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Multiple Genetic Polymorphisms of *GSTP1* 313AG, *MDR1* 3435CC, and *MTHFR* 677CC Highly Correlated with Early Relapse of Breast Cancer Patients in Taiwan

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Background: Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to inter-individual differences in the efficacy and toxicity of many medications. In the present study, multiple chemotherapeutic agent-related genetic polymorphisms, including *GSTP1, MDR1, MTHFR*, and *TS* tandem repeats, were analyzed in breast cancer patients and studied in correlation with the clinical outcome of patients receiving FEC adjuvant chemotherapy.

Methods: The genotypes from 192 stage II and III breast cancer patients who underwent operations and received six cycles of postoperative adjuvant chemotherapy (FEC) were determined by means of PCR-RFLP. The association of each genetic polymorphism with clinicopathological data of patients and early relapse status were analyzed.

Results: The results showed that the genotype distribution of *GSTP1 A313G*, *MTHFR C677T*, and *TS 3R3R* in Taiwanese subjects differed significantly from the distribution in Caucasians. After analysis of the relationship between the genotypes and clinicopathological data of the patients, a significant correlation was observed between postoperative early relapse in patients with genetic polymorphisms of both *MDR1 3435CC* and *MTHFR 677CC* (crude OR: 2.609, $P = .013$) and patients with additional *GSTP1 313AG* genetic polymorphism (crude OR: 2.833, $P = .017$).

Conclusions: The results of the present study highly suggest that *GSTP1*, *MDR1*, and *MTHFR* genotypes could be prognostic factors for Taiwanese patients with breast cancer.

Key Words: Breast cancer—Early relapse—Multiple genetic polymorphisms—*GSTP1*—*MDR1*—*MTHFR*.

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Adjuvant chemotherapy is defined as the administration of cytotoxic chemotherapy after primary surgery of cancer to kill or inhibit clinically occult micrometastases. Adjuvant chemotherapy imparted a

statistically significant reduction in the risk of breast cancer relapse and death at 5 years of follow-up (with a hazard reduction of approximately 25%), and combination chemotherapy was found to be significantly more effective than single-agent therapy.[1] Trials included more than 15 years of follow-up and led to the conclusion that adjuvant chemotherapy conferred benefit to both premenopausal and postmenopausal patients and also to node-positive and node-negative patients.[2] In general, approximately one of every four recurrences and one of seven deaths is avoided annually by adjuvant chemotherapy.[3]

Anthracycline-based chemotherapy (adriamycin or its analogue epirubicin) in combination with 5-fluorouracil (5-FU) and cyclophosphamide (FAC/FEC) comprises one of the standard adjuvant chemotherapeutic regimens for advanced breast cancer. The meta-analysis that evaluated a total of 11 trials at random between anthracycline- and nonanthracycline-containing adjuvant polychemotherapy revealed that anthracycline-containing regimens were modestly superior in reducing recurrence and death.[3] Epirubicin is one of the FEC combination chemotherapy regimens that is as active as doxorubicin and is significantly less myelotoxic and cardiotoxic.[4,5]

Significant variability in drug response may occur among cancer patients treated with the same medications.[6] Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to inter-individual differences in the efficacy and toxicity of many medications. Recently, pharmacogenomic studies have elucidated the inherited nature of these differences in drug disposition and effects, thereby providing a stronger scientific basis for optimizing drug therapy according to each patient's genetic constitution.[7]

In this study, we selected four candidate genes, including Glutathione S-transferase P1 (GSTP1), multidrug resistance 1 (MDR1), 5,10-methylenetetrahydrofolate reductase (MTHFR), and thymidylate synthetase (TS), involved in the pathways of FEC adjuvant chemotherapeutics drug transport, metabolism, and targeting from the previously published literature[8–11] as target genes in Taiwanese patients with breast cancer. Peripheral blood samples and clinicopathological data of 192 breast cancer patients were collected. These four genetic polymorphisms were analyzed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and confirmed by DNA sequencing. We reviewed the literature regarding the distribution of these four candidate genes in breast cancer patients among different ethnic groups and compared the differences between breast cancer patients of Taiwanese ethnicity and other races. Furthermore, the correlation between the single nucleotide polymorphisms of four candidate genes and clinicopathological features of 192 Taiwanese breast cancer patients were analyzed. In addition, the relationship between genetic variants and post-therapeutic early relapse were also evaluated. We believe this achievement could make a significant contribution to the improvement of breast cancer treatment.

PATIENTS AND METHODS

Sample Collection for Clinical Experiments

This prospective pilot study was conducted in the Kaohsiung Medical University Hospital from January 2003 to October 2005, including 192 consecutive stage II and III breast cancer patients (median age, 48.68 ± 9.64 years), who were receiving six cycles of postoperative adjuvant chemotherapy (FEC) for one day every three weeks. Written informed consent was obtained from all subjects and/or guardians for the use of their blood samples. Sample acquisition and subsequent use was also approved by the institutional review board of the Kaohsiung Medical University Hospital. 5-FU and cyclophosphamide were intravenously administered in doses of 500 mg/m^2 each, and epirubicin was administered intravenously in doses of 90 mg/m^2. There were 125 patients with estrogen receptor (ER) positive who received hormone treatment while they completed the chemotherapy regimen. Early relapse was designated as the development of new recurrent or distant metastatic lesions within 2 years after receiving postoperative chemotherapy. Constitutional genetic polymorphisms were analyzed by DNA extraction from 4 mL of peripheral blood using a PUREGENE DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN). Regular follow-up was performed according to our hospital's protocol, which includes monthly physical examinations, tumor-marker examinations in 3-month intervals, bilateral mammography, and breast and abdominal ultrasounds in 6-month intervals, as well as annual chest imaging and bone scans. The median follow-up occurred 26 months (range, 14–36 months) after the completion of chemotherapy. Clinical stage and pathological features of primary tumors were defined according to the criteria of the American Joint

TABLE 1. *Correlation between the clinicopathological features and early relapse status for 192 patients who received adjuvant FEC chemotherapy*

Characteristics	Total No. of patients (%)	No. of early relapse (%)	No. of non-early relapse (%)	P*
Age (years)				
< 50	112	19 (17.0)	93 (83.0)	.118
≥50	80	21 (26.3)	59 (73.7)	
Tumor site				
Unilateral	189	40 (21.2)	149 (78.8)	.370
Bilateral	3	0 (0)	3 (100)	
UICC stage				
II	160	25 (15.6)	135 (84.4)	< .0001
III	32	15 (46.9)	17 (53.1)	
ER				
Positive	125	21 (16.8)	104 (83.2)	.060
Negative	67	19 (28.4)	48 (71.6)	
PR				
Positive	120	20 (16.7)	100 (83.3)	
Negative	72	20 (27.8)	52 (72.2)	.066
HER2				
Positive	124	29 (23.4)	95 (76.6)	
Negative	68	11 (16.2)	57(83.8)	0.239

*Results of two-sided Pearson χ^2 test.

Commission on Cancer/International Union Against Cancer (AJCC/UICC).[12] Patient characteristics are depicted in Table 1.

Genotyping Analysis

All genomic DNA from the patients was analyzed using the PCR-RFLP technique for the determination of genotypes of *GSTP1*, *MDR1*, and *MTHFR* and the PCR technique for determination of *TS*. After digestion with appropriate corresponding restriction enzymes, PCR fragments were separated on a 2.5-3.0% agarose gel and visualized after staining with ethidium bromide. The results of PCR-RFLP were validated by the automated sequencing method. All primers used in this study were designed by using Primer 3 free software (http://www.web.umassmed.edu/bioapps/primer3_www.cgi). Table 2 shows the primer sequences and restriction enzymes in the study. The PCR reaction volume was 40 μL, and the PCR conditions for these polymorphisms are described as follows: for *GSTP1*, 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, annealing at 62°C for 10 seconds, and 72°C for 25 seconds; for *MDR1*, 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, annealing at 58°C for 15 seconds, and 72°C for 20 seconds; for *MTHFR*, 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, annealing at 70°C for 20 seconds, and 72°C for 25 seconds; for *TS*, 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, and 72°C for 35 seconds. *GSTP1 exon5 A313G*, *MDR1 C3435T*, and *MDR1 C3435T* were restricted by BsmAI, DpnII, and HinfI, respectively.

Statistical Analysis

All data were analyzed using Statistical Package for the Social Sciences Version 12.0 software (SPSS Inc., Chicago, IL). All genotypes were tested as to whether they were distributed according to the Hardy-Weinberg equilibrium. The Hardy-Weinberg equilibrium assumption was assessed by the standard method of matching the observed numbers of individuals in the different genotype categories with those expected under Hardy-Weinberg equilibrium for the estimated allele frequency. A χ^2 test for deviation from Hardy-Weinberg equilibrium was used to estimate differences in allele frequencies. The association of each polymorphism with early relapse status was analyzed using the relative risk ratio (RR), and a 95% confidence interval (CI) was calculated. To clarify the clinical significance of these genotypes combined as the predictor of early relapse, the crude odds ratio and a 95% CI were calculated using the risk estimate method. Furthermore, the two-sided Pearson χ^2 test was used to analyze the differences in the distribution of the genotypes between different races and to analyze the correlation between early relapse and clinicopathological data. A *P* value of less than .05 was considered statistically significant.

TABLE 2. *Characteristics of the studied polymorphisms with primer sequences and restriction enzymes*

Gene polymorphism	Primers	PCR size	PCR condition	Restriction enzyme
GSTP1Ile105Val (A313G)	[F]:5′-GTAGTTTGCCCAAGGTCAAG-3′ [R]:5′-AGCCACCTGAGGGGTAAG-3′	433	Annealing: 62°C, 20 sec	BsmAI
MDR1Ile1145Ile (C3435T)	[F]:5′-gatctgtgaactcttgttttc-3′ [R]:5′-GAAGAGAGACTTACATTAGGC-3′	244	Annealing: 58°C, 20 sec	DpnII
MTHFRAla222Val (C677T)	[F]:5′-TGGCAGGTTACCCCAAAGGC-3′ [R]:5′-ACTGTTGCTGGGTTTTGGGG-3′	400	Annealing: 70°C, 20 sec	HinfI
TS 28 bp tandem repeat	[F]:5′-GTGGCTCCTGCGTTTCCCCC-3′ [R]:5′-TCCGAGCCGGCCACAGGCAT-3′	240	Annealing: 72°C, 15 sec	–

RESULTS

Characteristics of Patients

There were 192 female patients recorded. Of these patients, 112 cases were younger than 50 years old and 80 cases were older than 50 years old, ranging in age from 23 to 77 years, with a median age of 48.68 years. Early relapse, whether local recurrence or distant metastasis, developed in 40 cases during the follow-up period. Primary tumor location included 189 unilateral cases and three bilateral cases. One hundred and sixty cases were UICC stage II, 32 cases were UICC stage III, and the other clinical data, such as ER, progesterone receptor (PR), and HER2 status, are shown in Table 1.

Correlation between Early Relapse and Clinicopathological Data

There were no statistical correlations between early relapse status and age, tumor location, ER status, PR status, or HER2 status ($P > .05$; Table 1). However, cancer stage was significantly related to tumor early relapse ($P < .0001$), the risk of which was higher in stage III than in stage II (RR, 4.76; $P < .0001$).

PCR-RFLP Analysis Results of Chemotherapeutic Agent-Related Genetic Polymorphisms

In our study, chemotherapeutic agent-related genetic polymorphisms are *GSTP1 A313G*, *MDR1 C3435T*, *MTHFR C667T*, and *TS* double or triple tandem repeat. The results of PCR-RFLP analysis of *GSTP1*, *MDR1*, and *MTHFR* single nucleotide polymorphisms, and *TS* tandem repeat are depicted in Fig. 1–3. The distribution of genotypes in our patients is shown in Table 3. The frequencies of genotypes *GSTP1 AA*, *AG*, and GG were 64.2%, 33.9%, and 1.9%, respectively. The frequencies of genotypes *MDR1 CC*, *CT*, and *TT* were 41.1%, 41.1%, and 17.8%, respectively. The frequencies of genotypes *MTHFR* CC, *CT*, and *TT* were 58.3%, 34.9%, and 6.8%, respectively. The frequencies of *TS* tandem repeat genotype distribution were 67.2% in *3R3R*, 31.8% in *3R2R*, and 1% in *2R2R*. As shown in Fig. 1–3, we confirm the above results of PCR-RFLP by DNA sequencing. The sequencing results were all comparable to the results of PCR-RFLP.

Comparison of Genotypes Distribution between Taiwanese Breast Cancer Patients and Other Races

We compared the distribution of genetic polymorphisms, such as *GSTP1 A313G (AA, AG, GG)*, *MDR1 C3435T (CC, CT, TT)*, *MTHFR C677T (CC, CT, TT)*, and *TS* tandem repeat *(3R3R, 2R3R, 2R2R)*, in various races, including Caucasian, Korean, African American, Chinese, Japanese American, and Taiwanese subjects from our work and literature reviews. The results are shown in Table 3. The distribution of *GSTP1 A313G* genotypes in our data show significant differences from that of Caucasian ($P = .001$) and African American populations ($P = .001$)[13] but no significant differences from Chinese ($P = .410$)[14] and Korean populations ($P = .242$).[15] The distribution of *MDR1 C3435T* genotypes also revealed significant differences from the Caucasian population ($P = .003$).[16] Significantly different distributions of *MTHFR C677T (CC, CT, TT)* genotypes are evident when comparing our data with Caucasian ($P = .001$),[17] Chinese ($P < .0001$),[18] Korean ($P < .0001$),[19] African American ($P < .0001$),[20] and Japanese American populations ($P < .0001$),[21] but our study is consistent with the other Taiwanese report ($P = .150$).[22] Our study and the another Taiwanese study[22] both showed that *MTHFR C677T CC* appeared more frequently than *CT* and *TT*, while another racial study showed that *MTHFR C677T CT* was the most frequent genetic polymorphism. The highest expression of *TS 3R3R* in our study was similar to that of the Chinese ($P = .042$)[23] but different from that of the Caucasian population ($P < .0001$).[24] Caucasian breast cancer

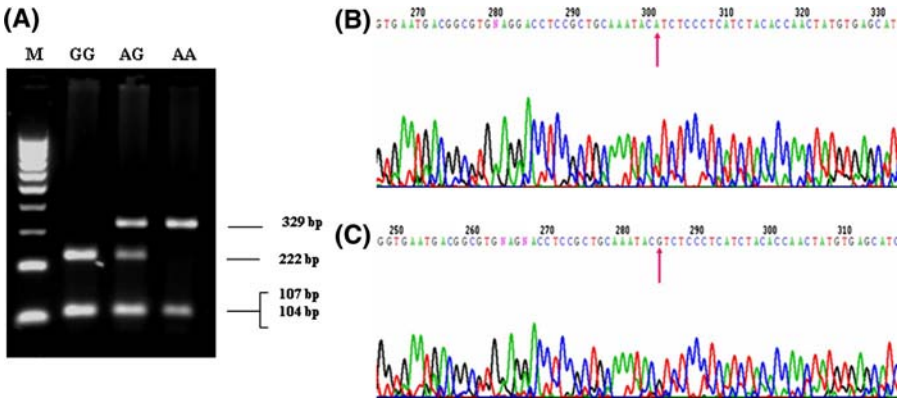

FIG. 1. Results of PCR-RFLP analysis of *GSTP1* single-nucleotide polymorphisms. *GSTP1 exon5 A313G* was restricted by BsmAI and resulted in: 329bp and 104bp *GSTP1 313AA* fragments; 329bp, 222bp, 107bp, and 104bp *GSTP1 313AG* fragments; and 222bp, 104bp, and 107bp *GSTP1 313GG* fragments. (**A**) Presentation of the results of RFLP of *GSTP1*. (**B**) Sequences of *GSTP1 313AA*. (**C**) Sequences of *GSTP1 313GG*.

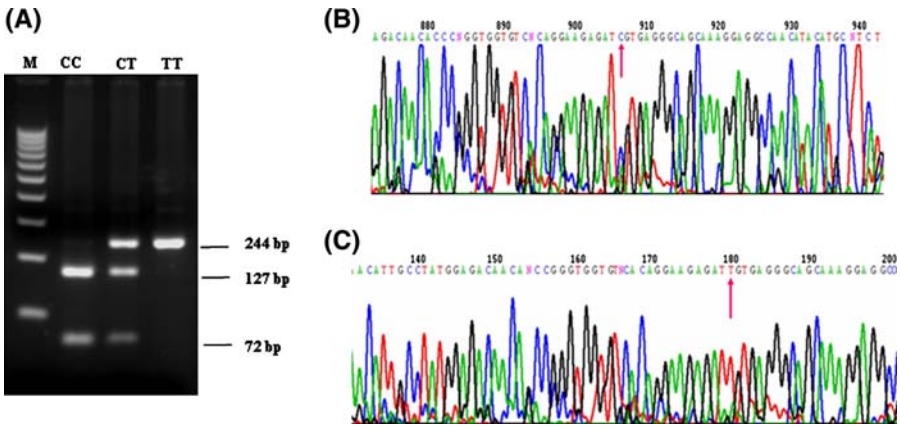

FIG. 2. Results of PCR-RFLP analysis of *MDR1* single nucleotide polymorphisms. *MDR1 C3435T* was restricted by DpnII and resulted in: 127bp and 72bp *MDR1 3435CC* fragments; 244bp, 127bp, and 72bp *MDR1 3435CT* fragments; and 244bp *MDR1 3435TT* fragment. (**A**) Presentation of the results of RFLP of *MDR1*. (**B**) Picture of sequences of *MDR1 3435CC*. (**C**) Picture of sequences of *MDR1 3435TT*.

patients presented *TS 2R3R* as the most frequent genetic polymorphism.

Correlation between Genotypes of Chemotherapeutic Agent-Related Genes with Clinicopathological Data

We analyzed the correlation between genetic polymorphisms and clinicopathological features of 192 breast cancer patients, as shown in Table 4. There was no statistically significant correlation between genotype distributions and age, tumor site, cancer stage, or HER2 status. The distribution frequency of the *GSTP1 AA* genotype was higher in PR positive than the *GSTP1 AG* or *GG* genotypes were ($P < .001$), and *MTHFR CT* or *TT* was higher in ER positive than the *MTHFR CC* genotype was ($P = .034$).

Correlation between Early Relapse and Genotypes of Chemotherapeutic Agent-Related Genes

The correlation between genetic polymorphisms (*MTHFR C677T*, *MDR1 C3435T*, *GSTP1 A313G*, and *TS* double or triple tandem repeat) and patients with or without early relapse was analyzed. Univariate statistical analysis showed that genetic polymorphisms of *GSTP1*, *MDR1*, and *MTHFR* have a statistically significant correlation between early relapse and non-early relapse patients ($P = .014$, .024, and .020, respectively). However, *TS* genetic polymorphisms have no statistical significance ($P > .05$), as shown in Table 5. In the univariate analysis, cancer stage was the clinical variable significantly associated with early relapse (Table 1). Therefore, we performed the risk estimate analysis of combined-risk genetic polymorphisms and early relapse status. Within 40 patients who had combined *MDR1 3435CC* and *MTHFR 677CC* gene polymorphisms, 14 of them (35%) had early relapse. On the other hand, there were 26 early relapse patients (17.1%) within 152 cases with other genotypes. The statistical results showed that breast cancer patients with *MDR1 3435CC* and *MTHFR 677CC* simultaneously have a risk of early relapse that is 2.609 times greater than that of other genotypes ($P = .013$; crude OR: 2.609; 95% CI: 1.202 - 5.663). However, the combi-

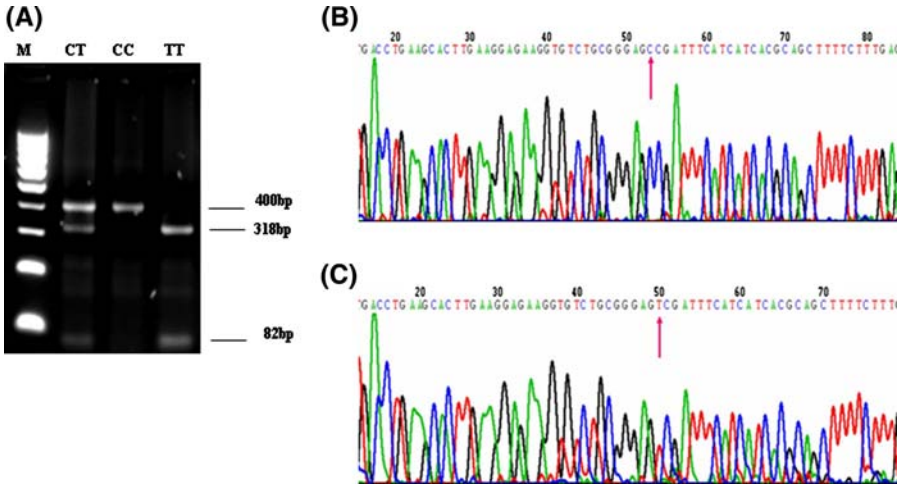

FIG. 3. Results of PCR-RFLP analysis of *MTHFR* single nucleotide polymorphisms. *MTHFR C677T* was restricted by HinfI and resulted in the following: 400bp *MTHFR 677CC* fragment; 400-bp, 318bp, and 82bp *MTHFR 677CT* fragments; and 318bp, 82bp *MTHFR677TT* fragments. (**A**) Presentation of the results of RFLP of *MTHFR*. (**B**) Sequences of *MTHFR 677CC*. (**C**) Sequences of *MTHFR 677TT*.

TABLE 3. *Comparison of distribution of genotypes between Taiwanese breast cancer patients and other races*[a]

Genotype	Caucasian No. (%)	Korean No. (%)	African American No. (%)	Chinese No. (%)	Taiwanese No. (%)	Japanese American No. (%)	Our data No. (%)
GSTP1 A313G	Sweeney[13]	Kim[15]	Sweeney[13]	Egan[14]			
AA	93 (48.4)	122 (71.3)	17 (35)	723 (63.5)	–	–	122 (64.2)
AG	80 (41.7)	44 (25.7)	27 (56)	363 (31.9)	–	–	65 (33.9)
GG	19 (9.9)	5 (2.9)	4 (8)	53 (4.6)	–	–	5 (1.9)
MDR1 C3435T	Kafka[16]						
CC	21 (21)	–	–	–	–	–	79 (41.1)
CT	57 (57)	–	–	–	–	–	79 (41.1)
TT	22 (22)	–	–	–	–	–	34 (17.8)
MTHFR C677T	Campbell[17]	Lee[19]	Martin[20]	Shrubsole[18]	Chou[22]	Le Marchand[21]	
CC	140 (41.8)	58 (31.2)	114 (81)	355 (34.2)	73 (51.4)	135 (42.5)	112 (58.3)
CT	162 (48.4)	96 (51.6)	27 (19)	507 (48.8)	51 (35.9)	140 (44.0)	67 (34.9)
TT	33 (9.8)	32 (17.2)	0 (0)	176 (17.0)	18 (12.7)	43 (13.5)	13 (6.8)
TS tandem repeat	Largillier[24]			Zhai[23]			
3R3R	27 (32.7)	–	–	279 (64.6)	–	–	129 (67.2)
2R3R	43 (50)	–	–	130 (30.1)	–	–	61 (31.8)
2R2R	13 (17.3)	–	–	23 (5.3)	–	–	2 (1)

[a] author[n] in this table are presented with reference number ([n]) and the abbreviated name of the first author in the previously reported paper.

nation of *GSTP1 313AG* with either *MDR1 3435CC* or *MTHFR 677CC* genotypes has no significant role in the prediction of early relapse. In addition, there were 10 early relapse patients (38.5%) within 26 cases who had *GSTP1 313AG*, *MDR1 3435CC*, and *MTHFR 677CC* gene polymorphisms simultaneously. On the other hand, 30 patients with early relapse (22.1%) were noted within 136 cases with other genotypes. Based on the results, adding *GSTP1 313AG* to *MDR1 3435CC* and *MTHFR 677CC* has a risk of early relapse that is 2.833 times greater than that of other genotypes ($P = .017$; crude OR: 2.833; 95% CI: 1.171– 6.855).

DISCUSSION

To date, most studies regarding prognosis of breast cancer have focused on tumor characteristics.[25,26] There is accumulated evidence suggesting that the genetic polymorphisms that are involved in drug-metabolizing enzymes, transporters, receptors, and drug targets have been linked to inter-individual differences in the efficacy and toxicity of many medications.[7] Pharmacogenetic analyses appear to be a promising tool in the development of personalized treatment plans. Recently, there is evidence that pharmacogenomic-related articles regarding the cor-

TABLE 4. *Correlation between gene polymorphism and clinicopathological features of 192 breast cancer patients*

	GSTP1			*MDR1*			*MTHFR*			*TS*		
Characteristics	*AA*	*AG or GG*	*P**	*CC*	*CT or TT*	*P**	*CC*	*CT or TT*	*P**	*3R3R*	*2R2R or 3R2R*	*P**
Age (years)												
< 50	74	38	.389	45	67	.747	67	45	.621	71	41	.185
≥50	48	32		34	46		45	35		58	22	
Tumor site												
Unilateral	119	70	.186	79	110	.144	111	78	.376	126	63	.222
Bilateral	3	0		0	3		1	2		3	0	
UICC stage												
II	101	59	.789	66	94	.948	95	65	.513	110	50	.303
III	21	11		13	19		17	15		19	13	
ER												
Positive	81	44	.621	53	72	.630	66	59	.034	84	41	.996
Negative	41	26		26	41		46	21		45	22	
PR												
Positive	88	32	< .001	51	69	.623	68	52	.454	80	40	.843
Negative	34	38		28	44		44	28		49	23	
HER2												
Positive	75	49	.219	56	68	.127	75	49	.414	79	45	.166
Negative	47	21		23	45		37	31		50	18	

* Results of two-sided Pearson χ^2 test.

TABLE 5. *Distribution of studied gene polymorphisms in 192 breast cancer patients in regard to the status of early relapse after FEC adjuvant chemotherapy*

Genotype	No. of patients (%)	No. of early relapse (%)	No. of non-early relapse (%)	*P**
GSTP1				
AA	122	19 (15.6)	103 (84.4)	.014
AG	65	21 (32.3)	44 (67.7)	
GG	5	0 (0)	5 (100)	
MDR1				
CC	79	24 (30.4)	55 (69.6)	.024
CT	79	11 (13.9)	68 (86.1)	
TT	34	5 (14.7)	29 (85.3)	
MTHFR				
CC	112	31 (27.7)	81 (72.3)	.020
CT	67	7 (10.4)	60 (89.6)	
TT	13	2 (15.4)	11 (84.6)	
TS				
3R3R	129	29 (22.5)	100 (77.5)	.598
2R3R	61	11 (18)	50 (82)	
2R2R	2	0 (0)	2 (100)	

* Results of two-sided Pearson χ^2 test.

relation between the prognosis or the survival and chemotherapeutic agent-related genetic polymorphisms have become more popular worldwide.[6,16,18] Nevertheless, information about Taiwanese breast cancer patients has remained rare. This study aimed to examine the feasibility of developing a multigene predictor of drug response to a complex chemotherapy regimen. We selected chemotherapeutic agent-related genes, including *GSTP1, MDR1, MTHFR*, and *TS*, as gene targets in Taiwanese breast cancer patients. The genotypes of *GSTP1* and *MDR1* are related to the drug transporter; the genotypes of *MTHFR* and *TS* are related to drug metabolism; and the genotype of *TS* is also related to the drug target.

In the current study, we found a significant correlation between early relapse status and the heterozygous *GSTP1 313AG* gene variant. In addition, Kafka et al. reported that a *MDR1 C3435T* gene polymorphism would predict a response to the neoadjuvant chemotherapy of anthracyclines (doxorubicine and epirubicin) combined with taxanes in locally advanced breast cancer.[16] The results of our study showed that *MDR1 3435CC* genetic polymorphisms had statistical significance in predicting early relapse

in breast cancer patients who had been treated with FEC chemotherapy. Meanwhile, Martin et al. reported that there is no statistically significant similarity between the survival rate of African American and Caucasian breast cancer patients in terms of the *MTHFR* at codon 677 (C/T or T/T) and C/C genotype.[20] In the present study, genetic polymorphism of *MTHFR 677CC* was statistically significant to breast cancer early relapse. While combining both *MDR1 3435CC* and *MTHFR 677CC* or adding the *GSTP1 313AG* genetic variant to our consideration, statistical results showed significant correlations to the prediction of early relapse.

After comparing the Taiwanese with other races with regard to genetic polymorphism, we found that the Taiwanese, Korean, and Chinese demonstrate a higher frequency of *GSTP1 A313G AA* than Caucasians and African Americans do. Taiwanese demonstrate a higher frequency of *MTHFR C677T CC* than other races do. Caucasians demonstrate a higher frequency of *MDR1 C3435T CT* and *TS* tandem repeat *2R* than the Taiwanese do. The differences in genetic polymorphisms may explain the variety of chemotherapeutic agent-related responses that occur worldwide.

In summary, the present pharmacogenetic research of combining *GSTP1*, *MDR1*, and *MTHFR* genotypes may be helpful in establishing powerful prognostic and predictive factors of FEC adjuvant chemotherapy, which subsequently may allow a better customization therapy for breast cancer patients. Meanwhile, a long-term follow-up of breast cancer patients in large population-based studies may be a prerequisite for further validation of the actual roles of these genetic polymorphisms.

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REFERENCES

1. Combination adjuvant chemotherapy for node-positive breast cancer Inadequacy of a single perioperative cycle. The Ludwig Breast Cancer Study Group. *N Engl J Med* 1988; 319:677–83.
2. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Early Breast Cancer Trialists' Collaborative Group. *Lancet* 1992;339:1–15.
3. Polychemotherapy for early breast cancer: An overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998;352:930–42.
4. Keiling R, Armand PP, Hurteloup P, et al. [French FAC vs FEC study in advanced breast cancer]. *Onkologie* 1986; 9:8–10.
5. Lopez M, Papaldo P, Di Lauro L, et al. 5-Fluorouracil, adriamycin, cyclophosphamide (FAC) vs. 5-fluorouracil, epirubicin, cyclophosphamide (FEC) in metastatic breast cancer. *Oncology* 1989; 46:1–5.
6. Choi JY, Nowell SA, Blanco JG, et al. The role of genetic variability in drug metabolism pathways in breast cancer prognosis. *Pharmacogenomics* 2006; 7:613–24.
7. Evans WE, Relling MV. Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science* 1999; 286:487–91.
8. Zhong S, Huang M, Yang X, et al. Relationship of glutathione S-transferase genotypes with side-effects of pulsed cyclophosphamide therapy in patients with systemic lupus erythematosus. *Br J Clin Pharmacol* 2006; 62:457–72.
9. Ambudkar SV, Dey S, Hrycyna CA, et al. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Ann Rev Pharmacol Toxicol* 1999; 39:361–98.
10. Weisberg IS, Jacques PF, Selhub J, et al. The 1298A–>C polymorphism in methylenetetrahydrofolate reductase (MTHFR): In vitro expression and association with homocysteine. *Atherosclerosis* 2001; 156:409–15.
11. Iacopetta B, Grieu F, Joseph D, Elsaleh H. A polymorphism in the enhancer region of the thymidylate synthase promoter influences the survival of colorectal cancer patients treated with 5-fluorouracil. *Br J Cancer* 2001; 85:827–30.
12. International Union Against Cancer. TNM *Classification of Malignant Tumors*. 6th ed: New York: Wiley-Liss, Inc., 2002.
13. Sweeney C, McClure GY, Fares MY, et al. Association between survival after treatment for breast cancer and glutathione S-transferase P1 Ile105Val polymorphism. *Cancer Res* 2000; 60:5621–4.
14. Egan KM, Cai Q, Shu XO, et al. Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: Results from the Shanghai Breast Cancer Study and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2004; 13:197–204.
15. Kim SU, Lee KM, Park SK, et al. Genetic polymorphism of glutathione S-transferase P1 and breast cancer risk. *J Biochem Mol Biol* 2004; 37:582–5.
16. Kafka A, Sauer G, Jaeger C, et al. Polymorphism C3435T of the MDR-1 gene predicts response to preoperative chemotherapy in locally advanced breast cancer. *Int J Oncol* 2003; 22:1117–21.
17. Campbell IG, Baxter SW, Eccles DM, et al. Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. *Breast Cancer Res* 2002; 4:R14.
18. Shrubsole MJ, Shu XO, Ruan ZX, et al. MTHFR genotypes and breast cancer survival after surgery and chemotherapy: A report from the Shanghai Breast Cancer Study. *Breast Cancer Res Treat* 2005; 91:73–9.
19. Lee SA, Kang D, Nishio H, et al. Methylenetetrahydrofolate reductase polymorphism, diet, and breast cancer in Korean women. *Exp Mol Med* 2004; 36:116–21.
20. Martin DN, Boersma BJ, Howe TM, et al. Association of MTHFR gene polymorphisms with breast cancer survival. *BMC Cancer* 2006; 6:257.
21. Le Marchand L, Haiman CA, Wilkens LR, et al. MTHFR polymorphisms, diet, HRT, and breast cancer risk: The multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2004; 13:2071–7.
22. Chou YC, Wu MH, Yu JC, et al. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: A case-control study in Taiwan. *Carcinogenesis* 2006; 27:2295–300.

23. Zhai X, Gao J, Hu Z, et al. Polymorphisms in thymidylate synthase gene and susceptibility to breast cancer in a Chinese population: A case-control analysis. *BMC Cancer* 2006; 6:138.
24. Largillier R, Etienne-Grimaldi MC, Formento JL, et al. Pharmacogenetics of capecitabine in advanced breast cancer patients. *Clin Cancer Res* 2006; 12:5496–502.
25. Neri A, Marrelli D, Rossi S, et al. Breast cancer local recurrence: Risk factors and prognostic relevance of early time to recurrence. *World J Surg* 2007; 31:36–45.
26. Livi L, Paiar F, Saieva C, et al. Survival and breast relapse in 3834 patients with T1-T2 breast cancer after conserving surgery and adjuvant treatment. *Radiother Oncol* 2007; 82:287–93.