Significant Association of XPD Codon 312 Single Nucleotide Polymorphism with Bladder Cancer Susceptibility in Taiwan

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Abstract. *Background: The DNA repair gene xeroderma pigmentosum group D (XPD), an important caretaker of the overall genome stability, is thought to play a major role in the development of human malignancy. Polymorphic variants of XPD, at codon 312 (rs1799793), 751 (rs13181) and promoter-114 (rs3810366), were chosen to be studied for their association with bladder cancer susceptibility in a central Taiwanese population. Patients and Methods: In this hospital-based case-control study, bladder cancer patients (308) and age- and gender-matched healthy controls (308) were recruited and their genotypes were analyzed by a polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) method. Results: A significant difference in the frequency of the XPD codon 312 genotype, but not the XPD codon 751 or promoter-114 genotypes, was found between the bladder cancer and control groups. Those who had G/A or A/A at XPD codon 312 showed a 1.85-fold (95% confidence interval=1.34-2.56) increased risk of bladder cancer compared to those with G/G. As for XPD codon 312 and promoter-114, there was no difference in distribution between the bladder cancer and control groups. Conclusion: The heterozygous and homozygous A allele of the XPD codon 312 may be responsible for bladder carcinogenesis and useful in the early detection and prediction of bladder cancer.*

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Bladder cancer is one of the most common urological malignancies in Taiwan, with an increasing incidence and death rate during the past decades (1). Bladder cancer is strongly affected by environmental carcinogens and some risk factors have been confirmed, such as cigarette smoking, exposure to aromatic amines and the intake of drugs such as phenacetine, chlornaphrazine and cyclophosphamide (2, 3). These environmental carcinogens may induce bladder cancer by causing DNA damage which is often regarded as an important originator of carcinogenesis in various sites or organs. Thus, the cellular capacities for repairing such DNA damage are closely correlated with the probabilities of cancer development. In previous studies, some genomic variants of DNA repair genes such as xeroderma pigmentosum group (XPG) and X-ray repair cross complementing protein 3 (*XRCC3*) have been proved to be risk factors or biomarkers of bladder cancer (4, 5), supporting the idea that DNA adducts that are repaired by nucleotide excision repair (NER) and that homologous recombination (HR) of DNA repair systems may also be involved. In addition to cigarette smoking which may induce substantial oxidative damage, causing DNA oxidative adducts together with single- and double-strand breaks (6-8), the BPDE which is mutagenic and highly carcinogenic compound may also induce some bulky adducts which should be removed by the NER system (6).

Sequence variants in DNA repair genes are also thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk (9). The Xeroderma pigmentosum group D (*XPD*) gene, also known as excision repair cross-complementing rodent repair deficiency Group 2 (*ERCC2*), is important in environmentally induced cancer (10). The *XPD* gene product is a helicase that is a component of the TFIIH (transcription factor II H) transcription factor complex. XPD plays a role in

transcription and NER, which removes bulky adducts, such as those caused by environmental agents, UV-induced DNA damage, crosslinks and oxidative damage (11, 12). Mutations in the *XPD* gene can diminish the helicase activity, resulting in a defect in NER, in transcription and in an abnormal response to apoptosis (13). Single nucleotide polymorphisms (SNPs) have been identified in several exons of the *XPD* gene, among which one in codon 312 of exon 10 and another in codon 751 of exon 23 are commonly studied and result in amino acid changes (Asp312Asn and Lys751Gln, respectively) (14). These SNPs are associated with lower DNA repair capacity and a higher level of DNA adducts (14,15). Some studies have reported significant associations between the Asp312Asn or Lys751Gln variants and predisposition to many types of cancer, including lung cancer (16), squamous cell carcinoma of the head and neck (17), melanoma (18) and breast cancer (19-22). A few studies have reported that *XPD* polymorphisms are associated with bladder cancer (23-27), but an investigation of *XPD* genotypes in bladder cancer in the Taiwanese population is still lacking.

Since DNA repair gene alterations have been shown to cause a reduction in DNA repair capacity, we hypothesized that *XPD* gene polymorphisms may be risk factors for bladder cancer. To test this hypothesis, DNA samples from bladder cancer patients and healthy controls, in a central Taiwan population were analyzed by a polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) method to determine the genotypic frequency of three SNPs of the *XPD* gene (Asp312Asn, Lys751Gln and promoter-114). To the best of our knowledge, this is the first study carried out to evaluate these polymorphisms at the same time and in a high prevalence of bladder cancer of Taiwanese population.

Patients and Methods

Study population and sample collection. Three hundred and eight patients diagnosed with bladder cancer were recruited at the outpatient clinics of general surgery between 2001-2008 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics were all defined by expert surgeons (Drs. Chang and Wu). All the patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of non-bladder 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 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 as described in previous papers (28-35). Briefly, the following primers were used: for *XPD* Asp312Asn: 5'-TGGCCCCTGTCTGACTTGTCCC-3' and 5'-GACGGGGAGG CGGGAAAGGGACT-3'; for *XPD* Lys751Gln: 5'-ACTTCATAA GACCTTCTAGC-3' and 5'-GATTATACGGACATCTCCAA-3' and for *XPD* promoter-114, 5'-ATGAATATTCAGCGAGAGGC-3' and 5'-CTGGGTTCGATCAATACTCAAT-3'.

The following cycling conditions were used: 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 Hpy99I, EarI, and Bme1580I, restriction enzymes for *XPD* Asp312Asn (cut from 250 bp A type into 188+62 bp G type), Lys751Gln (cut from 326 bp C type into 127+199 bp A type) and promoter -114 (cut from 303 bp G type into 101+202 bp C type), respectively.

Statistical analyses. Only those samples with complete DNA polymorphism data (control/case=308/308) were selected for final analyzing. 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 *XPD* 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 *XPD* genotypes between cases and controls. The cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. The data were recognized as significant when the statistical *p*-value was less than 0.05.

Results

The frequency of the alleles for the *XPD* Asp312Asn, Lys751Gln and promoter-114 in the bladder cancer and control groups is shown in Table I. The Asn allele at *XPD* Asp312Asn was significantly associated with bladder cancer risk (p=0.0001). In contrast, Lys or Gln at *XPD* Lys751Gln, or the C or G allele at *XPD* promoter-114, were not differently distributed in the oral cancer patient and control groups ($p>0.05$).

The frequency of the genotype of *XPD* Asp312Asn, Lys751Gln and promoter-114 polymorphisms in the bladder cancer and control groups is shown in Table II. Using 312G as the reference group, there was an obvious association between the homozygotes and heterozygotes of 312A of XPD and bladder cancer risk. A combination of the homozygotes and heterozygotes of A (with A) showed that the A allele at *XPD* Asp312Asn conferred a 1.85-fold risk factor for bladder cancer (Table II). Neither hetero- nor homozygotes of 751G of *XPD* seemed to be risky genotypes for bladder cancer, as was also the case in promoter-114 (Table II).

Discussion

In this study, the genotype frequency of the A allele at *XPD* Asp312Asn was significantly higher in the bladder cancer group (34.4%) than in the control group (24.5%) (Table

Table I. *Allele frequencies for XPD Asp312Asn, Lys751Gln and promoter-114 polymorphisms in the bladder cancer and control groups.*

Allele	Cases (%) N=616	Controls (%) N=616	*p*-Value[a]
XPD Asp312Asn			
Allele G (Asp)	404 (65.6)	465 (75.5)	0.0001
Allele A (Asn)	212 (34.4)	151 (24.5)	
XPD Lys751Gln			
Allele A (Lys)	581 (94.3)	578 (93.8)	0.7173
Allele C (Gln)	35 (5.7)	38 (6.2)	
XPD promoter-114			
Allele C	338 (54.9)	343 (55.7)	0.7745
Allele G	278 (45.1)	273 (44.3)	

[a]*p*-Value based on Chi-square test.

Table II. *Association of XPD Asp312Asn, Lys751Gln, promoter -114 polymorphisms and bladder cancer risk.*

Genotype	Cases (%)	Controls (%)	Odds ratio (95% CI)[a]	*p*-Value[b]
XPD Asp312Asn				0.0086
G/G	153 (49.7)	199 (64.6)	1.00 (ref)	
G/A	98 (31.8)	67 (21.8)	1.90 (1.31-2.77)	
A/A	57 (18.5)	42 (13.6)	3.81 (2.53-5.73)	
with A	155 (50.3)	109 (35.4)	1.85 (1.34-2.56)	
XPD Lys751Gln				0.9526
A/A	280 (90.9)	278 (90.3)	1.00 (ref)	
A/C	21 (6.8)	22 (7.1)	0.95 (0.51-1.76)	
C/C	7 (2.3)	8 (2.6)	0.87 (0.31-2.43)	
with C	28 (9.1)	30 (9.7)	0.93 (0.54-1.59)	
XPD promoter -114				0.9387
C/C	87 (28.2)	91 (29.5)	1.00 (ref)	
C/G	164 (53.3)	161 (52.3)	1.07 (0.74-1.54)	
G/G	57 (18.5)	56 (18.2)	1.06 (0.66-1.71)	
with G	221 (71.8)	217 (70.5)	1.07 (0.75-1.51)	

[a]CI, confidence interval; [b]*p*-value based on Chi-square test.

I). It was also found that participants homozygous for *XPD* Asp312Asn had a 3.81-fold higher risk of bladder cancer (Table II). As for the Asp/Asn heterozygotes, the risk was almost half of the level, a 1.90-fold increased risk. After combining the heterozygous and homozygous participants in both case and control groups, there was still an obvious increased risk of 1.85-fold (Table II). The data suggested that 312Asn was indeed a marker for bladder cancer in Taiwan. As long as 312Asn was detected, no matter whether as hetero- or homozygote, the carriers were more susceptible to bladder cancer. As for the role of *XPD* Asp312Asn in bladder carcinogenesis, the present findings were consistent with the previous study, which reported the Asn allele to be a risky genotype and the homozygous Asn/Asn genotype to have a significantly 4.62-fold higher risk than the combined group of hetero- and homozygous Asp with non-muscle-invasive bladder cancer (24). In addition, *XPD* Asp312Asn has also been reported to be associated with a 1.8-fold increased risk for lung cancer (16) and a 1.84-fold for prostate cancer (30). Interestingly, the two target populations in those studies (Han and Taiwanese) were very close to those here. However, SNP of *XPD* Asp312Asn have also been reported not to be associated with bladder cancer risk (27). As for *XPD* Lys751Gln, the present results indicated that this polymorphism was not associated with bladder cancer risk. The finding was consistent with another paper, investigating a smaller sample size of a very closely related population in central Taiwan (30) and two quite unrelated populations (36, 37). In contrast, some papers reported that *XPD* Lys751Gln was conditionally associated with bladder cancer risk (25-27). The inconsistencies among the reports may be due to the different ethnic groups investigated.

These results have added evidence showing that genetic variants involved in DNA repair pathways may also be involved in cancer etiology. We have previously reported that the non-homologous end-joining DNA repair capacities of each person may be associated with their susceptibility to breast cancer (38). Therefore, it would be interesting to investigate differences in NER repair capacities between individuals to preclude chance findings, particularly those among subgroups, and clarify the detail of the mechanisms involved.

In conclusion, in this large population study, focused on the SNPs of *XPD* and bladder cancer in Taiwan the presence of the A allele of Asp312Asn was associated with a higher risk of bladder cancer. The A allele of Asp312Asn may be a useful marker in bladder oncology for anticancer application and early cancer detection.

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