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Gene variants of *XRCC4* and *XRCC3* and their association with risk for urothelial bladder cancer

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Abstract The DNA double strand break repair gene *XRCC4*, an important caretaker of genome stability and *XRCC3* are suggested to play an imperative role in the development of carcinogenesis. However, no evidence has been provided showing that these genes are associated with risk of urinary bladder cancer (UBC). The study was designed to examine the polymorphisms associated with two genes namely *XRCC4* G1394T (rs6869366), intron 3 (rs28360317), intron 7 rs1805377 and rs2836007 and *XRCC3* (rs861539 and rs1799796), respectively and investigate their role as susceptible markers for UBC risk in North Indian cohort. In this hospital-based case–control study histologically confirmed 211 UBC patients and 244 age and gender matched controls of similar ethnicity were genotyped by means of PCR-RFLP. Significant different distributions in the frequency of the *XRCC4* intron 3 genotype, but not the *XRCC4* G1394T or intron 7 genotypes, between the UBC and control groups were observed. *XRCC4* intron 7 Del/Del conferred enhanced risk (OR 1.94; *P* 0.017) in UBC. Interestingly, *XRCC* −1394 G>T variant genotype GG was associated with reduced risk (OR 0.27; *P* 0.020). However, none of the four polymorphisms in *XRCC4* were associated with tobacco smoking and risk of recurrence in patients treated with BCG immunotherapy. Similarly, none of the *XRCC3* polymorphisms were associated with UBC susceptibility. Our results suggested that the *XRCC4* intron 3 rs6869366 genotype and intron 7 rs28360317 may be associated with UBC risk and may be a novel useful marker for primary prevention and anticancer intervention.

Keywords Polymorphism · Immunotherapy · *XRCC4* · *XRCC3*

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Introduction

Urinary bladder cancer (UBC) is considered a significant public health threat all over the world the low incidence of UBC in India is still a matter of health concern [1]. 70% recurrence rate within 1 year and 15–30% progression in such recurrent cases need attention. No adjuvant therapy consideration makes management difficult leading to decreased quality of life. Administration of intravesical Bacille Calmette-Guerin (BCG) substantially reduces recurrence and progression risk and has proved superior to intravesical chemotherapy [2]. Mutations or defects in the DNA repair system are likely to cause chromosomal aberrations which in turn lead to cell malfunctioning, cell death and tumorigenesis. Several studies have demonstrated that polymorphisms in DNA repair genes responsible for maintaining genomic integrity are modifiers of disease risk. Therefore, it is logical to postulate that some genetic variants of DNA repair genes might contribute to UBC pathogenesis. Sequence variants in DNA repair genes also are thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk.

Eukaryotic cells have developed two pathways to repair DNA double strand breaks (DSBs); the homologous recombination (HR) and the non-homologous end-joining (NHEJ) pathways *X-ray cross-complementing group 4* (*XRCC4*) gene, a key component of non-homologous end-

joining repair pathway, is found to restore DNA double strand break repair. It is basically required for precise end-joining of blunt DNA double strand breaks. End-joining of blunt DNA double strand breaks in mammalian fibroblasts is precise and requires DNA dependent protein kinase catalytic subunit (DNA-PKcs) and *XRCC4*. The gene targeted mutation studies demonstrate that differentiating lymphocytes and neurons strictly require the *XRCC4* end-joining proteins. The targeted inactivation of *XRCC4* gene leads to late embryonic lethality accompanied by defective lymphogenesis and neurogenesis manifested by extensive apoptotic death of newly generated cells [3, 4].

X-ray cross-complementing group 3 (*XRCC3*) gene is required for efficient repair of DSBs through the HRR pathway, for repair of DNA cross-linking, and for chromosomal segregation [5]. During HRR, the *XRCC3* protein interacts with the Rad51 protein, enabling Rad51 protein multimers to assemble at the site of damage. This gene functionally complements Chinese hamster irs1SF, a repair-deficient mutant that exhibits hypersensitivity to a number of different DNA-damaging agents and is chromosomally unstable. *XRCC3*-deficient cells were found to be unable to form Rad51 foci after radiation damage and demonstrated genetic instability and increased sensitivity to DNA damaging agents [6].

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 factor for UBC. Therefore, we analyzed the genotypic frequency of four polymorphisms of the *XRCC4* gene at G1394T (rs6869366), intron 3 (rs28360071), intron 7 (rs28360317), and intron 7 (rs1805377) and two polymorphisms in *XRCC3* at C18067T (T241M) (rs861539) and A17893G (IVS5-14) (rs1799796) using a polymerase chain reaction-based restriction fragment length polymorphism method. Further, we evaluated the outcome of BCG immunotherapy in terms of recurrence and whether it was associated with these genes. To the best of our knowledge, this is the first study carried out from North India to evaluate the influence of *XRCC4* and *XRCC3* polymorphisms in UBC.

Patients and methods

Study subjects

The present case–control study of UBC was conducted at Lucknow, India, at our Urology Center (Sanjay Gandhi Post Graduate Institute of Medical Sciences) during May 2007 to May 2010. 211 histologically confirmed transition cell carcinoma of UBC patients and 244 healthy, age, gender unrelated individuals visiting hospital for routine check-up or health awareness camps and hospital employees were recruited as controls. All the controls were from similar ethnicity and having no evidence of malignancy or chronic disease. All participants provided written informed consent for the study. The ethical committee of the institute approved the study.

Epidemiology and clinical data collection

The clinical information about tumor size, number, stage and grade, intravesical BCG immunotherapy, date of recurrence and histopathological findings, were obtained from our collaborator urologist of the department. The tumor stage was classified as per norms laid by American Joint Committee on Cancer's TNM staging system [7]. Seventy-five high risk superficial tumor patients (High Grade, multiple and >3 cm size tumor) were treated with live attenuated Danish 1331 strain (Guindi lab, Chennai, India) of Bacillus Calmette-Guerin (BCG) (BCG treated group). Intravesical BCG treatment consisted of either BCG six weekly instillations [Induction BCG (iBCG = 65)] or BCG induction plus monthly instillations [maintenance (mBCG = 8)]. Since the number of patients receiving mBCG was too low, we included them in BCG treated group for statistical calculations. Recurrence was considered as end point event and defined as a newly found bladder tumor following a previous negative cystoscopy.

Genotyping

Genomic DNA from all subjects was extracted from peripheral blood mononuclear cells using salting out method [8]. Table 1 represents detail of *XRCC4* and *XRCC3* polymorphisms studied in this study. Detection of genotype variants was performed by PCR-RFLP methodology, as described previously [9–11]. The PCR products of *XRCC4* intron-3 (rs28360071) polymorphism were 109 bp for the Del allele type and 139 bp for the Ins allele type, respectively. The PCR products of *XRCC4* intron-7 (28360317) polymorphism were 239 bp for CCT-positive form and no product for CCT-negative form. The PCR products were studied after digestion with *HincII*, and Tsp509I restriction enzymes for *XRCC4* −1394 G>T (rs6869366) (cut from 300 bp T type into 200 + 100 bp G type) and *XRCC4* intron 7 (rs1805377) (cut from 237 bp G type into 79 + 158 bp A type), respectively. For *XRCC3* codon 241 and IVS5-14 was analyzed by PCR followed by digestion by *NcoI* and *PVUII* (cut from 136 bp T type into 97 + 36 bp C type) and (AA-283/367, AG-283/367/650, GG-650), respectively. Five percent of DNA samples were replicated for genotyping and the concordance rate was 100% which was further validated by sequencing.

Table 1 *XRCC4* and *XRCC3* gene polymorphisms evaluated in North Indian population

Gene	Gene name	Chromosome	Location	Nucleotide change	Additional information	dbSNP	MAF in controls
XRCC4	X-ray cross-complementing group-4	5q13-14	Promoter	−1394 G>T	SNP	rs6869366	0.28
			Intron 3	Ins/del	30 bp insertion	rs28360071	0.12
			Intron 7	Ins/del	CCT insertion	rs28360317	0.47
			Intron 7	A>G	SNP	rs1805377	0.19
XRCC3	X-ray cross-complementing group-3	14q32.3	Exon7	C>T	SNP	rs861539	0.21
			IVS 5-14	A>G	SNP	rs1799796	0.19

MAF miner allele frequency, *SNP* single nucleotide polymorphism

Statistical analysis

Power of the study was calculated using Quanto software version 1.0 (http://hydra.usc.edu/gxe). Present study achieved 80% of the statistical power for odds ratio (OR) $\geq$1.5 at significance level (α) <0.05. Hardy–Weinberg equilibrium was checked in control by goodness of fit χ^2 test. Pearson χ^2 test was used to compare controls and patients. Multiple logistic regression analysis was also conducted to assess the risk (odds ratio) associated with UBC after adjusting for relevant biological variables (age, gender, and smoking). Pair wise linkage disequilibrium analysis and haplotypes of each individual consisting of four alleles of *XRCC4* was constructed by Expectation–Maximization algorithm using SNP Analyzer software version (1.2A). Further, multiple Cox regression analysis adjusted for age, gender and smoking was used to assess the effect of individual SNPs on the risk of recurrence in BCG treated patients. Kaplan–Meier analysis and Log-rank test was applied to determine recurrence free survival. Bonferroni's correction was applied in case of multiple comparisons using the formula $Pc = P \times n$ (Pc represents corrected value; P is χ^2 P value and n is the number of comparisons performed). In case of haplotypes, P value was corrected according to number of haplotypes compared ($n = 8$). A two tailed P and Pc value of less than 0.05 was considered statistically significant. All the statistical analysis was performed using SPSS software (version 11.5).

Results

A total of 455 individuals were genotyped in the study. Out of them 211 individuals were UBC patients. Table 2 represents all the clinical and demographical characteristics of the patients. No significant differences were observed between controls and patients in reference to age and

Table 2 Demographical details of study subjects

Characteristics	Controls ($n = 244$) n (%)	Patients ($n = 211$) n (%)	P value*
Mean age	60.8 ± 10.3	61.3 ± 13.2	0.654[a]
Gender			
Male	206 (84.4)	181 (85.8)	0.695
Female	38 (15.6)	30 (14.2)	
Smokers			
Non smokers	162 (74.7)	81 (44.0)	<0.001
Smokers	55 (25.3)	103 (56.0)	
Tumor stage			
Ta	–	71 (33.6)	
T1		86 (40.8)	
T2+ muscle invasive	–	54 (25.6)	
Grade			
G1	–	77 (36.2)	
G2		45 (21.4)	
G3	–	89 (42.4)	

* P value was calculated by chi-square test

[a] t-test was used to analyze the difference between the mean age

gender (Table 2). However, significantly higher percentage of smokers among cases (56%) as compared to the controls (25.3%, $P < 0.001$) was observed.

Influence of *XRCC4* and *XRCC3* polymorphisms and the risk of UBC

Of the four polymorphisms examined in *XRCC4*, reduced risk for UBC was seen in one (rs6869366); increased risk was seen in another (rs28260317) and rest two showed no association with risks. None of the polymorphism in *XRCC3* was associated with risk of UBC. The associations of *XRCC4* and *XRCC3* polymorphisms with UBC risk are presented in Table 3.

Age, gender and smoking adjusted logistic regression analysis in UBC patients revealed that the variant genotype of *XRCC4*-1397 (rs6869366) (GG) (OR 0.27; *P* 0.020; 95% CI 0.09–0.81) polymorphism was associated with reduced risk of UBC. In contrast, *XRCC4* intron 7 (rs28360317) variant genotype (Del/Del) demonstrated increased risk of

Table 3 Multiple logistic regression analysis of *XRCC4* and *XRCC3* gene variants and UBC risk

Polymorphism	Controls ($n = 244$) *n* (%)	Patients ($n = 211$) *n* (%)	*P* value	OR (95% CI)
XRCC4				
rs6869366				
TT	121 (49.6)	120 (60.0)	–	Ref
TG	106 (43.4)	83 (39.2)	0.421	0.83 (0.54–1.28)
GG	17 (7.0)	8 (3.8)	0.020	0.27 (0.09–0.81)
Allele T	348 (71.3)	323 (76.5)	–	Ref
Allele G	140 (28.7)	99 (23.5)	0.074	0.76 (0.56–1.02)
rs28360071				
I/I	188 (77.0)	153 (72.5)	–	Ref
I/D	50 (20.5)	47 (22.3)	0.754	1.08 (0.65–1.80)
D/D	6 (2.5)	11 (5.2)	0.202	2.03 (0.68–6.07)
Allele I	426 (87.3)	353 (83.6)	–	Ref
Allele D	62 (12.7)	69 (16.4)	0.119	1.34 (0.92–1.94)
rs28360317				
I/I	78 (32.0)	53 (25.1)	–	Ref
I/D	100 (41.0)	76 (36.0)	0.755	0.92 (0.54–1.55)
D/D	66 (27.0)	82 (38.9)	0.017	1.94 (1.12–3.34)
Allele I	256 (52.5)	182 (43.1)	–	Ref
Allele D	232 (47.5)	240 (56.9)	0.005	1.45 (1.12–1.89)
rs1805377				
AA	156 (64.0)	140 (66.4)	–	Ref
AG	79 (32.4)	70 (33.2)	0.574	1.13 (0.72–1.78)
GG	9 (3.6)	1 (0.5)	0.065	0.13 (0.01–1.14)
Allele A	391 (80.1)	350 (82.9)	–	Ref
Allele G	97 (19.9)	72 (17.1)	0.07	0.18 (0.06–1.94)
XRCC3				
rs861539				
CC	154 (63.1)	134 (63.5)	–	Ref
CT	79 (32.4)	68 (32.2)	0.941	0.97 (0.56–1.44)
TT	11 (4.5)	9 (4.3)	0.88	0.91 (0.33–2.01)
Allele C	387 (79.3)	336 (79.6)	–	Ref
Allele T	101 (20.7)	86 (20.4)	0.88	0.21 (0.12–1.34)
rs1799796				
AA	160 (65.5)	122 (57.8)	–	Ref
AG	77 (36.5)	83 (39.4)	0.085	1.51 (0.91–2.18)
GG	7 (3.0)	6 (2.8)	0.831	1.30 (0.36–3.43)
Allele A	397 (81.4)	327 (77.5)	–	Ref
Allele G	91 (18.6)	95 (22.5)	0.98	0.99 (0.09–2.14)

OR age, gender, and smoking adjusted odds ratio, *CI* confidence interval

UBC (OR 1.94; *P* 0.017; 95% CI 1.12–3.34). Similarly, Allele Del of *XRCC4* rs28360317 demonstrated enhanced chance of UBC (OR 1.45; *P* 0.005; 95% CI 1.12–1.89). Though, the *P* value was highly significant, odds ratio was not up to the statistical threshold (≥1.6 and ≤0.05) to demonstrate positive association. *XRCC4* intron-3 rs28360071 and intron-7 rs1805377 polymorphisms were not associated with UBC risk.

In case of *XRCC3*, codon 241 the frequency of the variant genotype was similar in controls and cases hence statistically no significant difference was observed. Another polymorphism of *XRCC3* IVS5-14 was also found not be associated with UBC susceptibility.

Further, to elucidate the combine influence of these polymorphisms, we constructed *XRCC4* haplotypes (Table 4). The haplotype containing wild type allele of all the four polymorphisms (T/Ins/Ins/A) was considered as reference. None of the haplotypes were associated with UBC risk. Though, the haplotype T/Ins/Ins/G demonstrated association with increased risk of UBC (OR 0.43; *P* 0.011, 95% CI, 0.23–0.82), the statistical significance was lost when Bonferoni's correction was applied for multiple comparisons (*Pc* 0.176).

Association of *XRCC4* and *XRCC3* polymorphisms with stage of UBC

To confirm whether the risk of UBC obtained was associated with invasive tumors, the patients were further categorized according to stage in Ta and T1 and muscle invasive tumors (≥T2 stage). The associations of stage with polymorphisms studied are represented in Table 5. Logistic regression analysis demonstrated statistically significant association of *XRCC4* rs28360071 heterozygous genotype (Ins/Del) with stage ≥T2 tumors (OR 2.74; *P* 0.035; 95% CI 1.07–7.00). Similarly variant genotype (Del/Del) was significantly associated with increased risk of ≥T2 stage tumors (OR 5.29; *P* 0.048; 95% CI 1.01–27.69). Similar trend of association was also observed in case of *XRCC4* rs28360071 Ins/Del genotype which demonstrated increased risk with stage T1 tumor (OR 4.29; *P* 0.017; 95% CI 1.29–14.25). None of the other polymorphisms in *XRCC4* and *XRCC3* were associated with bladder tumor stage.

Association of *XRCC4* and *XRCC3* genotypes with tobacco use as risk of UBC

The interaction of *XRCC4* genotypes with tobacco use was evaluated to confer risk of UBC. The *XRCC4* and *XRCC3* genotypes frequency distribution revealed that none of the polymorphisms were associated with UBC in individuals with tobacco habits (data not presented).

Association of *XRCC4* and *XRCC3* gene variants with recurrence after BCG immunotherapy

The potential association of variant genotypes of *XRCC4* and *XRCC3* and recurrence after BCG treatment are illustrated in Table 6. Multiple Cox regression hazards model revealed that none of the polymorphisms were associated with risk of recurrence in BCG treated patients.

Table 4 Haplotype analysis of *XRCC4* polymorphism and UBC risk

Haplotype	Controls ($n = 244$) *n* (%)	Patients ($n = 211$) *n* (%)	*P* value	OR (95% CI)
T/Ins/Ins/A	118 (24.2)	100 (23.7)	–	Ref
T/Ins/Ins/G	43 (8.8)	16 (3.8)	0.011*	0.43 (0.23–0.82)
T/Ins/Del/A	116 (23.8)	135 (32.0)	0.088	1.37 (0.95–1.97)
T/Ins/Del/G	27 (5.5)	16 (3.8)	0.298	0.70 (0.35–1.37)
T/Del/Ins/A	25 (5.1)	26 (6.2)	0.511	1.22 (0.66–2.56)
T/Del/Ins/G	1 (0.2)	4 (0.9)	0.168	4.72 (0.51–42.91)
T/Del/Del/A	13 (2.7)	18 (4.3)	0.206	1.63 (0.76–3.50)
T/Del/Del/G	5 (1.0)	8 (1.9)	0.278	1.88 (0.59–5.95)
G/Ins/Ins/A	50 (10.2)	26 (6.2)	0.078	0.61 (0.35–1.05)
G/Ins/Ins/G	8 (1.6)	9 (2.1)	0.574	1.32 (0.49–3.56)
G/Ins/Del/A	52 (10.7)	35 (8.3)	0.371	0.79 (0.48–1.31)
G/Ins/Del/G	12 (2.5)	15 (3.6)	0.344	1.47 (0.66–3.29)
G/Del/Ins/A	0	2 (0.5)	NC	NC
G/Del/Del/A	7 (1.4)	10 (2.4)	0.307	1.68 (0.61–4.60)
G/Del/Ins/A	11 (2.3)	0	NC	NC
G/Del/Del/G	0	2 (0.5)	NC	NC

Parenthesis represents percentage

NC Not calculated

* *Pc* 0.176

Table 5 Influence of *XRCC4* and *XRCC3* gene polymorphisms on tumor stage

Polymor phisms	Ta (a)	T1 (b)	≥T2 (c)	*P* value (a–b)	OR (95% CI) (a–b)	*P* value (a–c)	OR (95% CI) (a–c)
XRCC4							
rs6869366							
TT	38 (53.5)	50 (58.1)	32 (59.2)	–	Ref	–	Ref
TG	29 (40.8)	33 (38.3)	21 (38.8)	0.140	0.55 (0.25–1.21)	0.460	0.72 (0.30–1.72)
GG	4 (5.7)	3 (3.6)	1 (2.0)	0.080	0.09 (0.07–1.32)	0.256	0.22 (0.01–2.93)
rs28360071							
I/I	60 (84.6)	59 (68.6)	34 (63.0)	–	Ref	–	Ref
I/D	9 (12.6)	24 (27.9)	14 (25.9)	0.017	4.29 (1.29–14.2)	0.035	2.74 (1.07–7.00)
D/D	2 (2.8)	3 (3.5)	6 (11.1)	0.989	0.98 (0.12–7.95)	0.048	5.29 (1.01–27.69)
rs28360317							
I/I	15 (21.1)	23 (26.7)	15 (27.7)	–	Ref	–	Ref
I/D	29 (40.8)	33 (38.3)	14 (25.9)	0.099	0.39 (0.13–1.19)	0.067	0.32 (0.09–1.08)
D/D	27 (38.1)	30 (35.0)	25 (46.4)	0.090	0.39 (0.13–1.15)	0.381	0.60 (0.19–1.86)
rs1805377							
AA	42 (59.1)	62 (72.1)	36 (66.7)	–	Ref	–	Ref
AG	29 (40.9)	23 (26.7)	18 (33.3)	0.329	0.67 (0.30–1.49)	0.955	0.97 (0.40–2.32)
GG	0	1 (1.2)	0	–	–	–	–
XRCC3							
rs861539							
CC	38 (53.5)	54 (62.8)	35 (64.8)	–	Ref	–	Ref
CT	29 (40.8)	28 (32.6)	18 (33.3)	0.254	0.67 (0.35–1.34)	0.301	0.67 (0.32–1.41)
TT	4 (5.7)	4 (4.6)	1 (1.9)	0.634	0.71 (1.66–2.96)	0.254	0.27 (0.02–2.51)
rs1799796							
AA	40 (56.3)	46 (53.4)	36 (66.7)	–	Ref	–	Ref
AG	28 (39.4)	38 (44.1)	17 (31.4)	0.616	1.18 (0.61–2.25)	0.305	0.67 (0.31–1.44)
GG	3 (4.3)	2 (2.5)	1 (1.9)	0.561	0.58 (0.11–3.69)	0.399	0.38 (0.04–3.81)

Discussion

This study describes association of *XRCC4* and *XRCC3* polymorphisms with UBC risk and recurrence after BCG immunotherapy. To the best of our knowledge, this is the first report depicting association of *XRCC4* rs6869366 and rs28360317 polymorphisms with UBC risk. Simultaneously, *XRCC4* rs28360071 was conferring enhanced risk for invasive tumors. However, none of the polymorphisms were associated with risk of recurrence after BCG immunotherapy. In *XRCC3* none of the two polymorphisms were associated with UBC susceptibility. DNA damage due to environmental carcinogens, reactive oxygen species and other cancer causing insults remain one of the major cancer initiating events. Because DNA repair enzymes are correctives for DNA damage induced by carcinogens and environmental factors, it is very likely that SNPs in DNA repair genes may play an important part in both cancer susceptibility and anticancer treatment response.

XRCC4 (rs6869366) polymorphism was related with increased risk for UBC. This SNP may have functional regulatory significance since the nucleotide change from G to T in the promoter region may be susceptible to UBC risk. However, such observations need to be replicated in varied ethnic populations, since homozygote variants were low in the control populations (7% in our study). A study by Chang et al. (2009) [12] suggested *XRCC4* rs6869366 polymorphism as UBC risk factor, while *XRCC4* rs2836 0071 and rs28360317 were not associated with UBC. On the contrary, *XRCC4* rs6869366 demonstrated protective role, while *XRCC4* rs28360317conferred enhanced risk in our population. In a population based analysis conducted in Sweden and USA, investigating a SNP (rs1805377), located in the intron splice site of *XRCC4* gene, resulted in a non-significant association with UBC. Consistently, another group's study examining the same SNP in a larger USA population was also not associated with UBC risk [13, 14], which was compatible to our study. The ethnic variations may perhaps best explain these discrepancies observed with risk of UBC. Interestingly, in a population-based analysis of UBC susceptibility conducted in Spanish population, investigating the same SNP (rs1805377) reported significant

Table 6 Association of *XRCC4* and *XRCC3* gene polymorphisms with risk of recurrence in BCG treated UBC patients

Polymorphisms	Recurrence	Non recurrence	*P* value	HR (95% CI)
XRCC4				
rs6869366				
TT	9 (69.2)	34 (61.8)	–	Ref
TG	4 (30.8)	19 (34.5)	0.198	2.73 (0.59–12.63)
GG	0	2 (3.6)	–	–
rs28360071				
I/I	11 (84.6)	43 (78.2)	–	Ref
I/D	2 (15.4)	8 (14.5)	0.391	0.39 (0.04–3.26)
D/D	0	4 (7.3)	–	–
rs28360317				
I/I	5 (38.5)	15 (27.2)	–	Ref
I/D	6 (46.2)	20 (36.4)	0.256	4.00 (0.36–43.49)
D/D	2 (15.4)	20 (36.4)	0.877	1.22 (0.09–15.82)
rs1805377				
AA	10 (77.0)	36 (65.5)	–	Ref
AG	3 (23.0)	19 (34.5)	0.713	0.73 (0.13–3.87)
GG	0	0	–	–
XRCC3				
rs861539				
CC	8 (72.7)	39 (70.9)	–	Ref
CT	5 (27.3)	16 (29.1)	0.978	1.01 (0.42–2.39)
TT	0	0	NC	
rs1799796				
AA	6 (46.2)	31 (56.4)	–	Ref
AG	7 (53.8)	23 (41.8)	0.993	1.00 (0.46–2.18)
GG	0	1 (1.8)	NC	

HR Age, gender and smoking adjusted hazards ratio

association. Carriers of A allele had significantly increased 1.33-fold higher UBC risk compared with GG homozygotes [15]. This small inconsistency could be due to variant populations and/or sampling bias.

The *XRCC4* rs28360071 polymorphism was reported to be a risk factor for oral cancer in Taiwan whereas *XRCC4* rs6869366, 28360317 and 1805377 were not associated with oral cancer susceptibility [16]. In other studies, association of *XRCC4* rs6869366 with breast cancer, gastric cancer and oral cancer risk in Taiwan was reported [17–19]. The studies are limited regarding *XRCC4* polymorphisms for UBC susceptibility and results are sparse. Simultaneously, to analyze the combined influence of *XRCC4* polymorphisms, we constructed haplotypes and compared the frequency between controls and patients. The logistic regression analysis revealed that haplotype T/Ins/Ins/G was associated with UBC risk. However, after applying Bonferroni correction for multiple comparisons, this association became non significant (*Pc* 0.176). This observation suggested that the *XRCC4* rs6869366 and rs28360317 polymorphisms conferred UBC risk independently.

The *XRCC4* protein forms a complex with DNA ligase 4 and DNA-dependent protein kinase in the repair of DSBs by NHEJ, hence, altered repair capacity due to SNPs in *XRCC4* gene may modify an individual's susceptibility to cancers including UBC. The information about functional aspects of *XRCC4*-SNP selected in the present study is quite limited. There is a single study which suggests that *XRCC4* rs1805377 may have functional significance since the nucleotide change from G to A potentially abolishes an acceptor splice site at exon 8 [20]. Whether, rest of the polymorphisms modulate *XRCC4* protein activity and expression is unknown so far. The polymorphisms in upstream promoter region are generally known to regulate gene expression, while variations in introns may affect splicing sites and subsequently mRNA stability. Though, these possible mechanisms may partially explain modified UBC susceptibility with *XRCC4* rs6869366 and rs283 60317 polymorphisms, the functional aspects remains to be elucidated in physiological conditions. *XRCC3*. There is little evidence that any of the *XRCC3* polymorphisms alter protein function. The T241M polymorphism in *XRCC3* changes the amino acid from a neutral hydrophilic residue with a hydroxyl group to a hydrophobic one with a methyl sulfur group. This may result in a substantial change in protein structure and function, but no functional studies to

confirm this have yet been published. The *XRCC3* IVS5 A>G polymorphism is non-coding. We observed that both the polymorphism were not associated with UBC risk.

Similar results were seen in other studies, where no association was observed with susceptibility to UBC [21, 22]. In a metaanalysis study of 48 case control studies conducted by Shizhong et al. (2006) support that *XRCC3* could not be a major increased risk factor for cancer [23]. However, there are conflicting results also. Two case–control studies have found a significant association between the *XRCC3* codon 241 Met allele and melanoma [24] and UBC [25]. In case of *XRCC3* IVS5-14 we observed no significant difference in frequency distribution between cases and controls. Similarly, no association was observed with head and neck and lung cancer [11, 26]. A recent meta analysis in breast cancer showed that the variant G allele of *XRCC3* IVS5-14 polymorphism has a protective effect on breast cancer development [27].

In agreement with previous findings our results suggest that genetic variants in DNA repair genes are indeed involved in cancer etiology. Though, we did not observe any interaction of tobacco smoking and *XRCC4* and *XRCC3* variant genotypes, it is possible that some of the established UBC cancer related environmental risk factors such as industrial exposure to chemical carcinogens and tobacco smoking may modulate UBC risk in combination with other genetic variants of different repair pathways.

Further, we analyzed the association of these polymorphisms with clinical parameters such as tumor stage and risk of recurrence and with smoking habits. However, none of the polymorphisms were associated with tobacco smoking induced UBC or risk of recurrence in BCG treated patients. Immunological changes are considered as major events associated with anti-tumor activity of BCG immunotherapy. The molecular changes observed during antitumor activity of BCG are defined by recruitment of inflammatory cells and cytokine secretion resulting in strong immune response [28]. Less involvement of *XRCC4* and *XRCC3* in recurrence/progression may be a reason for no association observed with risk of recurrence in BCG treated patients.

In multi factorial diseases such as UBC, many genes together with other environmental factors may influence an individual's susceptibility. Though the sample size was small compared to other studies reported from western countries, nevertheless, based on low incidence rate of UBC in our population it is fairly optimal and could attain the required power statistically. Hence, the studies with large number of samples and functional assays are warranted to generate more significant outputs.

In conclusion, these findings suggest that *XRCC4* (rs6869366) and *XRCC4* (rs28360317) play a significant role in conferring risk for UBC and can be used as a marker for UBC screening. Simultaneously, we have reported that presences of these polymorphisms are not a risk factor for tobacco smoking induced UBC. Moreover, no associations of *XRCC4* polymorphisms with recurrence free survival were observed in patients treated with BCG immunotherapy.

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Conflict of interest None.

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