RESEARCH ARTICLE

Association between *ERCC1* Polymorphism and the Risk and Clinicopathological Features of Breast Cancer in Thai Women in the Lower Northeastern Region

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Abstract

Background: Breast cancer is a major public health problem around the world, including Thailand and it has the highest ranking among female cancer. Currently, the diversity or polymorphism of *ERCC1* gene (excision repair cross-complementary group 1 gene or *ERCC1*) was reported to associate with an increased risk of breast cancer. This study aims to investigate the relationship between *ERCC1* polymorphism and the breast cancer risk in the lower northeastern region women of Thailand. **Materials and Methods:** One hundred fifty one samples from breast cancer patients and 120 samples from healthy control group were analysed. Genomic DNA was extracted from white blood cell of all samples. The real-time polymerase chain reaction (qPCR) was used to demonstrate genetic polymorphism of *ERCC1*. **Results:** The results showed that the *ERCC1* rs11615 polymorphism variant AG was associated with an increased risk of breast cancer. This study demonstrated that the frequency of *ERCC1* rs11615 in patients with breast cancer was higher than healthy control group. The *ERCC1* polymorphism variant AG carrier presented 3.53-folds high risk of breast cancer [odds ratio (OR) = 3.53, 95% CI = 1.61-7.74, P = 0.001]. In addition, when age, menopause period, number of child, smoking and alcohol drinking were adjusted, the *ERCC1* rs11615 variant AG carrier was associated with increased breast cancer risk to 3.97 folds, with OR = 3.79, 95% CI = 1.62-8.84, P = 0.002. **Conclusions:** This study showed that *ERCC1* rs11615 genotype AG was associated with breast cancer risk in the lower northeastern region women of Thailand.

Keywords: Breast cancer- *ERCC1* rs11615-polymorphism- Thailand

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Introduction

Breast cancer is one of the most common malignancy diseases among women worldwide. It is an important problem in many countries including Thailand. About 1.38 million new breast cancer cases were diagnosed in 2008 which was accounted for 25% of all cancers (Ferley et al., 2010). In Thailand, the highest incidence of breast cancer in Thai women was 28.6 per 100,000 populations (Imsamran et al., 2015). The etiological data of breast cancer shows that alcohol drinking, having a family history of breast cancer, complex interplay of genetics, environmental exposures, hormones and behaviors may contribute to breast carcinogenesis (Benz, 2008). The DNA repair systems play an important role in maintaining the stability and integrity of human genome. High levels of DNA damage caused by excessive exposure to carcinogens might be responsible for increasing an incidence of breast cancer. Unrepaired DNA damage can result in apoptosis and may lead to unregulated cell growth and even cancer (Vispe et al., 2000). The excision repair cross-complementary group 1 gene (*ERCC1*) is part of the nucleotide excision repair (NER) pathway, which is usually indicated to be involved with repaired DNA. In recent years, several *ERCC1* variants such as rs11615, rs321986 and rs321961 have been reported to be associated with an increased risk of lung cancer, colorectal cancer and head and neck cancer (Zhang et al., 2012). *ERCC1* rs11615 AA genotype was associated with increased breast cancer risk at 1.62 fold in China (Zecheng et al., 2013).

However, there is no report about *ERCC1* polymorphism associated with breast cancer risk in Thailand and Southeast Asia. Therefore, study on *ERCC1* polymorphism and clinical pathology associated with breast cancer risk in the lower northeastern region women of Thailand is the main purpose of this research.

Materials and Methods

The study included 151 patients with diagnosed and histopathology confirmed with breast cancer and 120

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healthy controls who visited in Ubonratchathani Cancer Hospital during the year 2015 to 2016. All patients and healthy controls were asked to provide 5-10 ml of whole blood (1.5-2.2 mg/ml EDTA was used as anticoagulant) for genotyping and signed for a written informed consent. The EDTA blood was kept at -20°C. The DNA was extracted using QuickGene DNA whole blood kit S (DB-S) (Wako Chemicals GmbH, Germany) and QuickGene-810 FUJIFILM® equipment. DNA concentrations were conducted by spectrophotometer measurement of absorbance at 260 and 280 nm. by Nano Drop Technology. *ERCC1* polymorphism was detected using qPCR method. Two PCR primers of *ERCC1* rs11615 consisted of a forward primer, 5′-TAGTCGGGAATTACRTCGCCA-3′ and a reverse primer, 5′-CAATCCCGTACTGAAGTTCGTG-3′ (Xiaobo et al., 2013). The thermos cycling conditions were 95°C for 15 seconds and 60°C for 30 seconds in a total of 40 cycles followed by 60°C for 30 seconds. This method was able to detect all three possible genotypes of *ERCC1* rs11615: homozygous wild type (GG), heterozygous variant type (AG) and homozygous variant type (AA). The histopathological and immunohistochemical data were reported by the pathologists from Ubonrachathani Cancer Hospital. Demographic and clinicopathological data were collected from the medical records. This study was ethically approved by the local Ethical Committee of Thammasat University, Thailand (EC 072/2015) and Ubonratchathani Cancer Hospital, Thailand (EC 008/2015).

Statistical analysis

The genotype and allelic frequencies of *ERCC1* polymorphism in breast cancer cases and healthy controls were analyzed by STATA software (Version 11.0). Odds ratio (OR) and 95% confidence intervals (95% CI) were used to assess the effect of each SNPs on breast cancer risk and $P < 0.05$ was regarded as statistically significant.

Table 1. Clinical and Demographic Characteristic of Study Subjects

Variables	No.of cancer cases (%) (Total =151)	No.of control cases (%) (Total =120)	OR (95%) CI	P value
Age, years (Mean ± SD)	52.8 ±9.4	48.8±10.6		0.001
Menopausal status				
Pre-	102 (67.5)	59 (49.2)	1	
Post-	49 (32.5)	61 (50.8)	0.48 (0.29-0.79)	0.003
Number of child				
0	7 (4.6)	15 (12.5)	1	
1-2	80 (53.0)	74 (61.7)	2.13 (0.82-5.56)	0.115
≥3	64 (42.4)	31 (25.8)	4.13 (1.51-11.26)	0.003
Smoking behaviors				
Never	142 (94.1)	113 (94.2)		
Ever	9 (5.9)	7 (5.8)	1.84 (0.55-6.12)	0.414
Alcohol drinking				
Never	114 (75.5)	114 (95.0)		
Ever	37 (24.5)	6 (5.0)	6.17 (2.51-15.8)	0.001

Results

Clinicopathological and demographic characteristics

This study included 271 subjects with 151 patients of breast cancer (mean age 52.8 ± 9.4 years) and 120 healthy controls (mean age 48.8±10.6 years). Baseline demographic characteristics of study subjects were showed in Table 1. Decreased breast cancer risk was found in those who had post menopause (OR = 0.48, 95% CI = 0.29-0.79, P = 0.003) as compared with pre menopause. In contrast, an increased breast cancer risk was observed in those who had number of child ≥3 (OR = 4.13, 95% CI = 1.51-11.26, P = 0.003) as compared with those without child. In addition, such increased breast cancer risk was observed in those who had drinking alcohol in breast cancer group (OR = 6.17, 95% CI = 2.51-15.8, P = 0.001) as compared with those without drinking alcohol. However, there was no significant different for breast cancer risk between smoker and non-smoker.

The clinical data of 151 patients with breast cancer were shown in Table 2. It found that 80 patients (53%) were highly relevant to tumors size < 2 cm., 138 patients (91.4%) were highly relevant to invasive ductal carcinoma, 82 patients (54.3%) were grade 2, 87 patients (57.6%) were positive for estrogen receptor, 87 patients (57.6%) were negative for progesterone receptor and 92 patients (60.9%) were negative for Her-2.

Table 2. Clinical Data of Breast Cancer Cases

Clinical data	No. of breast cancer cases (Total = 151)	%
Tumor size		
< 2 cm	80	53
2-5 cm	13	8.6
> 5 cm	16	10.6
N/A	42	27.8
Type of breast cancer		
DCIS	10	6.6
Invasive ductal CA	138	91.4
Invasive mammary	1	0.7
Invasive with DCIS	2	1.3
Grade		
1	4	2.7
2	82	54.3
3	37	24.5
N/A	28	18.5
ER (estrogen receptor)		
Negative	64	42.4
Positive	87	57.6
PR (progesterone receptor)		
Negative	87	57.6
Positive	64	42.4
Her-2 (human epidermal growth factor receptor)		
Negative	92	60.9
Positive	48	31.8
N/A	11	7.3

DCIS , Ductal Carcinoma in Situ; CA, Cancer

Table 3. *ERCC1* Genotype Frequencies in Patients and Controls

Genotypes	No. of breast cancer patients (%)	No. of healthy controls (%)	Total (%)
GG	105 (69.6)	101 (84.2)	206 (76.1)
AG	33 (21.8)	9 (7.5)	42 (15.5)
AA	13 (8.6)	10 (8.3)	23 (8.4)
Total	151 (100)	120 (100)	271 (100)

Table 4. Comparison between Genotypes, OR and P Value with Breast Cancer Risk

Genotypes	Crude Odds ratio	95% CI	P
GG	1		
AG	3.53	1.61-7.74	0.001
AA	1.25	0.52-2.98	0.613
AA, AG	2.33	1.28-4.24	0.007

ERCC1 rs11615 *variants detection in breast cancer patients and healthy controls*

The study included 271 subjects with 151 breast cancer cases and 120 healthy controls. *ERCC1* genotype frequency data between breast cancer cases and healthy controls were shown in Table 3. It showed that *ERCC1* rs11615 AG had the highest frequency in breast cancer cases (21.8%) compared with healthy controls (7.5%). Heterozygous mutant allele affected breast cancer risk. The frequencies of *ERCC1* rs11615 AA in breast cancer cases and healthy controls were 8.6% and 8.3% respectively (Table 3).

ERCC1 was the important risk factor in our study population. The comparison between genotype, OR and P value revealed that the *ERCC1* rs11615 GG/AG genotype was the most important risk factor in this population. We found that GG/AG genotype had OR = 3.53, 95% CI = 1.61-7.74, P = 0.001 while AA/AG genotype had OR = 2.33, 95% CI = 1.28-4.24, P = 0.007 and genotype GG/AA had OR = 1.25, 95% CI = 0.52-2.98, P = 0.613. The *ERCC1* rs11615 variant AG genotype was associated with increased breast cancer risk in our Thai population study (Table 4).

The results of multivariate logistic regression analysis of the effects of *ERCC1* rs11615 genotypes on breast cancer risk, adjust for age, menopause period, number of child, smoking and alcohol drinking were analyzed by STATA software (Version 11.0). The *ERCC1* rs11615 variant AG genotype was associated with increased breast cancer risk: OR = 3.79; 95% CI = 1.62-8.84, P = 0.002 (Table 5).

Association between ERCC1 rs11615 *variants and clinicopathological features in breast cancer*

This study observed that *ERCC1* rs11615 variant AG genotype was associated with increased breast cancer risk (P= 0.002) when age, menopause period, number of child, smoking and alcohol drinking were adjusted. Tumor size was not associated with *ERCC1* rs11615 polymorphism (P = 0.114). Invasive ductal carcinoma and invasive with DCIS could be detected in 92.3% and 7.7% respectively in breast cancer cases who had variant AG genotype of rs11615. Invasive ductal carcinoma could be detected in the breast cancer patients who had *ERCC1* rs11615 genotype AG and AA variants for 92.3% and 90.9% respectively. This finding suggested that invasive ductal carcinoma was frequently occurred in *ERCC1* rs11615 genotype AG and AA variants of breast cancer patients. *ERCC1* rs11615 genotype AG and AA variants were associated with ER (P = 0.029). These genotype variants were not associated with PR (P = 0.090) and Her-2 (P = 0.407).

Table 5. Multivariate Logistic Regression Analysis of *ERCC1* rs11516 Polymorphism for Breast Cancer Risk

Genotypes	No.of cancer patients (%)	No.of healthy controls (%)	Adjusted OR[(a)] (95% CI)	P
GG	105 (69.6)	101 (84.2)	1	
AG	33 (21.8)	9 (7.5)	3.79 (1.62-8.84)	0.002
AA	13 (8.6)	10 (8.3)	1.14 (0.45-2.86)	0.772

Adjusted OR[(a)] for age, menopause period, number of child, smoking, drinking.

Discussion

Numerous studies revealed associations of polymorphisms in DNA repair genes and cancer risk (Goode et al., 2002). The DNA repair system is complex, governed by more than 125 genes (Ng and Henikoff, 2002). The *ERCC1* gene whose products are important in nucleotide excision repair (NER) and *ERCC1* contains 10 exons and encodes a 297 acetaldehyde ammonia product and has been mapped to chromosome 19q13.32 which is involved in correcting the excision repair deficiency of the NER pathway (Smith et al., 2006). It is reported *ERCC1* play a key role in the process of excising DNA lesion in the repair of DNA damage on the transcribed strand of the actively expressed gene and removing DNA damage from the remaining genome (Constantinou et al., 1999).

Previous studies have reported about the association between variants of *ERCC1* and breast cancer risk. Hosseini et al., (2012) studied about breast cancer in Iranian women and reported that *ERCC1* rs3212981 A/A genotype was associated with increasing of breast cancer risk. Pei et al., (2014) studied about *ERCC1* rs11615 in 417 breast cancer cases and healthy control. They reported that *ERCC1* rs11615 was associated with increased risk of breast cancer. Crew et al., (2007) studied in the United States with 1053 breast cancer cases and 1102 healthy controls. They reported that *ERCC1* rs3212986 C/A was associated with increased risk of breast cancer. Han et al., (2012) studied in Korean population and found that ERCC2 rs50872 TC genotype showed significant effects on breast cancer. Lee et al., (2005) reported the *ERCC1* 8092 AA genotype and the *ERCC1* 354T allele were associated with breast cancer risk. There was no association between the *ERCC1* polymorphisms, the haplotype and risk of colorectal cancer by Hansen et al., (2008) study. They found that *ERCC1* Asn118Asn has been associated with risk of breast cancer and lung cancer. However, the results were inconsistent with *ERCC1*

genotype that studied by Gomez-Diaz et al., (2015) and *ERCC2* rs1799793 showed associated breast cancer in Mexico. Our present study found that ER, PR and Her-2 negative subjects by immunohistochemical staining were high in breast cancer, 42.4%, 57.6% and 60.9%, respectively. Approximately 63% of women with triple-negative breast cancer were diagnosed before age 60 years and triple-negative breast cancer may be predictive of poor response to treatment (Bauer et al., 2007). Determination of estrogen receptor (ER) of invasive breast carcinoma is useful as a prognostic and predictive factor in the management of this neoplasm. ER positivity predicts for response to endocrine therapy such as antiestrogen (tamoxifen) administration. Similarly, human epidermal growth factor receptor 2 (Her-2) positivity is useful for selecting targeted therapy with monoclonal antibody (trastuzumab) against Her-2. Triple-Negative Breast Cancer (TNBCs) accounting for about 15% of breast cancer and characterized by negative for ER, PR and Her-2 is associated with aggressive histology, poor prognosis and unresponsiveness to the usual endocrine therapies and survival breast cancer (Perou et al., 2000).

Our study found that *ERCC1* rs11615 AG genotype was associated with a 3.53 folds of breast cancer risk and adjusting for the potential risk factor of age, menopause period, number of child, smoking, alcohol drinking was associated with a 3.79 folds of breast cancer risk. *ERCC1* rs11615 was associated with increased risk of breast cancer in Thai population. This is the first report about *ERCC1* rs11615 polymorphism and breast cancer risk in Thailand and Southeast Asia. Further studies with a large sample size may contribute to further elucidate the impact of *ERCC1* rs11615 polymorphism on the risk of breast cancer.

In conclusion, the AG genotype of *ERCC1* rs11615 was associated with an increased risk of breast cancer in Thai population.

Conflict of Interests

The authors declare that we have no conflict of interests.

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