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GENETIC ASSOCIATION OF *ABCB1* 1236 C>T POLYMORPHISM ON MALAY TRIPLE NEGATIVE BREAST CANCER (TNBC) SUSCEPTIBILITY RISK

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Abstract

Triple-negative breast cancer (TNBC) is characterized by the absence of estrogen receptors (ER), progesterone receptors (PR), and the lack of amplification of the human epidermal growth factor 2 (HER2) receptor. Adenosine triphosphate-binding cassette (ABC) subfamily B member 1 (ABCB1) serves as a drug efflux transporter, facilitating the translocation of various substrates (xenobiotics, toxins, carcinogens, etc.) across the membrane in an ATP-dependent manner. Genetic variations on *ABCB1* may lead to reduced substrate specificity, stability, and gene expression, thereby influencing the efflux activity of the protein. These variations can ultimately impact an individual's susceptibility to cancer. The present study aims to investigate the association of polymorphisms in *ABCB1* (1236 C > T, 2677 G > T/A, and 3435 C > T) in modulating the individual susceptibility risk to TNBC. DNA was extracted from blood samples collected from 75 TNBC patients and 100 healthy controls. Genotyping was performed using the PCR-RFLP technique, and the resulting genotype patterns were categorized into homozygous wild type, heterozygous, and homozygous variants. The association between genotype and TNBC and clinicopathological variables was assessed using the independent $\chi 2$ test. The strength of the association was determined by calculating the odds ratio (OR) with a 95% confidence interval. Subsequently, linkage disequilibrium and haplotype association analyses were performed to evaluate the association of the *ABCB1* haplotype with TNBC susceptibility. Overall, carriers of TT genotype and T allele of *ABCB1* 1236 C > T exhibited an increased OR of 2.750 (95% CI: 1.054–7.175) and 1.545 (95% CI: 1.001–2.385) for developing TNBC. Specifically, the *ABCB1* 1236 C > T variant is significantly associated with early age at diagnosis, advanced TNM staging, and the metaplastic/medullary subtype of carcinoma. Lastly, the haplotype 1236C/3435T/2677G was also associated with a reduced risk of TNBC. In summary, *ABCB1* 1236 C > T polymorphism was associated with an increased risk of TNBC susceptibility and correlated with early age diagnosis, high tumor staging, and the metaplastic/medullary subtype in TNBC patients. This suggests a potential predictive role in TNBC susceptibility and development for this polymorphism.

INTRODUCTION

Breast cancer stands as the most prevalent cancer in women, with an estimated 2.3 million cases worldwide, constituting 24.5% of all cancer instances [1]. The advent of molecular and genetic tools has enabled the characterization of breast cancer based on molecular and clinical manifestations. Typically, breast cancer is categorized by assessing the presence of estrogen receptor (ER), progesterone receptor (PR), and HER2 amplification. Triple-negative breast cancer (TNBC) represents a distinct subtype characterized by the absence of both ER and PR, along with a lack of HER2 amplification. TNBC constitutes approximately 10%–20% of all breast cancer cases [2]. Notably, significant racial disparities exist in TNBC incidence, with women of African ancestry exhibiting a higher prevalence (20%) among all breast cancer cases. In contrast, this prevalence is lower among women of European (9%), Hispanic ancestry (13%), and Asian descent (9%) [3].

TNBC is recognized for its aggressive manifestation and is associated with a worse prognosis compared to other subtypes of breast cancer. For example, a study by Dent et al. [4] revealed that TNBC poses a higher risk of lymph node involvement, with significantly larger size and higher tumor grading. Additionally, TNBC is linked to a higher risk of distant recurrence and death within five years of diagnosis, leading to overall poorer survival compared to other breast cancer types [4,5]. Due to the molecular nature of TNBC, endocrine and molecular targeted therapies rarely achieve clinical improvement in patients, resulting in limited treatment options for this subtype.

The *ABCB1* gene, also known as *MDR1,* encodes for P-glycoproteins (PGP), which are transporter and channel proteins forming a pore and nucleotide-binding domains for the ATP-dependent translocation of substrates across the membrane. The *ABCB1* gene is located in the chromosomal region 7q21 with a genomic sequence of 209 kb and a mRNA length of 4872 bp, resulting in a protein of 1280 amino acids [6]. In normal tissue, PGP is primarily expressed in a polarized cell and organ-specific manner. It protects by limiting xenobiotic absorption in the gastrointestinal tract and facilitating their excretion in the kidney and liver [7]. Consequently, genetic variations that alter protein function and expression can significantly impact the absorption, distribution, and elimination of xenobiotics and carcinogens. This notion is supported by a study conducted by Salama et al., which demonstrated reduced PGP activity in the variant *ABCB1*$_{1236T/2677T/3435T,}$ resulting in approximately 80%–100% loss of efflux activity on xenobiotics [8].

Among the variants, the single nucleotide polymorphisms (SNPs) 1236 C > T (rs1128503), 2677 G > T/A (rs2032582), and 3435 C > T (rs1045642) are the most prominent, located in the coding region of the gene. These SNPs account for 31.2% (1236 C > T), 52.2% (2677 G > T), 15.2% (2677 G > A), and 37.5% (3435 C > T) in the Asian population [9]. Several studies have suggested an association of these variants with different types of cancer, such as colorectal cancer and lung cancer [10,11]. In the case of TNBC, the haplotype *ABCB1* 3435T–1236T–2677T and *ABCB1* 3435T–1236G–2677T have been observed to be associated with an increased risk of chemoresistance [12].

Despite much evidence suggesting the association of *ABCB1* polymorphisms with various types of cancer, information regarding the association between these genes and TNBC predisposition is limited and still being explored. Thus, this study aimed to investigate the association between *ABCB1* polymorphisms and TNBC susceptibility risk, providing insight into the predictive value of *ABCB1* gene polymorphisms.

MATERIALS AND METHOD

Study population: This study received approval from the Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM) and complies with the Declaration of Helsinki (USM/JEPeM/20060343). This case-control study was conducted at Hospital Universiti Sains Malaysia in Kubang Kerian, Kelantan, Malaysia. A total number of 75 Malay breast cancer patients with histopathologically confirmed TNBC subtype (ER-, PR- and no amplification of HER2/neu) by immunohistochemistry (IHC) were recruited as the case subjects. The control group comprised 100 Malay cancer-free, healthy individuals biologically unrelated to the case subject. Informed consent was obtained from all the subjects before sample collection. Subsequently, 3 mL of peripheral blood was collected from all the subjects, and clinical and pathological variables, including age at diagnosis, histological type, staging according to TNM classification, lymph node involvement, menopausal status, and the presence of recurrence, were retrieved from their medical records.

SNP Genotyping: Genomic DNA from leukocytes in the blood was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany). The *ABCB1* 1236 C > T, 2677 G > T/A, and 3435 C > T polymorphisms were determined using the PCR-RFLP technique with specific restriction enzymes, following the protocol outlined by Abdul Aziz et al. [12]. Genotypes were classified into homozygous wild type, heterozygous, and homozygous variant. To validate the results, PCR products were chosen and sent for sequencing (1st BASE, Apical Scientific Sdn. Bhd., Malaysia).

Statistical analysis: The statistical analysis was primarily conducted using SPSS statistics 27 (IBM Corp., Armonk, NY, USA). The genotype distribution was examined for deviation from the Hardy-Weinberg equilibrium (HWE) using the $\chi 2$ test of goodness-of-fit. The association of genotypes and alleles for each SNP between TNBC patients and healthy controls was assessed using the independent $\chi 2$ test, and the strength of association was calculated by deriving odds ratio (ORs) with 95% confidence intervals through binary logistic regression analysis. The association between genotype and clinicopathological variables was explored using the independent $\chi 2$ test. A significance level of p <0.05 was considered for all the analyses. Linkage disequilibrium (LD) between SNPs was calculated by using SHEsis [13]. Evidence was presented for the haplotype block of *ABCB1* 1236 C > T, 2677 G > T/A, and 3435 C > T. Consequently, haplotype analysis was adopted to determine the association between the block and TNBC.

RESULT

Genotype and allele distribution of *ABCB1* polymorphisms

Genotype distribution in patients and controls conformed to the Hardy-Weinberg equilibrium ($p > 0.05$). Genotype and allele frequencies of *ABCB1* polymorphisms are presented in Table 1. The *ABCB1* 1236 CC genotype was significantly higher in controls (22.0%) compared to TNBC cases (10.7%) with $p = 0.049$. Conversely, the allele T of the *ABCB1* 1236 C > T polymorphism was found to be significantly higher in TNBC patients (64.0%) compared to controls (53.5%) with $p = 0.049$ respectively. Other SNPs did not exhibit significant differences in genotype and allele frequencies between TNBC patients and controls ($p > 0.05$).

Table 1. Genotype and allele frequencies of *ABCB1* polymorphisms in TNBC patients and healthy control.

	TNBC Patients, N=75 (%)	Controls, N=100(%)	χ^2	*p*	OR (95% CI)
ABCB1 1236 C > T					
Genotype					
CC	8 (10.7)	22 (22.0)	3.876	**0.049**	Ref
CT	38 (50.7)	49 (49.0)	0.048	0.827	2.133 (0.856–5.316)
TT	29 (38.7)	29 (29.0)	1.807	0.179	2.750 (1.054–7.175)
Allele					
C	54 (36.0)	93 (46.5)	3.879	**0.049**	Ref
T	96 (64.0)	107 (53.5)			1.545 (1.001–2.385)
ABCB1 3435 C > T					
Genotype					
CC	21 (28.0)	31 (31.0)	0.185	0.667	Ref
CT	45 (60.0)	56 (56.0)	0.281	0.596	1.186 (0.602–2.339)
TT	9 (12.0)	13 (13.0)	0.039	0.844	1.022 (0.371–2.819)
Allele					
C	87 (58.0)	118 (59.0)	0.035	0.851	Ref
T	63 (42.0)	82 (41.0)			1.042 (0.678–1.601)
ABCB1 2677 G > T/A					
Genotype					
GG	17 (22.7)	28 (28.0)	0.638	0.424	Ref
GT/GA	38 (50.7)	43 (43.0)	1.013	0.314	1.455 (0.692–3.063)
TT/TA/AA	12 (16.0)	14 (14.0)	0.136	0.713	1.412 (0.531–3.756)
Allele					
G	76 (50.7)	106 (53.0)	1.499	0.472	Ref
T	66 (44.0)	78 (39.0)			1.180 (0.760–1.834)
A	8 (5.3)	16 (8.0)			0.697 (0.284–1.713)

The bold values signify *p*-value < 0.05, statistically significant. Abbreviation: CI, confidence interval; N, number of individuals; OR, odds ratio; Ref, reference.

Genetic association analysis

The association test was conducted to analyze the correlation between genotype and allele and the susceptibility risk of TNBC. The results demonstrated that the genotype (TT) of *ABCB1* 1236 C > T significantly increased the risk of TNBC susceptibility, with an OR of 2.750 (95% CI: 1.054–7.175). Conversely, the T allele was found to increase the risk of TNBC susceptibility, with an OR of 1.545 (95% CI: 1.001–2.385). However, no significant association was observed between *ABCB1* 3435 C > T and 2677 G > T/A polymorphisms and TNBC susceptibility risk.

Subsequently, the association between *ABCB1* polymorphisms and clinicopathological variables was examined. The results revealed a significant association between the *ABCB1* 1236 C > T polymorphism and the age of diagnosis, TNM staging, and histologic type of carcinoma (Table 2). The heterozygous CT carrier was notably more prevalent in patients with age < 50 during diagnosis, almost doubling in proportion compared to patients aged > 50 ($p = 0.017$). Furthermore, a higher proportion of CT carriers was found in stage III patients compared to lower stages I and II ($p = 0.042$), with a calculated OR of 13.837 (95% CI: 0.745–256.865) versus CC carriers. In the TT versus CC model, it was 7.878 (95% CI: 0.411–151.142). A significant difference in genotype distribution was also observed when patients were further divided according to histologic types ($p = 0.024$). A higher frequency of CT carriers was observed in patients with metaplastic and medullary carcinoma compared to CC carriers. A higher frequency of TT carriers was observed in patients with IDC compared to metaplastic and medullary carcinoma. The calculated OR of CT carriers vs. CC for metaplastic and medullary versus IDC was 9.0 (95% CI: 0.481–168.131), and in the TT vs CC model, it was 2.245 (95% CI: 0.105–47.997).

Table 2. Correlation of *ABCB1* 1236 C > T polymorphisms with clinicopathological variables in TNBC patients.

			Genotype			
		N	CC	CT	TT	*p*
				N (%)		
Age at diagnosis	<50	42	2 (4.8)	27 (64.3)	13 (31.0)	**0.017**
	>50	33	6 (18.2)	11 (33.3)	16 (48.5)	
Menopausal status	Premenopause	49	5 (10.2)	28 (57.1)	16 (32.7)	0.302
	Menopause	26	3 (11.5)	10 (38.5)	13 (50.0)	
Histologic grade	G1 or G2	34	5 (14.7)	13 (38.2)	16 (47.1)	0.141
	G3	41	3 (7.3)	25 (61.0)	13 (31.7)	
TNM stage	Stage I or II	49	8 (16.3)	21 (42.9)	20 (40.8)	**0.042**
	Stage III	26	0	17 (65.4)	9 (34.6)	
Histologic type	IDC	59	8 (13.6)	25 (42.4)	26 (44.1)	**0.024**
	Metaplastic and medullary	16	0	13 (81.3)	3 (18.8)	
Recurrence status	Recurrent	25	2 (8.0)	17 (68.0)	6 (24.0)	0.122
	Not recurrent	50	6 (12.0)	21 (42.0)	23 (46.0)	
Lymph node involvement	Present	40	2 (5.0)	23 (57.5)	15 (37.5)	0.187
	Absent	35	6 (17.1)	15 (42.9)	14 (40.0)	

The bold values signify *p*-value < 0.05, statistically significant. Abbreviation: N, number of individuals.

On the other hand, *ABCB1* 3435 C > T and *ABCB1* 2677 G > T/A polymorphisms did not show any association with the age at diagnosis, menopausal status, histologic grade, TNM staging, histologic type of carcinoma, recurrence status, and lymph node involvement.

Table 3. *ABCB1* 2677 G > T/A polymorphisms correlate with clinicopathological variables in TNBC patients.

		N	Genotype GG	GT	GA	TT	TA	*p*
			N (%)					
Age at diagnosis	<50	42	11 (26.2)	22 (52.4)	1 (2.4)	4 (9.5)	4 (9.5)	0.102
	>50	33	6 (18.2)	16 (48.5)	3 (9.1)	8 (24.2)	0	
Menopausal status	Premenopause	49	12 (24.5)	24 (49.0)	3 (6.1)	6 (12.2)	4 (8.2)	0.517
	Menopause	26	5 (19.2)	14 (53.8)	1 (3.8)	6 (23.1)	0	
Histologic grade	G1 or G2	34	7 (20.6)	17 (50.0)	2 (5.9)	6 (17.6)	2 (5.9)	0.992
	G3	41	10 (24.4)	21 (51.2)	2 (4.9)	6 (14.6)	2 (4.9)	
TNM stage	Stage I or II	49	11 (22.4)	28 (57.1)	3 (6.1)	6 (12.2)	1 (2.0)	0.229
	Stage III	26	6 (23.1)	10 (38.5)	1 (3.8)	6 (23.1)	3 (11.5)	
Histologic type	IDC	59	15 (25.4)	28 (47.5)	3 (5.1)	11 (18.6)	2 (3.4)	0.268
	Metaplastic and medullary	16	2 (12.5)	10 (62.5)	1 (6.3)	1 (6.3)	2 (12.5)	
Recurrence status	Recurrent	25	7 (28.0)	10 (40.0)	2 (8.0)	3 (12.0)	3 (12.0)	0.239
	Not recurrent	50	10 (20.0)	28 (56.0)	2 (4.0)	9 (18.0)	1 (2.0)	
Lymph node involvement	Present	40	8 (20.0)	18 (45.0)	3 (7.5)	8 (20.0)	3 (7.5)	0.560
	Absent	35	9 (25.7)	20 (57.1)	1 (2.9)	4 (11.4)	1 (2.9)	

The bold values signify *p*-value < 0.05, statistically significant. Abbreviation: N, Number of individuals.

Table 4. Correlation of *ABCB1* 3435 C > T polymorphisms with clinicopathological variables in TNBC patients.

		N	Genotype CC	CT	TT	*p*
			N (%)			
Age at diagnosis	<50	42	12 (28.6)	25 (59.5)	5 (11.9)	1.000
	>50	33	9 (27.3)	20 (60.6)	4 (12.1)	
Menopausal status	Premenopausal	49	15 (30.6)	28 (57.1)	6 (12.2)	0.829
	Menopause	26	6 (23.1)	17 (65.4)	3 (11.5)	
Histologic grade	G1 or G2	34	9 (26.5)	21 (61.8)	4 (11.8)	0.944
	G3	41	12 (29.3)	24 (58.5)	5 (12.2)	
TNM stage	Stage I or II	49	15 (30.6)	30 (61.2)	4 (8.2)	0.383
	Stage III	26	6 (23.1)	15 (57.7)	5 (19.2)	
Histologic type	IDC	59	18 (30.5)	33 (55.9)	8 (13.6)	0.418
	Metaplastic and medullary	16	3 (18.8)	12 (75.0)	1 (6.3)	
Recurrence status	Recurrent	25	8 (32.0)	15 (60.0)	2 (8.0)	0.774
	Not recurrent	50	13 (26.0)	30 (60.0)	7 (14.0)	
Lymph node involvement	Present	40	11 (27.5)	25 (62.5)	4 (10.0)	0.891
	Absent	35	10 (28.6)	20 (57.1)	5 (14.3)	

The bold values signify *p*-value < 0.05, statistically significant. Abbreviation: N, Number of individuals.

Haplotype analysis

Next, haplotype analysis was employed to study further the association of *ABCB1* polymorphisms with TNBC susceptibility risk. The highest haplotype frequency was observed in the *ABCB1* 1236T/3435T/2677T haplotype among the TNBC patients and control groups, accounting for 40.0% and 34.0%, respectively. A significant association was found in the *ABCB1* 1236C/3435T/2677G haplotype, with a higher distribution observed in controls (6.0%) compared to patients (1.0%) with OR = 0.115, 95% CI: 0.014–0.903, $p = 0.013$.

Table 5. *ABCB1* haplotype analysis on TNBC patients and controls.

1236 C > T	3435 C > T	2677 G > T/A	TNBC Patients (frequency)	Controls (frequency)	$\chi 2$	*p*
C	C	G	45 (0.30)	66 (0.33)	0.356	0.550
T	T	T	60 (0.40)	68 (0.34)	1.330	0.248
T	C	G	28 (0.19)	26 (0.13)	2.109	0.146
T	C	T	6 (0.04)	10 (0.05)	0.196	0.657
C	C	A	8 (0.05)	16 (0.08)	0.954	0.328
C	T	G	1 (0.01)	11 (0.06)	6.047	**0.013**

The bold values signify *p*-value < 0.05, statistically significant.

DISCUSSION

ABCB1 encodes PGP, responsible for translocating various substrates across the membrane. The gene is highly polymorphic, consisting of approximately 66 SNPs in the coding region [14]. Among these variants, 1236 C > T (rs1128503), 2677 G > T/A (rs2032582), and 3435 C > T (rs1045642) have been extensively studied for their roles in cancer development and susceptibility. Earlier research suggests that *ABCB1* 1236 C > T and 3435 C > T polymorphisms reduce xenobiotic elimination by PGP through a 'silent mutation' [15], while 2677 G > T/A alters the amino acid sequence from alanine to serine. Consequently, PGP's compromised xenobiotic elimination activity contributes to carcinogenesis, considering its protective role against carcinogens [16].

Our study aimed to evaluate the association of *ABCB1* polymorphisms with TNBC susceptibility risk. The association analysis indicates the correlation of *ABCB1* with TNBC, particularly where 1236 C > T shows a significantly increased risk of TT genotype and T allele carrier developing TNBC. The present study's findings are consistent with a previous study conducted by Azevedo Delou et al. [17], suggesting that TT genotype carriers of 1236 C > T, exhibiting reduced expression of *ABCB1* mRNA, showed triple-negative tumor development and early onset of disease progression. In contrast, two studies observed that the *ABCB1* 1236 C > T polymorphism variant was associated with a reduced risk of breast cancer [18,19].

Additionally, our present investigation revealed that the *ABCB1* polymorphisms were differently represented among patients aged > 50 and < 50 during diagnosis. The incidence rate was more than double in patients < 50 years old carrying the heterozygote T compared to patients > 50 years old. Notably, TNBC occurs more frequently in the group under the age of 40 compared to other subtypes [20]. The age of diagnosis might reflect differences in molecular features and the level of exposure to certain risk factors, such as hormonal levels, oral contraceptive use practices, parity history, and the duration of breastfeeding. For instance, younger age TNBC patients are associated with higher expression of proliferation genes such as *CEP55*, *MYBL2,* and *KIF2C* and are more likely to be diagnosed with the basal-like subtype [21], as well as BRCA mutation since BRCA mutation is more often associated with younger age and TNBC [22,23]. Moreover, it is essential to note that PGP expression is modulated by the levels of steroid hormones such as estrogen and progesterone [24,25]. A study by Gutierrez-Rubio et al. [19] demonstrated that a higher distribution of TNBC in the

premenopausal group and T carrier of 3435 C > T were more prone to developing breast cancer than the postmenopausal group. However, our findings demonstrated a contrasting result where no correlation was observed between the menopausal status of TNBC patients and the presence of *ABCB1* 1236 C > T and 3435 C > T variants. This divergence in findings may be attributed to various factors, including the difference in premenopausal age (43.5 ± 6.2 in our study versus 39.5 ± 5.0 in the Mexican population), as well as other physical and gynecological variables like parity history [33], mammographic density [34], and weight gain [35].

A significant difference was observed in the distribution of genotypes when the patients were further divided based on TNM staging and histologic type. For instance, the heterozygote carrier T was more prominent in patients with stage III and rarer histologic types of carcinomas, such as metaplastic and medullary carcinoma. Findings from several studies suggested a significant connection of *ABCB1* polymorphisms with clinical parameters such as tumor grade [26], OS, and PFS [10,27,28].

Regarding haplotype analysis, the present study was concordant with other studies, where the frequency of *ABCB1* 1236T/2677T/3435T haplotypes was higher in breast cancer cases than in controls [27]. Additionally, the present study found that *ABCB1* 1236T/2677G/3435T haplotypes were associated with reduced TNBC susceptibility risk. This result aligned with a study conducted by Abuhaliema et al. [29], where the *ABCB1* 1236T/2677G/3435T haplotype was significantly associated with reduced risk in Jordanian breast cancer patients. In another disease, Phutong et al. [30] found that the *ABCB1* 1236C/2677G/3435T haplotype was significantly associated with an increased risk of cervical cancer in Northern Thailand.

However, this study also has limitations. First, there is a lack of TNBC patients due to the low incidence of TNBC cases. This limitation might affect the study's power, where the *p*-value was not significant despite a high OR value, and a wide 95% confidence interval (CI) was observed. The rarity of TNBC, making it the least common form among all other breast cancer subtypes, needs to be considered in this case. In Malaysia, a previous study demonstrated that the frequency of TNBC accounted for 12.3% to 17.6% of total breast cancer cases [31,32]. Thus, recruiting an adequate number of clinically and histopathologically confirmed patients as TNBC has been a challenge for the present study. On the other hand, differences in the genetic background of study subjects or populations might explain the differing results between the present study and previous studies. It is crucial to highlight that the current study mainly included subjects from the east coast region of Malaysia, which predominantly consists of Malay populations.

CONCLUSION

In conclusion, *ABCB1* 1236 C > T polymorphism was associated with an increased risk of TNBC susceptibility and correlated with early-age diagnosis, high tumor staging, and metaplastic/medullary subtype in TNBC patients. Thus, the results suggest a potential role of the *ABCB1* 1236 C > T polymorphism in modulating TNBC susceptibility risk. On the other hand, the present study also aims to include other ABC transporter genes, such as *ABCG2* and *ABCC1,* to observe their collective effects on TNBC. Understanding ABC transporter gene polymorphisms may offer a valuable predictive biomarker for identifying high-risk populations, which, in turn, could facilitate the implementation of early surveillance measures on high-risk individuals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Boyle P. Triple-negative breast cancer: Epidemiological considerations and recommendations. Ann Oncol [Internet]. 2012;23(SUPPL. 6):vi7–12.
3. Clarke CA, Keegan THM, Yang J, Press DJ, Kurian AW, Patel AH, et al. Age-specific incidence of breast cancer subtypes: Understanding the black-white crossover. J Natl Cancer Inst. 2012;104(14):1094–101.
4. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res. 2007;13(15):4429–34.
5. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: A population-based study from the California Cancer Registry. Cancer. 2007;109(9):1721–8.
6. Bodor M, Kelly EJ, Ho RJ. Characterization of the human MDR1 gene. AAPS J. 2005;7(1):1–5.
7. Brinkmann U. Polymorphisms in the ABC drug transporter gene MDR1. Pharmacogenomics J. 2001;1(1):59–64.
8. Salama NN, Yang Z, Bui T, Ho RJY. MDR1 Haplotypes Significantly Minimize Intracellular Uptake and Transcellular P-gp Substrate Transport in Recombinant LLC-PK1 Cells. J Pharm Sci. 2006;95(10).
9. Ieiri I. Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). Drug Metab Pharmacokinet [Internet]. 2012;27(1):85–105.
10. Yue AM, Xie Z Bin, Zhao HF, Guo SP, Shen YH, Wang HP. Associations of ABCB1 and XPC genetic polymorphisms with susceptibility to colorectal cancer and therapeutic prognosis in a chinese population. Asian Pacific J Cancer Prev. 2013;14(5):3085–91.

11. Zawadzka I, Jeleń A, Pietrzak J, Żebrowska-Nawrocka M, Michalska K, Szmajda-Krygier D, et al. The impact of ABCB1 gene polymorphism and its expression on non-small-cell lung cancer development, progression and therapy – preliminary report. Sci Rep. 2020;10(1):1–10.
12. Abdul Aziz AA, Md Salleh MS, Mohamad I, Bhavaraju VMK, Yahya MM, Zakaria AD, et al. Genotypes and haplotypes of ABCB1 contribute to TAC chemotherapy response in Malaysian triple negative breast cancer patients. Meta Gene [Internet]. 2018;16(October 2017):21–7.
13. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, et al. A partition-ligation-combination-subdivision em algorithm for haplotype inference with multiallelic markers: Update of the SHEsis (http://analysis.bio- x.cn). Cell Res. 2009;19(4):519–23.
14. Tulsyan S, Mittal RD, Mittal B. The effect of ABCB1 polymorphisms on the outcome of breast cancer treatment. Pharmgenomics Pers Med. 2016;9:47–58.
15. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar S V., et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science (80-). 2007;315(5811):525–8.
16. Mochida Y, Taguchi K, Tsuneyoshi M, Kuwano H, Tsuzuki T, Kuwano M, et al. The role of P-glycoprotein in intestinal tumorigenesis : disruption of mdr1a suppresses polyp formation in Apc Min a mice. 2003;24(7):1219–24.
17. de Azevedo Delou JM, Vignal GM, Índio-do-Brasil V, de Souza Accioly MT, da Silva TSL, Piranda DN, et al. Loss of constitutive ABCB1 expression in breast cancer associated with worse prognosis. Breast Cancer Targets Ther. 2017;9:415–28.
18. Alsaif AA, Hasan TN, Shafi G, Syed NA, Alsaif MA, Al-Assaf AH, et al. Association of multiple drug resistance-1 gene polymorphism with multiple drug resistance in breast cancer patients from an ethnic Saudi Arabian population. Cancer Epidemiol [Internet]. 2013;37(5):762–6.
19. Gutierrez-Rubio SA, Quintero-Ramos A, Durán-Cárdenas A, Franco-Topete RA, Franco-Topete RA, Castro-Cervantes JM, et al. 1236 C/T and 3435 C/T polymorphisms of the ABCB1 gene in Mexican breast cancer patients. Genet Mol Res. 2015;14(1):1250–9.
20. McGuire A, Brown JAL, Malone C, McLaughlin R, Kerin MJ. Effects of age on the detection and management of breast cancer. Cancers (Basel). 2015;7(2):908–29.
21. Gulbahce HE, Bernard PS, Weltzien EK, Factor RE, Kushi LH, Caan BJ, et al. Differences in molecular features of triple-negative breast cancers based on the age at diagnosis. Cancer. 2018;124(24):4676–84.
22. Billar JAY, Dueck AC, Stucky CCH, Gray RJ, Wasif N, Northfelt DW, et al. Triple-negative breast cancers: Unique clinical presentations and outcomes. Ann Surg Oncol. 2010;17(SUPPL. 3):384–90.
23. Malone KE, Daling JR, Doody DR, Hsu L, Bernstein L, Coates RJ, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in White and Black American women ages 35 to 64 years. Cancer Res. 2006;66(16):8297–308.
24. Coles LD, Lee IJ, Voulalas PJ, Eddington ND. Estradiol and progesterone-mediated regulation of P-gp in P-gp overexpressing cells (NCI-ADR-RES) and placental cells (JAR). Mol Pharm. 2009;6(6):1816–25.
25. Kim WY, Benet LZ. P-glycoprotein (P-gp/MDR1)-mediated efflux of sex-steroid hormones and modulation of P-gp expression in vitro. Pharm Res. 2004;21(7):1284–93.
26. Ghafouri H, Ghaderi B, Amini S, Nikkhoo B, Abdi M, Hoseini A. Association of ABCB1 and ABCG2 single nucleotide polymorphisms with clinical findings and response to chemotherapy treatments in Kurdish patients with breast cancer. Tumor Biol. 2016;37(6):7901–6.
27. Wu H, Kang H, Liu Y, Tong W, Liu D, Yang X, et al. Roles of ABCB1 gene polymorphisms and haplotype in susceptibility to breast carcinoma risk and clinical outcomes. J Cancer Res Clin Oncol. 2012;138(9):1449–62.
28. Li W, Zhang D, Du F, Xing X, Wu Y, Xiao D, et al. ABCB1 3435TT and ABCG2 421CC genotypes were significantly associated with longer progression-free survival in Chinese breast cancer patients. Oncotarget. 2017;8(67):111041–52.
29. Abuhaliema AM, Yousef AMF, El-Madany NN, Bulatova NR, Awwad NM, Yousef MA, et al. Influence of genotype and haplotype of MDR1 (C3435T, G2677A/T, C1236T) on the incidence of breast cancer - a case-control study in Jordan. Asian Pacific J Cancer Prev. 2016;17(1):261–6.
30. Phuthong S, Settheetham-Ishida W, Natphopsuk S, Settheetham D, Ishida T. Haplotype analysis of MDR1 and risk for cervical cancer in Northeastern Thailand. Asian Pacific J Cancer Prev. 2017;18(7):1815–9.
31. Tan GH, Taib NA, Choo WY, Teo SH, Yip CH. Clinical Characteristics of Triple-negative Breast Cancer: Experience in an Asian Developing Country. Asian Pacific J Cancer Prev. 2009;10:395–8.
32. Kanapathy Pillai SK, Tay A, Nair S, Leong CO. Triple-negative breast cancer is associated with EGFR, CK5/6 and c-KIT expression in Malaysian women. BMC Clin Pathol [Internet]. 2012;12(1):1. Available from: BMC Clinical Pathology
33. Slepicka PF, Cyrill SL, dos Santos CO. (2019). Pregnancy and Breast Cancer: Pathways to Understand Risk and Prevention. *Trends in Molecular Medicine*, *25*(10), 866–881.
34. Nazari SS, Mukherjee P. (2018). An overview of mammographic density and its association with breast cancer. *Breast Cancer*, *25*(3), 259–267.
35. Rosner B, Eliassen AH, Toriola AT, Hankinson SE, Willett WC, Natarajan L, Colditz GA (2015). Short-term weight gain and breast cancer risk by hormone receptor classification among pre- and postmenopausal women. *Breast Cancer Research and Treatment*, *150*(3), 643–653.