

## Association of XPD Polymorphisms with Prostate Cancer in Taiwanese Patients

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**Abstract.** *Background: The DNA repair gene 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, 751, and other sites, have been associated with cancer susceptibility, but few studies have investigated their effect on prostate cancer risk. Patients and Methods: In this hospital-based case-control study, the association of XPD codon 312, 751 and promoter-114 polymorphisms with prostate cancer risk in a Taiwanese population were investigated. In total, 123 patients with prostate cancer and 479 healthy controls recruited from the China Medical Hospital in Central Taiwan were genotyped. Results: We found a significant difference in the frequency of the XPD codon 312 genotype, but not the XPD codon 751 or promoter-114 genotypes, between the prostate cancer and control groups. Those who had G/A or A/A at XPD codon 312 showed a 1.81-fold (95% confidence interval=1.21-2.69) increased risk of prostate cancer compared to those with G/G. As for XPD codon 312 or promoter-114, there was no difference in distribution between the prostate cancer and control groups. Conclusion: Our findings suggest that the heterozygous and homozygous A allele of the XPD codon 312 may be associated with the development of prostate cancer and may be a useful marker for primary prevention and anticancer intervention.*

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Prostate cancer is one of the most commonly diagnosed cancers all over the world (1). The etiology of prostate cancer is largely unknown. Human DNA repair mechanisms protect the genome from DNA damage caused by endogenous and environmental agents. Mutations or defects in the DNA repairing system are essential for tumorigenesis. It is therefore logical to suspect that some genetic variants of DNA repair genes might contribute to prostate cancer pathogenesis.

Sequence variants in DNA repair genes also are thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk (2). *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 (3). The *XPD* gene product is a helicase that is a component of the TFIIH transcription factor complex. *XPD* 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 (4, 5). 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 (6). Single nucleotide polymorphisms (SNPs) have been identified in several exons of the *XPD* gene, among which one in codon 312 of exon 10 and the other in codon 751 of exon 23 are commonly studied and result in amino acid changes (Asp312Asn and Lys751Gln, respectively) (7). These polymorphisms are associated with lower DNA repair capacity and a higher level of DNA adducts (7, 8). Some studies have reported significant associations between the Asp312Asn or Lys751Gln variants and predisposition to many types of cancer, including lung cancer (9), squamous cell carcinoma of the head and neck (10), melanoma (11) and bladder cancer (12). There are few findings reporting the *XPD* polymorphisms in prostate cancer. The *XPD* codon 312 Asn/Asn genotype is itself associated with prostate cancer risk and it will markedly elevate prostate cancer risk when present in combination with the *XRCC1* codon 399

Gln/Gln genotype (13). The other polymorphism *XPD* codon 751 is negatively associated with prostate cancer (14).

Since DNA repair gene alterations have been shown to cause a reduction in DNA repair capacity, we hypothesized that DNA repair gene polymorphisms may be risk factors for prostate cancer. To test this hypothesis, DNA samples from 123 cases of prostate cancer and 479 age-matched healthy controls in a Central Taiwan population were analyzed using a polymerase chain reaction-based restriction fragment length polymorphism method to determine the genotypic frequency of three polymorphisms of the *XPD* gene (codon 312, 751 and promoter-114). To our knowledge, this is the first study carried out to evaluate the *XPD* codon 312, 751 and promoter-114 polymorphisms at the same time and in a high prevalence Taiwanese population.

### Patients and Methods

**Study population and sample collection.** One hundred and twenty-three patients diagnosed with prostate cancer were recruited at the outpatient clinics of general surgery between 1998-2006 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The mean age of the prostate cancer patients and the controls were 67.38 (SD=11.64) and 62.43 (SD=9.81) years, respectively. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Four hundred and seventy-nine non-prostate 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.

**Genotyping assays.** Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to our previous paper (15). Briefly, the following primers were used for *XPD* codon 312: 5'-TGGCCCCTGTCTGACTTGCCC-3' and 5'-GACGGGGAGGCGGGAAAGGGACT-3'; for *XPD* codon 751: 5'-ACTTCATAAGACCTTCTAGC-3' and 5'-GATTATACGGACATCTCCAA-3'; and for *XPD* promoter-114: 5'-ATGAATATTCAGCGAGAGGC-3' and 5'-CTGGGTTTCGATCAATACTCAAT-3'. 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 Hpy99I, EarI, and Bme1580I, restriction enzymes for *XPD* codon 312 (cut from 250 bp A type into 188+62 bp G type), 751 (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.** 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* 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  $\chi^2$  test or Fisher's exact test (when the expected number in any cell

Table I. Allele frequencies for *XPD* codon 312, 751 and promoter-114 polymorphisms in the prostate cancer and control groups.

Allele	Cases (%) N=246	Controls (%) N=958	p-value <sup>a</sup>
<i>XPD</i> codon 312			
Allele G (Asp)	163 (66.3)	726 (75.8)	0.003
Allele A (Asn)	83 (33.7)	232 (24.2)	
<i>XPD</i> codon 751			
Allele A (Lys)	232 (94.3)	915 (95.5)	0.533
Allele C (Gln)	14 (5.7)	43 (4.5)	
<i>XPD</i> promoter-114			
Allele C	133 (54.1)	513 (53.5)	0.942
Allele G	113 (45.9)	445 (44.5)	

<sup>a</sup>p-value based on  $\chi^2$  test.

was less than five) was used to compare the distribution of the *XPD* 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 was recognized as significant when the statistical p-value was less than 0.05.

### Results

The frequency of the alleles for the *XPD* codon 312, 751 and promoter-114 between prostate cancer and control groups is shown in Table I. The Asn allele at *XPD* codon 312 was significantly associated with prostate cancer risk ( $p=0.003$ ). In contrast, Lys or Gln at *XPD* codon 751, 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* codon 312, 751 and promoter-114 polymorphisms in the prostate 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 prostate cancer risk. A combination of the homozygotes and heterozygotes of A (with A) showed that the A allele at *XPD* codon 312 conferred a 1.81-fold risk factor for prostate cancer (Table II). Neither hetero- nor homozygotes of 751G of *XPD* seemed to be risky genotypes for prostate cancer, as was also the case for promoter-114 (Table II).

### Discussion

Regarding prostate cancer risk for *XPD* gene polymorphism, the results are very few. The *XPD* codon 312 Asn/Asn genotype is associated with prostate cancer risk (13), while *XPD* codon 751 polymorphism is negatively associated (14). There are no reports concerning the *XPD* promoter-114 polymorphism in prostate cancer. In this

Table II. Association of XPD codon 312, codon 751, promoter-114 polymorphisms and prostate cancer risk.

Genotype	Cases (%)	Controls (%)	Odds ratio (95% CI) <sup>a</sup>
<i>XPD</i> codon 312			
G/G	62 (50.4)	310 (64.7)	1.00 (ref)
G/A	39 (31.7)	106 (22.1)	1.84 (1.17-2.91) <sup>b</sup>
A/A	22 (17.9)	63 (13.2)	1.75 (1.01-3.05) <sup>b</sup>
with A	61 (49.6)	169 (35.3)	1.81 (1.21-2.69) <sup>b</sup>
<i>XPD</i> codon 751			
A/A	111 (91.1)	441 (92.1)	1.00 (ref)
A/C	10 (6.5)	33 (6.9)	1.20 (0.58-2.52)
C/C	2 (2.4)	5 (1.0)	1.59 (0.30-8.30)
With C	12 (8.9)	38 (7.9)	1.26 (0.64-2.48)
<i>XPD</i> promoter -114			
C/C	38 (27.7)	147 (30.7)	1.00 (ref)
C/G	57 (53.3)	219 (45.7)	1.01 (0.64-1.60)
G/G	28 (19.0)	113 (23.6)	0.96 (0.56-1.66)
with G	85 (72.3)	332 (69.3)	0.99 (0.65-1.52)

<sup>a</sup>CI, confidence interval; <sup>b</sup>*p*<0.05.

study, the genotype distribution of the A allele at *XPD* codon 312 (33.7%) was significantly higher in the prostate cancer group than in the control group (Table I). It was also found that participants homozygous for *XPD* codon 312Asn had 1.75-fold higher risk of prostate cancer (Table II). As for the Asp/Asn heterozygotes, the risk was almost the same level, a 1.84-fold increased risk. After combining the heterozygous and homozygous participants in both case and control groups, there was still an obvious increased risk of 1.81 fold (Table II). The data suggested that 312Asn was a marker for prostate cancer. As long as 312Asn was detected, no matter whether is hetero- or homozygotes, the carriers were more susceptible to prostate cancer. As for *XPD* codon 312, our findings are consistent with the previous study, which reported the Asn allele to be a risky genotype and the homozygous Asn/Asn genotype had a 1.61-fold higher risk than the combined group of hetero- and homozygous Asp (13). The Asn allele of *XPD* codon 312 has also been reported to be associated with a 1.8-fold increased risk for lung cancer (9). As for *XPD* codon 751, our results indicated that its polymorphism was not associated with prostate cancer risk. The finding is consistent with another paper, investigating a smaller sample size of a very closely related population, in Shanghai, China (14). The frequencies of the T allele of the *XPD* codon 751 in the Taiwanese and Chinese populations are very similar, both are about ninety percent (14). Although *XPD* codon 751 seems not to contribute much to prostate carcinogenesis, a slight decrease in risk for the *XPD* codon 751 Gln/Gln genotype compared with the Lys/Lys or Lys/Gln genotypes has been reported (14).

Furthermore, smokers with the Lys/Lys or Lys/Gln genotypes were twice as likely to have bladder cancer than smokers with the Gln/Gln genotype (12).

These results suggest that genetic variants involved in DNA repair pathways may also be involved in prostate cancer etiology. Some literature of case-control studies have shown that prostate cancer may be associated with polymorphisms of the androgen receptor, PSA, E-cadherin and cytochrome P450 family genes (16-20). Other risk factors, including consumption of preserved foods and insulin resistance, may also modulate prostate cancer risk in combination with genetic susceptibility in these repair pathways. Therefore, replication in larger studies and other functional repair assays may be performed in the future to preclude chance findings, particularly those among subgroups, and clarify the mechanisms involved.

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