Susceptibility of *XPD* and *RAD51* Genetic Variants to Carcinoma of Urinary Bladder in North Indian Population

Ranbir Chander Sobti,¹ Saranjeet Kaur,² Vijay Lakshmi Sharma,² Shrawan Kumar Singh,³
Sayed Ali Hosseini,¹ and Rupinder Kler¹

For the present study, two polymorphisms, xeroderma pigmentosum, complementation group D (*XPD*) Lys751Gln and *RAD51* 135G/C were studied with regard to bladder cancer. For *XPD* Lys751Gln polymorphism, an increased risk of bladder cancer was found to be associated with the Gln variant allele (odds ratio [OR] = 1.86, 95% confidence interval [CI] = 1.27–2.73), on taking AA (Lys/Lys) as the referent genotype. In males, the *XPD* 751C (Gln) allele was found to be associated with a significantly increased risk (OR = 2.33, 95% CI = 1.52–3.56). The inhabitants of rural areas showed a significantly increased risk with the *XPD* Gln allele (OR = 2.59, 95% CI = 1.46–4.62) when compared with those of urban areas. In smokers (OR = 5.30, 95% CI = 2.42–11.68), alcohol drinkers (OR = 4.33, 95% CI = 2.17–8.70), and nonvegetarians (OR = 2.21, 95% CI = 1.26–3.87), the *XPD* Gln allele showed a significantly increased risk toward bladder cancer. For *RAD51* 135G/C polymorphism, no significant difference was observed in the allelic and genotypic frequencies. Even after stratification, no significant association could be seen. After stratifying histopathologically, the *RAD51* CC genotype was associated with decreased risk in subjects having superficial stage (OR = 0.51, 95% CI = 0.27–0.99) and with those having G2 grade (OR = 0.24, 95% CI = 0.09–0.62) of bladder cancer. *XPD* polymorphism may be a predisposing factor, but the same cannot be said for *RAD51* gene polymorphism.

Introduction

BLADDER CANCER ILLUSTRATES the association of cancer with occupational exposure and tobacco smoking. Chemicals thought to be carcinogenic for bladder cancer include aniline dyes, which are often found in color fabrics, and cyclophosphamide, a chemotherapeutic drug (Vineis and Pirastu, 1997). Although the specific carcinogens in cigarette smoke responsible for the increased risk of bladder cancer are unknown, aromatic amines are thought to be the inciting factor (Vineis and Pirastu, 1997). Occupations with increased exposure to aromatic amines, such as beta-naphthylamine, 4-aminobiphenyl, and benzidine, and other potential bladder carcinogens are also responsible (Leppert *et al.*, 2006). Hair dyes have been also associated with an increased risk of bladder cancer (Gago-Dominguez *et al.*, 2003).

Molecular epidemiology studies have shown considerable interindividual variations in the DNA repair capacity (DRC) in the general population (Jeon *et al.*, 2003). The variation in the DRC may be the result of functional polymorphisms in the DNA repair genes (Jeon *et al.*, 2003). A number of studies have investigated the probable association between modulation of risk of bladder cancer and polymorphisms in DNA repair genes (Stern *et al.*, 2002). Xeroderma pigmentosum, complementation

group D (*XPD*) is one of the seven genetic complementation groups encoding for the proteins involved in the nucleotide excision repair (NER) pathway (O'Donovan and Wood, 1993). NER recognizes bulky, helix-distorting lesions such as pyrimidine dimers and 6,4-photoproducts. The *XPD* gene maps to 19q13.3 (Coin *et al.*, 1999). The *XPD* protein is a 760-amino-acid polypeptide that belongs to the RAD3/*XPD* subfamily of helicases. It has a dual function. It encodes a helicase, which participates in both NER, where it recognizes and repairs a wide range of structurally unrelated lesions such as bulky adducts and thymidine dimers, and also basal transcription, as a part of the transcription factor IIIH (TFIIH) (Drapkin *et al.*, 1994). Mutations destroying the enzymatic function of *XPD* protein are clinically manifested in combinations of three severe syndromes, cockayne syndrome, xeroderma pigmentosum, and trichothiodystrophy, depending on the location of the mutation (de Boer and Hoeijmakers, 2000). About 17 SNPs have been detected in the *XPD* gene, of which 6 were found in exons and 11 in introns (Shen *et al.*, 1998). Of the six coding region polymorphisms, two are silent, two are very rare, and the others, codon 312 and 751 polymorphisms, are common. In the Asp312Asn single-nucleotide polymorphism (SNP), the acidic moiety of the aspartic acid gets removed (converted to asparagine), whereas in the Lys751Gln SNP, there is a complete change of the electronic

Departments of ¹Biotechnology and ²Zoology, Punjab University, Chandigarh, India.

³Advanced Urology Centre, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

configuration of the amino acid (lysine to glutamine). This is a major change that is located in the important domain of interaction between XPD protein and its helicase activator, p44 protein, inside the TFIIH complex (Coin *et al.*, 1998). Hence, the consequence of the SNP at amino acid 751 should be the most important in terms of XPD function. In the present study, the susceptibility of carriers of the Lys751Gln polymorphism in exon 23 of XPD for the risk of bladder cancer has been evaluated.

The involvement of RAD51 in DNA repair determines its potential role in maintaining genetic stability, which is disturbed in cancer. RAD51 plays a central role in homologous recombination (HR), which repairs DNA double-strand break (Schild and Wiese, 2010). The RAD51 gene is located at position 15q15.1 (Entrez Gene Website, 2008). This gene encodes a 339 amino acid protein, which is the key enzyme for DNA double-strand break repair by HR. RAD51 proteins form nucleoprotein filaments on single-stranded DNA, mediating homologous pairing and strand exchange reactions between single-stranded DNA and homologous double-stranded DNA (Gupta *et al.*, 1997). In this process, an ATP-dependent DNA strand exchange takes place, in which a template strand invades base-paired strands of homologous DNA molecules. RAD51 is involved in the search for homology and strand-pairing stages of the process.

Observations that mammalian RAD51 protein associates with the tumor suppressors ATM (Chen *et al.*, 1999), p53 (Sturzbecher *et al.*, 1996), BRCA1 (Scully *et al.*, 1997), and BRCA2 (Sharan *et al.*, 1997), the *c-ABL* oncogene product (Yuan *et al.*, 1998), and BLM helicase (Wu *et al.*, 2001) suggest its possible role in tumor development. An SNP in the 5' untranslated (promoter) region of RAD51 (a G→C substitution at position 135, the G/C polymorphism) was reported to modulate breast cancer risk in BRCA1 and BRCA2 carriers (Wang *et al.*, 2001). To date, only one study on RAD51 and bladder cancer has been found in the literature (Figueroa *et al.*, 2007). It did not report any significant association. In the present study, the susceptibility of carriers of polymorphic forms of XPD Lys751Gln and RAD51 135 G/C to bladder cancer is reported.

Materials and Methods

Study design and study subjects

This retrospective case-control study comprised 270 histopathologically proven cases of urinary bladder cancer and 252 cancer-free controls. Peripheral blood samples from patients with urinary bladder cancer, treated at Advanced Urology Centre (AUC) of the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, were collected during routine investigations. The ethical

clearance for the present study was obtained from the institute's Ethics Committee. Cases having HIV, allergies, and other cancers or patients having received chemotherapy were excluded. Informed consents were obtained from all the participants. Data with respect to their age, sex, smoking status, alcohol consumption, occupational status, area inhabited, and eating habits were recorded. In patients with bladder tumor, the stage and grade of the tumor were noted.

Genotype analysis

Peripheral blood samples (2–4 mL) were collected from cases and controls in EDTA-coated vials. Genomic DNA was subsequently extracted from peripheral blood lymphocytes by the standard phenol-chloroform method. The XPD Lys751Gln (A→C) and RAD51 G→C polymorphisms were determined by the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay as per the conditions in Table 1 (Figs. 1 and 2). Independent repetition of genotyping in randomly selected samples ($n=125$) produced the same results.

Statistical analysis

Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies with the expected genotype frequencies among the cases and controls. The power calculations were conducted at 80% with a significance level of 0.05. The sample size used for the present study was adequate. The data showed normal distribution on applying one-sample Kolmogorov–Smirnov Z test when age was taken as the test variable. The data were age matched, as confirmed by *t*-test. The odds ratios (ORs) and 95% confidence intervals (CIs) were obtained using χ^2 test and Fisher's exact test for categorical variables. The ORs were calculated without adjustment for potential confounders, that is, sex, area, job status, smoking, alcohol consumption and diet. Based on previous studies, occupations related to automotive repair (Manju *et al.*, 2009), agricultural production, livestock and animal specialties, electrical assembly, installation and repair, health services (Cassidy *et al.*, 2009), printing industry, transportation equipment industry, and electrical/gas/sanitary services (Samani *et al.*, 2008) were altogether taken under the category of high-risk occupations and the rest under low-risk occupations.

To achieve an adequate sample size with the power of study at 80%, the various tumor stages were merged into two groups, superficial (Ta+T1) and muscle-invasive (T2+T3+T4). Both additive and dominant modes of inheritance were considered. The *p*-values were two-sided. Values

TABLE 1. CONDITIONS OF GENOTYPING ASSAYS FOR THE SELECTED POLYMORPHISMS OF REPAIR GENES

Single-nucleotide polymorphism	Primers	Polymerase chain reaction product	Enzyme	Gel band pattern
XPD Lys751Gln (Yeh <i>et al.</i> , 2005)	5'-CCTCTCCCTTTCCTCTGTTT-3' 5'-CAGGTGAGGGGGACATCT-3'	734 bp	<i>Pst</i> I (5 U, 4 h, 40°C)	C (Gln) allele: 646 bp, 88 bp A (Lys) allele: 734 bp (Fig. 1)
RAD51 135 G→C (Brooks <i>et al.</i> , 2008)	5'-TGGGAAGTCAACTCATCTGG-3' 5'-GCGCTCCTCTCTCCAGCAG-3'	157 bp	<i>Bst</i> NI (7 U, 3 h, 60°C)	G allele: 86 bp, 71 bp C allele: 157 bp (Fig. 2)

FIG. 1. A representative 1.5% gel showing RFLP product of *XPD* after digestion with *Pst*I: lane 4 = Lys/Lys (734 bp); lane 5 = Gln/Gln (646 bp); lanes 1–3, 6, and 7 = Lys/Gln (734 and 646 bp); and lane L = 100-bp DNA marker. Note that the 88-bp fragment for the *XPD* assay is not resolved on the gels used. RFLP, restriction fragment length polymorphism; *XPD*, xeroderma pigmentosum, complementation group D.

less than 0.05 were considered significant. All analyses were performed using SPSS, version 15.0 and Epi Info, version 3.4.3.

Results

A total of 270 consecutive cases of bladder cancer and 252 controls were recruited for the present study. Among the bladder cancer cases, 236 were males and 34 females. Among controls, there were 212 males and 40 females. The demo-

graphic characteristics of cases and controls are summarized in Table 2.

XPD Lys751Gln

The distribution of the genotypic and allelic frequencies of *XPD* Lys751Gln polymorphism among the cases and controls is shown in Table 3. The distribution of genotypes among cases and controls was in Hardy–Weinberg equilibrium. The allele frequencies were 46.70% for A (Lys) allele and 53.40% for C (Gln) in the cases. In the control group, the allele frequency for A (Lys) was 57.34% and that for C (Gln) was 42.66%. To see the overall effect of *XPD* polymorphism, both additive and dominant models were considered. When compared with AA (Lys/Lys) as the referent genotype, a significantly increased risk was associated with AC (Lys/Gln) (OR = 1.80, 95% CI = 1.16–2.80) and CC (Gln/Gln) (OR = 1.93, 95% CI = 1.22–3.05) genotypes (assuming an additive mode of inheritance). An increased risk was associated with the combined AC (Lys/Gln) and CC (Gln/Gln) genotypes (OR = 1.86, 95% CI = 1.27–2.73) (assuming a dominant mode of inheritance for the Gln variant allele).

The association between *XPD* polymorphism and the risk of bladder cancer was further examined after stratifying for potential confounding variables such as sex, area inhabited, occupational risk, smoking, alcohol consumption, and diet (Table 4). Once again, the AA genotypes were compared with the combined AC and CC genotypes (assuming a dominant model for the *XPD* Gln variant allele). In males, the C (Gln) allele (combined AC + CC genotypes) was associated with a significantly increased risk (OR = 2.33, 95% CI = 1.52–3.56). Similarly, in inhabitants of rural areas, a significantly increased risk was associated with the Gln allele (OR = 2.59, 95% CI = 1.46–4.62). The CC genotype (OR = 2.47, 95% CI = 0.97–6.38) was associated with an increased risk in subjects working under high-risk occupations, whereas the AC genotype (OR = 1.98, 95% CI = 1.17–3.35) was associated with an increased risk in subjects working under low-risk

FIG. 2. A representative 4% agarose gel showing RFLP product of *RAD51* after digestion with *Bst*NI: lanes 1 and 2 = G/G (86 and 71 bp); lanes 3 and 4 = C/C (157 bp); lanes 5–7 = G/C (157, 86, and 71 bp); and lane L = pBR322 DNA/*Hae*III digest.

TABLE 2. DISTRIBUTION OF DEMOGRAPHIC CHARACTERISTICS AMONG CASES AND CONTROLS

Variable	Cases (%)	Controls (%)
Average age	57.16 + 12.3	56.47 + 10.9
Sex		
Male	236 (87.4)	212 (84.1)
Female	34 (12.6)	40 (15.9)
Inhabitation		
Urban	132 (48.9)	153 (60.7)
Rural	138 (51.1)	99 (39.3)
Occupation		
Low risk	163 (60.4)	199 (79.0)
High risk	107 (39.6)	53 (21.0)
Smoking		
Nonsmokers	144 (53.3)	200 (79.4)
Smokers	126 (46.7)	52 (20.6)
Alcohol consumption		
No	157 (58.1)	167 (66.3)
Yes	113 (41.9)	85 (33.7)
Eating habits		
Vegetarian	141 (52.2)	135 (53.6)
Nonvegetarian	129 (47.8)	117 (46.4)

TABLE 3. DISTRIBUTION OF *XPD* CODON 751 GENOTYPIC AND ALLELIC FREQUENCIES AMONG CASES AND CONTROLS

Genotype	Cases (%)	Controls (%)	OR (95% CI)	p-Value
AA	74 (27.41)	104 (41.28)	1 (Ref.)	—
AC	104 (38.52)	81 (31.14)	1.80 (1.16–2.80)	0.005
CC	92 (34.07)	67 (26.59)	1.93 (1.22–3.05)	0.003
AA	74 (27.41)	104 (41.28)	1.00	—
AC+CC	196 (72.59)	148 (58.72)	1.86 (1.27–2.73)	0.0008
Allele A	252 (46.70)	289 (57.34)		
Allele C	288 (53.40)	215 (42.66)		

Significant *p*-values are in bold (*p* < 0.05).

OR, odds ratio; CI, confidence interval.

occupations. Again, with smokers (OR = 5.30, 95% CI = 2.42–11.68), alcohol drinkers (OR = 4.33, 95% CI = 2.17–8.70), and nonvegetarians (OR = 2.21, 95% CI = 1.26–3.87), the Gln allele showed a significantly increased risk toward bladder cancer. Such associations were not seen with nonsmokers, non-drinkers, and vegetarians.

The contribution of the *XPD* Lys751Gln polymorphism in each histological subcategory is shown in Table 5. There were 74 cases diagnosed as grade 1 (G1), 151 as grade 2 (G2), and 45 as grade 3 (G3). After stratifying according to stage, 212 (78.52%) cases had superficial bladder cancer, whereas the rest 58 (21.48%) had muscle-invasive bladder cancer. A similar approach was taken while analyzing the stages and grades of bladder cancer with respective genotypic divisions. Among stages, a highly significant risk was observed between the Gln allele (AC+CC) and patients having muscle-invasive stage of bladder cancer (OR = 4.39, 95% CI = 1.91–10.49). Among grades, the Gln allele (AC+CC) showed an increased risk with G2 grade (OR = 2.51, 95% CI = 1.55–4.09).

RAD51 G/C

The distribution of the genotypic and allelic frequencies of *RAD51* G→C polymorphism among the cases and controls is shown in Table 6. The distribution of genotypes among cases and controls was in Hardy–Weinberg equilibrium. The allele frequencies were 74.07% for G allele and 25.93% for C in the cases. In the control group, the allele frequency for G allele was 69.25% and that for C was 30.75%.

No significant differences were observed in the allelic and genotypic frequencies of this polymorphism. Because no association between the CC variant genotype and bladder cancer (additive mode of inheritance) was observed, these subjects were combined with those having GC genotype and this combined category was compared with subjects with GG genotype as the referent, thus making *RAD51* a dichotomous variable. Even using the dominant model, no significant association was seen with the variant C allele. The association between the *RAD51* polymorphism and the risk of bladder cancer was further examined after stratifying for similar variables as examined for *XPD* polymorphism (Table 7). No significant associations were found. The stratification was also considered histological categories (Table 8). Among the stages, the CC genotype showed a decreased risk in superficial stage (OR = 0.51, 95% CI = 0.27–0.99). Among the grades, the CC genotype showed a significantly decreased risk in G2-grade bladder cancer (OR = 0.24, 95% CI = 0.09–0.62). Significantly lower

association of CC genotype in patients with G2-grade bladder cancer when compared with controls suggested that the CC genotype might have a protective role. No other associations were found.

Discussion

The complex system of DNA repair enzymes plays a vital role in protecting the genome from the insults of cancer-causing agents, such as those found in tobacco smoke (Hoeijmakers, 2001). In humans, more than 70 genes are involved in the five major DNA repair pathways: NER, base excision repair, mismatch repair, homologous recombination repair, and nonhomologous end joining (Bernstein *et al.*, 2002).

A number of polymorphisms in the *XPD/ERCC2* gene have been reported. Polymorphisms in codons 199, 201, and 575 are rare, whereas those in codons 156, 312, 711, and 751 are common (Benhamou and Sarasin, 2005). The codon 751 polymorphism is located in the COOH-terminal domain of *XPD*. This region of the protein is essential for interaction with the p44 subunit of TFIIH, which stimulates *XPD* by increasing its helicase activity (Coin *et al.*, 1998, 1999). Several studies on different ethnic populations have investigated the possible association between *XPD* polymorphisms and risk of cancers of various other sites (Dufloth *et al.*, 2005; Yeh *et al.*, 2005; Le Morvan *et al.*, 2006). With regard to the Lys751Gln polymorphism, most of the reported data indicated that the Gln allele is associated with a higher DNA adduct level or lower DRC.

As far as we know, this is the second case–control study of *XPD* polymorphism in bladder cancer in a North Indian population. Before this study, an *XPD* polymorphism study was conducted on the same cancer in an Indian population (Gangawar *et al.*, 2010) but it studied recurrence and progression of cancer with post-BCG immunotherapy. Moreover, the latter did not show any significant genotypic difference. In the present study, the Gln allele (Lys/Gln + Gln/Gln) of *XPD* Lys751Gln polymorphism was found to have an increased risk toward bladder cancer (OR = 1.86, 95% CI = 1.27–2.73), when Lys/Lys was taken as the referent genotype.

As many as nine published reports on the *XPD* polymorphisms and risk of bladder cancer are in the literature. Of these, only two reports indicated some association between *XPD* Lys751Gln polymorphism and bladder cancer. According to a study conducted by Schabath *et al.*, (2005), a gender-specific role showing an increased risk for women,

TABLE 4. STRATIFICATION ANALYSIS OF XPD CODON 751 GENOTYPE FREQUENCIES IN CASES AND CONTROLS

Variable	Genotype	Cases (%)	Controls (%)	OR (95% CI)	p-Value
Sex					
Males	AA	56 (20.74)	89 (35.32)	1.00	—
	AC	95 (35.18)	66 (26.19)	2.29 (1.41–3.72)	0.0003
	CC	85 (31.48)	57 (22.62)	2.37 (1.44–3.92)	0.0003
	AC+CC	180 (66.67)	123 (48.81)	2.33 (1.52–3.56)	0.0000
Females	AA	18 (6.67)	15 (5.95)	1.00	—
	AC	9 (3.34)	15 (5.95)	0.50 (0.15–1.66)	0.203
	CC	7 (2.59)	10 (3.97)	0.58 (0.15–2.22)	0.370
	AC+CC	16 (5.93)	25 (9.92)	0.53 (0.19–1.49)	0.183
Inhabitation					
Rural	AA	39 (14.45)	50 (19.84)	1.00	—
	AC	50 (18.52)	29 (11.51)	2.21 (1.14–4.32)	0.012
	CC	49 (18.15)	20 (7.94)	3.14 (1.53–6.49)	0.0006
	AC+CC	99 (36.67)	49 (19.45)	2.59 (1.46–4.62)	0.0004
Urban	AA	35 (12.96)	54 (21.43)	1.00	—
	AC	54 (20.00)	52 (20.63)	1.60 (0.87–2.95)	0.104
	CC	43 (15.92)	47 (18.65)	1.41 (0.75–2.67)	0.254
	AC+CC	97 (35.92)	99 (39.28)	1.51 (0.88–2.60)	0.111
Occupation					
High risk	AA	26 (9.63)	19 (7.54)	1.00	—
	AC	37 (13.70)	21 (8.33)	1.29 (0.54–3.09)	0.534
	CC	44 (16.30)	13 (5.16)	2.47 (0.97–6.38)	0.036
	AC+CC	81 (30)	34 (13.49)	1.74 (0.80–3.78)	0.126
Low risk	AA	48 (17.80)	85 (33.73)	1.00	—
	AC	67 (24.81)	60 (23.81)	1.98 (1.17–3.35)	0.006
	CC	48 (17.80)	54 (21.43)	1.57 (0.90–2.76)	0.090
	AC+CC	115 (42.59)	114 (45.24)	1.79 (1.13–2.84)	0.009
Smoking					
Smokers	AA	20 (7.41)	26 (10.32)	1.00	—
	AC	43 (15.92)	14 (5.56)	3.99 (1.60–10.12)	0.0009
	CC	63 (23.33)	12 (4.76)	6.82 (2.70–17.54)	0.0000
	AC+CC	106 (39.26)	26 (10.32)	5.30 (2.42–11.68)	0.0000
Nonsmokers	AA	54 (20.00)	78 (30.95)	1.00	—
	AC	61 (22.59)	67 (26.59)	1.32 (0.78–2.21)	0.273
	CC	29 (10.74)	55 (21.82)	0.76 (0.41–1.40)	0.347
	AC+CC	90 (33.33)	122 (48.41)	1.07 (0.67–1.70)	0.778
Alcohol consumption					
Yes	AA	20 (7.41)	41 (16.27)	1.00	—
	AC	44 (16.30)	20 (7.94)	4.51 (2.00–10.31)	0.000
	CC	49 (18.15)	24 (9.52)	4.19 (1.91–9.25)	0.000
	AC+CC	93 (34.44)	44 (17.46)	4.33 (2.17–8.70)	0.000
No	AA	54 (20.00)	63 (25.00)	1.00	—
	AC	60 (22.22)	61 (24.21)	1.15 (0.67–1.97)	0.596
	CC	43 (15.93)	43 (17.06)	1.17 (0.64–2.12)	0.587
	AC+CC	103 (38.15)	104 (41.27)	1.16 (0.72–1.87)	0.533
Eating habits					
Vegetarian	AA	37 (13.70)	49 (19.44)	1.00	—
	AC	56 (20.74)	49 (19.44)	1.51 (0.82–2.80)	0.156
	CC	48 (17.80)	37 (14.68)	1.72 (0.90–3.30)	0.078
	AC+CC	104 (38.52)	86 (34.13)	1.60 (0.93–2.77)	0.071
Nonvegetarian	AA	37 (13.70)	55 (21.82)	1.00	—
	AC	48 (17.80)	32 (12.70)	2.23 (1.16–4.31)	0.009
	CC	44 (16.30)	30 (11.90)	2.18 (1.12–4.27)	0.013
	AC+CC	92 (34.10)	62 (24.60)	2.21 (1.26–3.87)	0.003

Significant *p*-values are in bold (*p* < 0.05).

but not for men, associated with the variant genotype was observed. When the exon 23 variant allele genotypes were combined (Lys/Gln+Gln/Gln), there was an increased bladder cancer risk in women, but not in men. On the contrary, Stern *et al.* (2002) found a slight decrease in risk for the Gln/Gln genotype compared with the subjects with the

Lys/Lys or Lys/Gln genotypes. No associations for the XPD polymorphism were found in a Turkish population (Narter *et al.*, 2009), in an Indian population (Gangawar *et al.*, 2010), in three Italian populations (Shen *et al.*, 2003; Matullo *et al.*, 2005; Covolo *et al.*, 2008), in American-Caucasians (Gu *et al.*, 2005), and in a German population (Sanyal *et al.*, 2004).

TABLE 5. DISTRIBUTION OF XPD CODON 751 GENOTYPES ACCORDING TO STAGES AND HISTOPATHOLOGICAL GRADES

Stages	Genotype	Cases (%)	OR (95% CI)	p-Value	
Superficial	AA	66 (24.44)	1	—	
	AC	76 (28.15)	1.48 (0.93–2.35)	0.081	
	CC	70 (25.93)	1.65 (1.02–2.66)	0.031	
	AC+CC	146 (54.07)	1.55 (1.04–2.33)	0.024	
Muscle invasive	AA	8 (2.96)	1	—	
	AC	28 (10.37)	4.49 (1.83–11.36)	0.000	
	CC	22 (8.15)	4.27 (1.68–11.14)	0.0005	
	AC+CC	50 (18.52)	4.39 (1.91–10.49)	0.000	
Grades	G1	AA	29 (10.74)	1	—
		AC	18 (6.70)	0.80 (0.39–1.61)	0.497
		CC	27 (10.00)	1.45 (0.75–2.77)	0.233
		AC+CC	45 (16.70)	1.09 (0.62–1.92)	0.748
	G2	AA	33 (12.22)	1	—
		AC	65 (24.07)	2.53 (1.47–4.35)	0.0003
		CC	53 (19.63)	2.49 (1.42–4.40)	0.0006
		AC+CC	118 (43.70)	2.51 (1.55–4.09)	0.0000
	G3	AA	12 (4.44)	1	—
		AC	21 (7.80)	2.25 (0.98–5.19)	0.035
		CC	12 (4.44)	1.55 (0.61–3.96)	0.312
		AC+CC	33 (12.22)	1.93 (0.91–4.17)	0.064

Significant *p*-values are in bold (*p* < 0.05).

Different population groups have different ethnicities. From an evolutionary standpoint, polymorphism represents the transient state between the origin of genetic variation by mutation and the loss of variation by fixation of either the ancestral or derived state. This transient state can, in certain circumstances, persist for quite some time (e.g., when there is a balancing selection that maintains polymorphism) (Kirk *et al.*, 2002). This could partially explain why only in certain population groups were significant associations observed between Lys→Gln polymorphism and increased risk of bladder cancer.

A multiple studies of the same polymorphism was carried out on lung cancer patients as well. The Gln/Gln genotype was associated with an increased risk for lung cancer when compared with the Lys/Lys genotype in Chinese (Liang *et al.*, 2003), Danish (Vogel *et al.*, 2004), Norwegian (Zienolddiny *et al.*, 2006), and German (Popanda *et al.*, 2004) populations. However, individuals with the Gln allele had a 61% reduction of lung cancer risk in a Chinese population (Shen *et al.*, 2005). No associations with the Lys751Gln polymorphism were seen in two Caucasian populations (Hou *et al.*, 2002; Zhou *et al.*, 2002), a mixed population (Spitz *et al.*, 2001), Finnish (Misra *et al.*, 2003), African-Americans (David-Beabes *et al.*, 2001), Chinese (Chen *et al.*, 2002),

Korean (Park *et al.*, 2002), European (Matullo *et al.*, 2006), and non-Hispanic Caucasian (United States) populations (Harms *et al.*, 2004). The inconsistent associations in previous studies of the XPD polymorphisms could be due to their small sample sizes, the ethnic differences in study populations, and possible environmental interactions.

No associations of XPD Lys751Gln were found with cervical (He *et al.*, 2008), renal (Lin *et al.*, 2008), and breast (Duflath *et al.*, 2005) cancers. Of three studies conducted on the same polymorphism in individuals having colorectal cancer, two did not show any significant association with XPD Lys751Gln polymorphism (Mort *et al.*, 2003; Yeh *et al.*, 2005). On the other hand, the Gln/Gln genotype had an increased risk in case of adenomatous polyps in a study done on adenomatous and hyperplastic polyps in individuals with colorectal cancer (Bigler *et al.*, 2005). A significantly increased risk of cutaneous melanoma was associated with XPD Lys/Gln and Gln/Gln genotypes compared with the XPD Lys/Lys genotype (Li *et al.*, 2006). No difference was found for XPD in a study on synovial sarcomas and myxoid liposarcomas (Le Morvan *et al.*, 2006).

In the present study, the inhabitants of rural areas having the Gln variant allele showed an increased risk towards bladder cancer. In a survey conducted on bladder cancer in

TABLE 6. DISTRIBUTION OF RAD51 G/C GENOTYPIC AND ALLELIC FREQUENCIES AMONG CASES AND CONTROLS

Genotype	Cases (%)	Controls (%)	OR (95% CI)	p-Value
GG	159 (58.89)	134 (53.17)	1 (Ref.)	—
GC	82 (30.37)	81 (32.15)	0.85 (0.57–1.28)	0.417
CC	29 (10.74)	37 (14.68)	0.66 (0.37–1.17)	0.129
GG	159 (58.89)	134 (53.17)	1.00	—
GC+CC	111 (41.11)	118 (46.83)	0.79 (0.55–1.14)	0.188
Allele G	400 (74.07)	349 (69.25)		
Allele C	140 (25.93)	155 (30.75)		

TABLE 7. STRATIFICATION ANALYSIS OF RAD51 G/C GENOTYPE FREQUENCIES IN CASES AND CONTROLS

Variable	Genotype	Cases (%)	Controls (%)	OR (95% CI)	p-Value
Sex					
Males	GG	135 (50.00)	112 (44.44)	1.00	—
	GC	77 (28.52)	71 (28.17)	0.90 (0.59–1.38)	0.612
	CC	24 (8.90)	29 (11.51)	0.69 (0.36–1.30)	0.214
	GC+CC	101 (37.41)	100 (39.68)	0.84 (0.57–1.24)	0.352
Females	GG	24 (8.90)	22 (8.73)	1.00	—
	GC	5 (1.85)	10 (3.97)	0.46 (0.11–1.78)	0.204
	CC	5 (1.85)	8 (3.17)	0.57 (0.14–2.35)	0.382
	GC+CC	10 (3.70)	18 (7.14)	0.51 (0.17–1.48)	0.168
Inhabitation					
Rural	GG	81 (30.00)	48 (19.05)	1.00	—
	GC	45 (16.67)	35 (13.90)	0.76 (0.41–1.40)	0.347
	CC	12 (4.44)	16 (6.35)	0.44 (0.18–1.10)	0.051
	GC+CC	57 (21.11)	51 (20.24)	0.66 (0.38–1.15)	0.119
Urban	GG	78 (28.89)	86 (34.13)	1.00	—
	GC	37 (13.70)	46 (18.25)	0.89 (0.50–1.56)	0.657
	CC	17 (6.30)	21 (8.33)	0.89 (0.41–1.92)	0.753
	GC+CC	54 (20.00)	67 (26.59)	0.89 (0.54–1.46)	0.623
Occupation					
High risk	GG	61 (22.59)	25 (9.92)	1.00	—
	GC	31 (11.48)	17 (6.75)	0.75 (0.33–1.70)	0.447
	CC	15 (5.56)	11 (4.36)	0.56 (0.21–1.52)	0.205
	GC+CC	46 (18.15)	28 (11.11)	0.67 (0.33–1.37)	0.240
Low risk	GG	98 (36.30)	109 (43.25)	1.00	—
	GC	51 (18.89)	64 (25.40)	0.89 (0.55–1.44)	0.605
	CC	14 (5.18)	26 (10.32)	0.60 (0.28–1.28)	0.151
	GC+CC	65 (24.07)	90 (35.71)	0.80 (0.52–1.25)	0.306
Smoking					
Smokers	GG	71 (26.30)	24 (9.52)	1.00	—
	GC	44 (16.30)	22 (8.73)	0.68 (0.32–1.43)	0.265
	CC	11 (4.07)	6 (2.38)	0.87 (0.60–1.25)	0.386
	GC+CC	55 (20.37)	28 (11.11)	0.66 (0.33–1.34)	0.215
Nonsmokers	GG	88 (32.59)	110 (43.65)	1.00	—
	GC	38 (14.07)	59 (23.41)	0.81 (0.48–1.36)	0.390
	CC	18 (6.67)	31 (11.48)	0.73 (0.36–1.45)	0.328
	GC+CC	56 (20.74)	90 (35.71)	0.78 (0.49–1.23)	0.257
Alcohol consumption					
Yes	GG	60 (22.22)	44 (17.46)	1.00	—
	GC	42 (15.55)	32 (12.70)	0.96 (0.50–1.84)	0.901
	CC	11 (4.07)	9 (3.57)	0.90 (0.31–2.60)	0.823
	GC+CC	53 (19.63)	41 (16.27)	0.95 (0.52–1.73)	0.852
No	GG	99 (36.67)	90 (35.71)	1.00	—
	GC	40 (14.81)	49 (19.44)	0.74 (0.43–1.27)	0.247
	CC	18 (6.67)	28 (11.11)	0.58 (0.29–1.18)	0.107
	GC+CC	58 (21.48)	77 (30.56)	0.68 (0.43–1.09)	0.094
Eating habits					
Vegetarian	GG	85 (31.48)	75 (29.76)	1.00	—
	GC	43 (15.93)	40 (15.87)	0.95 (0.54–1.67)	0.845
	CC	13 (4.81)	20 (7.94)	0.57 (0.25–1.31)	0.151
	GC+CC	56 (20.74)	60 (23.81)	0.82 (0.50–1.37)	0.426
Nonvegetarian	GG	74 (27.41)	59 (23.41)	1.00	—
	GC	39 (14.44)	41 (16.27)	0.76 (0.42–1.37)	0.329
	CC	16 (5.93)	17 (6.75)	0.75 (0.33–1.72)	0.460
	GC+CC	55 (20.37)	58 (23.02)	0.76 (0.44–1.29)	0.275

an Egyptian population (Zarzour *et al.*, 2008), residents of rural areas constituted 93.8% of cases, whereas only 6.2% of cases lived in urban areas. Use of smokeless tobacco by women is common in rural areas of Pakistan (Rafique, 2005). Here, the correlation between stage of bladder carcinoma and duration of smokeless tobacco use was significant ($p=0.03$) (Rafique, 2005). The patients having low-risk

occupations showed a significantly increased risk with the Gln variant (AC+CC) allele, whereas in patients having high-risk occupations, only the heterozygous AC genotype showed an increased risk. From such observations, it may be concluded that with XPD Lys751Gln polymorphism, the incidence of bladder cancer can occur irrespective of the type of occupation. The significant results with smokers, alcohol

TABLE 8. DISTRIBUTION OF *RAD51* G/C GENOTYPES ACCORDING TO STAGES AND HISTOPATHOLOGICAL GRADES

Stages	Genotype	Cases (%)	OR (95% CI)	p-Value	
Superficial	GG	127 (47.04)	1	—	
	GC	67 (24.81)	0.87 (0.57–1.33)	0.509	
	CC	18 (6.70)	0.51 (0.27–0.99)	0.031	
	GC+CC	85 (31.48)	0.76 (0.52–1.12)	0.145	
Muscle invasive	GG	32 (11.85)	1	—	
	GC	15 (5.55)	0.78 (0.37–1.59)	0.457	
	CC	11 (4.07)	1.24 (0.53–2.87)	0.579	
	GC+CC	26 (9.63)	0.92 (0.50–1.70)	0.783	
Grades	G1	GG	44 (16.30)	1	—
		GC	17 (6.30)	0.64 (0.33–1.24)	0.157
		CC	13 (4.81)	1.07 (0.49–2.31)	0.853
		GC+CC	30 (11.11)	0.77 (0.44–1.35)	0.339
	G2	GG	92 (34.07)	1	—
		GC	53 (19.63)	0.95 (0.60–1.51)	0.828
		CC	6 (2.22)	0.24 (0.09–0.62)	0.0008
		GC+CC	59 (21.85)	0.73 (0.47–1.12)	0.129
	G3	GG	23 (8.52)	1	—
		GC	12 (4.44)	0.86 (0.38–1.93)	0.7004
		CC	10 (3.70)	1.57 (0.63–3.85)	0.279
		GC+CC	22 (8.15)	1.09 (0.55–2.15)	0.798

Significant *p*-values are in bold (*p* < 0.05).

drinkers, and nonvegetarians developing bladder cancer were also in accord with other studies.

The potential modifying effect of *XPD* genotype in smokers having bladder cancer is of particular interest. Among the numerous repair genes that are known, *XPD* has been particularly analyzed in epidemiological studies on smoking-related cancers (e.g., lung cancer) (Liang *et al.*, 2003; Shen *et al.*, 2005). Because *XPD* helicase is a major protein in the NER pathway necessary for removal of tobacco-induced DNA lesions, *XPD* polymorphism is presumed to affect smoking-related bladder cancer risk by modulating the repair level (Benhamou and Sarasin, 2002). Smokers showed 5.30-fold increased risk for bladder cancer in the present study, which is supported by the observations of another study showing fourfold risk of bladder cancer (Morrison *et al.*, 1984). Evidence of gene–smoking interaction for bladder cancer was observed in two more studies (Matullo *et al.*, 2001; Shen *et al.*, 2003). Spitz *et al.* (2001) showed that lung cancer cases homozygous for the variant allele have a significantly reduced capacity for repair of BPDE-induced (benzo[*a*]pyrene diol epoxide) DNA damage. Because BPDE is a tobacco smoke metabolic product, this observation also supports the hypothesis that a gene–smoking interaction is more likely seen among individuals who smoke compared with those who do not (Schabath *et al.*, 2005), because cigarette smoking produces various types of DNA damage that requires repair.

Two lines of evidence support the possibility that the NER pathway may be involved in alcohol-related carcinogenesis. First, the NER pathway can remove certain types of small oxidative DNA lesions induced by chronic alcohol consumption (Brooks, 1997; Moller and Wallin, 1998). Second, alcohol consumption can generate lipid peroxidation products such as malondialdehyde (MDA), which is highly mutagenic and carcinogenic (Brooks, 1997). MDA interacts with DNA to form MDA-DNA adducts, which can be repaired via

the NER pathway (Marnett, 2002). As components of alcoholic drinks and their metabolites (such as acetaldehyde) are excreted through the urinary tract, a role of alcohol in bladder carcinogenesis is plausible.

An increased risk of the Gln (C) allele toward risk of bladder cancer was seen in nonvegetarians. According to Key *et al.* (2009), the risk for bladder cancer was lower among vegetarians than among meat eaters. Nitrosamines, which are known as bladder carcinogens or their precursors, are found in certain meat items (Scanlan, 1983; Dich *et al.*, 1996). A positive association with bladder cancer was detected in a cohort for intake of chicken without skin, but not for chicken with skin or other meats, including processed meats (Michaud *et al.*, 2006). Most case–control studies to date have reported no associations for meat intake and bladder cancer (La Vecchia and Negri, 1996; Steinmaus *et al.*, 2000). Red meat has also been implicated in colon and rectal cancer (Donaldson, 2004). Meat and the heterocyclic amines formed in cooking have been correlated to breast cancer in a case–control study in Uruguay as well (De Stefani *et al.*, 1997).

When stratified according to stages, the difference between the AA and AC+CC (dominant model) genotype frequencies was more pronounced in case of muscle-invasive (4.39-fold) rather than in superficial (1.55-fold) bladder cancer. In a study conducted by Narter *et al.* (2009), no significant differences were found between any of the stages for any of the genotypes. Among grades, the Gln variant (AC+CC) allele showed a 2.51-fold increased risk for grade G2 bladder cancer. Stern *et al.* (2002) have found no significant differences with respect to grades. The Gln variant showed increased risk in grade G3 too but, statistically, there was no significance. One possible reason could be that the *XPD* polymorphism might be working with other polymorphisms during progression toward higher grades, in which case, one or the other polymorphism might be masking the effect of the *XPD* 751 polymorphism.

Double-stranded DNA breaks (DSBs) may be the most detrimental form of DNA damage, because, if left unrepaired, broken chromosomes will lead to cell death. Additionally, if DSBs are improperly repaired, they can result in chromosomal translocations and cancer (Khanna and Jackson, 2001). Central to the repair of DSBs by HR is RAD51. Mammalian RAD51 appears to function as a part of a larger recombination complex that includes RAD52 (Liu *et al.*, 1999) and RAD54 (Tan *et al.*, 1999), which belong to the same epistasis group as RAD51 and replication protein A (RPA) (Golub *et al.*, 1998). Two SNPs in the 5' UTR of *RAD51*, 135 G→C and 172 G→T, were discovered by Wang *et al.* (2001). The latter SNP was found to have no effect on cancer risk, but carriers of the 135 C allele were reported to have an increased risk of breast cancer among a subset of *BRCA2* carriers (Wang *et al.*, 2001). No SNPs in the coding region were identified.

A plethora of literature is available on *RAD51* polymorphism in breast cancer, but there is only one study (Figueroa *et al.*, 2007) pertaining to such work on bladder cancer, which did not show any significant association. As far as we know, ours is the second study of *RAD51* polymorphism and bladder cancer.

In the present study, no significant difference was seen between the genotypic frequencies. No significant differences in the genotype distributions and allele frequencies were found in four studies conducted on breast cancer [of which two were conducted on Polish populations (Blasiak *et al.*, 2003; Romanowicz-Makowska *et al.*, 2006), one on an Australian population (Lose *et al.*, 2006), and one on a small population from New York (Brooks *et al.*, 2008)] and one on colorectal cancer in Poland (Garstka *et al.*, 2003). According to these studies, *RAD51* may not be a major familial breast cancer predisposition gene. Hence, the present study of *RAD51* polymorphism and bladder cancer is in concordance with earlier studies. *RAD51* 135C allele was associated with a reduced risk of breast cancer among *BRCA1* 5382insC carriers in Poland, but not among the Polish population in general (Jakubowska *et al.*, 2003). But in 2007, the *RAD51* 135 C allele was found to protect against both breast and ovarian cancers in a Polish population (Jakubowska *et al.*, 2007). In contrast, three previous studies found the *RAD51* 135 C allele to be associated with a higher risk of breast cancer (Levy-Lahad *et al.*, 2001; Wang *et al.*, 2001; Kadouri *et al.*, 2004).

The *RAD51* gene in breast cancer was studied mainly because of its potential to modify the penetrance of *BRCA1/BRCA2* mutations, which can increase susceptibility to breast cancer (Romanowicz-Makowska *et al.*, 2006). The product of *RAD51* protein functions with *BRCA1* and *BRCA2* in the repair of DSBs. The *BRCA2* protein binds to and regulates the *RAD51* protein to fix breaks in DNA (Lo *et al.*, 2003). The *BRCA2* protein transports the *RAD51* protein to the sites of DNA damage. *RAD51* then binds to the damaged DNA and encases it in a protein sheath, which is an essential first step in the repair process. In addition to its association with *BRCA2*, the *RAD51* protein also interacts with the *BRCA1* protein (Romanowicz-Makowska *et al.*, 2006). But, in case of bladder cancer, no such predispositions to genes such as *BRCA1* and *BRCA2* were reported.

Increased *RAD51* expression in a large range of cancer cell lines, including cervical cancer, prostate cancer, and breast cancer, has been reported (Raderschall *et al.*, 2002). Fluores-

cence *in situ* hybridization experiments did not detect *RAD51* gene amplification in tumors. Because northern blot analysis demonstrated highly elevated *RAD51* mRNA levels, they concluded that the increase in *RAD51* protein and nuclear foci formation in tumor cells were the result of transcriptional upregulation. Therefore, gene amplification did not seem to contribute to the elevated *RAD51* protein levels in tumor cells. Upregulation of the *RAD51* gene occurs at the transcriptional level (Raderschall *et al.*, 2002). This could be one reason as to why no associations could be detected with this polymorphism upon conducting PCR-RFLP assays.

HR is typified by homology search and DNA strand invasion (Schild and Wiese, 2010). *RAD51* is a major player in HR. One of the steps in HR is the formation of a single-stranded DNA coated by the protein RPA. A critical step in HR is the displacement of RPA by *RAD51* so that *RAD51* can recruit a double-stranded DNA to make a complementary strand to the single-stranded DNA already held by *RAD51* molecule. This step is highly regulated by cells to ensure that potentially deleterious events are avoided (Schild and Wiese, 2010). The 135 G/C polymorphism is located in the promoter region of the *RAD51* gene. Promoter region SNPs are capable of regulating the amount or timing of a protein's production. Any kind of error during the formation of this protein might disrupt the critical step of HR. Although the *RAD51* polymorphism appeared to have no effect on risk of bladder cancer, there was evidence of association of genotypes with histopathology of the cancer. Among stages, the CC genotype showed a significant protective association with superficial bladder cancer (OR=0.51, 95% CI=0.27–0.99). Among grades, the CC genotype showed a significant protective association with G2 grade (OR=0.24, 95% CI=0.09–0.62). No explanation can be offered for this discrepancy. This polymorphism may not be useful as an independent marker in bladder cancer.

Conclusion

Although polymorphism in *XPD* gene may be considered as a predisposition factor for bladder cancer, a concrete study on a larger population is still needed for the *RAD51* gene. Epidemiological studies of common polymorphisms in DNA repair genes, if large and unbiased, can provide insight into the *in vivo* relationships between DNA repair genes and bladder cancer risk. In addition, epidemiological studies of common polymorphisms can lead to an increased understanding of the public health dimension of DNA repair variation.

Acknowledgment

This study was supported by a grant (Grant number/CSIR sanction number: 09/135(0557)2008-EMR-1) for Junior Research Fellowship provided by Council of Scientific and Industrial Research (CSIR), Human Resource Development Group (Extra Mural Research Division), CSIR complex, Library Avenue, Pusa, New Delhi, India. The authors are indebted to the staff of PGIMER, Chandigarh, India, for their close cooperation and important contributions.

Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
 Ranbir Chander Sobti, D.Sc.
 Department of Biotechnology
 Punjab University
 Sector-14
 Chandigarh 160014
 India

E-mail: rcsobti@pu.ac.in

Received for publication March 19, 2011; received in revised form May 23, 2011; accepted May 23, 2011.