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PRECLINICAL STUDY

MDM2 SNP309 and *TP53* R72P associated with severe and febrile neutropenia in breast cancer patients treated with 5-FU/epirubicin/cyclophosphamide

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Abstract The aim of this study was to investigate the association of two genetic polymorphisms, *MDM2* SNP309 and *TP53* R72P, with incidence of neutropenia in breast cancer patients treated with 5-FU/epirubicin/cyclophosphamide (FEC). Primary breast cancer patients ($n = 216$) treated with adjuvant FEC (60, 75 or 100 mg/m^2) were included in this study. The association of genotypes of *MDM2* SNP309 and *TP53* R72P, determined by TaqMan SNP Genotyping Assays, with febrile neutropenia (FN) was investigated. In the patients treated with FEC100, G/G genotype for *MDM2* SNP309 (G/G genotype^{MDM2}) was significantly ($P < 0.01$) associated with a lower incidence (5.3 vs. 39.2%) of severe neutropenia (<100/mm^3) than with T/T + T/G genotypes^{MDM2}, and C/C genotype for *TP53* R72P (C/C genotype^{TP53}) was significantly ($P = 0.03$) associated with a higher incidence (58.3 vs. 27.3%) of FN than with G/G + G/C genotypes^{TP53}. The combination of C/C genotype^{TP53} and T/T + T/G genotype^{MDM2} showed the highest risk for developing severe neutropenia (83.3%) and FN (62.5%) than any other combinations. In the patients treated with FEC60 or FEC75, there was no significant association of *MDM2* SNP309 and *TP53* R72P with severe neutropenia and FN. *MDM2* SNP309 and *TP53* R72P are significantly associated with severe neutropenia and FN, respectively, in breast cancer patients treated with FEC100, and especially their combination may be a useful predictor of severe neutropenia and FN.

Keywords Breast cancer · FEC100 · *MDM2* SNP309 · Neutropenia · *TP53* R72P

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Introduction

The combination chemotherapy consisting of 5-FU, epirubicin, and cyclophosphamide (FEC) is often used for active treatment of breast cancer in neoadjuvant, adjuvant, and metastatic settings. The prescribed dose of epirubicin in FEC used to be 50–75 mg/m^2 every 3 weeks but recently a higher dose of 100 mg/m^2 every 3 weeks is used more often since this higher dose was clearly demonstrated to be more effective. However, one of the clinically most important and frequently observed adverse effects of FEC is bone marrow suppression. For Caucasian patients treated with FEC100, incidence of febrile neutropenia (FN) is reportedly 0–8% [1–3] while for Japanese patients it seems to be slightly higher (14–20%) [4, 5]. The ASCO guideline (2006 update) recommends the primary prophylactic use of colony stimulating factor (CSF) when the risk of FN is in the range of 20% or higher. Thus, the incidence of FN for Japanese patients treated with FEC100 can be considered to be close to the threshold for prophylactic use of CSF. For the adequate and safe administration of chemotherapy, it is therefore very important to predict who is more likely to develop FN since they can be candidates for prophylactic use of CSF.

Several factors such as higher age (>65 years), small body surface area (BSA), and pretreatment absolute neutrophile count (ANC) are reported to be associated with FN. But prediction of FN occurrence using these risk

factors is not accurate enough, so that more accurate predictors need to be developed. Nayak et al. [6] recently reported that *MDM2* SNP309 is associated with resistance to chemotherapy in vitro, i.e., G/G homozygotes up-regulate MDM2, which in turn down-regulates topoisomerase 2A (TOPO2A), rendering tumor cells less sensitive to anti-TOPO2A chemotherapy such as epirubicin-based regimens. Since this effect of *MDM2* SNP309 on tumor cells is speculated to be also operational in normal bone marrow hematopoietic cells, their sensitivity to epirubicin-based regimens may be influenced by *MDM2* SNP309 and such an association, if proved, might then be applicable to the prediction of FN occurrence.

TP53 R72P is one of the most thoroughly studied single nucleotide polymorphisms (SNPs) in breast cancer in terms of its association with resistance to chemotherapy [7–10]. 72P-allele has been shown to induce apoptosis less effectively than does R72-allele in vitro [11]. In fact, 72P-allele has been demonstrated to be associated with resistance to chemotherapy in vitro [12] and also in the neoadjuvant setting [8]. If this impact of *TP53* R72P on chemo-sensitivity is also operational in normal bone marrow hematopoietic cells, this polymorphism may well be associated with FN.

Although the association of *MDM2* SNP309 or *TP53* R72P with resistance to chemotherapy in breast tumors has been investigated by several investigators, conflicting results have been reported [7–10, 13, 14]. Since mechanisms of chemo-resistance are complicated and differ among tumors, it is very difficult to demonstrate in the clinical setting whether these SNPs, even though they have a certain impact, are associated with chemo-resistance. On the other hand, bone marrow hematopoietic cells are normal cells without any genetic changes such as somatic mutations and gene amplification/deletion, and thus, the influence of these SNPs on response to chemotherapy can be evaluated in a less complicated manner in normal bone marrow hematopoietic cells than in tumor cells. In the present study, we investigated whether *MDM2* SNP309 and *TP53* R72P are associated with the incidence of leucopenia and FN in breast cancer patients treated with FEC in the adjuvant setting.

Materials and methods

Patients

Primary breast cancer patients ($n = 216$), who underwent mastectomy or breast-conserving surgery and were treated with adjuvant FEC therapy (5FU 500 mg/m^2, epirubicin 60, 75 or 100 mg/m^2, and cyclophosphamide 500 mg/m^2, q3w× 6 cycles) at Osaka University Hospital between July 2001 and April 2010, were consecutively recruited for this study. Before FEC treatment, all the patients showed satisfactory organ (bone marrow, liver, and kidneys) function. After chemotherapy, the patients who underwent breast-conserving surgery received radiotherapy for the breast and patients with hormone-receptor-positive tumors received adjuvant hormonal therapy. The median follow–up was 55 months (range, 4–114 months). Characteristics of the patients are shown in Table 1. Informed consent was obtained from each patient.

Genotype analysis

Genomic DNA was extracted from peripheral whole blood mononuclear cells obtained before surgery, and TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) were used for genotyping *MDM2* SNP309 (genotype^{MDM2}) and *TP53* R72P (genotype^{TP53}).

Detection of neutropenia

All the patients had to have neutrophile counts >1,500/mm^3 before FEC was administered. For the first cycle, a complete blood count test was performed both before and about 7 days after FEC. When grade 4 (G4) neutropenia was found, the complete blood count test was repeated a few days later. A patient who developed FN was treated with G-CSF (lenograstim) until the neutrophile count improved to over 5,000/mm^3 and received a reduced dose (20–25% reduction) of FEC from the next cycle or the same dose combined with a prophylactic dose of G-CSF. For most patients, a complete blood count test was performed once before FEC from the second cycle thereafter.

Grades of neutropenia, infection, and FN were assessed according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 4) and G4 neutropenia with a neutrophile count <100/mm^3 was defined as severe neutropenia for the purposes of this study since severe neutropenia is considered to be a risk factor for the development of FN [15].

Estrogen receptor, progesterone receptor, HER2 and histological grade

Estrogen receptor and progesterone receptor were classified as positive when 10% or more of the tumor cells immunohistochemically stained positive (ER: Clone 6F11, Ventana, Tokyo, Japan; PR: Clone16, SRL Inc., Tokyo, Japan). The HER2 amplification was determined with FISH using the PathVysion HER-2 DNA Probe Kit (Abbott Molecular, Chicago, IL, USA and SRL) or immunohistochemically using anti-human c-erbB-2 polyclonal antibody (Nichirei Biosciences, Tokyo, Japan) as previously described [16]. FISH scores were obtained by counting

Table 1 Clinicopathological characteristics of patients enrolled in this study

	Total patients ($n = 216$)	FEC60, 75 ($n = 131$)	FEC100 ($n = 85$)
Age (years)			
≦60	168	105	63
>60	48	26	22
BSA (m^2)			
≦1.5	90	56	34
>1.5	126	75	51
Pretreatment (ANC/mm^3)			
≦3000	86	58	28
>3000	130	73	57
Tumor size (cm)			
≦2	94	50	44
>2	122	81	41
Lymph node status			
Positive	139	98	41
Negative	77	33	44
ER status			
Positive	139	84	55
Negative	77	47	30
PgR status			
Positive	112	73	39
Negative	104	58	46
HER-2 status			
Positive	44	19	25
Negative	143	83	60
Unknown	29	29	0
Histologic grade			
Grade I	48	32	16
Grade II	117	63	54
Grade III	51	36	15

BSA body surface area, *ANC* absolute neutrophile count, *ER* estrogen receptor, *PgR* progesterone receptor

fluorescence signals in at least 60 malignant cell nuclei per case, and for each specimen, the ratio of HER2 gene signals to chromosome 17 centromere signals was calculated. A tumor was considered to be HER2-amplified if the FISH ratio was ≥2.0. When a tumor showed +3 immunostaining, it was considered HER2 positive. The histological grade was determined with the Scarff-Bloom-Richardson grading system [17].

Statistical analysis

Associations between the clinicopathological parameters and genotypes were assessed with the χ^2 test or Fisher's exact test. A logistic regression model was used for univariate and multivariate analysis of the relationship between *the MDM2* SNP309 or *TP53* R72P and FN. All test results with a *P*-value of less than 0.05 were considered significant. All statistical analyses were performed with StatView software (Version 5.0 for Windows; SAS Institute, Cary, NC, USA).

Results

MDM2 SNP309 and neutropenia

Patients treated with FEC60 or FEC75 showed no significant differences in the incidence of any type of neutropenia among T/T, T/G, and G/G genotypes^{MDM2} or between G/G and T/T + T/G genotypes^{MDM2} (Table 2). For patients treated with FEC100, no significant differences were detected either in the incidence of any type of neutropenia among T/T, T/G, and G/G genotypes^{MDM2}. However, a comparison of G/G genotype^{MDM2} with T/T + T/G genotypes^{MDM2} found that the former showed a tendency ($P = 0.07$) toward a lower incidence (73.7 vs. 90.2%) of G4 neutropenia as well as a significantly ($P < 0.01$) lower incidence (5.3 vs. 39.2%) of severe neutropenia than did T/T + T/G genotypes ^{MDM2}.

TP53 R72P and neutropenia

Patients treated with FEC60 or FEC75 showed no significant differences in the incidence of any type of neutropenia among G/G, G/C, and C/C genotypes^{TP53} or between C/C and G/G + G/C genotypes^{TP53} (Table 3). For patients treated with FEC100, no significant differences were detected either in the incidence of any type of neutropenia among G/G, G/C, and C/C genotypes^{TP53}. However, a comparison of C/C genotype^{TP53} with G/G + G/C genotypes^{TP53}, found that the former showed a tendency ($P = 0.07$) toward a higher incidence (55.6 vs. 26.2%) of severe neutropenia as well as a significantly ($P = 0.03$) higher incidence (58.3 vs. 27.3%) of FN than did G/G + G/C genotypes^{TP53}.

Association of *MDM2* SNP309 and *TP53* R72P with neutropenia after adjustment for conventional risk factors

For the patients treated with FEC100, the associations of *MDM2* SNP309 and *TP53* R72P with severe neutropenia and FN after adjustment for conventional risk factors such as age, BSA, and pretreatment ANC were investigated (Table 4).

Table 2 Incidence of neutropenia in relation to *MDM2* SNP309 in patients treated with FEC60, 75 and 100

	No. of patients	G4 neutropenia			Severe neutropenia			No. of patients	Febrile neutropenia		
		Incidence (%)	OR (95%CI)[a]	*P*-value	Incidence (%)	OR (95%CI)	*P*-value		Incidence (%)	OR (95%CI)	*P*-value
FEC60, 75											
T/T	24	62.5	1		8.3	1		26	3.8	1	
T/G	69	59.4	0.87 (0.33–2.28)	0.79	8.7	1.04 (0.19–5.57)	0.99	76	10.5	2.94 (0.34–2.50)	0.44
G/G	23	52.2	0.65 (0.20–2.12)	0.47	0.0	–	0.48	29	3.4	0.89 (0.05–16.6)	0.99
T/T + T/G	93	60.2	1		8.6	1		102	8.8	1	
G/G	23	52.2	0.72 (0.28–1.81)	0.48	0.0	–	0.35	29	3.4	0.36 (0.04–3.12)	0.45
FEC100											
T/T	14	85.7	1		28.6	1		17	29.4	1	
T/G	37	91.9	1.96 (0.28–12.65)	0.50	43.2	1.90 (0.50–7.19)	0.33	45	37.8	1.45 (0.43–4.85)	0.53
G/G	19	73.7	0.46 (0.07–2.85)	0.66	5.3	0.13 (0.01–1.42)	0.13	23	21.7	0.66 (0.15–2.85)	0.57
T/T + T/G	51	90.2	1		39.2	1		62	35.5	1	
G/G	19	73.7	0.30 (0.07–1.21)	0.07	5.3	0.08 (0.01–0.69)	0.01>	23	21.7	0.50 (0.16–1.56)	0.21

[a] Odds ratio (95% confidence interval)

Table 3 Incidence of neutropenia in relation to *TP53 R72P* in patients treated with FEC60, 75 and 100

	No. of patients	G4 neutropenia			Severe neutropenia			No. of patients	Febrile nuetropenia		
		Incidence (%)	OR (95%CI)[a]	*P*-value	Incidence (%)	OR (95%CI)	*P*-value		Incidence (%)	OR (95%CI)	*P*-value
FEC60, 75											
G/G	44	61.4	1		2.3	1		51	5.9	1	
G/C	58	56.9	0.83 (0.54–2.67)	0.64	12.1	5.91 (0.69–50.00)	0.13	63	9.5	1.68 (0.40–7.09)	0.72
C/C	14	57.1	0.84 (0.24–2.84)	0.77	0.0	–	0.99	17	5.9	1.00 (0.09–10.30)	0.99
G/G + G/C	102	58.8	1		7.8	1		114	7.9	1	
C/C	14	57.1	0.93 (0.30–2.88)	0.91	0.0	–	0.59	17	5.9	0.72 (0.08–6.14)	0.99
FEC100											
G/G	33	93.9	1		24.2	1		37	32.4	1	
G/C	28	75.0	0.19 (0.03–1.02)	0.06	38.1	1.25 (0.39–3.92)	0.77	36	22.2	0.59 (0.20–1.69)	0.32
C/C	9	88.9	0.51 (0.04–6.43)	0.52	55.6	3.90 (0.83–18.18)	0.07	12	58.3	2.97 (0.76–11.12)	0.17
G/G + G/C	61	85.2	1		26.2	1		73	27.3	1	
C/C	9	88.9	1.38 (0.15–12.44)	0.99	55.6	3.52 (0.83–14.70)	0.07	12	58.3	3.71 (1.05–13.05)	0.03

[a] Odds ratio (95% confidence interval)

After these adjustments, G/G genotype^{MDM2} still showed a significantly lower incidence of severe neutropenia, and C/C genotype^{TP53} a significantly higher incidence of FN.

The percentage of patients who developed FN according to the treatment cycle at which FN was first seen is shown in Fig. 1. When FN occurred, it did so in the first cycle for almost all patients regardless of *MDM2* SNP309 or *TP53* R72P genotypes, so that there was no significant difference in the cycle at which FN occurred between the T/T + T/G genotype^{MDM2} and G/G genotype^{MDM2} or between the G/G + G/C genotype^{TP53} and C/C genotype^{TP53}.

Combination of *MDM2* SNP309 and *TP53* R72P as predictor of neutropenia

The combination of *MDM2* SNP309 and *TP53* R72P genotypes was subjected to analysis in terms of their association with neutropneia in FEC100 treated patients (Table 5). The combination of T/T + T/G genotype^{MDM2} and C/C genotype^{TP53} was associated with a significantly higher incidence of severe neutropenia (83.3%) ($P < 0.01$) and FN (62.5%) ($P = 0.02$) than the combination of G/G genotype^{MDM2} and G/G + G/C genotype^{TP53} (6.3 and

Table 4 Association of *MDM2* SNP309 and *TP53 R72P* with neutropenia for patients treated with FEC100

	No. of patients	G4 neutropenia			Severe neutropenia			No. of patients	Febrile neutropenia		
		Incidence (%)	OR (95%CI)[a]	*P*-value	Incidence (%)	OR[a] (95%CI)	*P*-value		Incidence (%)	OR[a] (95%CI)	*P*-value
MDM2 SNP309											
T/T + T/G	51	90.2	1		39.2	1		62	35.5	1	
G/G	19	73.7	0.25 (0.05–1.12)	0.07	5.3	0.07 (0.008–0.67)	0.02	23	21.7	0.44 (0.13–1.47)	0.18
TP53 R72P											
G/G + G/C	61	85.2	1		26.2	1		73	27.3	1	
C/C	9	88.9	1.34 (0.14–12.67)	0.79	55.6	3.26 (0.58–7.32)	0.13	12	58.3	4.73 (1.15–19.40)	0.03

[a] Odds ratio adjusted for age (≤60 vs. 60<), BSA (≤1.5 vs. 1.5<) and pretreatment ANC (≤3,000/mm^3 vs. 3,000/mm^3<) (95% confidence interval)

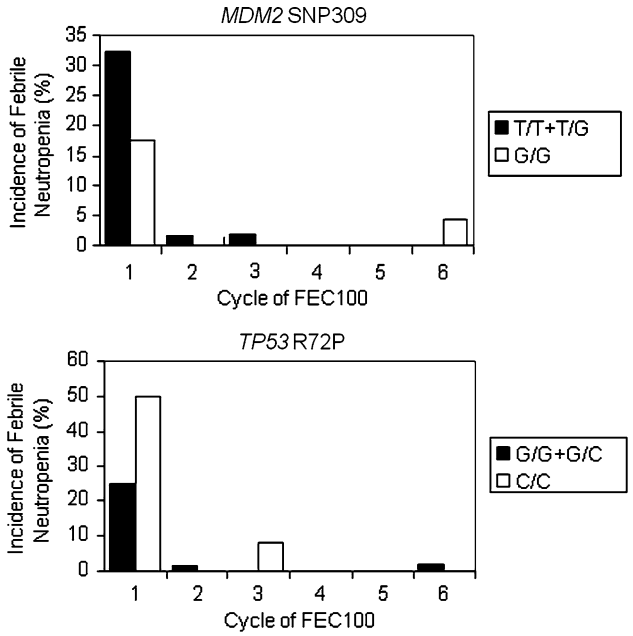

Fig. 1 Incidence of FN according to the cycle of FEC100 *MDM2* SNP309: Among the patients treated with FEC100, 22 (35.5%) of 62 patients with T/T + T/G genotypes^{MDM2} and 5 (21.7%) of 23 patients with G/G genotype^{MDM2} developed febrile neutropenia (FN). The *graph* shows distribution of the patients with FN according to the treatment cycle where FN first occurred. Frequency (*vertical axis*) means number of patients with FN as a percentage of the total number of patients in each genotype group (G/G genotype^{MDM2} or T/T + T/G genotypes^{MDM2}). *TP53* R72P**:** Among the patients treated with FEC100, 20 (27.3%) of 73 patients with G/G + G/C genotypes^{TP53} and 7 (58.3%) of 12 patients with C/C genotype^{TP53} developed FN. The *graph* shows distribution of the patients with FN according to the treatment cycle where FN first occurred. Frequency (*vertical axis*) means number of patients with FN as a percentage of the total number of patients in each genotype group (C/C genotypes^{TP53} or G/G + G/C genotypes^{TP53})

15.8%, respectively). Interestingly, the combination of G/G genotype^{MDM2} and C/C genotype^{TP53} and of T/T + T/G genotype^{MDM2} and G/G + G/C genotype^{TP53} were associated with an intermediate incidence of severe neutropenia and FN (31.3 and 32.3%, respectively).

Discussion

It has been reported that G/G genotype^{MDM2} is associated with an elevated expression of MDM2, which results in the down-regulation of TOPO2A, thus rendering tumor cells with G/G genotype^{MDM2} resistant to chemotherapeutic agents against TOPO2A [6]. Moreover, the elevated expression of MDM2 down-regulates TP53, which leads to chemoresistance because of the inhibition of the *TP53*-induced apoptotic pathway. Since bone marrow hematopoietic cells are thought to be similarly affected by *MDM2* SNP309, it is speculated that G/G genotype^{MDM2} would show resistance to anthracycline-based regimens such as FEC in terms of bone marrow suppression. In the study presented in this article, we could in fact demonstrate that G/G genotype^{MDM2} treated with FEC100 showed a significantly ($P < 0.01$) lower incidence (5.3%) of severe neutropnenia than did T/T + T/G genotypes^{MDM2} (39.2%). Although the difference was statistically not significant, the incidence of FN was also lower for G/G genotypes^{MDM2} (21.7%) than for T/T + T/G genotypes^{MDM2} (35.5%). However, such associations were not detected for patients treated with FEC60 or FEC75, probably because the incidence of severe neutropenia and FN was much lower in those patients, so that the impact of *MDM2* SNP309 on neutropenia, if any, is probably not detectable.

Since the apoptotic function of tumor cells with C/C genotype^{TP53} has been found to have diminished in association with an increased resistance to DNA-damaging agents in vitro [11, 12], it was expected that C/C genotype^{TP53} would show a lower incidence of severe neutropenia and FN. Unexpectedly, however, we obtained the opposite results. C/C genotype^{TP53} showed a significantly ($P = 0.03$) higher incidence (58.3 vs. 27.3%) of FN and a tendency ($P = 0.07$) toward an increase in the incidence (55.6 vs. 26.2%) of severe neutropenia than G/G + G/C genotypes^{TP53} when treated with FEC100. Recently, Khrunin et al. have reported a significant association of severe neutropenia with C/C genotype^{TP53} in ovarian cancer patients treated with cisplatin-based chemotherapy [18].

Table 5 Association of the combination of *MDM2* SNP309 and *TP53* R72P with neutropenia in patients treated with FEC100

Genotypes		No. of patients	Incidence (%)	OR (95%CI)[a]	*P*-value
MDM2 SNP309	*TP53* R72P				
G4 neutropenia					
G/G	G/G + G/C	16	75.0	1	
G/G	C/C	48[b]	87.5	2.33 (0.56–9.61)	0.25
T/T + T/G	G/G + G/C				
T/T + T/G	C/C	6	100.0	–	0.54
Severe neutropenia					
G/G	G/G + G/C	16	6.3	1	
G/G	C/C	48[b]	31.3	6.81 (0.82–56.48)	0.05
T/T + T/G	G/G + G/C				
T/T + T/G	C/C	6	83.3	75.00 (3.92–1434)	0.01 >
Febrile neutropenia					
G/G	G/G + G/C	19	15.8	1	
G/G	C/C	58[b]	32.3	2.59 (0.67–10.00)	0.15
T/T + T/G	G/G + G/C				
T/T + T/G	C/C	8	62.5	8.84 (1.34–58.82)	0.02

[a] Odds ratio (95% confidence interval)

[b] Patients with G/G genotype^{MDM2} and C/C genotype^{TP53} and those with T/T + T/G genotype^{MDM2} and G/G + G/C genotype^{TP53} are combined

These results put together suggest that C/C genotype^{TP53} is more sensitive to FEC100 than G/G + G/C genotypes^{TP53}.

The association between the *TP53* mutation and response to an anthracycline-based regimen is not straightforward. Although early studies reported on an association between the *TP53* mutation and resistance to an anthracycline-based regimen in various types of tumors in vitro [19] and several clinical studies also reported comparable findings [20–22], the opposite finding, namely that *TP53* mutation was associated with a better response to an anthracycline-based regimen was also reported both in vitro [23] and clinically [24]. The reason for these discrepant effects of *TP53* mutation on response to an anthracycline-based regimen is currently unknown. In tumor cells with *TP53* mutation, the apoptosis inducing function is impaired but so is the DNA repair function. It might thus be possible that tumor cells with DNA damage escape apoptosis and proceed to the G2/M phase where they meet with catastrophic cell death, resulting in a higher sensitivity to chemotherapy. Since the *TP53* function of bone marrow hematopoietic cells with C/C genotype^{TP53} is thought to be diminished, such cells may also show a high sensitivity to FEC through a mechanism analogous to the one described above. However, the precise mechanism through which C/C genotype^{TP53} acquires a high sensitivity to FEC in terms of bone marrow suppression will need to be investigated in a future study.

Since age, BSA, and pretreatment ANC are well-established risk factors for neutropenia, we examined the association of *MDM2* SNP309 and *TP53* R72P with neutropenia after adjustment for these risk factors (Table 4), but still the associations between *MDM2* SNP309 and severe neutropenia ($P = 0.02$) and between *TP53* R72P and FN ($P = 0.03$) remained significant. The combination of these two genetic polymorphisms was also investigated. For *MDM2* SNP309, T/T + T/G genotype^{MDM2} was found to be a high-risk genotype and G/G genotype^{MDM2} a low-risk genotype for neutropenia. For *TP53* R72P, C/C genotype^{TP53} was found to be a high-risk genotype and G/G + G/C genotype^{TP53} a low-risk genotype. In treatment with FEC100, we found that the combination of high-risk genotype^{MDM2} and high-risk genotype^{TP53} was associated with the highest incidence of severe neutropenia and FN and that of low-risk genotype^{MDM2} and low-risk genotype^{TP53} with the lowest incidence. Interestingly, the combination of high-risk genotype^{MDM2} and low-risk genotype^{TP53} and of low-risk genotype^{MDM2} and high-risk genotype^{TP53} was associated with an intermediate incidence of severe neutropenia and FN, suggesting that the combination of these two SNPs has an additive effect on their capability to predict severe neutropenia and FN.

In conclusion, we were able to show that the presence of *MDM2* SNP309 and *TP53* R72P is significantly associated

with, respectively, the frequency of severe neutropenia and FN in breast cancer patients treated with FEC100 but not with FEC60 or FEC75. These SNPs, especially in their combination, may be useful as predictors of risk for developing severe neutropenia and FN in patients treated with FEC100. However, our observations presented in this article need to be validated in a future study covering a larger number of patients.

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