RESEARCH ARTICLE

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Association of *ABCG2* Polymorphisms on Triple Negative Breast Cancer (TNBC) Susceptibility Risk

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Abstract

Objective: The aim of this study was to elucidate the association of ATP-binding cassette super-family G member 2 (*ABCG2*) gene polymorphisms with individual susceptibility to Triple Negative Breast Cancer (TNBC) as well as clinicopathological variables in TNBC patients. Two common polymorphisms in Asian population, *ABCG2* 34 G>A and 421 C>A was selected in this study. **Methods:** Blood samples were collected from 75 TNBC patients and 83 controls. Genomic DNA was extracted from blood samples and the SNP genotyping was performed by using PCR-RFLP technique. The genotypes were characterized and grouped into homozygous wildtype, heterozygote and homozygous variant based on the band size. The result was subjected to statistical analysis. **Results:** The A allele and AA genotype of *ABCG2* 421 C>A had OR of 3.011 (p=0.003, 95% CI: 1.417-6.398) and 9.042 (p=0.011, 95% CI: 1.640-49.837), to develop advanced staging carcinoma respectively. The AA genotype of *ABCG2* 421 C>A polymorphism was also associated with metaplastic and medullary carcinoma with an OR of 6.429 (p=0.018, 95% CI: 1.373-30.109). A significant association was also found in haplotype 34G/421A of *ABCG2* with advanced cancer staging as well as metaplastic and medullary carcinoma with OR of 2.347 (p=0.032, 95% CI: 1.010-5.560) and 2.546 (p=0.008, 95% CI: 1.005-6.447), respectively. **Conclusion:** The present study suggests that *ABCG2* 421 C>A polymorphism was associated with metaplastic and medullary histology and advanced cancer staging in TNBC patients.

Keywords: TNBC- *ABCG2* polymorphisms- susceptibility risk

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Introduction

Breast cancer is the most diagnosed cancer type and the leading cause of cancer related mortality in women. The World Cancer Report in 2020 reported that breast cancer has the highest incidence rate among female in developed countries, recorded about 54.4 cases per 100 000 women (Hankinson et al., 2020). In 2018, an estimated 2.1 million of new cases were recorded worldwide, accounted for 11.6% within 36 cancer types. Among the breast cancer subtypes, Triple Negative Breast Cancer (TNBC) is known for its aggressive nature and poor prognosis. TNBC is defined by the absence of estrogen receptor (ER), progesterone receptor (PR) and no amplification of human epidermal growth factor receptor 2 (HER2) under immunohistochemistry (IHC) staining (Kumar and Aggarwal, 2016).

In compared with other subtypes of breast cancer, inferior prognosis is often associated with TNBC patients. TNBC tend to show a relatively higher ration of axillary lymphatic invasion, as well as higher rate of distant recurrence and poorer survival (Bauer et al., 2007; Dent et al., 2007; Li et al., 2013; Qiu et al., 2016). Also, the histologic grade and stage with more aggressive phenotype are common in TNBC (Qiu et al., 2016). Due to the molecular phenotype of TNBC, the endocrine therapy and molecular targeted drug rarely achieve clinically improvement and thus, chemotherapy remain to be the main systemic treatment option.

The present study focuses on ATP-binding cassette super-family G member 2 (*ABCG2*). ABCG2 is an ATP dependent efflux transporter that translocate metabolites and xenobiotics from cells. The gene is located at chromosome 4q22 and encodes for a 72-kDa membrane protein with 655 amino acids (Ieiri, 2012). ABCG2 has been found to facilitate the efflux of anti-cancer agents such as Adriamycin, Daunorubicin, Topotecan, and Mitoxantrone, mediating multidrug resistance (MDR) of cancer cells (Ross and Nakanishi, 2010). In normal tissue, ABCG2 is prominently expressed in areas such as

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epithelium of colon and small intestines, bile canaliculi of liver, placenta Syncytiotrophoblact as well as lobules and ducts of mammary glands (Maliepaard et al., 2001).

Despite their role in MDR of cancer cells, their distribution in different tissue part might indicate important physiological function. For instance, ABCG2 transporter is responsible to the biliary excretion of toxic metabolites such as sulfate and glucuronide conjugates of xenobiotics and hormones (Dietrich et al., 2003; Mo and Zhang, 2012). ABCG2 in blood brain barrier can restrict the access of xenobiotics into the brain (Aronica et al., 2005; Mo and Zhang, 2012). While in placenta, the ABCG2 on the plasma membrane of chrionic villi offer the protective effect to the foetus against toxic materials ingested by mother (Jonker et al., 2000).

Genetic variation might influence the function of the protein in terms of substrate specificity, stability, drug efflux as well as cancer susceptibility and treatment response. The expanding knowledge on human genome promote the individual analysis of patient and help us to access the possible genes that associate with TNBC. *ABCG2* 34 G>A and 421 C>A polymorphisms are two common variants to be found in Asian, accounted for 19.3% to 35.7% of the population (Ieiri, 2012). Two of these variants are associated with reduced expression and activity of ABCG2 protein (Kasza et al., 2012; Kondo et al., 2004) and had been extensively studied for their role to affect the susceptibility of various cancer.

The 34G>A polymorphism is mapped at exon 2, changes the amino acid translation from valine to methionine (Val12Met) at codon 12. It is close to the N-terminus of the polypeptide in the intracellular part of the protein which could disrupt the pump localization and insertion into the membrane (Heyes et al., 2018). On the other hand, the 421 C>A polymorphism alter the amino acid from glycine to lysine (Gly141Lys) and is mapped at exon 5. This substitution might alter the tertiary structure of protein which led to a greater susceptibility to degradation. Therefore, localization of ABCG2 on plasma membrane are affected (Furukawa et al., 2009; Ingelheim et al., 2002). Other than that, it is also located between the Walker A and B that play a functional role for ATP binding. Thus, this substitution will reduce the ATPase activity of the protein domain causing the disruption of the protein function (Mizuarai et al., 2004).

However, the evidence to elucidate the association of *ABCG2* polymorphisms in TNBC is still inconclusive and limited. Thus, this study was intended to investigate the predictive value of *ABCG2* polymorphisms on TNBC susceptibility and corelate with clinicopathological parameters.

Materials and Methods

Study design

The study was approved by Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/20060343) under Declaration of Helsinki. This is a case-control study that conducted in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan. The sample size was calculated using the two-proportion formula; with Po= 0.33 (Wu et al., 2015), P1=0.56, power=0.8 and significance level= 0.05 (two-tailed) (Arifin, 2017). Thus, at least 72 TNBC patients and 72 controls were needed for this study.

The subjects were recruited from year 2017 to 2023 and were selected based on the following criteria. The TNBC patients must be female and showed the histopathological characteristics of (1) negative for ER, (2) negative for PR and (3) no amplification of HER2. The subjects that have history of previous cancer or malignancy were excluded from the study. While for the controls, the subjects were recruited based on the following criteria, (1) sex and age matched, (2) biologically unrelated to study subjects and (3) cancer free. The subjects that have family history of breast cancer or any other types of cancer were excluded from our study. The expected final ration of control per cases was 1:1.

The written informed consent was obtained from subjects, and all the individuals were labelled with a study code to protect their privacy and confidentiality. Peripheral blood samples were collected and the clinicopathological information of cases such as age at diagnosis, menopausal status, recurrent status, lymph node involvement, histologic grade, TNM staging , and histologic type, were access from patients' medical records.

The genomic DNA was extracted from the blood sample and the SNP genotyping was carried out to determine the genotype and allele pattern of the subjects. The outcomes of this study would be the TNBC susceptibility risk under different genotype patterns and the exposure group was the variant carrier of each SNPs. The risk was predicted by deriving odds ratio, under adjustment of age with 95% confidence interval (CI).

SNP Genotyping

The genomic DNA was extracted from peripheral blood samples of subjects using QIAGEN QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was employed to genotype *ABCG2* 34 G>A and 421 C>A polymorphisms. The PCR was conducted in a volume of 20 µL reaction mixture using MyTaq™ DNA Polymerase (meridian BIOSCIENCE, Ohio, USA). The forward and reverse primer sequence for 34 G>A was 5'-CAGTAATGTCGAAGTTTTTATCGCA-3' and 5'-AAATGTTCATAGCCAGTTTCTTGGA-3' respectively, and 421 C>A was 5'-GTTGTGATGGGCACTCTGATGGT-3' and 5'-CAAGCCACTTTTCTCATTGTT-3'. The amplification was carried out using Agilent SureCycler 8800 (Agilent Technologies, California, USA) with an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C (*ABCG2* 34 G>A)/58 oC (*ABCG2* 421 C>A) for 15 s and extension at 72 °C for 10 s, finished with final extension of 3 mins at 72 °C.

Next, the amplicons were incubated with restriction enzymes *BseMI* (*ABCG2* 34 G>A) and *TaaI* (*ABCG2* 421 C>A) under 55 °C and 65 °C respectively for 1 hour. The fragments of digestion were separated by using gel electrophoresis. The genotype was characterized into

homozygous wildtype, heterozygous, and homozygous variant based on the band size. For *ABCG2* 34 G>A, the homozygous wildtype was identified by a single band at 291 bp; the heterozygous genotype by 3 bands at 291 bp, 260 bp and 31 bp; and homozygous variants with 260 bp and 31 bp. While for *ABCG2* 421 C>A, the homozygous wildtype was identified by 2 bands at 252 and 37 bp; the heterozygous genotype with 3 bands at 289, 252 and 37 bp bp; and homozygous variants with a single band of 289 bp. The genotype of subjects was quantified and group according to the TNBC patients and controls group, as well as the subgroup under each clinicopathological variables.

Data Analysis

Distribution of genotype in both patient and control groups was tested for the deviation of Hardy-Weinberg equilibrium by using $\chi 2$ test of goodness-of-fit. To accessing the matching of age between cases and controls, the mean age of two groups was evaluated using independent T test and no significant difference was found between them (p=0.082).

The association of genotype and allele with TNBC susceptibility, as well as clinicopathological variables was tested by using independent $\chi 2$. P-value<0.05 (two-sided) indicates a statistical significance. The strength of association between genotype and TNBC susceptibility was calculated by deriving odds ratio, adjusted by age of subjects within 95% CI using logistic regression.

The TNBC patients were further separated into two categories for each clinicopathological variables. Their interaction with genotype was calculated by deriving odds ratio with 95% CI using binomial logistic regression. Multivariate analysis was performed to study the interaction between variables by using logistic regression with 95% CI. The statistical analysis was performed by using SPSS software version 27 (IBM, New York, USA).

Lastly, linkage disequilibrium (LD) of two SNPs was tested by using HaploView (Barrett et al., 2005). Since the LD was proven evident, the haplotype association test was adopted next to test for the effect of linked allele in polymorphisms and the risk of TNBC.

Results

Study Population

A total of 75 TNBC patients and 83 controls that fulfilled the selection criteria were recruited in this study. The mean age of the patients and controls were 49±9.58 and 46.3±9.56 respectively. The clinicopathological data of patients was summarized in Table S1.

Bivariate Analysis

The genotype frequencies among both patients and controls were in the Hardy Weinberg equilibrium (p>0.05). The allele G of *ABCG2* 34 G>A polymorphism attributed the most in both patients and controls, accounted for 64.7% and 62.7% respectively. The age adjusted OR of AA versus GG was 1.003 (95% CI: 0.391-2.575) and GA versus GG was 0.753 (95% CI: 0.379-1.497).

In the case of *ABCG2* 421 C>A, allele C contributed for 76.7% in TNBC patients and 70.5% in control group. The AA versus CC and CA versus CC model had the adjusted OR of 1.373 (95% CI: 0.459-4.104) and 0.605 (95% CI: 0.304-1.203) respectively. The genotype and allele distribution for both groups were summarized in Table 1. In general, no significant association was observed on these two polymorphisms and TNBC susceptibility risk (p>0.05).

The variant allele of *ABCG2* 34 G>A and 421 C>A also appear not to be associated with the age, nor menopausal status, recurrence status, lymph node involvement and histologic grade. Conversely, the variant allele was found to be significantly associated with tumour staging and histologic type in TNBC patients (Table 2). Indeed, the *ABCG2* 421 C>A variant genotype frequency was higher in TNBC patients with advanced staging (Stage III) and with histologic type of metaplastic and medullary carcinoma, while the frequency of CC genotype was more frequent in Stage I&II and IDC (p=0.015 and 0.028 respectively).

A binomial logistic regression analysis was further carried out to model the association of *ABCG2* 421 C>A with tumour staging and histologic type of carcinoma in patients. The patient with variant (AA) genotype had an increased risk of 9.042 (p=0.011, 95% CI: 1.640-49.837) times to develop advanced staging carcinoma (Stage III). As for the histologic type of carcinoma, the presence of variant genotype increases the risk of developing rarer

Table 1. The Genotype and Allele Frequency of *ABCG2* Polymorphisms and Risk of TNBC Susceptibility.

	TNBC Patients, N=75 (%)	Controls, N=83 (%)	p[a]	Adjusted OR[b] (95% CI)
ABCG2 34 G>A				
Genotype				
GG	34 (45.3)	33 (39.8)	0.66	1 (Ref)
GA	29 (38.7)	38 (45.8)		0.753 (0.379-1.497)
AA	12 (16.0)	12 (14.4)		1.003 (0.391-2.575)
Allele				
G	97 (64.7)	104 (62.7)	0.91	1 (Ref)
A	53 (35.3)	62 (37.3)		1.053 (0.668-1.658)
ABCG2 421 C>A				
Genotype				
CC	43 (57.3)	41 (49.4)	0.3	1 (Ref)
CA	23 (30.7)	35 (42.2)		0.605 (0.304-1.203)
AA	9 (12.0)	7 (8.4)		1.373 (0.459-4.104)
Allele				
C	109 (76.7)	117 (70.5)	0.96	1 (Ref)
A	41 (27.3)	49 (29.5)		1.036 (0.640-1.677)

Abbreviations: CI, Confidence interval; N, Number of individuals; OR, Odds ratio; Ref, reference.; a. P-value was assessed by independent $\chi 2$ test, p<0.05 indicates statistically significant; b. The calculated OR was adjusted by the age of subjects.

Table 2. Correlation of *ABCG2* Polymorphisms with Clinicopathological Variables in TNBC Patients

			ABCG2 34 G>A				*ABCG2* 421 C>A			
		N	Genotype			p[a]	Genotype			p[a]
			GG	GA	AA		CC	CA	AA	
			N (%)				N (%)			
Age at diagnosis	<50	42	20 (47.6)	16 (38.1)	6 (14.3)	0.864	23 (54.8)	14 (33.3)	5 (11.9)	0.847
	>50	33	14 (42.4)	13 (39.4)	6 (18.2)		20 (60.6)	9 (27.3)	4 (12.1)	
Menopausal status	Premenopausal	49	22 (44.9)	20 (40.8)	7 (14.3)	0.805	27 (55.1)	16 (32.7)	6 (12.2)	0.856
	Menopause	26	12 (46.2)	9 (34.6)	5 (19.2)		16 (61.5)	7 (26.9)	3 (11.5)	
Recurrence status	Recurrent	25	13 (52.0)	8 (32.0)	4 (16.0)	0.671	15 (60.0)	6 (24.0)	4 (16.0)	0.58
	Not recurrent	50	21 (42.0)	21 (42.0)	8 (16.0)		28 (56.0)	17 (34.0)	5 (10.0)	
Lymph node involvement	Present	40	20 (50.0)	14 (35.0)	6 (15.0)	0.683	22 (55.0)	11 (27.5)	7 (17.5)	0.283
	Absent	35	14 (40.0)	15 (42.9)	6 (17.1)		21 (60.0)	12 (34.3)	2 (5.7)	
Histologic grade	G1 or G2	34	17 (50.0)	11 (32.4)	6 (17.6)	0.593	21 (61.8)	11 (32.4)	2 (5.9)	0.331
	G3	41	17 (41.5)	18 (43.9)	6 (14.6)		22 (53.7)	12 (29.3)	7 (17.1)	
Staging	Stage I or II	49	20 (40.8)	21 (42.9)	8 (16.3)	0.525	31 (63.3)	16 (32.7)	2 (4.1)	**0.015**
	Stage III	26	14 (53.8)	8 (30.8)	4 (15.4)		12 (46.2)	7 (26.9)	7 (26.9)	
Histologic type	IDC	59	23 (39.0)	26 (44.1)	10 (16.9)	0.095	36 (61.0)	19 (32.2)	4 (6.8)	**0.028**
	Metaplastic and medullary	16	11 (68.8)	3 (18.8)	2 (12.5)		7 (43.8)	4 (25.0)	5 (31.3)	

Abbreviation: N, Number of individuals; a, P-value was assessed by independent $\chi 2$ test; Bold values signify $p<0.05$, statistically significant.

histologic type (metaplastic and medullary carcinoma) by 6.429-time (p=0.018, 95% CI: 1.373-30.109) (Table S2).

Multivariate Analysis

Next, multinomial logistic regression was adopted to test whether the tumour staging of patients could be predicted by *ABCG2* genotype and histologic type of carcinoma (Table S3). The analysis suggested the association of *ABCG2* 421 C>A with tumour staging, and histologic type did not act as a cofounding factor (p=0.484). Besides, the histologic type of carcinoma was also modelled by *ABCG2* 421 C>A genotype, recurrence status, histologic grade, tumour staging and lymph node involvement status to observe the interaction with these independent groups (Table S4). In consistent with bivariate analysis, significant association was found in between AA genotype and histologic type with OR: 11.301 (p=0.010, 95% CI: 1.775-71.954) for Metaplastic and Medullary Carcinoma versus Invasive Ductal Carcinoma (IDC) model. Besides, the Metaplastic and Medullary Carcinoma was also found to correlate with the reduced recurrence of patient with calculated OR of 0.216 (p=0.022, 95% CI: 0.058-0.802) with reference to IDC. In contrast, we found out that the patients with IDC have an increased OR of 4.633 (p=0.022, 95% CI: 1.247-17.218) to recurrent in compared to the Metaplastic and Medullary Carcinoma.

Haplotype and Allele Association Analysis

Haplotype and allele analysis were also carried out to further study the association of *ABCG2* polymorphisms

Table 3. Haplotype and Allele Association Analysis of Histologic Type and Tumor Staging in TNBC Patients.

				Histologic Type			Staging			
34 G>A	421 C>A	Frequency	IDC (frequency)	Metaplastic and medullary (frequency)	p[a]	OR (95%CI)	Stage I & II (frequency)	Stage III (frequency)	p[a]	OR (95% CI)
G	-	-	72 (0.610)	25 (0.781)	0.073	Ref	61 (0.622)	36 (0.692)	0.394	Ref
A	-	-	46 (0.390)	7 (0.219)		0.438 (0.175-1.095)	37 (0.378)	16 (0.308)		0.733 (0.358-1.500)
-	C	-	91 (0.771)	20 (0.625)	0.094	Ref	80 (0.816)	31 (0.596)	**0.003**	Ref
-	A	-	27 (0.229)	12 (0.375)		2.022 (0.878-4.659)	18 (0.184)	21 (0.404)		3.011 (1.417-6.398)
G	C	0.392	47.7 (0.404)	11.1 (0.347)	0.55	Ref	41.5 (0.423)	17.3 (0.333)	0.281	Ref
G	A	0.255	24.3 (0.206)	13.9 (0.435)	0.008	2.546 (1.005-6.447)	19.5 (0.199)	18.7 (0.359)	**0.03**2	2.347 (1.010-5.560)
A	C	0.335	43.3 (0.367)	6.9 (0.216)	0.109	0.71 (0.253-1.996)	36.5 (0.373)	13.7 (0.263)	0.176	0.935 (0.406-2.153)
A	A	0.019	2.7 (0.023)	0.1 (0.002)	0.446	1.021 (0.104-9.985)	0.5 (0.005)	2.3 (0.045)	0.087	4.941 (0.420-58.168)

Abbreviations: CI, Confidence interval; OR, Odds ratio; Ref, Reference; a.The p-value was assessed by independent $\chi 2$ test. Bold values signify $p<0.05$, statistically significant.

with tumour staging and histologic type. The allele A of *ABCG2* 421 C>A showed a significantly association with advanced staging (Stage III) with calculated OR: 3.011 (p=0.003, 95% CI: 1.417-6.398). Furthermore, a significant association was observed on 34G/421A and the histologic type with calculated OR of 2.546 (p=0.008, 95% CI: 1.005-6.447) for metaplastic and medullary carcinoma, in versus IDC (Table 3). Also, The OR of Stage III patient with haplotype of 34G/421A was 2.347 (p=0.032, 95% CI: 1.010-5.560).

Discussion

Overall, there is not enough evidence to illustrate the association of TNBC susceptibility. However, the AA genotype, variant allele of *ABCG2* 421 C>A and haplotype 34G/421A were found to associate with advanced staging and metaplastic and medullary carcinoma. To the best of the available knowledge, this is the first study investigating the *ABCG2* polymorphisms as a potential biomarker for the susceptibility risk among Malaysian TNBC patients and correlate the *ABCG2* polymorphisms to clinicopathological variables. The *ABCG2* polymorphisms had been extensively studied for their association with various cancer susceptibility, while for breast carcinoma, however, the evidence remains inconclusive and limited especially for TNBC.

Wu et al., (2015) showed that the variant allele carrier of 34 G>A (OR: 1.163, p=0.016, 95% CI: 1.028–1.316) and 421 C>A (OR: 1.130, p=0.048, 95% CI: 1.001–1.276) had an increased risk of developing breast carcinoma in a Chinese population based study. In contrast, a Turkish population based study by Zeliha et al., (2020) showed a contrasting result where higher odds of developing breast carcinoma was demonstrated in wildtype carrier of 421 C>A (OR: 3.06, p=0.002, 95% CI: 1.49–6.25). On the other hand, Li et al., (2017) found no association between *ABCG2* 421 C>T and breast cancer. This variation of result might be attributed by the several factors such as difference of variant number cause by the participants pool as well as the consideration of other gene mutation status that closely linked to breast cancer such as BRCA (Paul and Paul, 2015). Other than breast cancer, the *ABCG2* 421 C>A polymorphism was also found to associate with several cancer such as multiple myeloma (Niebudek et al., 2019), leukaemia (Salimizand et al., 2016) and lung cancer (Zhang et al., 2018).

Breast cancer is a heterogenous disease with various histologic types. The most common form is IDC which contributed up to 80% of overall breast cancer (Weigelt et al., 2010). In the present finding, a higher prevalence of IDC was observed in TNBC patients (78.7%) which in agreement with the study of Qiu et al., (2016) stating that IDC remain to be the major type of carcinoma in TNBC as in non-TNBC. Other than that, TNBC also display other histological types such as medullary, metaplastic, adenoid cystic, pleomorphic lobular and secretory (Weigelt et al., 2010).

Our finding presented higher distribution of *ABCG2* 421 C>A genotype CC in patients with IDC in compared to the patients with metaplastic and medullary carcinoma. The model of multivariate analysis corelate IDC with the increased odds of cancer recurrent with OR of 4.633 (p=0.022, 95% CI: 1.247-17.218). To the best of our knowledge, no conclusive observation was drawn on the correlation of *ABCG2* polymorphisms and histologic type of TNBC. A similar study conducted by Ghafouri et al., (2016) demonstrated an opposing result where higher prevalence of AA carrier was observed in IDC patients in compared to invasive lobular carcinoma (ILC). In contrast, Wu et al., (2015) reported no significant correlation of *ABCG2* 34 G>A and 421 C>A polymorphisms with histologic type when comparing IDC and ILC group. The discrepancy in our result might be attributed to the diversity of histologic type within the group since no patients with rare histologic type was included in their study, whereas no ILC patient was identified in our study. Since no significant relevance was found in *ABCG2* 421 C>A allele association analysis with histologic type but in genotype and haplotype analysis, we suggest that *ABCG2* 421 C>A might not play a main role in determining the development of IDC, rather as a cofounding role or the result of disease development.

Overall, *ABCG2* 421 C>A was found to be significantly correlated to the tumour staging and such correlation are not affected by histologic types based on the multivariate analysis. Our result shows that AA genotype of *ABCG2* 421 C>A was more frequent in patients with advanced staging, and the A allele carrier had an increased chance of developing Stage III TNBC in compared to C allele carrier. Similar result was shown by Ghafouri et al., (2016) in Kurdish population where higher progression of breast cancer was observed in AA carrier.

Amino acid substitution that caused by *ABCG2* 421 C>A polymorphism can reduce the expression of protein by promoting substantial degradation and influencing the protein localization (Furukawa et al., 2009), as well as impairing the ATPase activity of ABCG2 (Mizuarai et al., 2004). This reduced activity of ABCG2 might provide a hint since studies had suggested the protective role of ABCG2. With the idea that the cancer stem cells (CSC) functions as a tumour growth driver, Nedeljković et al., (2021) postulated the protective role of ABCG2 against the carcinogenesis and dissemination of CSC in TNBC. The increased level of ABCG2 is closely linked to CSC and render the 'side population' (SP) phenotype of them (Ding et al., 2010; Zhou et al., 2001). ABCG2 reduce ROS generation, reducing the activation of oncogene and prevent metastasis of tumour cells (Liao et al., 2019) caused by oxidative stress and inflammation (Nie et al., 2018; Shen et al., 2010). The upregulation of E-cadherin by nuclear *ABCG2* protein also play a part in preventing metastasis (Liang et al., 2015). Therefore, reduced level of ABCG2 expression might expose CSC to tumorigenesis and dissemination. However, since there is no case with clinical stage IV, we have limited evidence of correlation between ABCG2 polymorphism and distant metastasis.

The present study observed that ABCG2 421 C> A polymorphism was associated with metaplastic and medullary carcinoma and advanced cancer staging in TNBC, provide evidence to the contribution of *ABCG2* 421 C>A polymorphism in etiology of TNBC. Our result

suggests that genetic polymorphisms, especially *ABCG2* 421 C>A could potentially play a part as a prognosis factor for TNBC patients management, addressing the potential of *ABCG2* polymorphisms in TNBC progression.

However, there were a few limitations here. First, the observed variation in the association risk of current study and previous research could be due to the genetic backgrounds' difference in participants. It is important to note that the current study solely included individuals from the East Coast region of Malaysia, predominantly consisting of Malay ethnicity. Thus, caution should be exercised when generalizing the finding with other populations. On the other hand, low frequency of variant alleles due to the limited sample size might also contribute to differing results and lack of association.

For further studies, increasing the sample size, especially patients with clinical Stage IV will be needed to validate the association of *ABCG2* 421 C>A with metastasis. Other than that, we will further our study on other *ABCG2* polymorphism such as 376 C>T that causes premature stop codon and no protein expression (Kobayashi et al., 2005). Finally, other ACB transporter genes such as *ABCB1* and *ABCC1* polymorphisms also come into our notice to observe their collective effect on individual susceptibility towards TNBC.

Author Contribution Statement

Conceptualization, A.A.A.A. and R.A.; methodology, H.I.Y, R.A. and A.A.A.A.; analysis and interpretation, H.I.Y and A.A.A.A.; resources, M.S.M.S. and M.M.Y.; writing-original draft preparation, H.I.Y; supervision, A.A.A.A.; patient recruitment, M.S.M.S. and M.M.Y.; funding acquisition, A.A.A.A.

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Approval

This study is part of an approved student thesis that was submitted in fulfillment of the requirement for the degree of Master of Science.

Ethical Declaration

The study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/20060343) in accordance with the Declaration of Helsinki.

Conflict of Interest

All authors declare no conflict of interest.

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