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Original Article

ERCC5 rs751402 polymorphism is the risk factor for sporadic breast cancer in Thailand

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Abstract: Breast cancer is a complex disease. Single Nucleotide Polymorphisms (SNPs) can modify the risk of cancer. They may be regarded as potential markers of carcinogenesis. Currently, the diversity or polymorphism of ERCC5 gene (excision repair cross-complementary group 5 gene or *ERCC5*) was reported to associate with an increased risk of breast cancer. This study aims to investigate the relationship between *ERCC5* polymorphism and the breast cancer risk in the lower northeastern region women of Thailand. One hundred fifty five samples from breast cancer patients and 122 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 *ERCC5*. The results showed that the *ERCC5* rs751402 polymorphism variant AG was associated with an increased risk of breast cancer. The frequency of *ERCC5* rs751402 in patients with breast cancer was higher than healthy control group. The *ERCC5* rs751402 variant AG carrier was associated with increased breast cancer risk to 2.3 folds, with OR = 2.30, 95% CI = 1.22-4.35, *P* = 0.01, when age, menopause period, number of child, smoking and alcohol drinking were adjust. This study demonstrated that *ERCC5* rs751402 genotype AG was associated with breast cancer risk in the lower northeastern region women of Thailand.

Keywords: *ERCC5* rs751402, polymorphism, breast cancer

Introduction

Breast cancer is the most frequently diagnosed cancer in female worldwide. Single Nucleotide Polymorphisms (SNPs), DNA aberrant methylation, tumor suppressor gene transcription silencing and the inactivated DNA repairing process are appeared in epigenetic modification mechanism [1]. The DNA aberrant methylation approaches may show useful markers for cancer diagnostics, classification and prognostics as well as cancer chemotherapy treatment. Genetic polymorphisms of genes involved in multiple biological pathways, including DNA repair, have been identified as potential risk for breast cancer [2]. Polymorphisms can contribute to the differences between individuals in the susceptibility to a disease and its severity [3]. The regulation of DNA repair is a vital factor in the multistep process of carcinogenesis. DNA repairing is essential for maintain genomic stability in response to the assault of environmental carcinogens that causes DNA damage. If left unrepaired, such DNA damage can lead to mutation fixation and initiation of carcinogenesis [4]. The nucleotide-excision repair (NER) pathway is the most versatile and is particularly significant in association with cancer risk. Efficient DNA repair is required for preventing propagation of errors in the genome and for maintaining genomic stability. NER system acts to repair bulky lesions, such as thymine dimmers generated by ultraviolet irradiation [5].

Excision repair cross-complementing rodent repair deficiency, complementation group 5 (*ERCC5*) is an important member of a family of enzymes that includes the DNaseIV/flap structure-specific endonuclease 1 (FEN1) group of structure-specific nucleases, which function in NER. ERCC5 gene encodes a single strand specific DNA endonuclease that makes the 3 incision in DNA excision repair following UV-induced damage. The protein has the function in the cel-

Table 1. *ERCC5* specific primer sequences and product size

Primer-probe (5'→3')	Length (bp)	Tm	% GC	Product size
rs751402-FP	21	58.4	52	103 bp
CGCGTCGTATTAGACGGAAAC				
rs751402-RP	21	58.4	52	
CGGAAACAGCCAGAAGATGTC				
rs751402-WTP	17	66	41	
CAL560-CCATTTTTCGTGGGTTT-BHQplus				
rs751402-MTP	17	67	41	
FAM-CCCATTTTTCATGGGTT-BHQplus				

Table 2. Clinical and demographic characteristic of study subjects

Variables	No. of cancer cases (%) (Total = 155)	No. of control cases (%) (Total = 122)	OR (95% CI	*P value*
Age, years (Mean ± SD)	52.7 ± 9.3	48.8 ± 10.6		0.002
Menopausal status				
Pre-	106 (68.3)	59 (48.3)	1	
Post-	49 (31.7)	63 (51.7)	0.43 (0.27-0.71)	0.001
Number of child				
0	7 (4.5)	13 (10.7)	1	
1-2	84 (54.2)	78 (63.9)	1.35 (0.73-5.43)	0.177
≥3	64 (41.3)	31 (25.4)	2.59 (1.22-22.67)	0.465
Smoking behaviors				
Never	146 (94.1)	115 (94.2)		
Ever	9 (5.9)	7 (5.8)	1.01 (0.37-2.80)	0.814
Alcohol drinking				
Never	118 (76.1)	116 (95.1)		
Ever	37 (23.9)	6 (4.9)	6.06 (2.47-14.91)	0.001

lular processes, including RNA polymerase II transcription, and transcription-coupled DNA repair. Mutation in *ERCC5* gene cause xeroderma pigmentosum complementation group G, a skin disorder characterized by hypersensitivity to UV light and increased susceptibility for skin cancer development following UV exposure. *ERCC5* rs17655 was reported in bladder cancer [6].

Cancer susceptibility to cancers has attracted growing to investigate gene polymorphisms associated with cancer development. The capacity to repair DNA damage is under genetic control and may be an important endogenous factor influencing the susceptibility of cancer. In this study, we hypothesized that polymorphism in the ERCC5 gene is associated with the risk of breast cancer in northeastern part of Thailand. To test this hypothesis, a case-control study of 155 breast cancer patients and 122 controls were conducted to evaluate the association between *ERCC5* rs751402 polymorphism and breast cancer risk. The genotype frequencies of *ERCC5* polymorphism in the cancer patients and controls were investigated. We also study the association between *ERCC5* polymorphism and the clinicopathological characteristics of the patients and controls.

Materials and methods

Subjects

This study included 155 patients with breast cancer diagnosis and stay in the northeastern part of Thailand. The patients recruited from Ubonratchathani Cancer Hospital in the year 2017. 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 076/2015) and Ubonratchathani Cancer Hospital, Thailand (EC 007/2017).

SNPS selection and genotyping

All patients and healthy controls were asked to provide 5 mL of whole blood (1.5 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 absorban-

Table 3. Clinical data of breast cancer cases

Clinical data	No. of breast cancer cases (Total = 155)	%
Tumor size		
< 2 cm	84	54.2
2-5 cm	13	8.4
> 5 cm	16	10.3
N/A	42	27.1
Type of breast cancer		
DCIS	10	6.5
Invasive ductal CA	142	91.6
Invasive mammary	1	0.6
Invasive with DCIS	2	1.3
Grade		
1	4	2.6
2	87	56.1
3	37	23.9
N/A	27	17.4
ER (estrogen receptor)		
Negative	68	43.9
Positive	81	52.3
N/A	6	3.8
PR (progesterone receptor)		
Negative	85	54.8
Positive	64	41.3
N/A	6	3.9
Her-2 (human epidermal growth factor receptor)		
Negative	96	61.9
Positive	48	31.0
N/A	11	7.1

DCIS, Ductal Carcinoma in Situ; CA, Cancer.

Table 4. *ERCC5* genotype frequencies in patients and controls

Genotypes	No. of breast cancer patients (%)	No. of healthy controls (%)	Total (%)
GG	39 (25.2)	40 (32.8)	79 (28.5)
AG	83 (53.5)	51 (41.8)	134 (48.3)
AA	33 (21.3)	31 (25.4)	64 (23.2)
Total	155 (100)	122 (100)	277 (100)

ce at 260 and 280 nm by Nano Drop Technology. *ERCC5* rs751402 polymorphism was detected using qPCR technique.

Total RNA was extracted and checked for the quality. One microgram of total RNA was reverse transcribed for first strand cDNA synthesis. All reactions were done in triplicate to analyze expression using gene specific primers (**Table 1**) by qPCR technique where *β*-actin was taken as an endogenous reference control. 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 *ERCC5* rs751402: homozygous wild type (GG), heterozygous variant type (AG) and homozygous variant type (AA).

Samples were coded for case-control status, and at least 10% of the samples were randomly selected and subjected to repeat analysis as quality control for verification of genotyping procedure.

Statistical analysis

The differences in distributions of demographic, epidemiologic and clinical variables as well as genotypes between breast cancer cases and healthy controls were calculated for percentage, odds ratio (OR) and 95% confidence intervals (95% CI). These data were analyzed by STATA software (Version 11.0) to assess the effect of each SNPs on breast cancer risk and $P < 0.05$ was regarded as statistically significant.

Results

Clinicopathological and demographic characteristics

This study included subjects with 155 patients of breast cancer and 122 healthy controls. The mean age of patients was 52.7 ± 9.3 years and the mean age of controls was 48.8 ± 10.6 years. Baseline demographic characteristics of study subjects were showed in **Table 2**. Decreased breast cancer risk was found in those who had post menopause (OR = 0.43, 95% CI = 0.27-0.71, $P = 0.001$) as compared with pre menopause. In addition, such increased breast cancer risk was observed in those who had

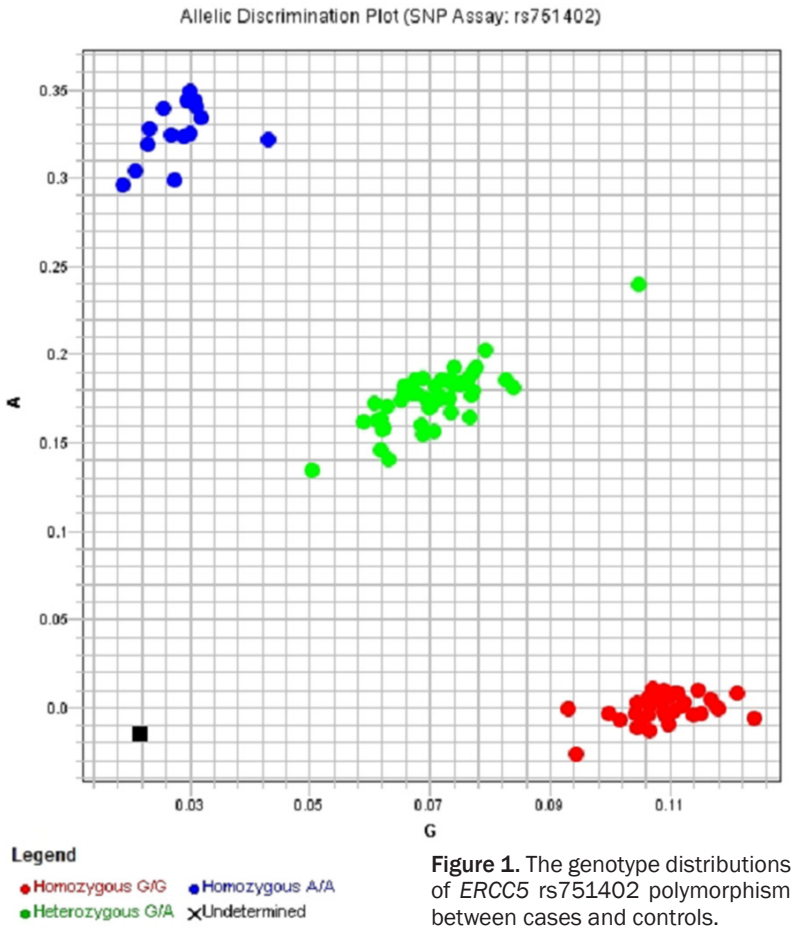

Figure 1. The genotype distributions of *ERCC5* rs751402 polymorphism between cases and controls.

Table 5. Comparison between genotypes, OR and *P* value with breast cancer risk

Genotypes	Crude odds ratio	95% CI	*P*
GG	1		
AG	1.66	0.95-2.92	0.074
AA	1.09	0.56-2.11	0.794

drinking alcohol in breast cancer group (OR = 6.06, 95% CI = 2.47-14.91, *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. An increased breast cancer risk was not significant about number of child.

The summary of the clinicopathological characteristics of the patients and controls, including age, menopause, number of children, tobacco smoking, alcohol drinking, were shown in **Table 2**.

The clinical data of 155 patients with breast cancer were shown in **Table 3**. It found that 84 patients (54.2%) were highly relevant to tumors size < 2 cm., 142 patients (91.6%) were highly relevant to invasive ductal carcinoma, 87 patients (56.1%) were grade 2, 81 patients (52.3%) were positive for estrogen receptor, 85 patients (54.8%) were negative for progesterone receptor and 96 patients (61.9%) were negative for Her-2.

Genotype study

The genotype distributions of *ERCC5* rs751402 polymorphism between cases and controls were shown in **Table 4** and **Figure 1**. Statistical analysis for comparison between genotypes, OR and *P* value with breast cancer risk was shown in **Table 5**.

It showed that *ERCC5* rs751402 AG had the highest frequency in breast cancer cases (53.5%) compared with healthy controls (41.8%). Heterozygous mutant allele affected breast cancer risk. The frequencies of *ERCC5* rs751402 AA in breast cancer cases and healthy controls were 21.3% and 25.4% respectively (**Table 3**). Individuals with AG genotype were associated with increased risk of breast cancer when compared with wild type genotype. We found that GG/AG genotype had OR = 1.66, 95% CI = 0.95-2.92, *P* = 0.074 and genotype GG/AA had OR = 1.09, 95% CI = 0.56-2.11, *P* = 0.794. The *ERCC5* was the important risk factor in our study population (**Table 5**).

The results of multivariate logistic regression analysis of the effects of *ERCC5* rs751402 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 *ERCC5* rs751402 variant AG genotype was associated with increased breast cancer risk: OR = 2.30; 95% CI = 1.22-4.35, *P* = 0.010 (**Table 6**).

Table 6. Multivariate logistic regression analysis of *ERCC5* rs751402 polymorphism for breast cancer risk

Genotypes	No. of cancer patients (%)	No. of healthy controls (%)	Adjusted OR[(a)] (95% CI)	*P*
GG	39 (25.1)	40 (32.7)	1	
AG	83 (53.5)	51 (41.8)	2.30 (1.22-4.35)	0.010
AA	33 (21.2)	31 (25.4)	1.46 (0.71-3.00)	0.300

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

Association between ERCC5 rs751402 variants and clinicopathological features in breast cancer

This study observed that *ERCC5* rs751402 variant AG genotype was associated with increased breast cancer risk (*P* = 0.010) when age, menopause period, number of child, smoking and alcohol drinking were adjusted. Invasive ductal carcinoma and invasive with DCIS could be detected in 91.6% and 6.5% respectively in breast cancer cases (**Table 3**). Invasive ductal carcinoma and invasive with DCIS could be detected in the breast cancer patients who had *ERCC5* rs751402 genotype AG for 67.4% and 6.9% respectively. The breast cancer patients who had *ERCC5* rs751402 genotype AG had estrogen receptor positive for 50%. This finding suggested that invasive ductal carcinoma and estrogen receptor (ER) positive were frequently occurred in *ERCC5* rs751402 genotype AG of Thai breast cancer patients.

Discussion

Breast cancer is a complex multifactorial disease with unclear etiology. DNA damage and genomic instability, a potential risk of breast cancer, are induced by common environmental factors. Polymorphisms can contribute to the difference between individuals and the susceptibility to a diseases and its severity. Polymorphism in many genes were reported that they involved in cancer risk. The nucleotide-excision repair (NER) pathway is the mechanism for the repair of bulky and helical distorting DNA adducts generated by cigarette smoke. NER proteins can play an important role in repairing some form of oxidative damage, basic sites and C-C mismatches. NER repairs damage introduced by ultraviolet radiation, products of organic combustion, intrastrand DNA cross-links, heavy metals, and oxidative stress. The polymorphisms could change the NER ability by influencing the expression and function of important proteins. Polymorphisms in nucleotide excision repair (NER) pathway genes are associated with the risk of lymphoma and breast cancer [7]. Lys939Gln genotype in XPC gene was associated with bladder cancer risk [6]. Genetic variability of genes in the NER pathway can influence the treatment outcome of many types of cancer [5]. Genetic variants of ERCC genes were widely studied as risk factors for cancer as well as predictors of treatment outcomes in cancer. Ying et al., (2016) found that the presence of *ERCC1* rs3212986 polymorphism was correlated with an increased risk of pancreatic cancer [8]. It has been observed that the SNPs of *ERCC5* are associated with the development of certain cancers. Lu et al., (2014) reported the association between *ERCC5* rs17655 polymorphism and laryngeal cancer risk and found that they confer more risk among smokers and drinkers [9]. *ERCC5* rs17655 status represented a potential DNA repair signature and it could be used for the production of clinical response to trabectedin in patients with soft tissue sarcoma [10]. Qin et al., (2013) reported that association between DNA repair genes and risk of glioma, and found that *ERCC5* Asp1558His are associated with risk of this cancer [11]. Na et al., (2015) reported *ERCC5* rs2094258 was associated breast cancer risk in China [12]. Guo et al., (2016) investigated the role of *ERCC5* polymorphisms (rs17655 and rs751402) in the development of gastric cancer and found that AA genotype of rs751402 significantly increased gastric cancer risk compared to the GG genotype but rs17655 did not [13]. Hussain et al., (2009) examined an American population, in which they found that the *ERCC5* rs1047768, rs17655 and rs2227869 polymorphisms were associated with gastric cancer [14]. Joo et al., (2016) showed highly significant associations of rs454421 in *ERCC2* and rs17655 in *ERCC5* with cervical cancer [15]. *ERCC5* may contribute to the etiology of cervical cancer. The genetic polymorphisms in nucleotide excision repair pathway influences response to chemotherapy in bone cancer. *ERCC1* rs11615 and rs2298881 genetic polymorphisms were significantly associated with poor response to chemotherapy and unfavourable survival of osteosarcoma [16].

ERCC5 rs751402 had interaction with alcohol drinking in breast cancer risk in this study. Invasive ductal carcinoma and ER positive were frequently found among these Thai breast cancer patients. ER was more affected in breast cancer risk than PR and Her-2. Our study indicated that *ERCC5* rs751402 genotype AG was associated with breast cancer risk. This polymorphism may contribute to the etiology of breast cancer in Thailand. This is the first report about the association between *ERCC5* rs751402 genotype AG and breast cancer risk in Thailand and Southeast Asia. More comprehensive screening based on a large number of genetic variants related to breast cancer susceptibility is expected to provide evidence for addressing breast cancer-related public health issues such as defining the high risk population for aggressive cancer screening and the use of prevention and treatment.

Conclusion

The AG genotype of *ERCC5* rs751402 was associated with an increased risk of breast cancer in Thai population.

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Disclosure of conflict of interest

None.

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