

Significant Association of Ku80 Single Nucleotide Polymorphisms with Bladder Cancer Susceptibility in Taiwan

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Abstract. The aim of the present study was to evaluate the association between the polymorphisms of the Ku80 DNA repair gene, which plays an important role in maintaining genome stability, and the risk of bladder cancer in Central Taiwan. **Materials and Methods:** In this hospital-based case-control study, the association of Ku80 G-1401T rs828907, Ku80 C-319T rs11685387 and Ku80 intron 19 rs9288518 polymorphisms with bladder cancer risk in a central Taiwanese population was investigated. In total, 288 patients with bladder cancer and 288 age- and gender-matched healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped. **Results:** A significantly different distribution was found in the frequency of the Ku80 G-1401T polymorphism genotypes, but not the Ku80 C-319T or intron 19 polymorphism genotypes, between the bladder cancer and control groups. The T allele of Ku80 G-1401T conferred a significant ($p=0.0055$) increased risk of bladder cancer. Gene-environment interactions with smoking, but not with alcohol consumption, 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 2.053-fold (95% confidence interval=1.232-3.419) for bladder cancer. **Conclusion:** The first evidence that the T allele of the Ku80 G-1401T may be associated with the development of bladder cancer and may be a novel useful marker for primary prevention and anticancer intervention is provided.

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Bladder cancer is the most serious urinary neoplasm worldwide and the majority (70%) of cases occur in men (1). In the Western world, bladder cancer has become the fourth most common cancer among men, account for 7% of total malignancies (2). In Taiwan, the incidence and mortality of bladder cancer takes seventh place among the common carcinomas (3). Bladder carcinogenesis is a complex, multistep and multifactorial process resulting from the interactions between environmental and genetic factors. The risk factors for bladder cancer include cigarette smoking, exposure to carcinogenic aromatic amines and the uptake of harmful drugs, such as phenacetine, chlornaphazine and cyclophosphamide (4, 5). Those carcinogens thought of as DNA damage inducers induce various types of DNA adducts, such as DNA base damage, DNA single-strand breaks and double-strand breaks (DSBs) (6). The DSBs may lead to dramatic genome instability, which is closely related to carcinogenesis (7, 8). There are two specific DNA repair pathways responsible for DSBs repair: homologous recombination (HR) repair and the non-homologous end-joining (NHEJ) (7). Most DSBs are repaired by NHEJ and several key components are involved (9). Once DSBs occur in the genomic DNA, they are first recognized by a heterodimeric DNA-binding component KU of a DNA-dependent protein kinase (DNA-PK), which is formed from Ku70 and Ku80 (10). The Ku80 gene is located on chromosome 2q35 and has 21 exons (11). Former studies have indicated that mutation of Ku80 may affect the age at cancer onset (12).

Some single nucleotide polymorphisms (SNPs) have been confirmed as genetic risk factors for cancer (13-17). Recently, the Ku80 gene has been reported to play a role in cancer development (18), but the association of its SNPs with any cancer has not been investigated yet. In this study, for the first time, the role of Ku80 in a central Taiwanese population was investigated by the analysis of SNPs of Ku80 in bladder cancer.

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

Characteristics	Controls (n=288)			Patients (n=288)			<i>P</i> ^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			62.8 (9.4)			63.1 (11.2)	0.68
Age group (years)							0.73
≤55	117	40.6%		113	39.2%		
>55	171	59.4%		175	60.8%		
Gender							0.84
Male	220	76.4%		218	75.7%		
Female	68	23.6%		70	24.3%		
Habits							
Cigarette smokers	140	48.6%		156	54.2%		0.18
Alcohol drinkers	135	46.9%		152	52.8%		0.17

^a*P*-value based on Chi-square test.

Materials and Methods

Study population and sample collection. The study population consisted of 288 patients and 288 cancer-free control volunteers. The patients, diagnosed with bladder cancer, were recruited at the outpatient clinics of general surgery between 2004 and 2007 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of the patients including their histological details were all graded and defined by expert surgeons. All the patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of cancer-free healthy volunteers, as controls, were selected by matching for age, gender and some habits after initial random sampling, from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin 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 methods (19, 20). Briefly, the following primers were used: for *Ku80* G-1401T rs828907, 5'-TAGCTGACAACCTCACAGAT-3' and 5'-ATTCAG AGGTGCTCATAGAG-3'; for *Ku80* C-319T rs11685387, 5'-TCTAACTCCAGAGCTCTGAC-3' and 5'-AACTCTGAGCAT GCGCAGAT-3', and for *Ku80* intron 19 rs9288518, 5'-GGTGT GAAGACCTATCAATC-3' and 5'-TTACAGAACAGCCTTGC AC-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, SpeI, and 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 SNP data were selected for the 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 *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. The data were recognized as significant when the statistical *p*-value was less than 0.05.

Results

The frequency distributions of the selected characteristics of the bladder cancer patients and the controls are shown in Table I. The characteristics of the patients and controls were all well matched and none of the differences between the groups was statistically significant (*p*>0.05) (Table I).

The frequency distributions of the *Ku80* G-1401T, C-319T and intron 19 genotypes in the controls and the bladder cancer patients are shown in Table II. The genotype distribution of the genetic polymorphisms of *Ku80* G-1401T was significantly different between the bladder cancer and control groups (*p*=0.0294), while those for C-319T and intron 19 were not significant (*p*>0.05) (Table II). The heterozygous *Ku80* G-1401T genotype was significantly associated with bladder cancer susceptibility. The representative PCR-based restriction analyses for the *Ku80* G-1401T polymorphisms are shown in Figure 1.

The frequency distributions of the alleles for *Ku80* G-1401T, C-319T and intron 19 in the controls and bladder cancer patients are shown in Table III. The distributions of all these polymorphisms were in Hardy-Weinberg equilibrium and were similar between controls and bladder

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

Genotype	Controls	%	Patients	%	P ^a
G-1401T rs828907					0.0294
GG	218	75.7%	189	65.6%	
GT	49	17.0%	70	24.3%	
TT	21	7.3%	29	10.1%	
C-319T rs11685387					0.4983
CC	39	13.5%	33	11.5%	
CT	68	23.6%	79	27.4%	
TT	181	62.9%	176	61.1%	
Intron 19 rs9288518					0.3375
AA	28	9.7%	38	13.2%	
AG	98	34.0%	87	30.2%	
GG	162	56.3%	163	56.6%	

^aP-value based on Chi-square test.Table III. Distribution of *Ku80* alleles among bladder cancer patients and controls.

Allele	Controls	%	Patients	%	P ^a
G-1401T rs828907					0.0055
Allele G	485	84.2%	448	77.8%	
Allele T	91	15.8%	128	22.2%	
C-319T rs11685387					0.9459
Allele C	146	25.3%	145	25.2%	
Allele T	430	74.7%	431	74.8%	
Intron 19 rs9288518					0.5527
Allele A	154	26.7%	163	28.3%	
Allele G	422	73.3%	413	71.7%	

^aP-value based on Chi-square test.

cancer patients. The T allele of the *Ku80* G-1401T polymorphism was significantly associated with bladder cancer ($p=0.0055$) (Table III).

The genotype distribution of the various genetic polymorphisms of *Ku80* G-1401T was significantly different between the bladder cancer and control groups who had a smoking habit ($p=0.0053$) (Table IV), while those for C-319T and intron 19 were not significant ($p>0.05$). The T allele frequency was significantly higher in the bladder cancer patients who smoked than in the non-cancer controls and the patients who did not smoke. In Central Taiwan, individuals with *Ku80* G-1401T GT or TT who smoked were approximately 2-fold more likely to have bladder cancer than those who did not smoke (Table IV).

Table IV. *Ku80* G-1401T genotype and bladder cancer stratified by cigarette smoking.

Variable	<i>Ku80</i> G-1401T genotype		P ^a	OR (95% CI) ^b
	GG	GT+TT		
Smokers			0.0053	
Controls	108	32		1.00
Patients	97	59		2.053 (1.232-3.419) ^c
Non-smokers			0.3886	
Controls	110	38		1.00
Patients	92	40		1.259 (0.746-2.124)

^aP-value based on Chi-square test; ^bORs (odds ratios) were estimated by multivariate logistic regression analysis; ^cStatistically identified as significant.

Discussion

This was the first study which focused on the association between *Ku80* polymorphisms and bladder cancer susceptibility. Only *Ku80* G-1401T had statistical significance in association with increased bladder cancer, while the *Ku80* C-319T and *Ku80* intron 19 genotypes had no effect (Tables II and III). In the population with a smoking habit, the genetic effect of *Ku80* G-1401T on the bladder cancer risk was much more significant. In the smoking groups, the T allele clearly raised the bladder cancer risk (Table IV). According to these findings, the T allele of the *Ku80* G-1401T polymorphism may play a role in carcinogenesis. Those people carrying the T allele may have similar efficiency in removing DSBs if non-smokers, but in smokers, DNA damage increases significantly and individuals carrying the T allele may not have enough capacity to remove all the DSBs promptly and efficiently, thus increasing their bladder cancer risk.

In this study, a novel potential biomarker of bladder cancer, *Ku80* G-1401T, was found and the importance of smoking in bladder cancer was also shown. Carcinogenesis is indeed a complex and multistep process, and it is difficult to explain the causes of bladder cancer with simply one hypothesis. Thus, the findings in this study only reveal part of the process of bladder carcinogenesis, but we strongly believe that the findings can help the fight against bladder cancer and help to lower its prevalence.

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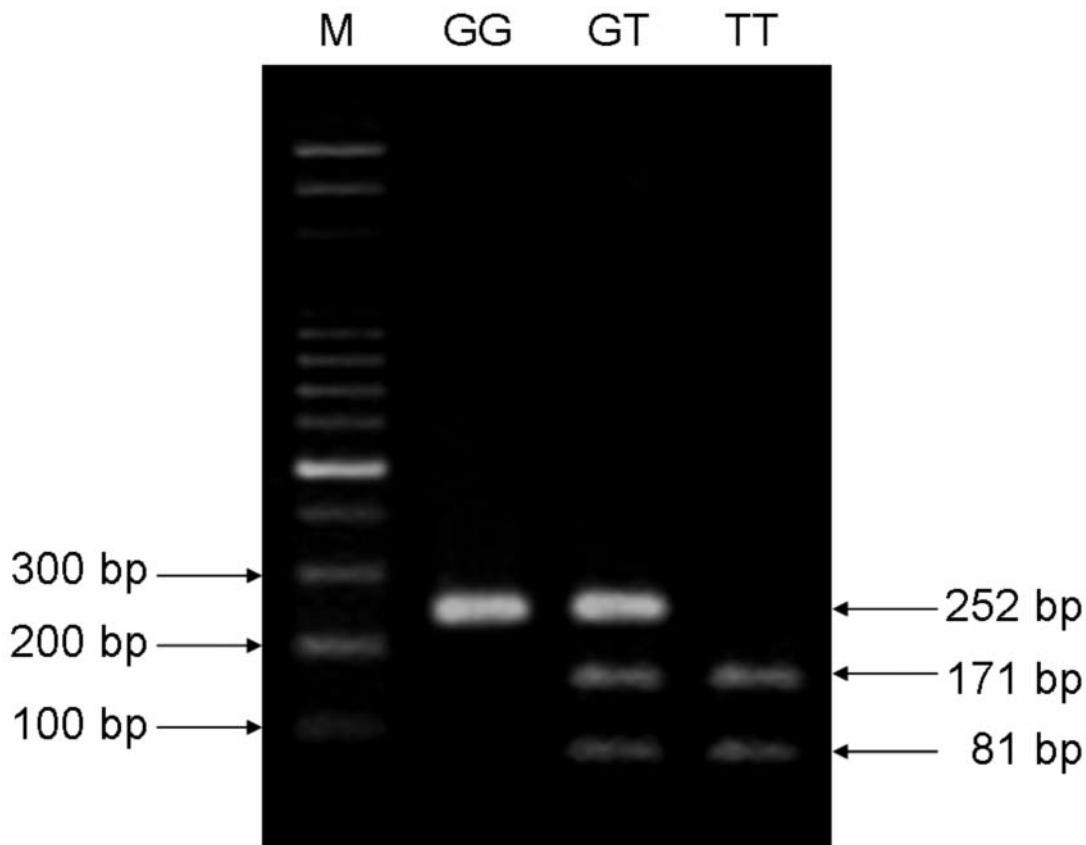


Figure 1. PCR-based restriction analysis of the *Ku80* G-1401T polymorphism shown on 2.5% agarose electrophoresis. M: 100 bp DNA size marker, G/G: enzyme indigestible homozygote, G/T: heterozygote, and T/T: enzyme digestible homozygote.

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