Significant Association of DNA Repair Gene *Ku80* Genotypes with Breast Cancer Susceptibility in Taiwan

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Abstract. *Aim: To evaluate the association between the genotypes of the heterodimeric DNA-binding component, Ku80 gene and the breast cancer risk in Taiwan. Patients and Methods: In this hospital-based case-control study, the association of Ku80 G-1401T rs828907, Ku80 C-319T rs11685387 and Ku80 intron19 rs9288518 polymorphisms with breast cancer risk in a Taiwanese population was investigated. In total, 1272 patients with breast cancer and the same number of age- and gender-matched healthy controls were genotyped. Results: A significantly different distribution was found in the frequency of the Ku80 G-1401T genotype, but not the Ku80 C-319T or intron 19 genotypes, between the breast cancer and control groups. The T allele Ku80 G-1401T conferred a significant (p=0.0069) increased risk of breast cancer. Gene interactions with smoking, but not with breastfeeding, were significant for the Ku80 G-1401T polymorphism. The Ku80 G-1401T GT and TT genotypes in association with smoking conferred an increased risk of 3.162 (95% confidence interval=2.275-4.393) for breast cancer. Conclusion: The T allele of Ku80 G-1401T may be associated with the development of breast cancer and may be a novel useful marker for breast cancer detection and prediction.*

Breast cancer is the most prevalent female cancer worldwide (1), and 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 the use of hormones or oral contraceptives. However, only a small proportion of women thus exposed 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). Among the types of DNA damage, double-strand breaks (DSBs) may lead to dramatic genome instability, which is closely related to carcinogenesis (5, 6). There are two specific DNA repair pathways responsible for DSB repair, homologous recombination (HR) repair and the non-homologous end-joining (NHEJ) (5). Most DSBs are repaired by NHEJ, involving several key components (7). Once DSBs occur in genomic DNA, they are first recognized by a heterodimeric DNA-binding component KU, which is formed from Ku70 and *Ku80* (8). The Ku80 gene is located on chromosome 2q35 and has 21 exons (9). Former studies have indicated that mutation of *Ku80* may affect the age of cancer onset (10).

Mounting increasing number of single nucleotide polymorphisms (SNPs) have been confirmed as genetic factors associated with carcinogenesis (11-18). Recently, the *Ku80* gene has been reported to play a role in cancer development, but the association of its SNPs with breast cancer has not been investigated yet. In this study, the role of three SNPs of *Ku80*, G-1401T, C-319T and intron 19 in breast cancer in a central Taiwanese population was investigated for the first time.

Patients and Methods

Study population and sample collection. The study population consisted of 1272 patients and 1272 cancer-free control volunteers. Patients diagnosed with breast cancer were recruited at the Outpatient Clinics of General Surgery between 2002-2008 at the China Medical University Hospital, Taichung, Taiwan, People's

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Table I. *Frequency distributions of characteristics among breast cancer patients and controls.*

Characteristic	Controls (n=1272)			Patients (n=1272)			*P*-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			53.9 (12.5)			54.7 (12.2)	0.099
Age group (years)							0.096
≤50	517	40.6%		475	37.3%		
>50	755	59.4%		797	62.7%		
Breastfeeding							0.342
Yes	446	35.1%		469	36.9%		
No	826	64.9%		803	63.1%		
Cigarette smoking							
Yes	315	24.8%		329	25.9%		0.553
No	957	75.2%		943	74.1%		

Breastfeeding: Breast feeding for at least ten days, cigarette smoking: smoking habit previously or currently for at least one year.

Republic of China. The clinical characteristics of the patients, including histological details, were all graded and defined by expert surgeons (Dr. Wang's surgical team). All the patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of non-cancer healthy volunteers as controls were selected by matching for age, gender and some indulgences after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included any previous or current malignancy, metastasized cancer from other or unknown origin (other cancers with previous diagnosis), and any familial or genetic diseases. The study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all the participants.

Genotyping assays. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous papers (19, 20). Briefly, the following primers were used for *Ku80* G-1401T rs828907: 5'-TAGCTGACAACCTCACAGAT-3' and 5'-ATTCAGAGGTGCT CATAGAG-3'; for *Ku80* C-319T rs11685387: 5'-TCTAACTCC AGAGCTCTGAC-3' and 5'-AACTCTGAGCATGCGCAGAT-3'; and for *Ku80* intron19 rs9288518: 5'-GGTGTGAAGACCTATCAATC-3' and 5'-TTACAGAACAAGCCTTGCAC-3'. The following cycling conditions were performed: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s and a final extension at 72˚C for 10 min. The PCR products were studied after digestion with BfaI, SpeIor BsrI, restriction enzymes for *Ku80* G-1401T rs828907 (cut from 252 bp G type into 81+171 bp T type), *Ku80* C-319T rs11685387 (cut from 311 bp C type into 108+203 bp T type) and *Ku80* intron19 rs9288518 (cut from 275 bp A type into 110+165 bp G type), respectively.

Statistical analyses. Only those matches with all the SNPs data available were selected for the final analysis. To ensure that the controls were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *Ku80* SNPs 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 *Ku80* genotypes between cases and controls. Data was recognized as significant when the statistical *p*-value was less than 0.05.

Results

The frequency distributions of the selected characteristics of the breast cancer patients and controls are shown in Table I and were all well matched. None of these comparisons between the two groups was statistically significant ($p>0.05$) (Table I).

The frequency distributions of the genotypes for the *Ku80* G-1401T, C-319T and intron 19 in the controls and breast cancer patients are shown in Table II. The genotype distribution of the genetic polymorphisms of *Ku80* G-1401T was significantly different between the breast cancer and control groups ($p=5.05\times10^{-7}$), while those for C-319T and intron 19 were not significantly different ($p>0.05$) (Table II). To sum up, the *Ku80* G-1401T heterozygous and homozygous TT genotypes were significantly associated with breast cancer susceptibility.

The frequency distributions of the alleles for *Ku80* G-1401T, C-319T and intron 19 in the controls and breast cancer patients are shown in Table III. The distributions of all these polymorphisms were in Hardy-Weinberg equilibrium and were similar between the controls and breast cancer patients. The T allele of the *Ku80* G-1401T polymorphism was significantly associated with breast cancer ($p=2.56\times10^{-9}$) (Table III).

The genotype distribution of the various polymorphisms of *Ku80* G-1401T was significantly different between the breast cancer and control groups who had a smoking habit ($p<0.0001$) (Table IV), while those for C-319T and intron 19 were not ($p>0.05$). The T allele frequency was significantly higher in the breast cancer patients who smoked

Table II. *Distribution of Ku80 genotypes among breast cancer patients and controls.*

Genotype	Controls	%	Patients	%	*P*-value[a]
G-1401T rs828907					5.05^{-7}
GG	956	75.2%	833	64.5%	
GT	230	18.1%	309	24.3%	
TT	86	6.7%	130	10.2%	
C-319T rs11685387					0.5273
CC	170	13.4%	155	12.2%	
CT	303	23.8%	322	25.3%	
TT	799	62.8%	795	62.5%	
Intron19 rs9288518					0.4384
AA	132	10.4%	141	11.1%	
AG	402	31.6%	373	29.3%	
GG	738	58.0%	758	59.6%	

[a]Based on Chi-square test.

Table III. *Distribution of Ku80 alleles among breast cancer patients and controls.*

Allele	Controls	%	Patients	%	*P*-value[a]
G-1401T rs828907					2.56^{-9}
Allele G	2142	84.2%	1975	77.6%	
Allele T	402	15.8%	569	22.4%	
C-319T rs11685387					0.7219
Allele C	643	25.3%	632	24.8%	
Allele T	1901	74.7%	1912	75.2%	
Intron 19 rs9288518					0.7250
Allele A	666	26.2%	655	25.8%	
Allele G	1878	73.8%	1889	74.2%	

[a]Based on Chi-square test.

than in the non-cancer controls, and the patients who did not smoke. In Taiwan, individuals with *Ku80* G-1401T GT or TT who smoked were approximately 3.2-fold more likely to have breast cancer than those who did not smoke (Table IV). There was no significant joint effect between *Ku80* G-1401T and breastfeeding on breast cancer risk.

Discussion

This was the first study which focused on the association between the polymorphisms of *Ku80* and breast cancer susceptibility. Only *Ku80* G-1401T was found to have statistical significance in association with increased breast cancer, while the *Ku80* C-319T and *Ku80* intron 19 genotypes had no effect. In the population with a smoking habit, the genetic effect of the *Ku80* G-1401T on breast cancer risk was much more significant. In the smoking

Table IV. *Ku80 G-1401T genotype and breast cancer after stratification by cigarette smoking.*

Variable	*Ku80* G-1401T genotype			
	GG	GT+TT	*P*-value[a]	OR (95% CI)[b]
Smokers			<0.0001	
Controls	170	145		1.00
Patients	89	240		3.162 (2.275-4.393)[c]
Non-smokers			0.0821	
Controls	786	171		1.00
Patients	744	199		1.229 (0.979-1.544)

[a]Based on Chi-square test; [b]odds ratios (OR) were estimated by multivariate logistic regression analysis; [c]statistically identified as significant.

groups, the T allele clearly raised the breast cancer risk. Accordingly, we propose that the T allele of *Ku80* G-1401T polymorphism may play a role in carcinogenesis. Non-smokers carrying the T allele may have similar efficiency compared to non-T allele carriers in removing DSBs, but in smokers, the DNA damage increases significantly, and people with the T allele may not have sufficient capacity to remove all the DSBs promptly and efficiently, thus increasing their breast cancer risk.

A limitation of this hospital-based case-control study was that the results might not be representative of the total breast cancer population overall in Taiwan. However, recording of the risk factors, such as smoking, and the genotyping methods were stably and reliably performed by well-trained scientists to minimize any possible bias. Additionally, the study population was large enough that no re-evaluation of a larger sample size is needed. In this study, a novel potential biomarker of breast cancer, *Ku80* G-1401T, was found, and the importance of smoking in breast cancer was also demostrated. The findings in this paper can only reveal part of the complex and multistep process of breast carcinogenesis. In the future, functional studies on the polymorphic variants as in our previous papers (21, 22), and further studies on other genotypic variants involved in the DSB and other repair pathways are warranted.

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