

A New Single Nucleotide Polymorphism in XRCC4 Gene is Associated with Breast Cancer Susceptibility in Taiwanese Patients

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Abstract. *Background:* The DNA repair gene XRCC4, an important caretaker of the overall genome stability, is thought to play a major role in the human carcinogenesis. Some new and important polymorphic variants of XRCC4, at codon 247 (rs 3734091), G-1394T (rs 6869366), and Intron 7 (rs 28360317), and their association with breast cancer susceptibility was investigated in a Taiwanese population. *Materials and Methods:* In a hospital-based case-control study, 432 female patients with breast cancer and 432 age-matched healthy controls recruited from the China Medical Hospital in Central Taiwan were genotyped. *Results:* A significant difference in the frequency of the XRCC4 G-1394T genotype, but not the XRCC4 codon 247, or intron 7 genotypes was found between the breast cancer and control groups. Individuals with G/T or T/T at the XRCC4 G-1394T locus showed a 2.33-fold (95% confidence interval=1.37-3.98) increased risk of breast cancer compared to those with G/G. For XRCC4 codon 247 or intron 7, there was no difference in distribution between the breast cancer and control groups. *Conclusion:* Our findings suggest that the heterozygous and homozygous T allele of the XRCC4 G-1394T may be associated with the development of breast cancer and may be a useful biomarker for anticancer prevention and intervention.

Breast cancer is the most prevalent cancer over the world and the most common female cancer (1). The etiology of breast cancer is largely unknown. Epidemiological studies suggest that the etiology of breast cancer is multi-factorial, including exposure to ionizing radiation, high-fat dietary intake, alcohol consumption and use of hormones or oral contraceptives. However, only a small proportion of women exposed to these external factors develop breast cancer (2, 3), suggesting that genetic susceptibility plays a role in the individual risk of breast cancer. The appropriate response of the cell to genetic injury and its ability to maintain genomic stability by means of a variety of DNA repair mechanisms are essential in preventing tumor initiation and progression. Mutations or defects in the DNA repairing system are essential for tumorigenesis (4). It is therefore logical to suspect that some genetic variants of DNA repair genes, such as X-ray cross-complementing group 4 (XRCC4), might contribute to breast cancer pathogenesis.

Sequence variants in DNA repair genes are also thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk (5). Because single-nucleotide polymorphism (SNP) is the most frequent and subtle genetic variation in the human genome and has great potential for application to association studies in complex disease (6), SNPs in the XRCC4 gene were used to define their tumorigenic contribution to breast cancer development.

The XRCC4 gene, which is important in the non-homologous end-joining (NHEJ) repair pathway, has been found to restore DNA double-strand breaks and to have the ability to support V(D)J recombination of transiently introduced substrates in the XR-1 CHO cell line (7). The XRCC4 gene product interacts directly with Ku70/Ku80 (8), and it is hypothesized that XRCC4 serves as a flexible tether between Ku70/Ku80 and its associated protein, ligase 4 (8).

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Key Words: XRCC4, polymorphism, breast cancer, carcinogenesis.

XRCC4 has been shown to be required for precise end-joining of blunt DNA double-strand breaks in mammalian fibroblasts (9). In a gene-targeting mutation mouse model, *XRCC4* gene inactivation led to late embryonic lethality accompanied by defective lymphogenesis and defective neurogenesis manifested by extensive apoptotic death of newly generated postmitotic neuronal cells (10, 11). These findings demonstrated that differentiating lymphocytes and neurons strictly require the *XRCC4* end-joining proteins. Thus, it is reasonable to suppose, that only polymorphisms of *XRCC4* gene and not mutations, can be sustained in the genome for the lengthy duration of carcinogenesis. For this reason, few studies have investigated the single-gene role of the *XRCC4* gene in breast cancer. A significant association between increased breast cancer risk and a cooperative effect of SNPs in non-homologous end-joining genes has been reported (12), and the well-documented breast cancer susceptibility *BRCA1* gene, has been found to possibly contribute to breast cancer risk via its modification of cellular non-homologous end-joining capacity (13, 14). It has also been reported that *XRCC4* may play a role in the age at diagnosis and risk of breast cancer in non-*BRCA1/2*, heritable breast cancer cases (15).

Because of the potential contribution of the NHEJ pathway to breast cancer and the need for further investigation of new genetic variants in this pathway, the aim of the current study was to determine whether genetic polymorphisms in one of the NHEJ pathway genes, *XRCC4*, are associated with breast cancer. To test this hypothesis, the genotypic frequency of three polymorphisms of the *XRCC4* gene at codon 247 (rs 3734091), G-1394T (rs 6869366), and Intron 7 (rs 28360317), which will hereafter be referred to as X1, X2 and X3, respectively, were determined. To our knowledge, this is the first study carried out to evaluate at the same time the *XRCC4* X1, X2 and X3 polymorphisms in high prevalence Taiwanese population.

Materials and Methods

Study population and sample collection. Four hundred and forty patients diagnosed with breast cancer were recruited at the outpatient clinics of general surgery between 1998 and 2006 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The mean age of the breast cancer patients and the controls were 56.38 (SD=10.31) and 54.28 (SD=8.64) years, respectively. All patients and controls voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Four hundred and forty non-breast cancer healthy people as controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the Hospital. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Table I. Allele frequencies for *XRCC4* X1, X2, and X3 polymorphisms in the breast cancer and control groups.

Allele	Controls (%) N=864	Cases (%) N=864	p-value ^a
X1: rs 3734091			
Allele A (Ser)	849 (98.3)	839 (97.1)	0.10965
Allele C (Ala)	15 (1.7)	25 (2.9)	
X2: rs 6869366			
Allele G	843 (97.6)	814 (94.2)	0.00044
Allele T	21 (2.4)	50 (5.8)	
X3: rs 28360317			
Insertion	636 (73.6)	634 (73.4)	0.91319
Deletion	228 (26.4)	230 (26.6)	

^ap-value based on χ^2 test.

Genotyping assays. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) and further processed according to our previous papers (16). Briefly, the following primers were used for *XRCC4* X1: 5'-GCTAATGAGTTGCTGCATTAA-3' and 5'-TTCTAGGGAAACTGCAACTGTG-3'; for *XRCC4* X2: 5'-GATGCGAACTCAAAGATACTGA-3' and 5'-TGTAAGCCAGTACTCAAACCT-3'; and for *XRCC4* X3: 5'-ATACTGTGTTTGGAACTCCT-3' for CCT-positive forward primer, 5'-ATACTGTGTTTGGAACTAGA-3' for CCT-negative forward primer, and 5'-TATCCTATCATCTCTGGATA-3' as reverse common primer. The following cycling conditions were performed: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 10 min. The PCR products were studied after digestion with BbsI, and Hinc II, restriction enzymes for *XRCC4* X1 (cut from 308 bp C type into 204+104 bp A type), and X2 (cut from 300 bp T type into 200+100 bp G type), respectively.

Statistical analyses. Only those samples with complete DNA polymorphism data (case/control=432/432) were selected for final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *XRCC4* single nucleotide polymorphisms in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *XRCC4* genotypes between cases and controls. The cancer risk associated with the genotypes was estimated as odds ratio (OR) with 95% confidence intervals (CI) using unconditional logistic regression. Data was recognized as significant when the statistical p-value was less than 0.05.

Results

The frequency of the alleles for *XRCC4* X1, X2, and X3 between breast cancer and control groups is shown in Table I. The distributions of all these polymorphisms were in Hardy-

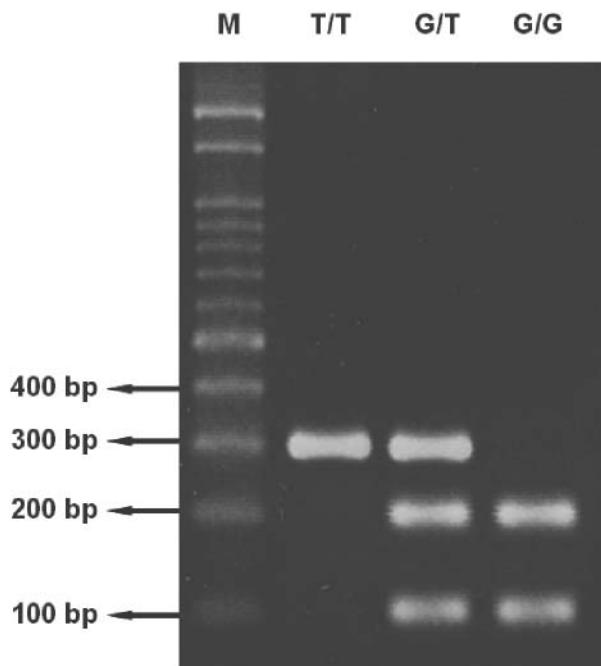


Figure 1. PCR-based restriction analysis of the X2 polymorphism of the *XRCC4* gene shown on 3% agarose electrophoresis. M: 100 bp DNA size marker, T/T: indivisible homozygote, G/T: heterozygote, and G/G: divisible homozygote.

Weinberg equilibrium and were similar between breast patients and controls (data not shown). The T allele at *XRCC4* X2 was found to be significantly associated with breast cancer risk ($p=0.00044$). In contrast, the A or C at *XRCC4* X1, or the insertion or deletion allele at *XRCC4* X3, were not differently distributed in the breast cancer patient and control groups ($p>0.05$). The representative PCR-based restriction analyses for the *XRCC4* X2 polymorphisms are shown in Figure 1.

The frequency of the genotypes of the *XRCC4* X1, X2, and X3 polymorphisms in the breast cancer and control groups is shown in Table II. Using the G allele as the reference group, there was an obvious association between the heterozygotes of the T allele of *XRCC4* X2 and breast cancer risk (OR, 2.13, 95% CI, 1.24-3.66). A combination of the homozygotes and heterozygotes of T showed a further increase of the T allele at *XRCC4* X2 as a 2.33-fold risk factor for breast cancer (Table II). Neither hetero- nor homozygotes of the C allele of *XRCC4* X1, seemed to be risky genotypes for breast cancer, as was also the case in the deletion allele of *XRCC4* X3 (Table II).

Discussion

There are no reports concerning any *XRCC4* polymorphism and risk of in other types of cancer. In the present study the T allele of X2 was positively associated with breast cancer

Table II. Association of *XRCC4* X1, X2, and X3 polymorphisms and breast cancer risk.

Genotype	Controls (%)	Cases (%)	Odds Ratio (95% CI) ^a
X1: rs 3734091			
A/A	417 (96.5)	410 (94.9)	1.00 (ref)
A/C	15 (3.5)	19 (4.4)	1.29 (0.65-2.57)
C/C	0 (0)	3 (0.7)	7.12 (0.37-138.27)
with C	15 (3.5)	22 (5.1)	1.49 (0.76-2.92)
X2: rs 6869366			
G/G	411 (95.1)	386 (89.4)	1.00 (ref)
G/T	21 (4.9)	42 (9.7)	2.13 (1.24-3.66)^b
T/T	0 (0)	4 (0.9)	9.58 (0.51-178.57)
with T	21 (4.9)	46 (10.6)	2.33 (1.37-3.98)^b
X3: rs 28360317			
I/I	249 (57.6)	240 (55.6)	1.00 (ref)
I/D	138 (31.9)	154 (35.6)	1.16 (0.87-1.55)
D/D	45 (10.4)	38 (8.8)	0.88 (0.55-1.40)
with D	183 (42.4)	192 (44.4)	1.09 (0.83-1.43)

^aCI, confidence interval; ^b $p<0.05$.

risk, while other polymorphisms of the *XRCC4* gene were not associated with increased risk (Tables I and II), the genotype distribution of the T allele at *XRCC4* X2 (5.8%) was more than 2-fold higher in the breast cancer group than in the control group (Table I). It was also found that participants heterozygous for *XRCC4* X2 had a 2.13-fold higher risk of breast cancer (Table II). Although there was no homozygous T/T in the control group, when the heterozygous and homozygous T groups were combined, the risk was of almost the same level, a 2.33-fold increased risk (Table II). The data suggested that the T allele at *XRCC4* X2 could be a novel marker for breast cancer. Since whenever the T allele was detected, whether as hetero- or homozygote, the individuals were more susceptible to breast cancer. Other polymorphisms of *XRCC4* were also investigated, including those of rs 1805377, rs 2075585, and rs 2075686 (data not shown), and the findings were consistent with the previous study (12), which reported that the polymorphism of rs 2075585, but not those of rs 1805377 and rs 2075686, was a biomarker of breast cancer. Another study of *XRCC4* association with breast cancer reported that the polymorphisms of rs 1478485 and rs 13180316 played a role in the age at diagnosis and risk of breast cancer in non-BRCA1/2, heritable breast cancer cases in a Northern European population (15).

These results also suggest that genetic variants involved in DNA repair pathways may be involved in breast cancer etiology. The possible mechanism is that women with the risky genotypes, such as the hetero- or homozygous T allele of *XRCC4* X2, may have a lower capacity for NHEJ repair of DNA damage caused by carcinogens. Over time, subtle

genetic defects accumulate more easily in their genome, which may lead to carcinogenesis. In conclusion, in this first report of the association between new *XRCC4* gene polymorphisms, X1, X2, and X3, and breast cancer, the T allele of *XRCC4* X2, was associated with higher susceptibility to breast cancer, and is a novel useful marker for the early detection, prevention and anticancer intervention of breast cancer.

Acknowledgements

We thank Chia-Wen Tsai, Hsiu-Yun Liang, and Chiao-Lin Lin for their technical assistance. This study was supported by research grants from the China Medical University Hospital (DMR-94-020), Terry Fox Cancer Research Foundation and the National Science Council (NSC 95-2320-B-039-014-MY3, second year).

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Received June 27, 2007

Revised October 25, 2007

Accepted November 1, 2007