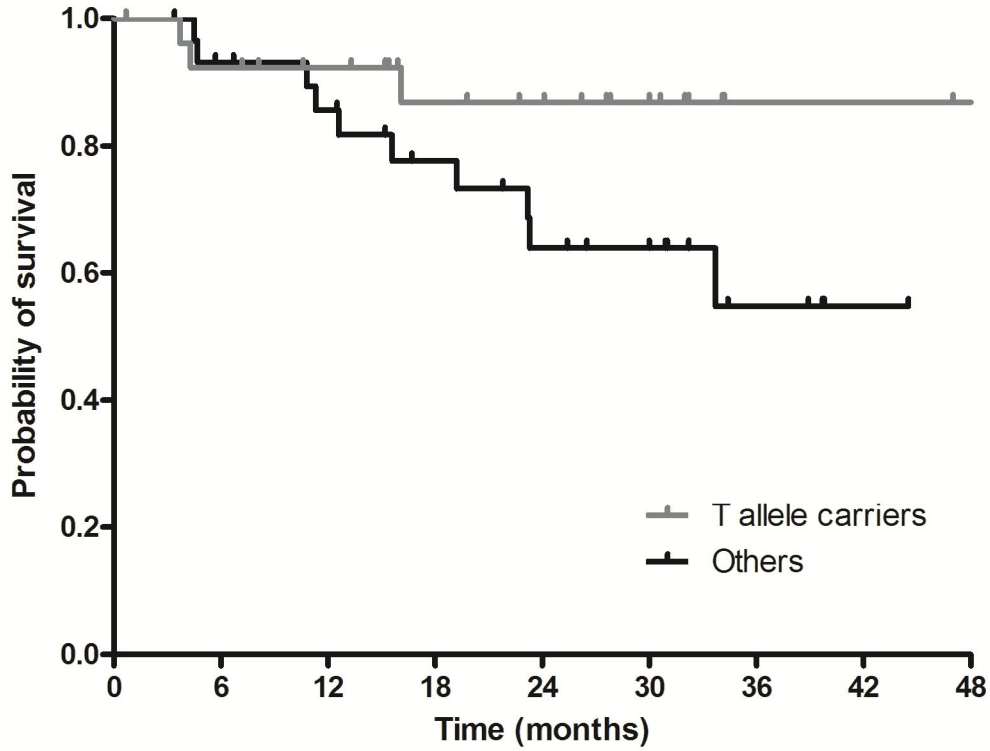

Figure 2. OS according to *ABCB1* 2677G>T/A polymorphism

ABCB1 2677T allele carriers tended to have longer OS compared to the others (p=0.057).

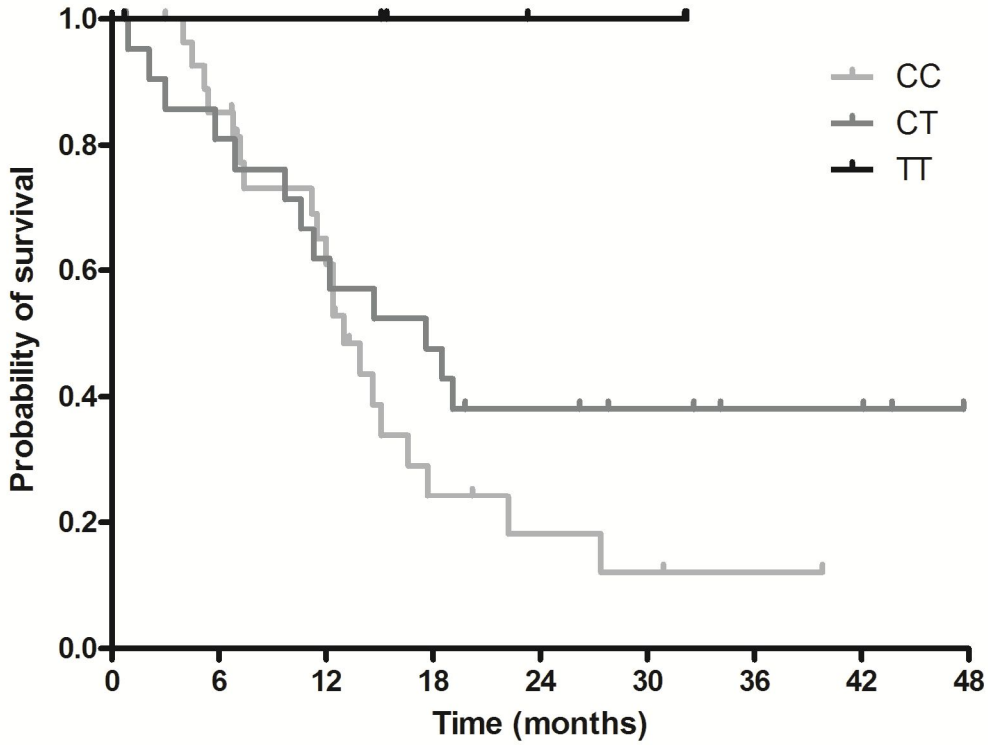

Figure 3. PFS according to *ABCB1* 3435C>T polymorphism

PFS was significantly different according to *ABCB1* 3435C>T genotypes (p=0.026).

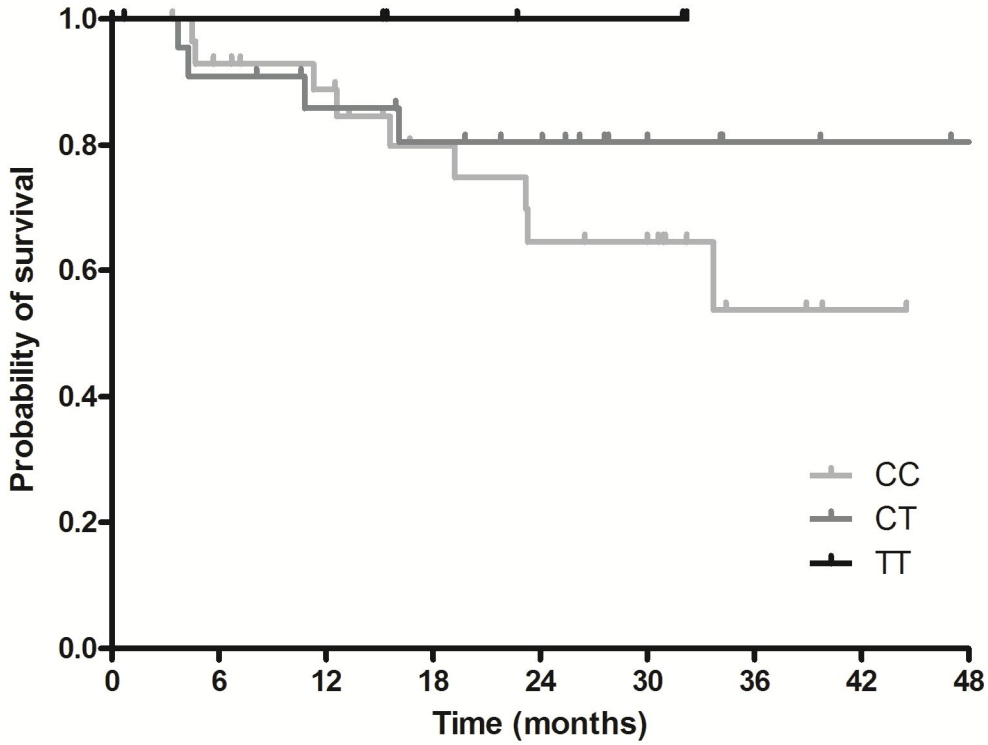

Figure 4. OS according to *ABCB1* 3435C>T polymorphism

OS tended to be different according to *ABCB1* 3435C>T genotypes (p=0.199).

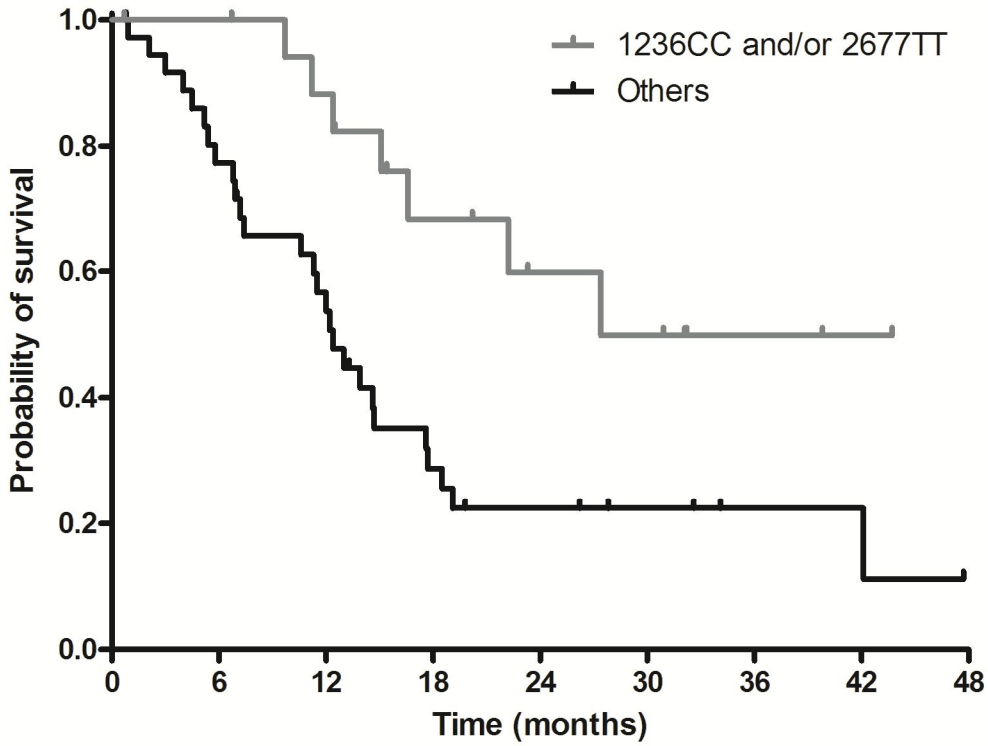

Figure 5. Combined effect of *ABCB1* 1236C>T and 2677G>T/A polymorphisms on PFS

PFS was significantly longer in *ABCB1* 1236CC and/or 2677TT carriers compared to the others (p=0.006).

Discussion

Previously, *ABCB1* 2677G>T/A polymorphism was found to correlate significantly with RR to paclitaxel in combination with carboplatin in patients with ovarian cancer: homozygously-mutated patients (TT or TA carriers) had a higher RR (10). In this study, for the first time, we demonstrated that *ABCB1* 2677T allele carriers had a significantly longer PFS along with a tendency toward a longer OS among patients with HER2-positive MBC receiving TH chemotherapy. Moreover, the combined analysis of *ABCB1* 1236C>T and 2677G>T/A polymorphisms more clearly demonstrated that *ABCB1* 1236CC and/or 2677TT carriers had a significantly longer PFS compared to the others. This result suggests that *ABCB1* 1236C>T and 2677G>T/A polymorphisms may be a predictive marker of TH chemotherapy in patients with HER2-positive MBC.

P-gp, which is encoded in the *ABCB1* gene, acts as an energy-dependent drug efflux pump for taxanes. P-gp overexpression can induce chemotherapy resistance in patients with malignancies (8). The substitution of *ABCB1* 3435C (glutamate) to T (glutamate) cannot change the amino acid sequence, nor is it located in a regulatory region (9). However, this substitution is known to decrease mRNA stability (25). This finding suggests that altered mRNA expression due to polymorphic alleles may change P-gp expression *in vivo*. Previous studies showed that *ABCB1* 3435CC genotype carriers had significantly higher P-gp expression in duodenum and breast cancer tissue (26, 27), which may result in decreased drug

concentration in cells. From these results, we hypothesized that *ABCB1* 3435CC genotype carriers may have worse treatment response and survival. In our results, this genotype was significantly associated with a worse PFS along with a concordant tendency toward a shorter OS. In a previous study performed in patients with MBC who received paclitaxel monotherapy, the *ABCB1* 3435CC genotype also tended to be associated with a lower RR (11). However, the authors concluded that heterozygous 3435CT genotype was associated with a lower disease control rate and a shorter OS. Since *ABCB1* 3435CC genotype carriers, not 3435CT individuals, showed the highest P-gp expression (25, 26), it is much easier and more appropriate to conclude that the *ABCB1* 3435CC genotype is a poorer predictive marker of taxane therapy than the heterozygous 3435CT genotype. To our knowledge, this is the first study demonstrating that *ABCB1* 3435C>T polymorphism is significantly associated with PFS after first-line TH combination chemotherapy in patients with MBC. Moreover, the trends of treatment outcomes according to genotypes were in accordance with previous study results regarding the association between P-gp expression and *ABCB1* 3435C>T polymorphism.

FCGR2A 131H/R and *FCGR3A* 158V/F polymorphisms are known to be associated with binding affinity of effector cells to the Fc receptor of IgG (28-30). In a previous *in vitro* study, phagocytes obtained from *FCGR2A* 131H/H carriers are reported to ingest IgG2-coated erythrocytes much more efficiently than phagocytes obtained from 131R/R carriers (28, 29). *FCGR3A* 158V carriers also have a higher affinity for both IgG1 and IgG3 than 158F carriers (28, 30). We hypothesized that these polymorphisms may also influence trastuzumab-mediated cellular cytotoxicity

resulting in change in clinical outcomes after TH chemotherapy in patients with HER2-positive MBC. A previous study by Musolino A, *et al.* demonstrated that *FCGR2A* 131H/R and *FCGR3A* 158V/F polymorphisms were significantly associated with better RR and PFS after TH chemotherapy in 54 patients with HER2-positive MBC as well as higher trastuzumab-dependent cellular cytotoxicity to HER2-overexpressing human breast cancer cells *in vitro* (20). In contrast, Hurvitz S, *et al.* recently studied the same genetic variants in a much larger cohort from BCIRG006 study (n=1286), and no correlation was found between the variants and clinical outcomes of patients with both early stage and metastatic breast cancer (31). Also, we could not find any tendency or a significant association between these polymorphisms and clinical outcomes after TH therapy in this study. This is of interest as this is the first study on *FCGR2A* and *FCGR3A* polymorphisms in relation to trastuzumab in an Asian population. However, in our study, the *FCGR2A* 131R/R genotype and the *FCGR3A* 158V/V genotype that demonstrated significantly distinct RR and PFS in a previous study were observed in only 4 patients (7.0%) and 2 patients (3.7%), respectively, which did not provide enough statistical power. Given that both the previous study and our study were performed retrospectively in a relatively small sample size, it is still uncertain whether these polymorphisms influence treatment results after trastuzumab-based chemotherapy in patients with HER2-positive MBC. Further investigations are warranted in a prospectively-accrued large population.

Besides, it is conceivable that altered drug clearance or ADCC activity owing to *ABCB1*, *FCGR2A*, and *FCGR3A* polymorphisms may possibly influence adverse

events as well as RR, PFS, and OS in our study. A previous study demonstrated that *ABCB1* 3435CT and TT genotypes were significantly associated with mucositis, and that variant genotypes at the 2677 loci were significantly associated with diarrhea (12). In our study, the effect of these polymorphisms on hematologic or cardiac adverse events was evaluated. However, no significant association was found.

In anti-cancer treatment, a variety of genetic polymorphisms are known to contribute to treatment outcome as exploratory biomarkers (32). However, most of these polymorphisms are still in the process of evaluation without sufficient evidence of biologic mechanism. Unfortunately, biologic mechanisms how these polymorphisms influence individual outcome were not fully elucidated although some of them have already been studied as previously described (25-30). Moreover, in some studies, the effects of polymorphic genotypes on clinical outcome were not even consistent (11, 20, 31). Nevertheless, genetic polymorphism has a strong point as a biomarker compared to other biomarkers utilizing tumor tissues since it can be analyzed with peripheral blood samples which are relatively easier to obtain as compared to tumor tissues.

This study has several limitations because of its retrospective nature and a relatively small sample size. In general, the RR to TH chemotherapy is approximately 60% in patients with HER2-positive MBC (5, 33, 34). In contrast, the RR of our study population, 76.0%, seems quite higher. In addition, during a median follow-up of 30.6 months, the OS did not reach the median value, which is much longer than that of previous studies (5, 33, 34). Besides, patients who were treated with three different kinds of regimens using TH were included in the present

study although treatment outcomes were not statistically significant according to the treatment regimens. In addition, because of the low statistical power resulting from a relatively small sample size, the result drawn from this study could not maintain statistical significance after Bonferroni correction. The combined analysis of *ABCB1* 1236C>T and 2677G>T/A polymorphisms yielded a much smaller *p*-value of 0.006. However, considering numerous combinations among different genotypes, this value may not also have statistical significance after Bonferroni correction. Nevertheless, we suggest that our results are clinically meaningful because this is the first study presenting the clinical role of *ABCB1* 2677G>T/A and 3435C>T polymorphisms in relation to clinical outcomes of TH chemotherapy in patients with HER2-positive MBC. Therefore, further prospective studies with a bigger patient cohort are necessary to clarify and validate the clinical significance of these promising biomarkers.

Conclusions

Our data support that *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T polymorphisms may predict PFS after first-line TH chemotherapy in patients with HER2-positive MBC. In contrast, *FCGR2A* 131H/R and *FCGR3A* 158V/F polymorphisms were not significantly correlated to clinical outcomes after TH chemotherapy. Further studies with a more statistical power are necessary to validate our findings.

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국문초록

본 연구의 목표는 HER2-양성 전이성 유방암 환자에서 탁센과 트라스투주맙 치료 후 *ABCB1*, *FCGR2A*, *FCGR3A* 유전자 다형성이 갖는 임상적 의미를 밝히는 것이다. 1차 화학요법으로 탁센과 트라스투주맙 치료를 받은 HER2-양성 전이성 유방암 환자들의 말초 혈액 단핵구로부터 추출한 DNA 검체를 이용하여 *ABCB1* 1236C>T, *ABCB1* 2677G>T/A, *ABCB1* 3435C>T, *FCGR2A* 131H/R, *FCGR3A* 158V/F 유전자 다형성을 분석하였고, 이 결과에 따른 환자들의 반응률, 무진행 생존기간, 전체 생존기간을 비교 분석하였다. 총 57명의 여성 환자들을 분석하였다. 연령의 중앙값은 46세 (범위, 27-72세)였다. *ABCB1* 2677T를 가진 환자들에서 무진행 생존기간이 통계적으로 유의하게 길었고 (p=0.037), 전체 생존기간도 긴 경향을 보였다 (p=0.057). *ABCB1* 3435CC 유전형을 보유한 환자군은 무진행 생존기간이 통계적으로 유의하게 짧았고 (p=0.039), 전체 생존기간도 더 짧은 경향을 보였다 (p=0.093). 또한, *ABCB1* 1236CC나 2677TT 유전형을 보유한 환자들에서는 그렇지 못한 환자들에 비해 유의하게 무진행 생존기간이 더 길었다 (p=0.006). *FCGR2A* 131H/R과 *FCGR3A* 158V/F 유전자 다형성은 환자들의 반응률, 무진행 생존기간, 전체 생존기간과 유의한 관계가 없었다. 결론적으로, HER2-양성 전이성 유방암 환자에서 1차 요법으로 탁센 및 트라스투주맙 병합요법을 시행하였을 때, *ABCB1* 유전자 다형성 분석을 통

해 환자들의 무진행 생존기간을 예측할 수 있을 가능성을 제시하였다. 반면에, *FCGR2A* 131H/R과 *FCGR3A* 158V/F 유전자 다형성 분석 결과로는 치료 성적을 예측할 수 있을 가능성을 제시할 수 없었다.

주요어: *ABCB1*; *FCGR2A*; *FCGR3A*; 다형성; 탁센; 트라스투주맙

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