RESEARCH ARTICLE

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The Dual Role of *ABCG2* Copy Number Variation: A Protective Factor in Cancer Risk, and a Prognostic Marker for Survival in Thai Breast Cancer

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Abstract

Objective: This study aimed to investigate CNVs of *MDR1* and *ABCG2* and their associations with clinicopathological characteristics and survival outcomes in Thai breast cancer patients. **Methods:** Genomic DNA from 126 breast cancer patients and 162 healthy controls was analyzed. CNVs were determined using real-time PCR and the ΔCt method. Associations with clinicopathological parameters were assessed using the Chi-square test. Kaplan–Meier survival analysis and the log-rank test, were used to evaluate overall survival. **Result:** The *ABCG2* >1/1 genotype was significantly more common in controls than in patients (OR = 0.32, 95% CI: 0.19–0.525, P < 0.001), suggesting a protective role. *MDR1* CNVs showed no significant difference between groups. Among breast cancer patients, the *MDR1* >1/1 genotype was associated with larger tumor size (>3 cm) and distant metastasis (P = 0.037, 0.008), while the *ABCG2* >1/1 genotype was correlated with progesterone receptor positivity and distant metastasis (P = 0.005, 0.046). Survival analysis revealed that *ABCG2* >1/1 was associated with shorter overall survival (P = 0.013), whereas *MDR1* CNVs showed no significant association with survival (P = 0.127). **Conclusion:** These findings suggest that copy number variations (CNVs) in *ABCG2* may serve as both protective and prognostic markers in breast cancer, while *MDR1* CNVs may be associated with tumor aggressiveness. Both genes hold potential as biomarkers for breast cancer pathogenesis, clinicopathological characteristics, and survival outcomes in Thai breast cancer patients.

Keywords: *MDR1*- *ABCG2*- copy number variation- breast cancer- real-time PCR

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Introduction

Breast cancer is the most common cancer worldwide and continues to have a large impact on the global number of cancer deaths. Approximately 670,000 female breast cancer deaths and 2.3 million new cases were reported worldwide in 2022 [1, 2]. For Thailand, the incidence of breast cancer in female has increased. Based on new cases registered in 2020, the age-standardized incidence rate (ASR) of all cancer sites in females was 166.1 per 100,000, with breast cancer ranking first (ASR = 40.7), followed by cancers of the trachea, bronchus and lung (ASR = 19.1) and colon and rectum (ASR = 15.8) [3]. Both genetic predisposition and environmental variables contribute to the etiology of breast cancer [4]. A growing interest in epigenetics and gene regulation, particularly copy number variations (CNVs), has led to studies on potential genes to better understand the pathophysiology of breast cancer [5, 6].

The ATP-binding cassette (ABC) transporters, particularly Multidrug Resistance Protein 1 (*MDR1*, also known as ABCB1) and *ABCG2* (also known as breast cancer resistance protein, BCRP), are key contributors to multidrug resistance in breast cancer cells, significantly affecting the efficacy of chemotherapy [7]. *MDR1*, also referred to as P-glycoprotein (P-gp) or ABCB1, plays a crucial role in drug–drug interactions involving anticancer agents. It functions as an efflux pump, actively transporting a broad spectrum of structurally diverse cytotoxic drugs out of cancer cells, thereby reducing intracellular drug concentrations and diminishing their therapeutic effects [8]. This resistance mechanism is particularly relevant in cancers treated with chemotherapeutic agents such as doxorubicin, etoposide, and paclitaxel, all of which are substrates for P-gp. Similarly, *ABCG2*, also known as breast cancer resistance protein (BCRP), mediates the efflux of various anticancer drugs, including imatinib, nilotinib, and dasatinib, thereby limiting their effectiveness against cancer cells [9, 10]. The overexpression of *MDR1* and *ABCG2* in cancer cells is frequently observed, often driven by genetic and epigenetic mechanisms, and is associated with poor clinical outcomes due to

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reduced chemotherapy sensitivity [11, 12]. The ability of cancer cells to develop resistance to multiple drugs simultaneously, a phenomenon known as multidrug resistance (MDR), remains a major challenge in cancer treatment, often leading to treatment failure and disease progression [13].

The Real-time PCR was employed to analyze the DNA copy number variations (CNVs) of *MDR1* and *ABCG2* in Thai breast cancer patients. The associations between CNVs frequencies in patients and the control group were assessed using binary logistic regression, while the correlations between CNVs and clinicopathological features were analyzed using the Chi-square test. The relationship between CNVs and survival status was evaluated using the Kaplan-Meier survival analysis and multivariate Cox regression analysis. A P-value ≤ 0.05 was considered statistically significant. The aim of this study was to investigate the impact of *MDR1* and *ABCG2* copy number variations on clinicopathological characteristics and survival outcomes in Thai breast cancer patients.

Materials and Methods

Specimen recruitment

The sample size for this study was estimated using the Epitools Epidemiological Calculators [14]. According to the TCGA database (https://www.cancer.gov/tcga), data from project ID TCGA-BRCA indicated that the proportions of *MDR1* and *ABCG2* copy number gains were 17.90% and 8.39%, respectively. Using an assumed odds ratio of 4, a confidence level of 95%, a desired power of 80%, and expected proportions exposed in controls of 0.179 and 0.0839 for *MDR1* and *ABCG2* CNVs respectively, the estimated sample size required per group at a P-value of 0.05 was 98. In the actual sample recruitment, a total of 288 retrospective specimens were recruited from 126 breast cancer blood samples and 162 blood samples from normal individuals, which was sufficient to meet the sample size requirements. All specimens were collected from the National Cancer Institute, Bangkok, Thailand, during the period from 2009 to 2013. This study was approved by the Rangsit University Ethics Committee (DPE.NO.RSUERB2020-079) and the Ethics Committee of the National Cancer Institute, Thailand (Project Number: 024_2020RB_OUT667). Patients' clinicopathological data such as age at diagnosis, tumor size, histological grade, tumor stage, lymph-node status, number of lymph nodes, immunohistochemical profile of estrogen receptor (ER) status, progesterone receptor (PgR) status, human epidermal growth factor receptor 2 (HER2) status, and triple-negative tumor status, chemotherapy treatment (anthracycline and anthracycline+taxane), and distant metastasis status were collected from patient files.

DNA extraction

Genomic DNA from both breast cancer and normal control samples was isolated from EDTA-anticoagulated blood using the High Pure PCR Template Preparation Kit (Roche Molecular Diagnostics, Mannheim, Germany) following the manufacturer's instructions. The purity of the extracted DNA was initially assessed using a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples with an absorbance ratio (A260/A280) between 1.8 and 2.0 were considered suitable for quantitative PCR (qPCR) analysis.

Determination of copy number variation of MDR1 gene and ABCG2 gene

Copy number variation (CNVs) analysis of the *MDR1* and *ABCG2* genes was performed on genomic DNA using real-time PCR with the Luna® Universal qPCR Master Mix Kit (New England Biolabs, Inc., MA, USA). The GAPDH gene was used as a reference. The primer sequences used for qPCR were as follows: *MDR1* (Forward: 5'-TTG ATG GCA AAG AAA TAA AGC-3', Reverse: 5'-CTT ACA TTA GGC AGT GAC TCG-3'), *ABCG2* (Forward: 5'-GCT ACA CCA CCT CCT TCT GT-3', Reverse: 5'-GGA AGA AGA GAA CCC CAG CT-3'), and GAPDH (Forward: 5'-AGG TCG GAG TCA ACG GAT TT-3', Reverse: 5'-TAG TTG AGG TCA ATG AAG GG-3'). Real-time PCR was conducted using the CFX96 Connect™ Real-Time PCR Detection System with CFX Maestro™ Software (Bio-Rad Laboratories, Inc., CA, USA). The thermal cycling conditions included initial denaturation at 95°C for 1 minute, followed by 40 cycles of denaturation at 95°C for 5 seconds, and annealing/extension at 60°C for 30 seconds. Gene copy numbers were determined using the ΔCt method as described by Nørskov et al. [15], calculated as ΔCt = Ct,target gene – Ct,reference gene. Based on the ΔCt values, samples were categorized into genotype groups for statistical analysis as follows: "0/0": ΔCt not detected, "1/0": ΔCt between -2.0 and 0.5, "1/1": ΔCt between 0.5 and 1.9, and ">1/1": ΔCt > 2.0. Subsequently, data analysis was performed to assess correlations between CNVs and the clinicopathological characteristics of breast cancer patients.

Statistical analysis

The Chi-square test was used to compare the frequencies of CNVs between breast cancer patients and the control group. The association between CNVs genotypes and disease risk was evaluated using Cross-tabulation analysis. Additionally, the Chi-square test was applied to analyze correlations between *MDR1* and *ABCG2* CNVs and clinicopathological characteristics, including gender, tumor stage, tumor size, differentiation, and age at diagnosis. Survival analysis was conducted using the Kaplan–Meier method, and differences in survival curves were assessed using the log-rank test. Multivariate Cox regression analysis was then employed to evaluate the prognostic impact of *MDR1* and *ABCG2* copy number variations on breast cancer survival. The follow-up period for patients ranged from 1 to 102 months. A P-value < 0.05 was considered statistically significant.

Results

Detection of MDR1 and ABCG2 CNVs

The copy number variations (CNVs) of *MDR1* and *ABCG2* relative to the reference gene were determined using the obtained Ct values and expressed as ΔCt values. Samples were then categorized into CNV genotypic

groups based on these values. In breast cancer patients, the *MDR1* gene exhibited a combined 1/0 + 1/1 genotype in 88.10% of cases, while the >1/1 genotype was observed in 11.90%. For the *ABCG2* gene, the 1/0 + 1/1 genotype was present in 58.70% of cases, whereas the >1/1 genotype accounted for 41.30%. In the control group, the *MDR1* gene showed a 1/0 + 1/1 genotype in 92.00% of individuals and a >1/1 genotype in 8.00%. Meanwhile, for *ABCG2*, the 1/0 + 1/1 genotype was found in 31.50%, while the >1/1 genotype was observed in 68.50% of controls. Since only one case in the breast cancer group exhibited the 1/0 genotype for *ABCG2*, and no 1/0 genotype was detected for *MDR1* in either group, the 1/0 and 1/1 genotypes were combined for statistical analysis.

The correlation between patients' and the control group's MDR1 and ABCG2 CNVs frequencies

The analysis of *MDR1* and *ABCG2* CNVs relative to the reference gene GAPDH in both the control and breast cancer patient groups revealed no significant difference in *MDR1* CNVs between the two groups. However, *ABCG2* CNVs exhibited a statistically significant difference ($P \leq 0.05$) between the groups, with an odds ratio (OR) [95% CI] of 0.32 (0.19–0.525). This suggests that the >1/1 genotype of *ABCG2* serves as a protective factor against breast cancer, meaning that individuals with a higher copy number of *ABCG2* in the control group tend to have a lower likelihood of developing breast cancer. Conversely, the *MDR1* CNVs, with an odds ratio (OR) [95% CI] of 1.55 (0.71–3.39), suggest a potential increased risk of breast cancer associated with the >1/1 genotype of *MDR1*, although this finding was not statistically significant. These results are summarized in Table 1.

Statistical Analysis of MDR1 and ABCG2 CNVs and Their Association with Patients' Clinicopathological Parameters

The association between clinicopathological parameters and *MDR1* and *ABCG2* CNVs in breast cancer patients was analyzed and is presented in Tables 2 and 3, respectively. The results indicated that *MDR1* CNVs were significantly associated with tumor size (P = 0.037) and distant metastasis (P = 0.008) in breast cancer patients. However, no significant associations were observed between *MDR1* CNVs and other clinicopathological factors, including age, histological differentiation, cancer stage, lymph node involvement, immunohistochemical biomarker expression, and chemotherapy treatment.

The analysis of *ABCG2* CNVs revealed a significant association with progesterone receptor (PgR) status, distinguishing between PgR-negative and PgR-positive patients (P = 0.005). Additionally, *ABCG2* CNVs were significantly associated with distant metastasis (P = 0.046). However, no significant associations were observed between *ABCG2* CNVs and other clinicopathological factors, including age, tumor size, histological differentiation, cancer stage, lymph node involvement, other immunohistochemical biomarkers, and chemotherapy treatment.

Survival Analysis of Breast Cancer Patients in Relation to MDR1 and ABCG2 CNVs

The Survival analysis of breast cancer patients based on *MDR1* and *ABCG2* copy number variations (CNVs) was performed using the Kaplan-Meier method, with comparisons made using the log-rank test, as shown in Figures 1 and 2, respectively. The results showed that *MDR1* CNVs were not significantly associated with overall survival in breast cancer patients (P = 0.127). In contrast, *ABCG2* CNVs were significantly associated with patient survival (P = 0.013). Specifically, patients with the >1/1 genotype of *ABCG2* who received chemotherapy exhibited a shorter overall survival compared to those with the 1/0 or 1/1 genotypes. Additionally, multivariate Cox regression analysis revealed a significant association between breast cancer survival and *ABCG2* CNVs (HR = 6.203, 95%

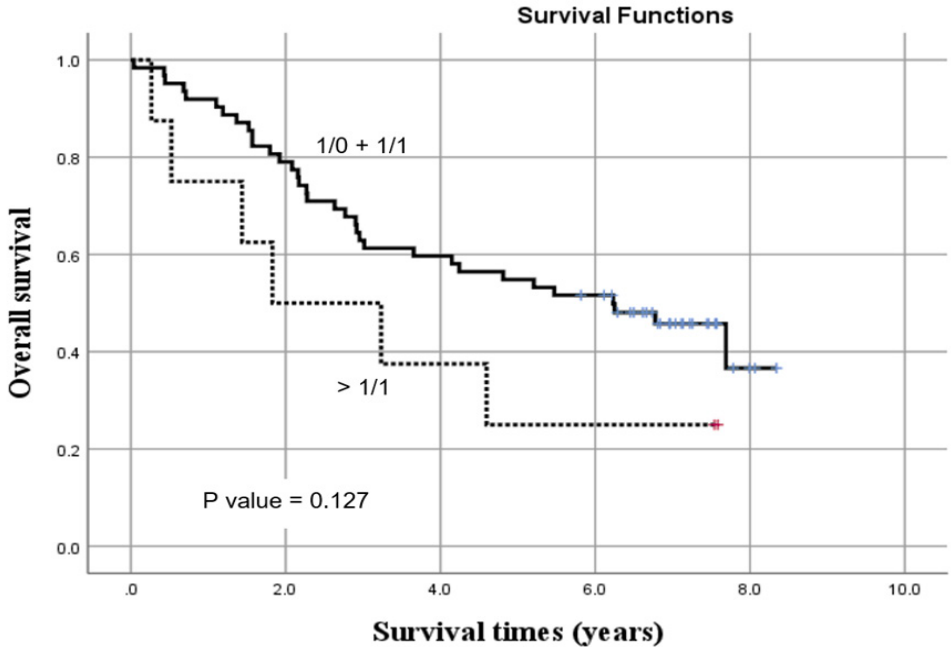

Figure 1. Kaplan-Meier Survival Curve of Breast Cancer Patients According to *MDR1* CNVs

Table 1. The *MDR1* and *ABCG2* CNVs in Breast Cancer Patients and Controls

CNVs status	MDR1		ABCG2	
	Control	Case	Control	Case
1/0 + 1/1	149 (92)	111 (88.1)	51 (31.5)	74 (58.7)
>1/1	13 (8)	15 (11.9)	111 (68.5)	52 (41.3)
Total	162	126	162	126
Odds ratio (95%CI)	1.55 (0.71-3.39)		0.32 (0.19-0.525)	
P value	0.27		0.000*	

CNVs, Copy number variants; CI, confidence interval

CI = 1.957-19.658, P = 0.002), as shown in Table 4. Among other clinical variables, age (HR = 3.389, 95% CI = 1.003-11.446, P = 0.049), tumor stage (HR = 11.236, 95% CI = 2.771-45.555, P = 0.001), distant metastasis (HR = 22.765, 95% CI = 3.992-129.808, P = 0.000), and Triple-negative status (HR = 12.452, 95% CI = 1.115-139.09, P = 0.041) were also significantly associated with breast cancer survival.

Discussion

Breast cancer is among the most commonly diagnosed cancers in women worldwide. Multiple risk factors are associated with its development, one of the most important being genetic alterations that contribute to tumorigenesis. Following a breast cancer diagnosis, chemotherapy is commonly administered as part of the standard treatment. However, individual responses to chemotherapy vary significantly, and such variation is often linked to genetic differences in drug metabolism pathways. In particular, genes encoding ATP-binding cassette (ABC) transporter proteins play a crucial role in drug resistance by facilitating the efflux of chemotherapeutic agents out of cancer cells, thereby reducing intracellular drug concentrations. Key proteins in this family include P-glycoprotein (P-gp1), encoded by the *MDR1* gene; breast cancer resistance protein (BCRP), encoded by *ABCG2*; and multidrug resistance-associated protein 1 (MRP1), encoded by ABCC1 [16]. Given this context, our study focused on analyzing copy number variations (CNVs) of the *MDR1* and *ABCG2* genes, which are hypothesized to influence gene expression levels, and drug handling in breast cancer cells, potentially affecting treatment outcomes. We also investigated the association between these CNVs and various clinicopathological parameters, including tumor size, metastasis, and receptor status. Furthermore, comparisons were made with a normal control group to evaluate the potential of *MDR1* and *ABCG2* CNVs as predictive biomarkers for breast cancer development.

Both the *MDR1* and *ABCG2* genes, which encode P-glycoprotein (P-gp1) and breast cancer resistance protein (BCRP), respectively, play crucial roles in the efflux of drugs and xenobiotics from cells [17, 18]. These transporters influence the intracellular accumulation of carcinogenic xenobiotics in normal cells and chemotherapeutic agents in breast cancer cells. However, the results demonstrated that copy number variation (CNVs) of the *MDR1* gene did not differ significantly between breast cancer patients and the control group. In contrast, *ABCG2* CNVs showed

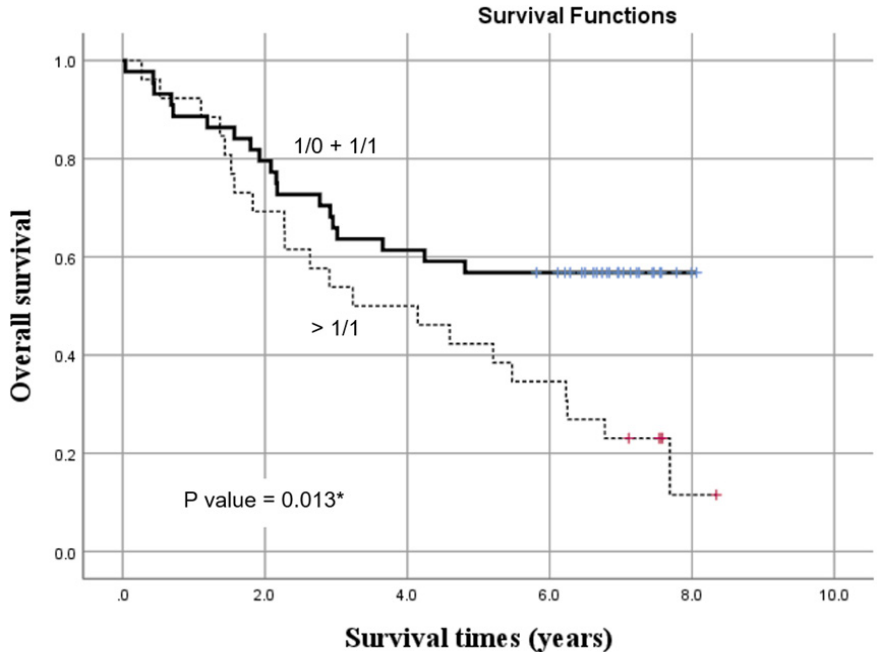

Figure 2. Kaplan–Meier Survival Curve of Breast Cancer Patients According to *ABCG2* CNVs. * indicates statistical significance at P < 0.05.

Table 2. *MDR1* CNVs and Their Association with Clinicopathological Parameters in Breast Cancer Patients

Parameter	Numbe of case	CNV status of MDR1		Odds ratio (95%CI)	P value
		1/0 + 1/1; n (%)	>1/1; n (%)		
Age					
≤50	67	57(85.1)	10(14.9)	0.53,(0.17-1.64)	0.287
>50	59	54(91.5)	5(8.5)		
Tumor size(cm)					
≤3	101	92(91.1)	9(8.9)	3.23,(1.03-10.14)	0.037*
>3	25	19(76.0)	6(24.0)		
Histologic grade					
I	11	11(100)	0	-	0.402
II	48	41(85.4)	7(14.6)		
III	35	30(85.7)	5(14.3)		
Tumor stage					
I, IIA, IIB	58	51(87.9)	7(12.1)	1.75,(0.53-5.75)	0.354
IIIA, IIIB	31	25(80.6)	6(19.4)		
Lymph-node status					
Negative	55	50(90.9)	5(9.1)	2.06,(0.60-7.03)	0.242
Positive	41	34(82.9)	7(17.1)		
Lymph Nodes (no.)					
0-3 positive	75	68(90.7)	7(9.3)	3.04,(0.85-10.81)	0.76
>3 positive	21	16(76.2)	5(23.8)		
Immunohistochemical					
ER status					
Negative	42	39(92.9)	3(7.1)	2.36,(0.628-8.89)	0.193
Positive (1+,2+,3+)	78	66(84.6)	12(15.4)		
PgR status					
Negative	55	51(92.7)	4(7.3)	2.56,(0.78-8.68)	0.111
Positive (1+,2+,3+)	65	54(83.1)	11(16.9)		
HER2 status					
Negative	76	69(90.8)	7(9.2)	1.92,(0.62-5.89)	0.25
Positive (1+,2+,3+)	43	36(83.7)	7(16.3)		
Triple negative tumor					
ER, PR, HER2 positive	106	92(86.8)	14(13.2)	0.55,(0.67-4.54)	1
ER, PR, HER2 negative	13	12(92.3)	1(7.7)		
Chemotherapy treatment					
Antracycline	36	29(80.6)	7(19.4)	0.59,(0.17-2.06)	0.532
Antracycline+taxane	40	35(87.5)	5(12.5)		
Distant metastasis					
No	72	65(90.3)	7(9.7)	7.74,(1.87-32.01)	0.008*
Yes	11	6(54.5)	5(45.5)		

a statistically significant difference between the two groups, with an odds ratio (OR) [95% CI] of 0.32 (0.19–0.525). Interestingly, the >1/1 genotype of *ABCG2* was found to act as a protective factor against breast cancer development. This observation suggests that the >1/1 genotype of *ABCG2* may serve as a protective factor against the development of breast cancer. This may be explained by the physiological role of BCRP in normal breast epithelial cells, where it functions in eliminating xenobiotic substances and potentially carcinogenic agents, thereby protecting cells from malignant transformation [19]. Because *ABCG2* plays a role in the elimination of drugs and xenobiotics, including carcinogenic xenotoxins implicated in breast cancer, an increased copy number of *ABCG2* may enhance the clearance of such harmful agents [20]. This mechanism may contribute to a protective effect against the development of breast cancer. Therefore, a gain in *ABCG2* copy number may indicate reduced susceptibility to breast cancer. Furthermore, analysis of the association between *MDR1* and *ABCG2* CNVs and

Table 3. *ABCG2* CNVs and Their Association with Clinicopathological Parameters in Breast Cancer Patients

Parameter	Number of case	CNV status of ABCG2		Odds ratio (95%CI)	P value
		1/0 + 1/1; n (%)	>1/1; n (%)		
Age					
≤50	67	41 (61.2)	26 (38.8)	1.24,(0.61-2.53)	0.549
>50	59	33 (55.9)	26 (44.1)		
Tumor size(cm)					
≤3	101	61 (60.4)	40 (39.6)	1.41,(0.58-3.39)	0.445
>3	25	13 (52.0)	12 (48.0)		
Histologic grade					
I	11	7 (63.6)	4 (36.4)	-	0.276
II	48	26 (54.2)	22 (45.8)		
III	35	25 (71.4)	10 (28.6)		
Tumor stage					
I, IIA, IIB	58	36 (62.1)	22 (37.9)	1.98,(0.82-4.81)	0.126
IIIA, IIIB	31	14 (45.2)	17 (54.8)		
Lymph-node status					
Negative	55	33 (60.0)	22 (40.0)	1.29,(0.57-2.93)	0.534
Positive	41	22 (53.7)	19 (46.3)		
Lymph Nodes (no.)					
0-3 positive	75	45 (60.0)	30 (40.0)	1.65,(0.62-4.37)	0.311
>3 positive	21	10 (47.6)	11 (52.4)		
Immunohistochemical					
ER status					
Negative	42	28 (66.7)	14 (33.3)	1.63,(0.75-3.56)	0.22
Positive (1+,2+,3+)	78	43 (55.1)	35 (44.9)		
PgR status					
Negative	55	40 (72.7)	15 (27.3)	2.93,(1.36-6.03)	0.005*
Positive (1+,2+,3+)	65	31 (47.7)	34 (52.3)		
HER2 status					
Negative	76	46 (60.5)	30 (39.5)	1.10,(0.52-2.36)	0.799
Positive (1+,2+,3+)	43	25 (58.1)	18 (41.9)		
Triple negative tumor					
ER, PR, HER2 positive	106	62 (58.5)	44 (41.5)	0.63,(0.18-2.16)	0.456
ER, PR, HER2 negative	13	9 (69.2)	4 (30.8)		
Chemotherapy treatment					
Antracycline	36	19 (52.8)	17 (47.2)	0.83,(0.33-2.04)	0.678
Antracycline+taxane	40	23 (57.5)	17 (42.5)		
Distant metastasis					
No	72	45 (62.5)	27(37.5)	4.44,(1.08-18.21)	0.046*
Yes	11	3 (27.3)	8(72.7)		

the clinicopathological features and survival outcomes of breast cancer patients revealed that the >1/1 genotype of *MDR1* was significantly associated with larger tumor size (>3 cm) and distant metastasis (P = 0.037 and 0.008, respectively), although it showed no significant association with overall survival (P = 0.127). On the other hand, the >1/1 genotype of *ABCG2* was significantly associated with progesterone receptor (PgR) status and distant metastasis (P = 0.005 and 0.046, respectively), and more importantly, with a significantly shorter overall survival among breast cancer patients (P = 0.013). This paradox may be partially explained by the known role of BCRP in drug efflux, which could theoretically reduce intracellular concentrations of chemotherapeutic agents, such as mitoxantrone and topotecan [20], potentially compromising treatment efficacy. This mechanism likely contributes to the poor survival outcomes observed in patients with increased copy number of *ABCG2* gene. These findings are consistent with a study by Robey et al. [21], which demonstrated overexpression of

Table 4. Multivariate Cox Regression Analysis of Prognostic Biomarker for Survival of Breast Cancer Patients

Clinical variables	HR	95% CI		P value
		Lower	Upper	
Age; ≤50 vs >50	3.389	1.003	11.446	0.049*
Tumor size(cm); ≤3 vs >3	1.037	0.246	4.382	0.96
Tumor stage; I,IIA, IIB vs IIIA, IIIB	11.236	2.771	45.555	0.001*
Regimen; antracycline vs antracycline+taxane	0.901	0.448	1.812	0.77
Distant metastasis; no vs yes	22.765	3.992	129.808	0.000*
ER status; negative vs positive	1.477	0.292	7.46	0.637
HER2 status; negative vs positive	0.55	0.172	1.763	0.315
Triple-negative status; positive vs negative	12.452	1.115	139.09	0.041*
MDR1 CNVs; 1/0+1/1 vs >1/1	0.171	0.023	1.275	0.085
ABCG2 CNVs; 1/0+1/1 vs >1/1	6.203	1.957	19.658	0.002*

HR, Hazard ratio; CI, confidence interval; CNVs, Copy number variants

ABCG2 in MCF-7 breast cancer cells resistant to the chemotherapeutic agent flavopiridol, leading to enhanced drug efflux and increased resistance. Similarly, Yin et al. [22] reported that inhibition of *MDR1* and *ABCG2* in lung cancer cells significantly enhanced the cytotoxic effect of doxorubicin, suggesting that these transporters play a critical role in chemoresistance across cancer types. In relation to their association with clinicopathological characteristics, our study identified significant correlations between CNVs and specific pathological features. The >1/1 genotype of *MDR1* was associated with larger tumor size and an increased risk of tumor progression. Similarly, the >1/1 genotype of *ABCG2* was significantly correlated with progesterone receptor (PgR) positivity, which may influence responsiveness to hormone-targeted therapies. Moreover, both *MDR1* and *ABCG2* >1/1 genotypes were significantly associated with a higher risk of distant metastasis, underscoring their potential roles in disease progression and prognosis. Regarding survival outcomes, multivariate Cox regression analysis revealed that an increased copy number of *ABCG2*, but not *MDR1*, along with higher tumor stage and the presence of distant metastasis, were significantly associated with shorter overall survival. Although the direct correlation between *MDR1* and *ABCG2* CNVs and overall survival in breast cancer has not been widely reported, variations in the expression levels of these genes have been shown to influence chemotherapeutic response, which may impact overall patient survival [23, 24]. These findings underscore the dual role of *ABCG2* CNVs as both a protective biomarker for breast cancer susceptibility and a prognostic indicator for disease progression and patient outcomes.

In conclusion, the findings of this study suggest that *ABCG2* copy number variation (CNVs) may serve as a useful predictive biomarker for breast cancer. The presence of the >1/1 genotype appears to act as a protective factor against the development of breast cancer. However, among patients already diagnosed with the disease, this genotype is associated with shorter overall survival, and may also serve as a prognostic marker for progesterone receptor (PgR) status and the likelihood of distant metastasis. Similarly, the >1/1 genotype of *MDR1* was associated with an increased risk of tumor growth and metastasis, indicating its potential utility in predicting disease aggressiveness. Nonetheless, due to the lack of complete and standardized treatment response data, a formal analysis of chemotherapeutic response in relation to *MDR1* and *ABCG2* CNVs could not be performed in this study.

Author Contribution Statement

TS and TP designed the study and provided critical revisions. TS and PS performed statistical analysis, recruited specimens, and carried out DNA preparation, real-time PCR experiments, and data interpretation. The first draft of the manuscript was written by TS, and both TP and PS contributed to reviewing and editing the manuscript. All authors have read and approved the final version of the manuscript.

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General

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Funding Statement

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Ethical Declaration

This study was approved by the Rangsit University Ethics Committee (Approval No. DPE. NO.RSUERB2020-079) and the Ethics Committee of the National Cancer Institute, Thailand (Project No. 024_2020RB_OUT667). As this was a retrospective study, the requirement for informed consent was waived.

Conflict of Interest

All authors declare that there are no conflicts of interest

References

1. Kim J, Harper A, McCormack V, Sung H, Houssami N, Morgan E, et al. Global patterns and trends in breast cancer incidence and mortality across 185 countries. Nat Med. 2025;31(4):1154-62. https://doi.org/10.1038/s41591-025-03502-3.
2. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 2022;66:15-23. https://doi.org/10.1016/j.breast.2022.08.010.
3. Rojanamatin J, Ukranun W, Supaattagorn P, Chiawiriyabunya M, Wongsena A, Chaiwerawattana P, et al., editors. Cancer in thailand, volume x, 2016-2018 [internet]. Bangkok: Medical record and databased cancer unit; 2021 [cited 2025 apr 18]. Available from: Https://www.Nci.Go.Th/e_book/cit_x/index.Html.
4. Thakur C, Qiu Y, Fu Y, Bi Z, Zhang W, Ji H, et al. Epigenetics and environment in breast cancer: New paradigms for anti-cancer therapies. Front Oncol. 2022;12:971288. https://doi.org/10.3389/fonc.2022.971288.
5. Shahrouzi P, Forouz F, Mathelier A, Kristensen VN, Duijf PHG. Copy number alterations: A catastrophic orchestration of the breast cancer genome. Trends Mol Med. 2024;30(8):750-64. https://doi.org/10.1016/j.molmed.2024.04.017.
6. Troester MA, Hoadley KA, D'Arcy M, Cherniack AD, Stewart C, Koboldt DC, et al. DNA defects, epigenetics, and gene expression in cancer-adjacent breast: A study from the cancer genome atlas. NPJ Breast Cancer. 2016;2:16007. https://doi.org/10.1038/npjbcancer.2016.7.
7. Kovalev AA, Tsvetaeva DA, Grudinskaja TV. Role of abc-cassette transporters (mdr1, mrp1, bcrp) in the development of primary and acquired multiple drug resistance in patients with early and metastatic breast cancer. Exp Oncol. 2013;35(4):287-90.
8. Mealey KL, Fidel J. P-glycoprotein mediated drug interactions in animals and humans with cancer. J Vet Intern Med. 2015;29(1):1-6. https://doi.org/10.1111/jvim.12525.
9. Eadie LN, Hughes TP, White DL. Interaction of the efflux transporters abcb1 and abcg2 with imatinib, nilotinib, and dasatinib. Clin Pharmacol Ther. 2014;95(3):294-306. https://doi.org/10.1038/clpt.2013.208.
10. Chen R, Yu Y, Liu R, Chen Q. Targeting breast cancer resistance protein (bcrp/abcg2) in cancer. Transl Cancer Res. 2024;13(11):6550-64. https://doi.org/10.21037/tcr-24-1129.
11. Wu CP, Hung CY, Lusvarghi S, Huang YH, Tseng PJ, Hung TH, et al. Overexpression of abcb1 and abcg2 contributes to reduced efficacy of the pi3k/mtor inhibitor samotolisib (ly3023414) in cancer cell lines. Biochem Pharmacol. 2020;180:114137. https://doi.org/10.1016/j.bcp.2020.114137.
12. Wu ZX, Yang Y, Wang JQ, Narayanan S, Lei ZN, Teng QX, et al. Overexpression of abcg2 confers resistance to mln7243, a ubiquitin-activating enzyme (uae) inhibitor. Front Cell Dev Biol. 2021;9:697927. https://doi.org/10.3389/fcell.2021.697927.
13. Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, et al. Resistance to cancer chemotherapy: Failure in drug response from adme to p-gp. Cancer Cell Int. 2015;15:71. https://doi.org/10.1186/s12935-015-0221-1.
14. Sergeant ES, 2018. Epitools epidemiological calculators. [internet]. Australia: Ausvet; c2025 [updated 2025; cited 2025 may 1]. Available from: Http://epitools.Ausvet.Com.Au.
15. Nørskov MS, Frikke-Schmidt R, Loft S, Tybjaerg-Hansen A. High-throughput genotyping of copy number variation in glutathione s-transferases m1 and t1 using real-time pcr in 20,687 individuals. Clin Biochem. 2009;42(3):201-9. https://doi.org/10.1016/j.clinbiochem.2008.10.020.
16. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of abc transporters in multidrug-resistant cancer. Nat Rev Cancer. 2018;18(7):452-64. https://doi.org/10.1038/s41568-018-0005-8.
17. Kónya A, Andor A, Sátorhelyi P, Németh K, Kurucz I. Inhibition of the mdr1 transporter by new phenothiazine derivatives. Biochem Biophys Res Commun. 2006;346(1):45-50. https://doi.org/10.1016/j.bbrc.2006.05.058.
18. Kukal S, Guin D, Rawat C, Bora S, Mishra MK, Sharma P, et al. Multidrug efflux transporter abcg2: Expression and regulation. Cell Mol Life Sci. 2021;78(21-22):6887-939. https://doi.org/10.1007/s00018-021-03901-y.
19. Nakanishi T, Ross DD. Breast cancer resistance protein (bcrp/abcg2): Its role in multidrug resistance and regulation of its gene expression. Chin J Cancer. 2012;31(2):73-99. https://doi.org/10.5732/cjc.011.10320.
20. Mao Q, Unadkat JD. Role of the breast cancer resistance protein (bcrp/abcg2) in drug transport--an update. Aaps J. 2015;17(1):65-82. https://doi.org/10.1208/s12248-014-9668-6.
21. Robey RW, Medina-Pérez WY, Nishiyama K, Lahusen T, Miyake K, Litman T, et al. Overexpression of the atp-binding cassette half-transporter, abcg2 (mxr/bcrp/abcp1), in flavopiridol-resistant human breast cancer cells. Clin Cancer Res. 2001;7(1):145-52.
22. Yin W, Xiang D, Wang T, Zhang Y, Pham CV, Zhou S, et al. The inhibition of abcb1/mdr1 or abcg2/bcrp enables doxorubicin to eliminate liver cancer stem cells. Sci Rep. 2021;11(1):10791. https://doi.org/10.1038/s41598-021-89931-9.
23. Spitzwieser M, Pirker C, Koblmüller B, Pfeiler G, Hacker S, Berger W, et al. Promoter methylation patterns of abcb1, abcc1 and abcg2 in human cancer cell lines, multidrug-resistant cell models and tumor, tumor-adjacent and tumor-distant tissues from breast cancer patients. Oncotarget. 2016;7(45):73347-69. https://doi.org/10.18632/oncotarget.12332.
24. Litviakov NV, Cherdyntseva NV, Tsyganov MM, Slonimskaya EM, Ibragimova MK, Kazantseva PV, et al. Deletions of multidrug resistance gene loci in breast cancer leads to the down-regulation of its expression and predict tumor response to neoadjuvant chemotherapy. Oncotarget. 2016;7(7):7829-41. https://doi.org/10.18632/oncotarget.6953.