Significant Association of *ERCC6* Single Nucleotide Polymorphisms with Bladder Cancer Susceptibility in Taiwan

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Abstract. *Aim: To evaluate the association between the polymorphisms of the ERCC6 DNA repair gene, which plays an important role in maintaining genome stability, and the risk of bladder cancer in Taiwan. Materials and Methods: In this hospital-based case–control study, the association of ERCC6 codon 399, 1097 and 1413 polymorphisms with bladder cancer risk in a Central Taiwanese population was first investigated. In total, 288 patients with bladder cancer and 288 age- and gender-matched healthy controls recruited from the China Medical Hospital in Taiwan were genotyped. Results: A significantly different distribution was found in the frequency of the ERCC6 codon 399 genotypes, but not the ERCC6 codon 1097 or 1413 genotypes, between the bladder cancer and control groups. Those who had homozygous A/A or heterozygous A/G at ERCC6 codon 399 showed a 1.97- and 1.04-fold (95% confidence interval=1.29-3.01 and 0.71-1.53, respectively) increased risk of bladder cancer compared to those with G/G. As for ERCC6 codon 1097 or 1413, there was no difference in distribution between the bladder cancer and control groups. Conclusion: The first evidence that the homozygous A allele of the ERCC6 codon 399 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 occurs in men (1). In the western world, bladder cancer has become the fourth most common cancer among men, accounting 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, chlornaphrazine and cyclophosphamide (4). These carcinogens, thought of as DNA damage inducers, induce various types of DNA adducts, leading to DNA base damage, DNA single-strand breaks and double-strand breaks (DSBs) (5).

Sequence variants in DNA repair genes also are thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk (6). Excision repair cross-complementing group 6 (*ERCC6*) gene, alternatively named *CSB* gene, is important in age-related macular degeneration (7), and is known to function extensively in repair of damaged DNA (8-10). The disruption of *ERCC6* is causal to a subtype of Cockayne syndrome, an autosomal, recessive human disorder marked by striking somatic and neurological impairment (11). ERCC6 plays a role in transcription and nucleotide excision repair (NER), which removes bulky adducts, such as those caused by environmental agents, UV-induced DNA damage, crosslinks and oxidative damage (12, 13). It is possible that mutations in the *ERCC6* gene can diminish its activity, further resulting in a defect in overall NER. Single nucleotide polymorphisms have been identified in several exons of the *ERCC6* gene, among which, one in codon 399 (rs 2228528) and others in codon 1097 (rs 2228526) and codon 1413 (rs2228529) are beginning to be investigated; all of these polymorphisms result in amino acid

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

Characteristic	Controls (n=288)			Patients (n=288)			*P*-value[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%			39.2%		
>55	171	59.4%			60.8%		
Gender							0.84
Male	76			75			
Female	23			24			
Habits							
Cigarette smokers	140			54			0.18
Alcohol drinkers	135			52			0.17

[a]*P*-value based on chi-square test.

Table II. *Allele frequencies for ERCC6 codon 399, 1097 and 1413 polymorphisms in the bladder cancer and control groups.*

Allele	Cases (%) N=576	Controls (%) N=576	*P*-value[a]
ERCC6 codon 399			
Allele G (Gly)	283 (49.1)	340 (59.0)	**0.0009**
Allele A (Asp)	293 (50.9)	236 (41.0)	
ERCC6 codon 1097			
Allele A (Met)	532 (92.4)	527 (91.5)	0.6653
Allele G (Val)	44 (7.6)	49 (8.5)	
ERCC6 codon 1413			
Allele A (Gln)	535 (92.9)	526 (91.3)	0.3822
Allele G (Arg)	41 (7.1)	50 (8.7)	

[a]*P*-value based on χ^2 test with Yate's correction.

changes (Asp399Gly, Met1097Val and Gln1413Arg, respectively). Among these polymorphisms, only a study on NER gene polymorphisms and recurrence after treatment for superficial bladder cancer revealed that the G allele of codon 1097 is associated with better DNA repair capacity (14). There were significant associations between the Met1097Val variants and predisposition to cytokinesis-block micronucleus frequency in coke-oven workers (15) and superficial bladder cancer treatment outcome (14).

In this study, we hypothesized that DNA repair gene polymorphisms may be risk factors for bladder cancer. To test this hypothesis, DNA samples from 288 cases of bladder cancer and 288 age- and gender-matched healthy controls were genotyped of three SNPs for these the *ERCC6* gene (codon 399, 1097 and 1413). To our knowledge, this is the first study carried out to evaluate the *ERCC6* codon 399, 1097 and 1413 polymorphisms at the same time in a high prevalence Taiwanese population.

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 2008 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 non-cancer 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 disease. 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 (16-23). Briefly, the following primers were used: for *ERCC6* codon 399, 5'-TGAAGAGTCTGAGTATTTCC-3' and 5'-ATCTTCATCTCCATC ATCTC-3'; for *ERCC6* codon 1097, 5'-CCTGCTTCTAACATAT CTGT-3' and 5'-AATCACTGACAACTCTTCTG -3', and for *ERCC6* codon 1413, 5'-AAGAGCAGAAGATGCAGACT-3' and 5'-GTTTCT CATCTCCACCAGAA-3'. The PCR products were studied after digestion with *Rsa I, Nla III* and *Pst I*, restriction enzymes for *ERCC6* codon 399 (cut from 271 bp A type into 180+91 bp G type), codon 1097 (cut from 201 bp G type into 123+78 bp A type) and codon 1413 (cut from 184 bp G type into 123+61 bp A type), respectively. Ten percent of DNA samples were genotyped for a second time and the concordance rate was 100%.

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 *ERCC6* SNPs in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's χ^2 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 *ERCC6* genotypes between cases and controls. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. 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).

Table III. *Association of ERCC6 codon 399, codon 1097, codon 1413 polymorphisms and bladder cancer risk.*

Genotype	Cases (%)	Controls (%)	Odds ratio (95% CI)
ERCC6 codon 399			
G/G	92 (31.9)	112 (38.9)	1.00 (ref)
G/A	99 (34.4)	116 (40.3)	1.04 (0.71-1.53)
A/A	97 (33.7)	60 (20.8)	1.97 (1.29-3.01)*
With A	196 (68.1)	176 (61.1)	1.36 (0.96-1.91)
ERCC6 codon 1097			
A/A	248 (86.1)	242 (84.0)	1.00 (ref)
A/G	36 (12.5)	43 (15.0)	0.82 (0.51-1.32)
G/G	4 (1.4)	3 (1.0)	1.30 (0.29-5.87)
With G	40 (13.9)	46 (16.0)	0.85 (0.54-1.34)
ERCC6 codon 1413			
A/A	251 (87.2)	243 (84.4)	1.00 (ref)
A/G	35 (12.1)	40 (13.9)	0.84 (0.52-1.37)
G/G	2 (0.7)	5 (1.7)	0.39 (0.07-2.02)
With G	37 (12.8)	45 (15.6)	0.80 (0.50-1.27)

CI, Confidence interval. *$p<0.05$.

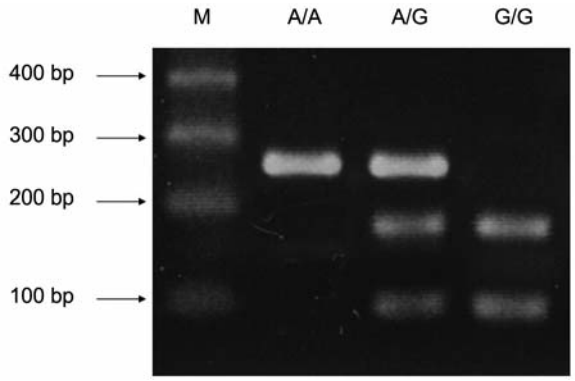

Figure 1. *PCR-based restriction analysis of the Asp399Gly polymorphism of ERCC6 gene shown on 3% agarose electrophoresis. M: 100 bp DNA size marker, A/A: indivisible homozygote, A/G: heterozygote and G/G: divisible homozygote.*

The frequencies of the alleles for the *ERCC6* codon 399, 1097 and 1413 between bladder cancer and control groups are shown in Table II. The distributions of all these polymorphisms were in Hardy-Weinberg equilibrium and were similar between bladder patients and controls (data not shown). The Asp allele at *ERCC6* codon 399 was significantly associated with bladder cancer risk ($p=0.0009$). In contrast, Met or Val at *ERCC6* codon 1097, and Gln or Arg at *ERCC6* codon 1413 were not differently distributed in the bladder cancer patient and control groups ($p>0.05$). Representative PCR-based restriction analyses for the *ERCC6* codon 399 polymorphisms are shown in Figure 1.

The genotype frequencies of *ERCC6* codon 399, 1097 and 1413 polymorphisms in the bladder cancer and control groups are shown in Table III. Using 399G as the reference group, there was an obvious association between homozygosity of 399A of *ERCC6* and bladder cancer risk. Combination of the homozygotes and heterozygotes of A (with A) showed that the A allele at *ERCC6* codon 399 conferred a 1.36-fold risk factor for bladder cancer (Table III). Neither hetero- nor homozygotes of 1097G of *ERCC6* seemed to be risky genotypes for bladder cancer, as was also the case in codon 1413 (Table III). Thus, among the three *ERCC6* polymorphisms investigated, only *ERCC6* codon 399 seems to be associated with bladder cancer risk.

Discussion

The present study has investigated the role of polymorphisms of *ERCC6* gene, a gene which has never been reported to be associated with bladder cancer risk. Among the *ERCC6* gene polymorphisms, Met1097Val is most commonly studied, and its variants are only reported to be associated to cytokinesis-block micronucleus susceptibility in coke-oven workers (8), and superficial bladder cancer treatment outcome (9).

Our larger sample size and concise data analysis strengthen the accuracy and reliability of our study, and the frequencies of *ERCC6* codon 1097 variant alleles were similar to those reported in the literature for the Chinese population, which suggest no selection bias for the participant enrolment in terms of genotype (8, 10, 11). Therefore, the need for the present conclusion to be verified in further larger studies is less. In the future, the joint effects of *ERCC6* genotypes and individual habits, such as smoking, can be further investigated.

In this study, the genotype distribution of the A allele at *ERCC6* codon 399 (50.9%) was significantly higher in the bladder cancer group than in the control group (Table II). It was also found that participants homozygous for *ERCC6* codon 399A had 1.97-fold higher risk of bladder cancer (Table III). As for the A/G heterozygotes, the risk was of a lower and non-significant level, a 1.04-fold increased risk. After combining the heterozygous and homozygous participants in both case and control groups, there was still a non-significant increased risk of 1.36-fold (Table III). This can be explained that the heterozygotes of A were not so obvious that the partial contribution diluted the significance of the homozygotes. All these data suggest that 399A may be novel marker for bladder cancer, and only the homozygotes of 399A was detected were the carriers more susceptibled to bladder cancer. As for *ERCC6* codon 1097, our results indicated that its polymorphism was not associated with bladder cancer risk. The frequencies of the G allele of the *ERCC6* codon 1097 in the Taiwanese and China populations are very similar (8). As for *ERCC6* codon 1413, there was no association either.

In conclusion, this is the first report to investigate the association between *ERCC6* gene polymorphisms and bladder cancer. Our findings suggest that A allele of *ERCC6* codon 399 was associated with higher susceptibility to bladder cancer.

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