RESEARCH ARTICLE

Association of *CYP2E1*, *STK15* and *XRCC1* Polymorphisms with Risk of Breast Cancer in Malaysian Women

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Abstract

Background: Breast cancer is the most common type of cancer affecting Malaysian women. Recent statistics revealed that the cumulative probability of breast cancer and related deaths in Malaysia is higher than in most of the countries of Southeast Asia. Single nucleotide polymorphisms (SNPs) in *CYP2E1* (rs6413432 and rs3813867), *STK15* (rs2273535 and rs1047972) and *XRCC1* (rs1799782 and rs25487) have been associated with breast cancer risk in a meta-analysis but any link in Southeast Asia, including Malaysia, remained to be determined. Hence, we investigated the relationship between these SNPs and breast cancer risk among Malaysian women in the present case-control study. Materials and Methods: Genomic DNA was isolated from peripheral blood of 71 breast cancer patients and 260 healthy controls and subjected to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Results: Our study showed that the c1/c2 genotype or subjects with at least one c2 allele in *CYP2E1* rs3813867 SNP had significantly increased almost 1.8-fold higher breast cancer risk in Malaysian women overall. In addition, the variant Phe allele in *STK15* rs2273535 SNP appeared to protect against breast cancer in Malaysian Chinese. No significance association was found between *XRCC1* SNPs and breast cancer risk in the population. Conclusions: This study provides additional knowledge on *CYP2E1*, *STK15* and *XRCC1* SNP impact of risk of breast cancer, particularly in the Malaysian population. From our findings, we also recommend Malaysian women to perform breast cancer screening before 50 years of age.

Keywords: Breast cancer - *CYP2E1* - Malaysian women - single nucleotide polymorphisms - *STK15* - *XRCC1*

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Introduction

Breast cancer research remains a challenging subject for researcher worldwide (Hutchinson et al., 2010). It is a highly heterogeneous disease that consists of more than 20 diverse subtypes, complicated with different insinuations of its histopathological, molecular, and clinical stage (Perou et al., 2000; Malhotra et al., 2010; Mangia et al. 2011; Liu et al., 2014). Basically, breast cancer can be non-invasive in which the cancer cells remain in the milk ducts or lobules in the breast but in most of the cases, it is invasive where the cancer cells do invade into normal breast tissue and later metastasize to the lymph nodes or other organs of the body.

Despite a number of targeted therapies have been conducted in treating breast cancer (Higgins and Baselga, 2011), the incidence and mortality rates of this disease remain increasing globally. The incidence of breast cancer was reported with 3.1% increase rate yearly and from 600 thousand cases in 1980 to 1.6 millions cases in 2010. The cumulative probability of breast cancer incidences and deaths in Malaysians were 7.3% and 1.5% in 2010, respectively, and apparently higher than most of the Southeast Asia countries (Forouzanfar et al. 2011). Breast cancer is the most common cancer among Malaysian women, contributed to about 31% of all newly diagnosed cancer cases in 2003 (Lim and Yahaya, 2004). The unpromising situation of breast cancer in Malaysia should be seriously monitored, and urging an effective early detection system for breast cancer in the country in order to reduce the mortality rate.

Amplified and over-expressed of *STK15* was previously reported in breast tumors (Zhou et al., 1998; Staff et al., 2010). Besides, recent studies also showed that the progression or phenotype of breast cancer can be amended by *CYP2E1* and *XRCC1* genes (Sultana et al., 2012; Leung et al., 2013). Taken together, there is significant evidence showing that these three genes are involve in modulating the pathway in the development of breast cancer. Single nucleotide polymorphisms

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(SNPs) in *CYP2E1* (rs6413432 and rs3813867), *STK15* (rs2273535 and rs1047972) and *XRCC1* (rs1799782 and rs25487) were inconclusively associated to breast cancer risk in case-control or meta-analysis studies in different populations (Wu et al., 2006; Bu et al., 2014; Dai et al., 2014; Ding et al., 2014; Feng et al., 2014; Guo et al., 2014; Qin et al., 2015), but association of these SNPs to breast cancer in Southeast Asia populations, especially in Malaysian women was unquestionably insufficient. Therefore, this pilot study investigates the association of these SNPs, together with etiology factors such as age and ethnicity, to breast cancer risk in Malaysian women.

Materials and Methods

Subjects and blood sample collection

Blood sample was collected in a BD Vacutainer® Plus Plastic K2-EDTA tube (Becton Dickson, USA) from 260 healthy volunteers (mean age=31.7 ± 9.8) and 71 breast cancer patients (52.2 ± 9.9) who were admitted to Queen Elizabeth Hospital, Kota Kinabalu, Sabah and University Malaya Medical Centre (UMMC), Kuala Lumpur with written consent from 2010 to 2014. The clinicopathological characteristics of breast tumors were recorded. This study was approved by the Sabah State Health Department and the ethical approval was obtained from Universiti Malaysia Sabah Medical Ethics Committee with reference JKEtika 1/15 (8).

CYP2E1 genotyping

CYP2E1 rs6413432 and rs3813867 SNPs were determined as previously described by Chong et al. and Goh et al. (Chong et al., 2014; Goh et al., 2014).

STK15 genotyping

STK15 polymorphisms were determined using specific primer sets: 5'-CTT TCA TGA ATG CCA GAA AGT T-3'/5'-CTG GGA AGA ATT TGA AGG ACA-3' for Phe31Ile (rs2273535) SNP and 5'-CTT TCA TGA ATG CCA GAA AGT T-3'/5'-CTG CTT CTG ATT CTG AAC CGG CTT G-3' for Val57Ile (rs1047972) SNP, respectively. The PCR reaction was carried out in a 25 *µl* final reaction volume with 100 ng of extracted DNA, 0.2 *µM* of each primer, 0.2 mM of each dNTPs, 1.5 mM of $MgCl_2$ solution, 1.0 unit of *GoTaq®* Flexi DNA polymerase (Promega, USA) and 1X reaction buffer. The mixture was subjected to cycling conditions set at: 1 cycle for 4 min at 95°C; 35 cycles for 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and a final elongation for 7 min at 72°C. The resulting PCR product was digested overnight using 5 units of *ApoI* (New England Biolabs® Inc., Ipswich, MA) at 50°C for Phe31Ile SNP and 5 units of *BstUI* (New England Biolabs® Inc., Ipswich, MA) at 60°C for Val57Ile SNP. The digested fragment was analyzed in 3% agarose gel electrophoresis to determine the genotype of the subject.

XRCC1 genotyping

PCR was performed in a final volume of 25 µl mixture with 100 ng of DNA, 0.5 µM of each primer, 0.2 mM of each dNTPs, 2.0 mM of $MgCl_2$ solution, 1.0 unit of *GoTaq®* Flexi DNA polymerase (Promega, USA) and 1X of reaction buffer with cycling conditions of 95°C for 4 min, followed by 35 cycles of 94°C for 30 s, 65°C for 30 s and 72°C for 45 s, and a final extension at 72°C for 7 min. The primer sequences were 5'-GCC AGG GCC CCT CCT TCA A-3' (forward) and 5'-TAC CCT CAG ACC CAC GAG T-3' (reverse) for codon 194 (rs1799782 SNP) whereas primers used for codon 399 (rs25487 SNP) were 5'-TCC TCC ACC TTG TGC TTT CT-3' (forward) and 5'-AGT AGT CTG CTG GCT CTG GG-3' (reverse). PCR product was digested at 37°C overnight using 4 units of *PvuII* (New England Biolabs® Inc., Ipswich, MA) for Arg194Trp SNP and 2 units of *NciI* (New England Biolabs® Inc., Ipswich, MA) for Arg399Gln SNP, and were electrophoresized on 2% agarose gel for genotype determination.

Direct sequencing

Direct sequencing was performed to confirm all SNPs. In brief, PCR product was purified using QIAquick PCR Purification Kit (QIAGEN, USA) according to manufacturer's instructions and were sequenced using ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA).

Table 1. Clinicopathological Characteristics of Breast Tumors

Characteristics	Overall percentages (%)
Lesion site	
Left	63.6
Right	36.4
Tumor diameter (cm)	
≤ 2	18.2
> 2 and ≤ 5	36.4
> 5	45.4
Differentiation	
Poor	54.5
Moderate	45.5
TNM staging	
1	27.3
2	9.1
3	45.4
4	18.2
Tumour invasion	
T1	18.2
T2	36.3
T3	27.3
T4	18.2
Lymph node metastasis	
N0	36.3
N1	27.3
N2	9.1
N3	27.3
Metastasis	
M0	54.5
M1	27.3
M2	18.2
HER2 status*	
Negative	9.1
1+	27.3
2+	27.3
3+	36.3

*HER2=human epidermal growth factor receptor 2

Statistical analysis

Genotype distribution for all SNPs was tested and considered within Hardy-Weinberg equilibrium (HWE) when $\chi^2<3.84$ with degree of freedom=1. Odd's ratio (OR) and 95% confidence interval (95% CI) were calculated using SPSS V17.0 (SPSS Inc, Chicago, Illinois, USA) with a significant level at $p < 0.05$ (Fisher's exact-test). Significant level for sub-group analysis (e.g. age and ethnicity) was adjusted to Bonferroni correction. The reference genotype for *CYP2E1* rs6413432, *CYP2E1* rs3813867, *STK15* rs2273535 and *STK15* rs1047972 were D/D, c1/c1, Ile/Ile, and Val/Val, respectively, whereas Arg/Arg was the reference genotype for both *XRCC1* rs1799782 and *XRCC1* rs25487 SNPs.

Results

Homozygosity and heterozygosity of all SNPs in this study were confirmed using sequencing in addition to PCR-RFLP. In clinicopathological analysis, majority of breast cancer patients had > 2 cm tumor in size (81.8%), T2 to T3 tumor invasion (63.6%) and TNM staging of 3 to 4 (63.6%). Table 1 lists the clinicopathological characteristics of breast tumor in this study.

Our study revealed that Malaysian women with heterozygous c1/c2 genotype or at least one c2 allele in *CYP2E1* rs3813867 SNP had about 1.8-fold higher risk to breast cancer when compared to Malaysian women with common (c1/c1) genotype (HWE, $\chi^2=1.59$) (Table 2).

In etiology factor analysis, age was not an interacting factor for breast cancer in all SNPs studied (Table 3) but Malaysian Chinese women with Phe/Ile genotype or at least one Phe allele was seemed to protect against breast cancer even after Bonferroni correction (HWE, $\chi^2=0.06$) (Table 4). No significance association was found between *XRCC1* SNPs and breast cancer among Malaysian women

Table 2. Risk Association of *CYP2E1*, *STK15* and *XRCC1* SNPs to Breast Cancer

SNPs	Cases, *N*	Controls, *N*	OR (95% CI)	*p*
CYP2E1 (rs6413432)	$\chi^2=0.02$			
D/D	38	149	1.00 (Reference)	-
D/C	28	95	1.16 (0.67-2.01)	0.671
C/C	5	16	1.23 (0.42-3.56)	0.776
D/C + C/C	33	111	1.17 (0.69-1.98)	0.591
CYP2E1 (rs3813867)	$\chi^2=1.59$			
c1/c1	40	181	1.00 (Reference)	-
c1/c2	29	74	1.77 (1.02-3.07)	0.043*
c2/c2	2	5	1.81 (0.34-9.67)	0.616
c1/c2 + c2/c2	31	79	1.78 (1.04-3.04)	0.046*
STK15 (rs2273535)	$\chi^2=13.61$**			
Ile/Ile	29	85	1.00 (Reference)	-
Phe/Ile	21	110	0.56 (0.30-1.05)	0.081
Phe/Phe	21	65	0.95 (0.50-1.81)	1
Phe/Ile + Phe/Phe	42	175	0.70 (0.41-1.21)	0.207
STK15 (rs1047972)	$\chi^2=1.46$			
Val/Val	56	178	1.00 (Reference)	-
Val/Ile	12	73	0.52 (0.27-1.03)	0.064
Ile/Ile	3	9	1.06 (0.28-4.05)	1
Val/Ile + Ile/Ile	15	82	0.58 (0.31-1.09)	0.106
XRCC1 (rs1799782)	$\chi^2=0.43$			
Arg/Arg	40	152	1.00 (Reference)	-
Arg/Trp	26	97	1.02 (0.58-1.78)	1
Trp/Trp	5	11	1.73 (0.57-5.26)	0.347
Arg/Trp + Trp/Trp	31	108	1.09 (0.64-1.85)	0.787
XRCC1 (rs25487)	$\chi^2=0.38$			
Arg/Arg	25	114	1.00 (Reference)	-
Arg/Gln	33	114	1.32 (0.74-2.36)	0.38
Gln/Gln	13	32	1.85 (0.85-4.03)	0.139
Arg/Gln + Gln/Gln	46	146	1.44 (0.83-2.48)	0.223

*Statistically significant ($p < 0.05$); **Not in Hardy-Weinberg equilibrium ($\chi^2 > 3.84$); SNP=single nucleotide polymorphism; *N*=number of sample; OR=odd's ratio; CI=confidence interval, Ile=isoleucine, Phe=phenylalanine, Val=valine, Arg=arginine, Trp=tryptophan, Gln=glutamine

Table 3. Age Association of *CYP2E1, STK15* and *XRCC1* SNPs to Breast Cancer Risk

SNPs	<50				≥50			
	Cases	Controls	OR (95%CI)	*p*	Cases	Controls	OR (95%CI)	*p*
CYP2E1 (rs6413432)	χ^2=0.30				χ^2=1.49			
D/D	12	145	1.00 (Reference)	-	26	4	1.00 (Reference)	-
D/C	14	89	1.90 (0.84-4.29)	0.14	14	6	0.36 (0.09-1.49)	0.171
C/C	1	13	0.93 (0.11-7.72)	1	4	3	0.21 (0.03-1.28)	0.108
D/C + C/C	15	102	1.78 (0.80-4.00)	0.218	18	9	0.31 (0.08-1.15)	0.114
CYP2E1 (rs3813867)	χ^2=0.55				χ^2=1.81			
c1/c1	14	174	1.00 (Reference)	-	26	7	1.00 (Reference)	-
c1/c2	12	68	2.19 (0.97-4.98)	0.071	17	6	0.76 (0.22-2.66)	0.753
c2/c2	1	5	2.49 (0.27-22.77)	0.387	1	0	-	-
c1/c2 + c2/c2	13	73	2.21 (0.99-4.94)	0.078	18	6	0.81 (0.23-2.81)	0.759
STK15 (rs2273535)	χ^2=8.03**				χ^2=7.07**			
Ile/Ile	11	79	1.00 (Reference)	-	18	6	1.00 (Reference)	-
Phe/Ile	8	105	0.55 (0.21-1.42)	0.233	13	5	0.87 (0.22-3.46)	1
Phe/Phe	8	63	0.71 (0.35-2.40)	1	13	2	2.17 (0.38-12.50)	0.45
Phe/Ile + Phe/Phe	16	168	0.68 (0.30-1.54)	0.391	26	7	1.24 (0.34-4.30)	0.759
STK15 (rs1047972)	χ^2=0.50				χ^2=1.83			
Val/Val	23	170	1.00 (Reference)	-	33	8	1.00 (Reference)	-
Val/Ile	2	70	0.21 (0.05-0.92)	0.031	10	3	0.81 (0.18-3.64)	1
Ile/Ile	2	7	2.11 (0.41-10.79)	0.308	1	2	0.12 (0.01-1.51)	0.125
Val/Ile + Ile/Ile	4	77	0.38 (0.13-1.15)	0.118	11	5	0.53 (0.14-1.98)	0.483
XRCC1 (rs1799782)	χ^2=0.49				χ^2=0.02			
Arg/Arg	16	146	1.00 (Reference)	-	24	6	1.00 (Reference)	-
Arg/Trp	9	91	0.92 (0.38-2.13)	1	17	6	0.71 (0.20-2.58)	0.743
Trp/Trp	2	10	1.83 (0.37-9.07)	0.359	3	1	0.75 (0.07-8.55)	1
Arg/Trp + Trp/Trp	11	101	0.94 (0.44-2.23)	1	20	7	0.71 (0.21-2.47)	0.754
XRCC1 (rs25487)	χ^2=0.37				χ^2=0.02			
Arg/Arg	7	109	1.00 (Reference)	-	18	5	1.00 (Reference)	-
Arg/Gln	14	107	2.04 (0.79-5.25)	0.171	19	7	0.75 (0.20-2.81)	0.748
Gln/Gln	6	31	3.01 (0.94-9.63)	0.084	7	1	1.94 (0.19-19.74)	1
Arg/Gln + Gln/Gln	20	138	2.26 (0.92-5.53)	0.099	26	8	0.90 (0.25-3.21)	1

**Not in Hardy-Weinberg equilibrium ($\chi 2 > 3.84$); SNP=single nucleotide polymorphism; OR=odd's ratio; CI=confidence interval, Ile=isoleucine, Phe=phenylalanine, Val=valine, Arg=arginine, Trp=tryptophan, Gln=glutamine

in this study.

Discussion

Malaysia is a multiethnic country with majority of Malays, Chinese, and Indians whereas some indigenous ethnic groups (e.g. KadazanDusun, Bajau, Rungus, Sungai, etc.) are dominant in East Malaysia. This study investigated the association of *CYP2E1*, *STK15* and *XRCC1* SNPs to breast cancer risk by including indigenous ethnic groups among Malaysian women.

CYP2E1 encodes an enzyme that metabolizes more than eighty low molecular weight substrates to highly reactive metabolites. It is also able to reduce oxygen molecule to highly active form, which is carcinogenic, and may leads to cancer development (Guengerich et al., 1991). We have previously reported that *CYP2E1* gene polymorphism elevated the risk of gastrointestinal

Table 4. Ethnicity Association of *CYP2E1*, *STK15* and *XRCC1* SNPs to Breast Cancer Risk

SNPs	Malaysian Chinese				Malaysian non-Chinese			
	Cases	Controls	OR (95% CI)	p	Cases	Controls	OR (95% CI)	p
CYP2E1 (rs6413432)	χ^2=6.55**				χ^2=2.58			
D/D	6	30	1.00 (Reference)	-	32	119	1.00 (Reference)	
D/C	9	29	1.55 (0.49-4.91)	0.567	19	66	1.07 (0.56-2.04)	0.87
C/C	0	1	-	-	5	15	1.24 (0.42-3.67)	0.773
D/C + C/C	9	30	1.50 (0.48-4.74)	0.571	24	81	1.10 (0.61-2.01)	0.761
CYP2E1 (rs3813867)	χ^2=1.78				χ^2=0.47			
c1/c1	9	38	1.00 (Reference)	-	31	143	1.00 (Reference)	-
c1/c2	6	21	1.21 (0.38-3.86)	0.771	23	53	2.00 (1.07-3.74)	0.031
c2/c2	0	1	-	-	2	4	2.31 (0.40-13.16)	0.303
c1/c2 + c2/c2	6	22	1.15 (0.36-3.67)	1	25	57	2.02 (1.10-3.72)	0.034
STK15 (rs2273535)	χ^2=0.06				χ^2=18.58**			
Ile/Ile	9	14	1.00 (Reference)	-	20	71	1.00 (Reference)	-
Phe/Ile	2	36	0.09 (0.02-0.45)	0.001*	19	74	0.91 (0.45-1.85)	0.858
Phe/Phe	4	10	0.62 (0.15-2.60)	0.724	17	55	1.10 (0.53-2.29)	0.852
Phe/Ile + Phe/Phe	6	46	0.20 (0.06-0.67)	0.011*	36	129	0.99 (0.53-1.84)	1
STK15 (rs1047972)	χ^2=0.11				χ^2=2.02			
Val/Val	13	43	1.00 (Reference)	-	43	135	1.00 (Reference)	-
Val/Ile	1	17	0.20 (0.02-1.61)	0.165	11	56	0.62 (0.30-1.28)	0.228
Ile/Ile	1	0	-	-	2	9	0.70 (0.15-3.35)	1
Val/Ile + Ile/Ile	2	17	0.39 (0.08-1.91)	0.328	13	65	0.63 (0.32-1.25)	0.194
XRCC1 (rs1799782)	χ^2=3.96**				χ^2=0.11			
Arg/Arg	9	32	1.00 (Reference)	-	31	120	1.00 (Reference)	-
Arg/Trp	6	27	0.79 (0.25-2.50)	0.776	20	70	1.11 (0.59-2.09)	0.748
Trp/Trp	0	1	-	-	5	10	1.94 (0.62-6.08)	0.321
Arg/Trp + Trp/Trp	6	28	0.76 (0.24-2.41)	0.775	25	80	1.21 (0.67-2.20)	0.542
XRCC1 (rs25487)	χ^2=1.12				χ^2=0.01			
Arg/Arg	6	32	1.00 (Reference)	-	19	82	1.00 (Reference)	-
Arg/Gln	7	21	1.78 (0.52-6.03)	0.369	26	93	1.21 (0.62-2.34)	0.618
Gln/Gln	2	7	1.52 (0.25-9.19)	0.639	11	25	1.90 (0.80-4.52)	0.163
Arg/Gln + Gln/Gln	9	28	1.71 (0.54-5.42)	0.399	37	118	1.35 (0.73-2.52)	0.358

*Statistically significant with Bonferroni correction ($p < 0.025$); **Not in Hardy-Weinberg equilibrium ($\chi2 > 3.84$); SNP=single nucleotide polymorphism; OR=odd's ratio; CI=confidence interval, Ile=isoleucine, Phe=phenylalanine, Val=valine, Arg=arginine, Trp=tryptophan, Gln=glutamine

cancer in Malaysian population (Chong et al., 2014). Interestingly, the heterozygous c1/c2 genotype or present of c2 allele in *CYP2E1* rs3813867 SNP was also associated with higher breast cancer risk in this study, and was in agreement with a study conducted in Lebanese women (Zgheib et al., 2013). However, some studies claimed that the c1/c2 genotype was protected against breast cancer (Khedhaier et al., 2008; Sangrajrang et al., 2010). Although we do not have enough evidence to link between the variant c2/c2 genotype in *CYP2E1* rs3813867 and breast cancer risk, it was denoted as a risk reducer for breast cancer in Taiwanese (Wu et al., 2006).

A review of breast cancer research in Malaysia revealed that late clinical stages presentation of the disease was common in the population (Yip et al., 2014), and in line with the present study with more than 60% of breast cancer patients were presented in late clinical stages. This discouraging situation might due to strong traditional

medicine dependency, negative view of the disease, poor education, fear and neglect to the disease (Hisham and Yip, 2004). Besides, various histological types of breast cancer were regularly being observed in both age < 50 years and ≥ 50 years among Malaysian women (Sheikh et al., 2009). Based on that, we categorized our subjects according to their age for SNPs association analysis and we found no evidence to associate age differences to breast cancer risk in Malaysian women. However, we strongly recommend Malaysian women to perform their breast cancer screening in younger age as our data reveals that more than 38% of the breast cancer subjects are < 50 years old.

STK15 is a kinase correlated with centrosome maturation and spindle formation (Sen et al., 1997). Overexpression of *STK15* in breast cancer cell line has been previously reported (Sen et al., 1997; Zhou et al., 1998), indicating that *STK15* may involve in breast cancer development. *STK15* rs2273535 SNP has been associated to breast cancer risk in different populations (Cox et al., 2006; Fletcher et al., 2006; Ruan et al., 2011), and the Ile allele in this SNP was used as the reference group in this study since it was reported to be more common in Asian populations (Ewart-Toland et al., 2005). Our results showed that subjects with Phe/Ile genotype or at least one Phe allele had a significant reduced risk to breast cancer in Malaysian Chinese women even after Bonferroni correction, suggesting that it can be act as a strong protective biomarker for breast cancer in the population. This finding was similar to recent meta-analysis studies claiming that the Ile allele was an increase risk factor for breast cancer (Qin et al., 2013; Tang et al., 2013; Dai et al., 2014; Guo et al., 2014; Xu et al., 2014; Qin et al., 2015). In Malaysia, Malaysian Chinese women has the highest breast cancer incidences when compared to other ethnic groups (Leong et al., 2007; Pathy et al., 2011), and it is yet to clarify that the role of genetic abnormality contribute to the high incidence rate in this ethnic.

For *XRCC1*, it encodes a scaffolding protein which involves in single-strand break repair and base excision repair mechanisms (Kubota et al., 1996). Polymorphisms in *XRCC1* have been connected to functional changes at the protein level by influencing the DNA repair rate and may results in carcinogenesis (Vodicka et al., 2007). In this study, we have no statistical evident to associate the variant in *XRCC1* rs1799782 and rs25487 SNPs to breast cancer risk in Malaysian women but latest meta-analysis studies showed that variant in both SNPs were significantly increased the risk of breast cancer (Wu et al., 2011; Liu et al., 2013; Bu et al., 2014; Feng et al., 2014). Interestingly, a study conducted by Ye et al. revealed that breast cancer patients with Gln/Gln genotype in *XRCC1* rs25487 SNP were associated with a longer survival rate after chemotherapy treatment when compared to wild-type genotype (Ye et al., 2012), indicating the prognostic value of this SNP in breast cancer patients receiving chemotherapy.

In summary, this is the first significant study to associate the *CYP2E1* and *STK15* SNPs to breast cancer risk by including indigenous ethnic groups in Malaysian women. This study provides additional knowledge and more precise breast cancer risk estimation in future meta-analysis related to *CYP2E1*, *STK15* and *XRCC1* SNPs. Nevertheless, more education campaigns on breast cancer care should be conducted in Malaysia as breast cancer awareness remains poor among Malaysian women and breast cancer screening should begin in younger age for better survival rate from breast cancer.

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