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International Journal of Cancer Research and Treatment

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ANTICANCER RESEARCH *32*: xxx-xxx (2012)

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Evaluation of Breast Cancer Susceptibility Loci on 2q35, 3p24, 17q23 and *FGFR2* Genes in Taiwanese Women with Breast Cancer

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Abstract. *Aim: Breast cancer is the most common line of cancer in women. In recent years, mounting evidence has identified the possibility that 2q35, 3p24, 17q23 and fibroblast growth factor receptor 2 (FGFR2) may be genetic susceptibility loci for breast cancer. This study aimed to evaluate the association of four polymorphic genotypes in these loci with breast cancer in Taiwanese women. Materials and Methods: Eighty-eight patients with breast cancer and 70 controls without breast cancer were selected. Polymorphic variants of 2q35-rs13387042, 3p24-rs4973768, 17q23-rs650490 and FGFR2-rs2981578 were analyzed to test for their association with breast cancer susceptibility. The 2q35, 17q23 and FGFR2 polymorphisms were detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) and the 3p24 polymorphism was detected using an amplification-created restriction site method. Results: The distribution of genotypes of 2q35 were significantly different between the breast cancer group and the control group (p=0.035), while the distributions for 3p24, 17q23, and FGFR2 were not significantly different (p>0.05). In addition, allele A of 2q35 conferred a higher risk for breast cancer risk than allele G (odds ratio, OR=2.95, 95% confidence interval, CI=1.29-6.71, p=0.008). Furthermore, the genotypic distribution of 2q35 was not significantly different among patients with different tumor stages, or from different specimen type. Conclusion: The 2q35 allele A may be a potential biomarker for breast cancer risk, but further confirmation is needed to determine its role in breast carcinogenesis. Blood samples can be used for determining the genotypes for 2q35-rs13387042 in patients for risk of breast cancer.*

Breast cancer is the most prevalent type of cancer in women, with an estimated 1,384,155 new cases in 2008 according to the global cancer statistics from the International Agency for Research on Cancer [IARC, Globocan http://globocan.iarc.fr/factsheet.asp]. The etiology of breast cancer is very complicated and in addition to estrogen exposure (1), scientists are keen to determine the extent to which genetic factors contribute to breast cancer. Some genes, such as breast cancer type 1 (*BRAC1*), *BRAC2*, Human Epidermal growth factor Receptor 2/receptor tyrosine-protein kinase erbB-2 (*HER-2/neu*), and *p53*, have been linked to breast cancer susceptibility and development, but they are not sufficient to explain breast cancer etiology completely (2-6).

In recent years, the genome-wide association studies (GWAS) have highlighted promising single nucleotide polymorphisms (SNPs) on specific loci for further investigation (7-13). For instance, variants on rs2981578 of the *FGFR2* gene on chromosome 10q, which encodes fibroblast growth factor receptor 2, were reported to be associated with breast cancer in GWAS of African American (13) and European populations (7, 8). In addition, the SNP on 2q35, rs13387042, was associated with an increased risk of breast tumors that were positive for estrogen receptor (ER) staining (14) in both ER-positive and ER-negative

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Key Words: Breast cancer, 2q35, 3p24, 17q23, *FGFR2*, Taiwanese.

Table I. *Primer pairs and general information for polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) and amplification-created restriction site (ACRS) for fibroblast growth factor receptor 2 (FGFR2), 2q35, 3q24, and 17q23.*

Locus and rs number	Primer sequences	Restriction enzyme	SNP sequence	Product size (bp)
FGFR2, rs2981578	F: 5'-AATGCTGCTTTGGAGGATTG-3'	*Aci* I	A>G	173
	R: 5'-CCAGAGGACTGAAACCCACA-3'			89+84
2q35, rs13387042	F: 5'-CCCTGTTTTGTTGCAGTGAA-3'	*Mnl* I	G>A	173
	R: 5'-ACGGAGCACTCTCAACATCC-3'			102+71
3p24, rs4973768	F: 5'-CTACTGCTTACATACACTTATTTAAG-3'	*Bfa* I[a]	C>T	181
	R: 5'-TAAGAGCAAAGGTAACTCATGTCTA-3'			156+25
17q23, rs6504950	F: 5'-AATCACTCCTTGCCAACCAC-3'	*Bsr* I	A>G	105
	R: 5'-GGGAAATGGGATATCAGCAAT-3'			64+41

F and R indicate forward and reverse primers, respectively. [a]Primers used in the PCR– ACRS (C instead of A).

tumor (15). These GWAS have made a great deal of progress in stratifying patients with breast cancer according to ER status, and investigating the correlation between the rs13387042 genotypes and ER phenotypes. In 2009, to identify additional loci with a two-stage GWAS method, Ahmed *et al*. evaluated over 800 promising associations from GWAS involving 37,012 cases and 40,069 controls gathered from 33 individual studies. Their work provided strong evidence for additional susceptibility loci on 3p-rs4973768 and 17q-rs6504950, with potential causative genes including solute carrier family 4, sodium bicarbonate cotransporter, member 7 (*SLC4A7*) and Never-in mitosis related kinase 10 (*NEK10*) on 3p and cytochrome C assembly protein 11 (*COX11*) on 17q (16).

Although most GWAS of breast cancer have been conducted among women of Caucasian and European ancestry, which may not be extrapolated to Asian populations which are of different genetic backgrounds, re-evaluation of previously reported loci in the Taiwanese population is still valuable and could also help in finding additional SNPs near the previous loci, as in the case of 16q12 for breast cancer (7). In Taiwan, breast cancer is the second most common type of cancer and the fourth leading cause of cancer death in women, uniquely characterized by early onset compared to Western populations (17). In this study, we aimed to evaluate the potential susceptibility loci on *FGFR2*-rs2981578, 2q35-rs13387042, 3p24-rs4973768 and 17q23-rs6504950 in breast cancer in Taiwanese women, and the association of different genotypes with diagnostic features, such as breast cancer stage and menopause status.

Materials and Methods

Study population and sample collection. Eighty-eight patients diagnosed with breast cancer together with seventy age-matched healthy individuals without breast cancer were recruited at the China Medical University Hospital (2003-2009). All participants completed an informed consent form prior to participation and provided their blood and tissue samples for genotyping. The research project was reviewed and approved by the Institutional Review Board of our hospital (DMR99-IRB-108).

Table II. *Clinical characteristics of the breast cancer patients.*

Characteristic	Number (%)
Age (years)	
Mean±SD	53.1±12.42
Range	33-85
TNM stage[a]	
0	0 (0.0%)
I	8 (11.2%)
IIa/IIb	32 (44.4%)
III/IV	32 (44.4%)
ER-positive	51 (58.0%)
PR-positive	71 (80.7%)
HER2-positive	29 (33.0%)

[a]TMN stage based on the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 6th (2002). Only for 72 individuals were recorded.

Genomic DNA extraction. Genomic DNA was extracted from tissue using a commercially available kit (GE Healthcare, Little Chalfont, UK). After extraction, DNA was quantified using a conventional spectrophotometric method with absorbance measurements at 260 nm (A_{260}). The DNA concentration was between 50 and 300 ng/μl.

Polymerase chain reaction-restriction fragment length polymorphism and amplification-created restriction site. 2q35, 17q23, and *FGFR2* genotypes were detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), and 3p24 was detected using an amplification-created restriction site (ACRS) method. All PCR conditions and procedures were performed as follows: Each PCR reaction contained 5 ng of DNA, 2.0× Master Mix RED (150 mM Tris-HCl pH 8.5, 40 mM $(NH_4)_2SO_4$, 3.0 mM $MgCl_2$, 0.2% Tween 20, 0.4 mM dNTPs, 0.05 units/μl Ampliqon Taq DNA polymerase; Ampliqon, Skovlunde, Denmark), and 0.2 μM forward and reverse primers in a 30 μL reaction volume. The cycling parameters were as follows: 7 min initial denaturation at 94˚C; 35

Table III. *Distribution of fibroblast growth factor receptor 2 (FGFR2)-rs2981578, 2q35-rs13387042, 3q24-rs4973768, and 17q23-rs6504950 genotypic frequencies among breast cancer patients and age-matched controls.*

Genotypic frequency	Cases n=88 (%)	Controls n=70 (%)	OR (95% CI)[a]	*P*-value[b]
FGFR2-rs2981578				
GG	35 (40.2%)	21 (30.0%)	1.0	0.407
AG	39 (44.8%)	36 (51.4%)	0.65 (0.32-1.31)	
AA	13 (14.9%)	13 (18.6%)	0.60 (0.23-1.54)	
2q35-rs13387042				
GG	64 (72.7%)	61 (88.4%)	1.0	0.035
AG	21 (23.9%)	8 (11.6%)	2.50 (1.03-6.07)	
AA	3 (3.4%)	0 (0.0%)		
3p24-rs4973768				
CC	58 (65.9%)	45 (64.3%)	1.0	0.196
CT	22 (25.0%)	23 (32.9%)	0.74 (0.37-1.50)	
TT	8 (9.1%)	2 (2.9%)	3.10 (0.63-15.34)	
17q23-rs6504950				
GG	64 (72.7%)	49 (70.0%)	1.0	0.347
AG	22 (25.0%)	21 (30.0%)	0.80 (0.40-1.62)	
AA	2 (2.3%)	0 (0%)		

[a]Odds ratios (OR) and 95% confidence intervals (CI) calculated by logistic regression. [b]By chi-square test or Fisher's exact test when the cell expectation was less than five.

Table IV. *Distribution of fibroblast growth factor receptor 2 (FGFR2)-rs2981578, 2q35-rs13387042, 3q24-rs4973768, and 17q23-rs6504950 allelic frequencies among breast cancer patients and age-matched controls.*

Allelic frequency	Cases n=176 (%)	Controls n=140 (%)	OR (95% CI)[a]	*P*-value
FGFR2-rs2981578				
Allele G	109 (62.6%)	78 (55.1%)	1.0	0.214
Allele A	65 (37.4%)	62 (44.3%)	0.75 (0.48-1.18)	
2q35-rs13387042				
Allele G	149 (84.7%)	130 (94.2%)	1.0	0.008
Allele A	27 (15.3%)	8 (5.8%)	2.95 (1.29-6.71)	
3p24-rs4973768				
Allele C	138 (78.4%)	113 (80.7%)	1.0	0.615
Allele T	38 (21.6%)	27 (19.3%)	1.15 (0.66-2.00)	
17q23-rs6504950				
Allele G	148 (84.1%)	119 (85.0%)	1.0	0.424
Allele A	28 (15.9%)	21 (15.0%)	1.07 (0.58-1.98)	

[a]Odds ratios (OR) and 95% confidence intervals (CI) calculated by logistic regression.

cycles of 30 s at 94˚C, 30 s at 57˚C for *FGFR2*, 52˚C for 2q35 and 17q23, and 50˚C for 3p24, 40 s at 72˚C; followed by a final extension of 7 min at 72˚C.) For 25% of samples in which SNPs were detected, the genotypes were confirmed by direct sequencing (Figure 1). The primer sequences and general information for PCR-RFLP/ACRS for each SNP are briefly summarized in Table I.

Statistical analysis. A Pearson's chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distributions of each genotype between cases and controls. Cancer risk associated with the genotypes was calculated with odds ratios (ORs) with 95% confidence intervals (CIs) using unconditional logistic regression. The data was determined to be statistically significant when the *p*-value was less than 0.05.

Results

The clinical characteristics of the female breast cancer patients are presented in Table II. The clinical indices include mean age, age range, TNM stage, ER-positivity, progesterone receptor (PR)-positivity, and HER2-positivity (Table II). We have further compared the status of hormone receptor expression and TNM stage to menopause age (≤49 or >49 years old; the age cut-off point was based on the average menopause age for Taiwanese women in data from the Department of Health, executive Yuan, Taiwan, 2005), and the results showed that there was no significant difference among ER-positive and ER-negative (p=0.723), PR-positive and PR-negative (p=0.490), HER2-positive and HER2-negative (p=0.590), and TNM stage (p=0.923) subgroups (data not shown).

The genotypic frequencies of the breast cancer susceptibility loci between breast cancer patients and controls are shown and compared in Table III. Among the four SNPs investigated, the genotypes of 2q35-rs13387042 had significantly different distribution between the breast cancer and control groups (p=0.035), while those for *FGFR2*-rs2981578, 3p24-rs4973768, 17q23-rs6504950 were not significantly different (p=0.407, 0.196 and 0.347, respectively).

The frequencies of the alleles for the breast cancer susceptibility loci in breast cancer patients and controls are shown and compared in Table IV. Allelic frequency distributions of the 2q35-rs13387042 allele A were 15.3% and 5.8% in the patient and control groups, respectively, and people carrying the A allele had a 2.95-fold increased risk of developing breast cancer (OR=2.95, 95% CI=1.29-6.71, p=0.008). To summarize the findings in Tables II and III, the A allele at 2q35-rs13387042 appears to be associated with a higher susceptibility for breast cancer.

We are interested in examining whether the genotypes of 2q35-rs13387042 could be a valuable marker in clinical prediction for different subgroups of breast cancer patients. In Table V, the contribution of *FGFR2*-rs2981578 genotypes to breast cancer susceptibility in women at different ages and with different tumor stages were evaluated. The breast cancer patients were stratified into two groups according to their menopausal age (≤49 or >49 years old), tumor stage (early or late phase), or specimen (blood or tissue) (Table V). The results showed that there was no significant difference in the genotype distribution between breast cancer patients of

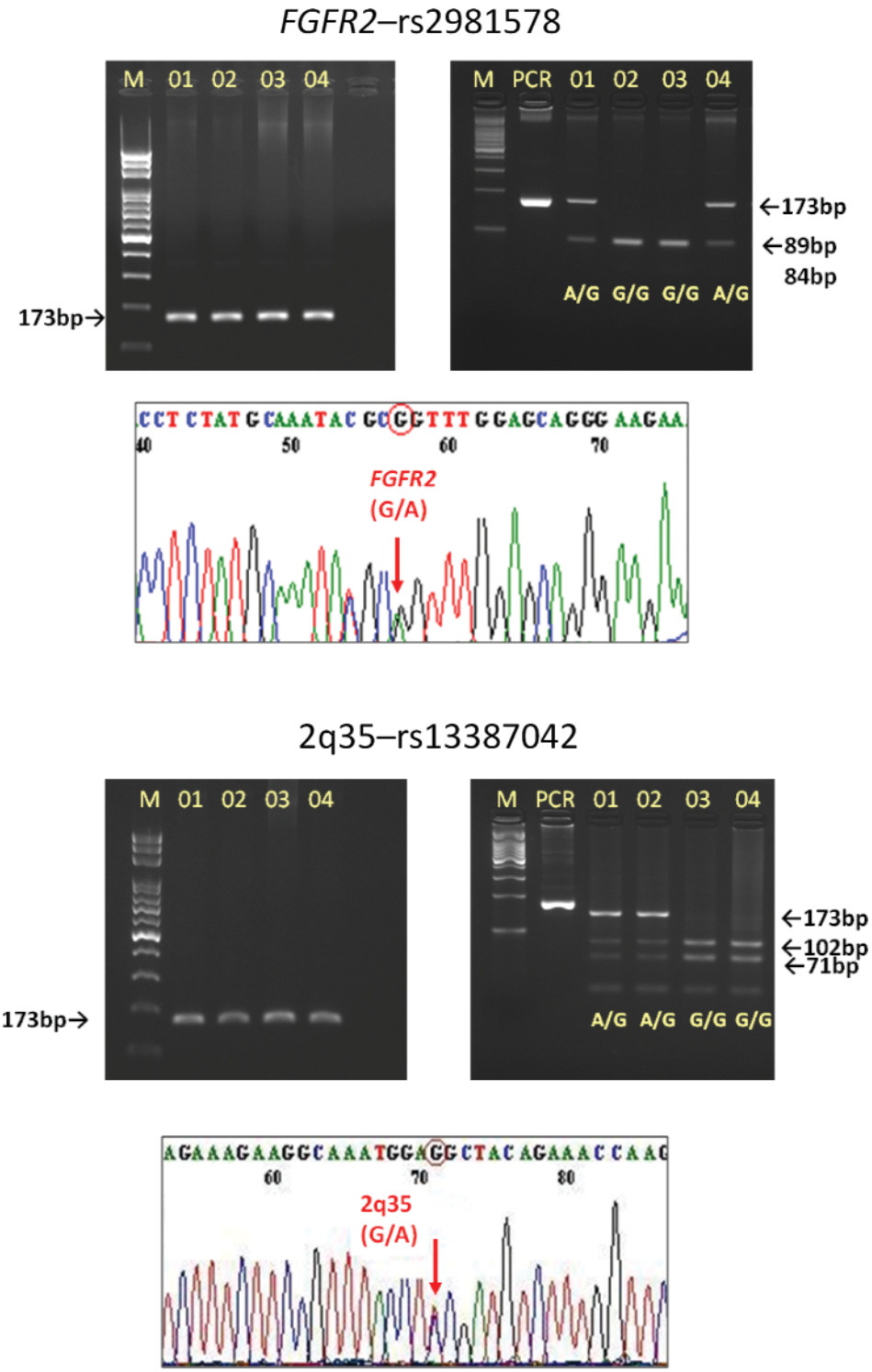

Figure 1 Continued

Figure 1. *Gel electrophoresis of polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP)/amplification-created restriction site (ACRS) for fibroblast growth factor receptor 2 (FGFR2)-rs2981578, 2q35-rs13387042, 3q24-rs4973768, and 17q23-rs6504950 genotypes and confirmation by direct sequencing.*

Figure 1 Continued

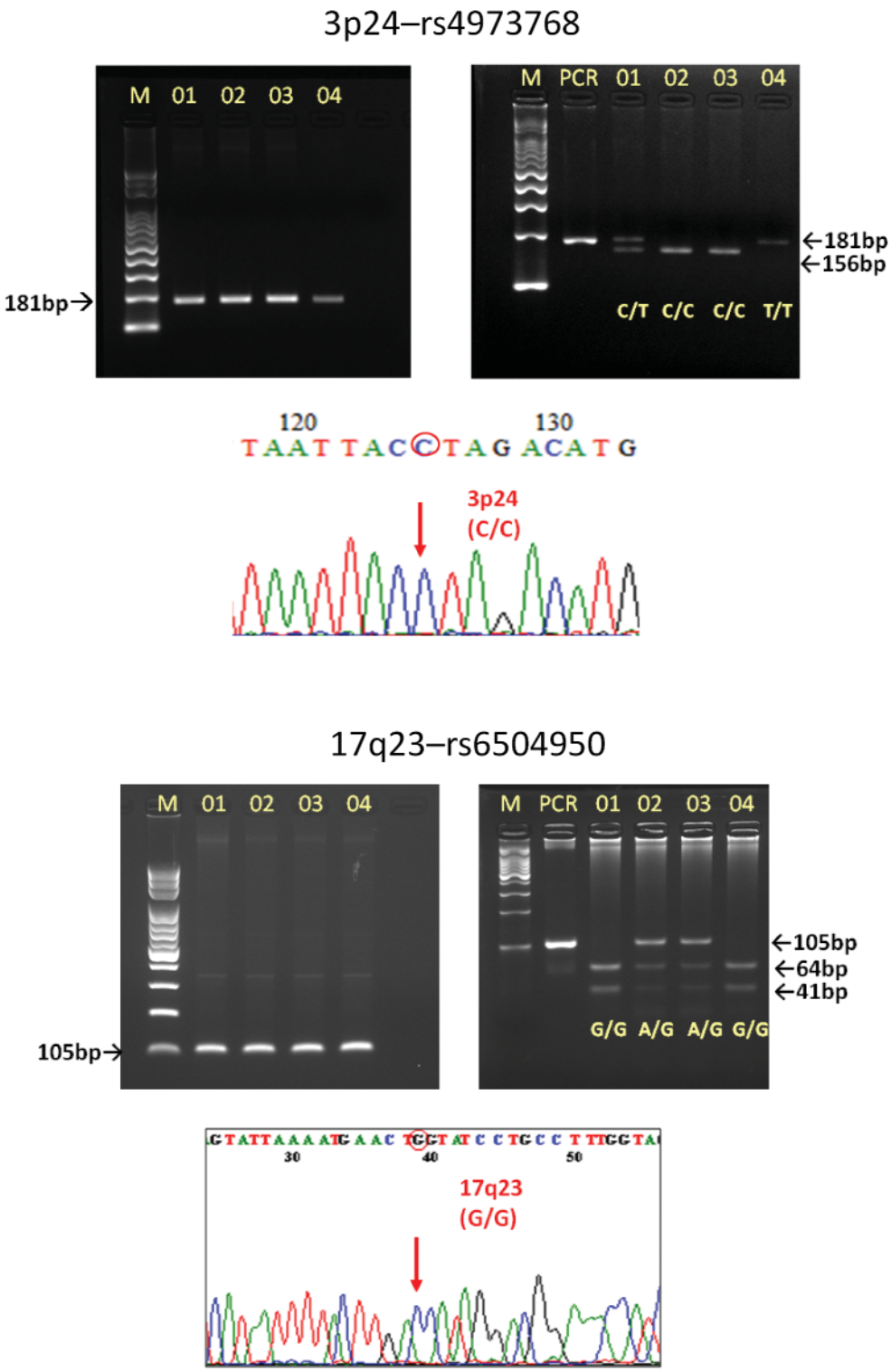

Table V. *Distribution of 2q35-rs13387042 genotypes among breast cancer patients of different menopausal age, tumor stage, and specimen type.*

Genotype	Menopause age[a]			Tumor stage[b]			Specimen		
	≤49 years, n (%)	>49 years, n (%)	*P*-value[c]	Early phase, n (%)	Late phase, n (%)	*P*-value[c]	Blood, n (%)	Tissue, n (%)	*P*-value[c]
2q35-rs13387042			0.542			0.334			1.00
GG	27 (67.5%)	37 (77.1%)		42 (79.2%)	22 (68.8%)		64 (72.7%)	64(72.7%)	
AG	11 (27.5%)	10 (20.8%)		10 (18.9%)	10 (31.3%)		21 (23.9%)	21(23.9%)	
AA	2 (5.0%)	1 (2.1%)		1 (1.9%)	0 (0.0%)		3 (3.4%)	3 (3.4%)	

[a]The menopausal age cut-off point is based on data from the Department of Health, Executive Yuan, Taiwan, R.O.C. [b]The patients with tumor stages I and II were grouped as early phase, and those with tumor stages III and IV were grouped as late phase. [c]*P*-value by chi-square test or Fisher's exact test when the cell expectation was less than five.

different menopausal age, tumor stage, or specimen type ($p>0.05$). We also examined the distribution patterns of the other three SNPs and no significant difference was found (data not shown).

Discussion

The GWAS performed in Western countries have provided a wealth of useful information with respect to the breast cancer susceptibility loci that have been identified, such as *FGFR2*-rs2981578, 2q35-rs13387042, 3p24-rs4973768, and 17q23-rs6504950 (7-11, 13-16, 18, 19). However, the associations of the genotypes of these loci with breast cancer had never previously been examined in the Taiwanese population, where the prevalence of breast cancer is high and characterized by early onset age. Thus, we aimed to verify the relationship of these genotypes with breast cancer risk in Taiwan. Among the four SNPs investigated in this study, only the genotypes of 2q35-rs13387042, not those of 3p24-rs4973768, 17q23-rs650490 or *FGFR2*-rs2981578, were found to be associated with breast cancer susceptibility (Tables III and IV). This is consistent with previous findings in European population (14, 15). Interestingly, in a GWAS investigation of a Chinese population with 6498 cases and 3999 controls, the genotype of 2q35-rs13387042 was not associated with breast cancer risk (15). The explanation for the different findings between our study and the Chinese study, which examine individuals with much closer genetic backgrounds than these in the Western studies, may be the interaction among the genotype of 2q35-rs13387042 with other genetic and life style environmental factors, such as estrogen exposure status and smoking habit.

It should be noted that 2q35-rs13387042 genotype was associated with an increased risk of breast cancer only in some specific subgroups of women, for instance, women with tumors that showed positive staining for ER (14) and in both ER-positive and ER-negative tumors (15). Therefore, we have also investigated the association of 2q35-rs13387042 genotypes in subgroups of our breast cancer patients with different ER statuses, finding that there was no significant difference in risk (data not shown). This is consistent with a report finding that the association with 2q35-rs13387042 was apparent for both ER-positive and ER-negative breast cancer patients (15). We further investigated the possibility of 2q35-rs13387042 genotype as biomarker for specific subgroups in our breast cancer patients with different PR or Her2 statuses, but no significant association was found (data not shown). Also, we have investigated the association of 2q35-rs13387042 genotypes in patients at different menopause ages and with different TNM stages (early and late phase), but made no significant findings (Table V).

2q35-rs13387042 is located in a 90-kb region of high linkage disequilibrium that contains neither known genes nor non coding RNAs (12, 14, 20, 21). The causal variant (or variants) in this region has (have) not been determined, and it is possible that one or more SNPs may confer a higher risk than 2q35-rs13387042. Elucidating the causal mechanism may improve our understanding of the etiology of breast cancer. However, we found it was possible to use blood samples for detecting the genotype of 2q35-rs13387042 in patients with breast cancer. One limitation of this study is the small sample size, and further investigations with larger sample populations would be helpful for both verifying the findings and allowing for further stratification of the sample populations.

In conclusion, this was a pilot report to evaluate the associations of breast cancer susceptibility loci in Taiwan. Our findings suggested that 2q35-rs13387042, but not 3p24-rs4973768, 17q23-rs650490 or *FGFR2*-rs2981578, was associated with breast cancer susceptibility. The 2q35-rs13387042 A allele might become a potential biomarker for breast cancer prediction. Blood sample can be used for detecting the genotype of 2q35-rs13387042 in breast cancer risk patients.

Acknowledgements

This study was supported by research grants from the Taiwan Department of Health, China Medical University Hospital Cancer Research Center of Excellence (DOH101-TD-C-111-005), and China Medical University Hospital (DMR-97-151).

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Received November 12, 2011
Revised December 19, 2011
Accepted December 21, 2011

ANTICANCER RESEARCH

International Journal of Cancer Research and Treatment

ISSN: 0250-7005

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ANTICANCER RESEARCH

International Journal of Cancer Research and Treatment

ISSN: 0250-7005

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