Int J Clin Exp Pathol 2015;8(3):3192-3197
www.ijcep.com /ISSN:1936-2625/IJCEP0005626

Original Article

Association between ERCC5 gene polymorphisms and breast cancer risk

Nari Na[1,2], Eer Dun[3], Lidong Ren[2], Guoxin Li[1]

[1]Department of General Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China; [2]Department of Thyroid, Breast, Hernia and Vascular Surgery, Inner Mongolia People's Hospital, Hohhot, China; [3]Department of Respiratory, Inner Mongolia People's Hospital, Hohhot, China

Received January 7, 2015; Accepted February 27, 2015; Epub March 1, 2015; Published March 15, 2015

Abstract: We conducted a case-control study to evaluate the association between ERCC5 polymorphism and breast cancer risk. 325 breast cancer patients and 325 controls were recruited in our study between January 2011 and March 2014. ERCC5 rs1047768, rs2094258, rs2296147, rs751402 and rs873601 polymorphisms were genotyped, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. By logistic regression analysis, we found that individuals with AA genotype of rs2094258 was associated with increased risk of breast cancer when compared with wide-type genotype, and the OR (95% CI) was 1.80 (1.12-2.92) for AA genotype. Individuals with GA + GG genotype of rs2094258 were significantly correlated with increased risk of breast cancer in tobacco smokers, and the OR (95% CI) was 7.35 (1.21-47.20). In conclusion, our study indicated that ERCC5 rs2094258 polymorphism may contribute to the risk of breast cancer.

Keywords: ERCC5, polymorphism, breast cancer

Introduction

Breast cancer is the most frequently diagnosed cancer in females worldwide, with ~1.38 million new cases estimated to have occurred in 2008, making it the second most common malignancy among females worldwide (10.9% of all cancers) [1]. The incidence of BC in China rises rapidly from 7/100,000 to 28/100,000 in the past decades. Pathogenesis of BC that involves multiple environmental and hereditary factors is complex and poorly understood [2].

DNA repair is essential for maintaining 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. At present, there are at least five known major DNA repair pathways, including more than 150 human DNA repair genes, among which the nucleotide-excision repair (NER) pathway is the most versatile and is particularly significant in association with cancer risk [3]. It has been reported that high levels of damaged DNA and deficient repaired DNA are associated with breast cancer [4, 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 [6]. Recently, genetic polymorphisms of genes involved in multiple biological pathways, including DNA repair, have been identified as potential risk factors for breast cancer [7]. Certain studies have additionally reported the association between ERCC5 and risk of breast cancer [8, 9], but the results are inconsistent.

Therefore, we hypothesized that polymorphisms in the ERCC5 gene are associated with the risk of breast cancer. To test this hypothesis, a case-control study of 325 patients with breast cancer and 325 controls were conducted to evaluate the association between ERCC5 polymorphism and breast cancer risk.

Table 1. Clinicopathological characteristics of the patients and controls

Characteristics	Cases	%	Controls	%	χ^2 test	*P* value
Age						
< 50	156	47.9	164	50.4		
≥ 50	169	52.1	161	49.6	0.39	0.53
Menopause						
No	255	78.6	199	61.2		
Yes	70	21.4	126	38.8	22.91	< 0.05
Age at menarche						
< 15	232	71.4	218	67.2		
≥ 15	93	28.6	107	32.8	1.42	0.23
Age at first live birth, years						
No	33	10.3	18	5.6		
< 30	106	32.5	137	42.1		
≥ 30	186	57.2	170	52.3	9.09	0.01
Tobacco smoking						
Never	299	92.1	314	96.7		
Ever	26	7.9	11	3.3	6.45	0.01
Alcohol drinking						
Never	236	72.6	243	74.8		
Ever	89	27.4	82	25.2	0.39	0.53
Family history of cancer						
No	296	91.2	314	96.5		
Yes	29	8.8	11	3.5	8.63	0.003

Materials and methods

Study population

A hospital-based case-control study was conducted. All subjects were genetically unrelated Han Chinese females from Inner Mongolia People's Hospital. 325 patients who were newly diagnosed with histopathologically confirmed primary breast cancer were recruited from Inner Mongolia People's Hospital between January 2011 and March 2014. 325 control subjects were collected from health check center of Inner Mongolia People's Hospital, who came to receive health examination in our hospital. Control subjects who had any primary tumors were excluded from our study. The control subjects were matched to cases by age (± 5 years) and sex, and one control was matched to one case. At recruitment, each participant was scheduled for an interview once written informed consent had been obtained.

Cases and controls were investigated by doctors to obtain demographic parameters. For the cases, clinical and pathological information were collected from the medical records.

SNPs selection and genotyping

Genomic DNA was isolated from 5 ml peripheral blood of each blood sample, using the TIANGEN DNA Blood Mini Kit (Tiangen Biotech CO., LTD, Beijing, China) according to the manufacturer's instructions. ERCC5 rs1047768, rs2094258, rs2296147, rs751402 and rs873601 polymorphisms were genotyped, using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay. PCR amplification was performed in a mixture containing 100 ng genomic DNA, 300 nM each primer, 200 nM dNTPs, and 0.5U Taq polymerase in PCR buffer (TaKaRa Biotechnology Co., Ltd, Dalian, China). The primers and probes for PCR amplification were designed using Primer 5.0 software (PREMIER Biosoft, Palo Alto, CA). The reaction condition was as follows: 94°C for 4 min; 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec with 35 cycles; 72°C for 7 min. 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 procedures. Some samples were also confirmed by DNA sequencing analysis. Two researchers independently reviewed all genotyping results.

Statistical analysis

The differences in distributions of demographic, epidemiologic, and clinical variables as well as genotypes between the two groups were assessed using a χ^2 test or Fisher exact test. The Hardy Weinberg equilibrium for genotype distribution in the controls was tested by a goodness-of-fit χ^2 test. The association between ERCC5 rs1047768, rs2094258,

Table 2. Information of five SNPs in NER pathway

SNPs	Base change	SNP location	Hardy–Weinberg Equilibrium	MAF	
				In database	In controls
rs1047768	T > C	Exon	0.35	0.241	0.269
rs2094258	G > A	Promoter	0.58	0.383	0.371
rs2296147	T > C	5'Upstream	< 0.05	0.201	0.238
rs751402	C > T	Promoter	0.87	0.367	0.353
rs873601	G > A	3'Untranslated region	0.28	0.496	0.434

Table 3. Association between ERCC5 polymorphisms and breast cancer risk

ERCC5	Cases	%	Controls	%	OR (95% CI)[1]	*P* value
rs1047768						
TT	161	49.54	171	52.62	1.0 (Ref.)	
TC	140	43.08	134	41.23	1.11 (0.80-1.55)	0.52
CC	24	7.38	20	6.15	1.27 (0.65-2.53)	0.45
rs2094258						
GG	102	31.38	131	40.31	1.0 (Ref.)	
GA	157	48.31	147	45.23	1.37 (0.96-1.96)	0.07
AA	66	20.31	47	14.46	1.80 (1.12-2.92)	0.01
rs2296147						
TT	188	57.85	199	61.23	1.0 (Ref.)	
TC	104	32.00	98	30.15	1.12 (0.79-1.60)	0.50
CC	33	10.15	28	8.62	1.25 (0.70-2.23)	0.42
rs751402						
CC	128	39.38	137	42.15	1.0 (Ref.)	
CT	152	46.77	147	45.23	1.11 (0.78-1.56)	0.55
TT	45	13.85	41	12.62	1.17 (0.70-1.97)	0.52
rs873601						
GG	99	30.46	109	33.54	1.0 (Ref.)	
GA	156	48.00	150	46.15	1.15 (0.79-1.65)	0.45
AA	70	21.54	66	20.31	1.17 (0.74-1.84)	0.48

[1]Adjusted for age, menopause, age at menarche, age at first live birth, tobacco smoking, alcohol drinking, and family history of cancer.

rs2296147, rs751402 and rs873601 polymorphisms and risk of breast cancer were analyzed using conditional logistic regression, and the results were expressed using odds ratio (OR) and their confidence intervals (CI). All tests were 2-sided, and probability values less than 0.05 were considered statistically significant. Analyses were performed using SPSS 13.0 software (SPSS Inc, IL, USA).

Results

Clinical characteristics

Table 1 summarizes the clinicopathological characteristics of the patients and controls, including age, menopause, age at menarche, marital status, age at first pregnancy, tobacco smoking, alcohol drinking and family history of cancer. The mean ages of patients and controls were 51.4 ± 8.3 and 49.8 ± 9.1 years old, respectively. No significant differences were found between cases and controls in terms of age, age at menopause and alcohol drinking. Compared with the control subjects, the breast cancer cases were more likely to have earlier menarche age, lower age of first live birth, more tobacco smokers, and more family history of cancer.

The genotype distributions of ERCC5 rs1047768, rs2094258, rs751402 and rs873601 were found to in line with Hardy–Weinberg equilibrium in the control group, while rs2296147 were not (**Table 2**). The minor allele frequencies (MAF) in controls of the six SNPs were similar to those in database (http://www.ncbi.nlm.nih.gov/snp).

The genotype distributions of six SNPs in ERCC5 between cases and controls are shown in **Table 3**. By logistic regression analysis, we found that individuals with AA genotype of rs2094258 was associated with increased risk of breast cancer when compared with wide-type genotype, and the OR (95% CI) was 1.80 (1.12-2.92) for AA genotype. However, no significant difference was found between polymorphisms in rs1047768, rs2296147, rs751402 and rs873601 and breast cancer risk.

We further analyzed the association between rs2094258 polymorphism and breast cancer risk stratified by menopause, live birth, tobacco smoking and family history of cancer (**Table 4**). Individuals with GA + GG genotype of rs2094258 were significantly correlated with increased risk of breast cancer in tobacco smokers, and the OR (95% CI) was 7.35 (1.21-47.20).

Table 4. Association between rs2094258 gene polymorphisms and clinicopathological characteristics on the breast cancer risk

Variables	Cases		Controls		OR (95% CI)[1]	*P* value
	GG	GA + GG	GG	GA + GG	GA + GG vs. GG	
Menopause						
No	83	172	81	118	1.42 (0.95-2.13)	0.07
Yes	19	51	50	76	1.77 (0.90-3.55)	0.08
Live birth						
No	7	26	8	10	2.97 (0.71-12.40)	0.08
Yes	95	197	123	184	1.39 (0.98-1.97)	0.06
Tobacco smoking						
Never	97	202	124	190	1.36 (0.96-1.92)	0.07
Ever	5	21	7	4	7.35 (1.21-47.20)	0.01
Family history of cancer						
No	94	202	125	189	1.36 (0.96-1.92)	0.07
Yes	8	21	6	5	7.35 (1.21-47.20)	0.01

[1]Adjusted for age.

However, we did not find significant interaction between rs2094258 polymorphism and menopause, live birth and family history of cancer in breast cancer risk.

Discussion

Polymorphisms, which have an effect on the regulation of gene expression, can contribute to the differences between individuals in the susceptibility to a disease and its severity. The regulation of DNA repair is a vital factor in the multistep process of carcinogenesis, and the ERCC5 gene is a crucial part of the DNA repair machinery. It has been observed that the SNPs of ERCC5 are associated with the development of certain cancers, including breast cancer [10].

The ERCC5 polymorphism may result in a defect in nucleotide excision repair, and the role of insufficient DNA repair in carcinogenesis has been extensively studies. For example, a failure in DNA repair has been identified as a risk factor for the development of many cancers [11-17]. Liang et al. assessed the association between ERCC5 rs17655 polymorphism and lung cancer risk, and found that ERCC5 rs17655 polymorphism may not contribute to genetic susceptibility for lung cancer [11]. Lu et al. reported the association between SNPs in DNA repaired genes and risk of laryngeal cancer, and found that ERCC5 rs17655 polymorphisms are associated with increased risk of laryngeal cancer, and that they confer more risk among smokers and drinkers [12]. Luo et al. reported that association between DNA repair genes and risk of glioma, and found that ERCC5 Asp1558His are associated with risk of this cancer [14]. Duan et al. conducted a case-control study to assess the association between ERCC5 polymorphisms and susceptibility to gastric cancer, and they found that ERCC5 rs751402 and rs2296147 polymorphisms were associated with increased gastric cancer risk [15]. Zavras et al. suggested that there was an association between rs751402 polymorphism and oral cancer risk [16]. However, a meta-analysis reported the association between ERCC5 polymorphisms and cancer risk, and did not find that ERCC5 polymorphism may contribute to individual susceptibility to cancer risk [17]. The results of these studies are inconsistent and came to a different conclusion. The discrepancies between these studies may be caused by differences in tumor types, study design and populations.

Despite a growing number of epidemiologic studies on the association of the DNA repaired gene polymorphisms with susceptibility to brain tumors, four studies reported the significant association between ERCC5 polymorphisms and breast cancer risk [18-21]. Kumar et al. found that XPG Asp1104His polymorphism was associated with increased risk of breast cancer [18]. However, other two studies did not find significant association between XPG Asp1104His polymorphism and breast cancer risk [19, 21]. One meta-analysis with

eight published articles suggested that XPG Asp1104His polymorphism is not associated with breast cancer risk [20]. Our study found that rs2094258 polymorphism was associated with breast cancer risk, but no previous study reported similar results with ours. Therefore, further large sample size studies are greatly needed to confirm our results.

Genetic factors could interact with environmental factors in the pathogenesis of breast cancer. NER may be an important pathway modulating susceptibility to breast cancer, since NER is the primary mechanism for the repair of bulky and helical distorting DN adducts generated by cigarette smoke [22-24]. NER proteins can play an important role in repairing some forms of oxidative damage, basic sites and C-C mismatches [25-27]. Our study found that rs2094258 polymorphism had interaction with tobacco smoking in breast cancer risk, which suggested that reduced efficiency of NER could increase susceptibility to DNA damage caused by cigarette smoke, and thus increase the breast cancer risk in smokers.

There are two limitations in our study. First, cases and controls were selected from one hospital, and rs2296147 were not in line with Hardy–Weinberg equilibrium. Selection bias may be considered in our study. Second, smoking and drinking behaviors were recalled by participants, and information bias may be existed. Third, the sample size of this study is relatively small, and small sample size may limit the statistical power to find differences between groups.

In conclusion, this study showed that ERCC5 rs2094258 polymorphism was associated with breast cancer risk, especially in smokers. Therefore, our study indicated that ERCC5 rs2094258 polymorphism may contribute to the etiology of breast cancer. Further large sample studies are greatly warranted to elucidate our finding.

Acknowledgements

We thanks for the funding from Key Clinical Speciality Discipling Construction Program.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Guoxin Li, Department of General Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China. Tel: +86-13802771450; Fax: +86-13802771450; E-mail: guoxinli92@126.com

References

[1] Globocan 2012. Breast Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr/factsheet.asp. International Agency for Research on Cancer.

[2] DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin 2014; 64: 52-62.

[3] Grossman L, Riazuddin S, Haseltine WA, Lindan C. Nucleotide excision repair of damaged DNA. Cold Spring Harb Symp Quant Biol 1979; 43: 947-55.

[4] Smith TR, Miller MS, Lohman KK, Case LD, Hu JJ. DNA damage and breast cancer risk. Carcinogenesis 2003; 24: 883-9.

[5] Ramos JM, Ruiz A, Colen R, Lopez ID, Grossman L, Matta JL. DNA repair and breast carcinoma susceptibility in women. Cancer 2004; 100: 1352-7.

[6] Hohl M, Dunand-Sauthier I, Staresincic L, Jaquier-Gubler P, Thorel F, Modesti M, Clarkson SG, Schärer OD. Domain swapping between FEN-1 and XPG defines regions in XPG that mediate nucleotide excision repair activity and substrate specificity. Nucleic Acids Res 2007; 35: 3053-63.

[7] McCullough LE, Santella RM, Cleveland RJ, Millikan RC, Olshan AF, North KE, Bradshaw PT, Eng SM, Terry MB, Shen J, Crew KD, Rossner P Jr, Teitelbaum SL, Neugut AI, Gammon MD. Polymorphisms in DNA repair genes, recreational physical activity and breast cancer risk. Int J Cancer 2014; 134: 654-63.

[8] Rajaraman P, Bhatti P, Doody MM, Simon SL, Weinstock RM, Linet MS, Rosenstein M, Stovall M, Alexander BH, Preston DL, Sigurdson AJ. Nucleotide excision repair polymorphisms may modify ionizing radiation-related breast cancer risk in US radiologic technologists. Int J Cancer 2008; 123: 2713-6.

[9] Ming-Shiean H, Yu JC, Wang HW, Chen ST, Hsiung CN, Ding SL, Wu PE, Shen CY, Cheng CW. Synergistic effects of polymorphisms in DNA repair genes and endogenous estrogen exposure on female breast cancer risk. Ann Surg Oncol 2010; 17: 760-71.

[10] Ding DP, He XF, Zhang Y. Lack of association between XPG Asp1104His and XPF Arg415GIn polymorphism and breast cancer risk: a meta-analysis of case-control studies. Breast Cancer Res Treat 2011; 129: 203-209.

[11] Liang Y, Deng J, Xiong Y, Wang S, Xiong W. Genetic association between ERCC5 rs17655 polymorphism and lung cancer risk: evidence based on a meta-analysis. Tumour Biol 2014; 35: 5613-8.
[12] Lu B, Li J, Gao Q, Yu W, Yang Q, Li X. Laryngeal cancer risk and common single nucleotide polymorphisms in nucleotide excision repair pathway genes ERCC1, ERCC2, ERCC3, ERCC4, ERCC5 and XPA. Gene 2014; 542: 64-8.
[13] Li X, Xu J, Yang X, Wu Y, Cheng B, Chen D, Bai B. Association of single nucleotide polymorphisms of nucleotide excision repair genes with laryngeal cancer risk and interaction with cigarette smoking and alcohol drinking. Tumour Biol 2014; 35: 4659-65.
[14] Luo KQ, Mu SQ, Wu ZX, Shi YN, Peng JC. Polymorphisms in DNA repair genes and risk of glioma and meningioma. Asian Pac J Cancer Prev 2013; 14: 449-52.
[15] Duan Z, He C, Gong Y, Li P, Xu Q, Sun LP, Wang Z, Xing C, Yuan Y. Promoter polymorphisms in DNA repair gene ERCC5 and susceptibility to gastric cancer in Chinese. Gene 2012; 511: 274-9.
[16] Zavras AI, Yoon AJ, Chen MK, Lin CW, Yang SF. Association between polymorphisms of DNA repair gene ERCC5 and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol 2012; 114: 624-9.
[17] Zhu ML, Wang M, Cao ZG, He J, Shi TY, Xia KQ, Qiu LX, Wei QY. Association between the ERCC5 Asp1104His polymorphism and cancer risk: a meta-analysis. PLoS One 2012; 7: e36293.
[18] Kumar R, Höglund L, Zhao C, Försti A, Snellman E, Hemminki K. Single nucleotide polymorphisms in the XPG gene: determination of role in DNA repair and breast cancer risk. Int J Cancer 2003; 103: 671-5.
[19] Ding DP, He XF, Zhang Y. Lack of association between XPG Asp1104His and XPF Arg415Gln polymorphism and breast cancer risk: a meta-analysis of case-control studies. Breast Cancer Res Treat 2011; 129: 203-9.
[20] Xu XM, Xie LC, Yuan LL, Hu XL, Jin JQ, Niu YM. Association of xeroderma pigmentosum complementation group G Asp1104His polymorphism with breast cancer risk: A cumulative meta-analysis. Mol Clin Oncol 2014; 2: 1177-1181.
[21] Smith TR, Levine EA, Freimanis RI, Akman SA, Allen GO, Hoang KN, Liu-Mares W, Hu JJ. Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk. Carcinogenesis 2008; 29: 2132-8.
[22] Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 2004; 73: 39-85.
[23] DeMarini DM, Shelton ML, Levine JG. Mutation spectrum of cigarette smoke condensate in Salmonella: comparison to mutations in smoking-associated tumors. Carcinogenesis 1995; 16: 2535-42.
[24] Asami S, Manabe H, Miyake J, Tsurudome Y, Hirano T, Yamaguchi R, Itoh H, Kasai H. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. Carcinogenesis 1997; 18: 1763-6.
[25] Reardon JT, Bessho T, Kung HC, Bolton PH, Sancar A. In vitro repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. Proc Natl Acad Sci U S A 1997; 94:9463-8.
[26] Torres-Ramos CA, Johnson RE, Prakash L, Prakash S. Evidence for the involvement of nucleotide excision repair in the removal of abasic sites in yeast. Mol Cell Biol 2000; 20: 3522-8.
[27] Fleck O, Lehmann E, Schär P, Kohli J. Involvement of nucleotide-excision repair in msh2 pms1-independent mismatch repair. Nat Genet 1999; 21: 314-7.