Original Article

Genetic variation in nucleotide excision repair pathway genes influence prostate and bladder cancer susceptibility in North Indian population

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BACKGROUND: Inherited polymorphisms of XPD and XPC genes may contribute to subtle variations in NER DNA repair capacity and genetic susceptibility to development of urological cancer such as prostate and bladder cancer.
MATERIALS AND METHODS: We genotyped four Single Nucleotide Polymorphs (SNPs) of the DNA repair gene XPD and XPC in 195 prostate cancer (PCa) and 212 bladder cancer (BC) patients and 250 healthy controls from the same area. XPD Exon 10 (G>A) by amplification refractory mutation system and Exon 23 (A>C), XPC Intron 9 (Ins/Del) and Exon 15 (A>C) were genotyped by PCR-RFLP.
RESULTS: Variant genotype of XPC demonstrated association with PCa as well as in BC (*P*, 0.013; *P*, 0.003). Combined genotype (GA+AA) revealed association with PCa and in BC (*P*, 0.012, *P*, 0.002). Variant allele also demonstrated risk in both the cancer. Diplotype of XPD and XPC was associated with a significant increase in PCa and BC risk. Variant (+/+) genotype of XPC intron 9 shown increased risk with PCa and in BC (*P*, 0.012; *P*, 0.032). CC genotype of XPC exon 15 revealed increase risk (*P*, 0.047) with PCa not in BC. In clinopathological grade variant allele of XPC intron 9 and 15 demonstrated risk with high grade of tumor and bone metastasis of PCa. In BC variant allele of XPD exon 10 and 15 also shown association with tumor grade. XPC intron 9 influences the risk of BC in former tobacco users in BC.
CONCLUSIONS: Our result support that SNPs in XPD and XPC gene may reduce NER repair capacity and play a major role for PCa and BC in North India.

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Key words: Bladder cancer, Prostate cancer, diplotype, NER genes, polymorphism

Introduction

Cancer is a multifactorial disease that results from complex interactions between the genetic background and environmental factors. Tumors of the urinary tract contribute significantly to the overall human cancer burden.[1] The incidence rate of prostate and bladder cancer in India is low compared to the Western countries, and is the sixth most commonly diagnosed cancer.[2] A wide variability in the incidence of urinary cancer reflects its multi-factorial etiology involving genetic and ethnic backgrounds, which further complexes by the effect of environmental factors.

Carcinogenesis is a multistep, polygenic, and multicausal process. Tobacco smoke and environmental and occupational activities involve several potent chemical carcinogens, which have shown to increase DNA adducts levels and to be associated with many cancers. An accumulation of genetic abnormalities and a decline in DNA repair may lead to carcinogenesis.[3]

Nucleotide excision repair (NER) is one of the most important pathways and eliminates a wide variety of DNA damage, including UV photoproducts by involving the protein products of >30 genes.[4] Mutations and single nucleotide polymorphisms (SNPs) in NER genes may contribute to deficient NER capacity and human cancer

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risk.[5,6] Among the many genes the XPD and XPC gene play a major role in NER pathway.

The XPD also called ERCC2 gene, is located at chromosome 19q13.3 The XPD protein is a component of the core transcription factor IIH, involved in repair of DNA by opening DNA around the damage. it also acts in the initiation of RNA transcription by RNA polymerase II by anchoring the cyclin-dependent kinase activating kinase complex to the core transcription factor IIH complex.[7] Mutations in the XPD gene can completely prevent DNA opening and dual incision, steps that lead to the repair of DNA adducts.[8] The two (XPD) polymorphic loci that have been of particular interest in molecular epidemiological studies are exon 10 (G>A) (Asp312Asn) and exon 23 (A>T) (Lys751Gln) polymorphisms.[9] These polymorphisms are associated with lower DNA repair capacity (DRC) and a higher level of DNA adducts.[10] Studies have reported significant associations between the Asp312Asn (G>A) or Lys751Gln (A>C) variants and predisposition to many types of cancers.

The XPC gene is located on chromosome 3p25 and encodes a 940-amino acid protein involved in DNA damage recognition during the early steps of the NER process. Sequence variants of the XPC gene may alter NER capacity and modulate cancer risk. An intronic biallelic poly (AT) insertion/deletion polymorphism (PAT) in intron 9 and non-synonymous SNPs, Lys939Gln (an A>C transversion) in exon 15 of XPC have been identified. Although the variant alleles of the intron 9 polymorphism and of exon 15 have been associated with reduced DRC.[11,12] XPC-PAT polymorphism has been reported to be in linkage disequilibrium with XPC exon 15 that causes an amino acid change Lys939Gln (A33512C). The effects of all three of these XPC polymorphisms on cancer risk have been extensively studied, but with inconsistent results. In some studies, the three polymorphisms were found to significantly modify cancer risk, but other studies showed a lack of association between the polymorphisms and cancer risk.

We believe that in addition to the base excision repair and mismatch repair pathways, NER should also be evaluated for its role in urothelial cancer such as prostate cancer (PCa) and bladder cancer (BC) risk. To test this hypothesis, we investigate a possible association between NER repair gene polymorphisms (XPD and XPC*)* and their diplotypes with PCa and BC risk in north Indian population.

Materials and Methods

Study subjects

The study subjects were enrolled in the Department of Urology (Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow) between January 2006 and February 2009. The participants in this study were unrelated individuals of similar ethnicity from Lucknow and other adjoining cities of North India. A total histologically confirmed 195 (prostate) and 212 (bladder) cancer patients were enrolled for the study. Tumor grade was evaluated in PCa samples by the Gleason scoring system[13] and BC has been classified as per the American Joint Committee on Cancer's TNM staging system.[14] A total of 250 cancer free, unrelated, age- and sex-matched healthy control individuals of similar ethnicity, randomly from general population of Lucknow and adjoining areas, were recruited as controls. PSA was done in all the control individuals. Individuals with total PSA $\geq$4 ng/ml and/or any irregularity in DRE or with history of cancer were excluded from the PCa study. Serum PSA was assayed by sandwich ELISA using CanAg PSA kit, Sweden. The study was approved by the Ethical Committee Review Board of our Institute and informed written consent was obtained from each participant.

DNA extraction and genotype analysis

Five milliliters of peripheral blood samples was collected in ethylenediaminetetraacetic acid vials from both the patients as well as controls. Genomic DNA was extracted from the stored peripheral blood by salting out method.[15] Genotyping was performed using amplification refractory mutation-specific polymerase chain reaction (PCR) methodology for XPD exon 10 (G>A).[16] PCR was used to amplify regions of XPC-PAT.[17] XPD exon 23 (A>C) and XPC exon 15 was genotyped by PCR–restriction fragment length polymorphism methods using PstI and PVUII (New England Biolabs, Beverly, MA) restriction enzyme.[17,18] All the genes polymorphisms

were successfully genotyped in both the patients and controls.

Quality control procedures

Precise quality control procedures were applied during the genotyping process. As a negative control, PCR mix without DNA sample was used to ensure contamination-free PCR product. Samples that failed to genotype were scored as missing and subjected to repetition. Ten percent of samples from patients and controls were repeated to evaluate the quality of genotyping, which showed 100% concordance. Genotyping was performed without knowledge of the case or control status.

Statistical analyses

The sample size was calculated and was found to be adequate using QUANTO software, version 1.0 (http://hydra.usc.edu/gxe) for the genetic marker. Sample size achieved 80% of the statistical power. A two-tailed *P* value <0.05 was considered statistically significant. Student's t-test was used to assess the significance of differences between the case and control subjects' Chi-square (χ^2) analysis was used to assess deviation from Hardy–Weinberg's equilibrium and to compare the genotype/allele/haplotype frequency between patients and controls. Odds ratios (ORs) were obtained by unconditional logistic regression analysis and adjusted for age and smoking as a continuous variable. All the statistical analyses were conducted using the SPSS software, version 11.5 (SPSS, Chicago, IL, USA). All the statistical tests were two-sided. Values of $P < 0.05$ were considered statistically significant.

Table 1: Demographical characteristics of controls and PCa study subjects

	Controls (n = 250)	PCa (n = 195)	χ^2 *P* value
Age (years ± SD)	64.7 ± 5.71	66.0 ± 5.46	0.286 δ
Total PSA (mean± SD) ng/ml	2.3 ± 0.8	221 ± 57.4	<0.0001
Demographic Details	n (%)	n **(%)**	
Cigarette/*bidi* smoking*			
Non smokers	174 (71.6)	126 (64.9)	
Smokers	69 (28.4)	68 (35.1)	0.136
Clinical Details	n **(%)**	n **(%)**	
Bone Metastasis			
Bone Mets (-)	-	91 (47.3)	
Bone Mets (+)	-	80 (41.8)	
Bone Scan not done		24 (10.9)	
Gleason Grade			
<7	-	54 (27.7)	
7	-	57 (29.2)	
>7	-	84 (43.1)	

*Numbers may not add to the total because of some missing data, δ-Student-test was used to determine the *P* value. PCa, prostate cancer; PSA, prostate-specific antigen

Diplotype analysis

Diplotypes were constructed and their frequencies assessed using the maximum-likelihood method, using an expectation–maximization algorithm by performing 100,000 permutations through software Arlequin (Version 2.0). OR was calculated using unconditional logistic regression for risk haplotypes taking the wild-type haplotype as reference.

Results

Demographical and clinical details of PCa and BC and controls

There was no statistical difference between ages of the PCa and BC patients and healthy controls. The average age in the control group was 64.7 ± 5.71 years as compared to 66.0 ± 5.46 years for PCa patients. In case of BC the average age in the control group was 58.8 ±10.8 years as compared BC patients 59.6 ± 12.4 years. The demographical characteristics of study subjects have been summarized in Tables 1 and 2.

Table 2: Demographical characteristics of controls and BC study subjects

Variable	BC (212) n (%)	Controls (250) n (%)	χ^2 *P* value
Sex			
Female	25(11.8)	35(14.0)	0.068
Male	187(88.2)	215(86.0)	
Age (years)			
≤ 60	132(62.3)	153(61.2)	0.107$^{\$}$
> 60	80(37.7)	97(38.8)	
Tobacco users			
Non Users	90(42.4)	173(69.2)	0.001
Former Users	85(40.4)	44(17.6)	
Current Users	37(17.2)	33(13.2)	
Tumor number			
Single	120(56.6)	-	-
Multiple	92(43.4)	-	
Tumor Size (cm)			
< 1	60(28.3)	-	-
1-3	103(48.6)	-	
>3	49(23.1)	-	
Stage			
Ta	61(28.8)	-	-
T1	86(40.6)	-	
T2	65(30.6)	-	
Grade			
G1	78(36.7)	-	-
G2	41(19.5)	-	
G3	93(43.8)	-	

$^{\$}$Student's *t* test for mean age comparison between patients and controls

Association of XPD genotype variants with PCa and BC risk

The genotypic distributions of XPD gene polymorphisms in the controls were in Hardy–Weinberg equilibrium (HWE). The genotypic frequency distribution between cases and controls is given in Tables 3 and 4, respectively. The variant allele frequency (A) of XPD exon 10 was significantly higher in both cases as compared to controls and exhibited 1.54 folds risk (*P*, 0.013) with PCa and 1.68 folds risk (*P*, 0.0003) for BC. Subsequent variant genotype (AA) demonstrated significant risk associated with PCa and BC (*P*, 0.013 and *P*, 0.0003, respectively). Variant allele carrier (GA+AA) also showed increased risk of PCa and BC with significant *P* value (*P*, 0.012). In contrast, XPD exon 23 was not differently distributed in the PCa and BC and controls.

Association of XPC genotype variants with PCa and BC risk

XPC intron 9 homozygous variant (+/+) demonstrated 2.57 folds statistically significant risk with PCa (*P*, 0.012) and 2.34 folds increased risk with BC (*P*, 0.032). Interestingly variant genotype CC of XPC exon 15 revealed 1.99 fold increased risk for PCa only (*P*, 0.047) not with BC [Tables 5 and 6].

Association of XPD and XPC diplotypes with PCa and BC risk

To elucidate the combined influence of these polymorphisms, we constructed diplotypes of XPD and XPC [Figures 1-4]. Diplotype analysis of these two polymorphisms revealed significant *P* values. The

Table 3: Genotype frequency distribution of XPD gene polymorphisms and association in controls and PCa cases

	Controls n (%)	Patients n (%)	OR (95%CI)	*P* value
XPD Exon 10				
GG	127(50.8)	84(43.1)	Reference	
GA	94(37.6)	65(33.3)	0.97(0.62-1.49)	0.895
AA	29(11.6)	46(23.6)	2.04(1.16-3.58)	0.013
GA+AA	123(49.2)	111(56.9)	1.66(1.12-2.47)	0.012
Allele G	348(69.6)	233(59.7)	Reference	
Allele A	152(30.4)	157(40.3)	1.54(1.16-2.03)	0.002
XPD Exon 23				
AA	89(44.5)	73(42.7)	Reference	
AC	94(47.0)	84(49.1)	1.07(0.69-1.67)	0.735
CC	17(8.5)	14(8.2)	0.83(0.36-1.89)	0.661
AC+CC	111(55.5)	98(57.3)	1.04(0.68-1.58)	0.857
Allele A	272(68.0)	230(67.3)	Reference	
Allele C	128(32.0)	112(32.7)	1.03(0.76-1.40)	0.828

Odds ratio adjusted with age and smoking

Table 4: Genotype frequency distribution of XPD gene polymorphisms and association in controls and BC cases

	Controls n (%)	Patients n (%)	OR (95%CI)	*P* value
XPD Exon 10				
GG	128(51.2)	78(36.8)	Reference	
GA	104(41.6)	100(47.2)	1.58(1.06-2.36)	0.023
AA	18(7.2)	34(16.0)	3.21(1.68-6.14)	0.0003
GA+AA	122(48.8)	134(63.2)	1.81(1.24-2.64)	0.002
Allele G	360(72.0)	256(60.4)	Reference	
Allele A	140(28.0)	168(39.6)	1.68(1.28-2.23)	0.0001
XPD Exon 23				
AA	110(44.0)	90(42.5)	Reference	
AC	121(48.4)	105(49.5)	0.99(0.67-1.47)	0.995
CC	19(7.6)	17(8.0)	1.14(0.54-2.41)	0.721
AC+CC	14056.0)	122(57.5)	1.02(0.70-1.50)	0.882
Allele A	341(68.2)	285(67.3)	Reference	
Allele C	159(31.8)	139(32.7)	1.04(0.79-1.37)	0.750

Odds ratio adjusted with age and smoking

Table 5: Genotype frequency distribution of XPC gene polymorphisms and association in controls and PCa cases

	Controls n (%)	Patients n (%)	OR (95%CI)	*P* value
XPC Intron 9				
-/-	140(56.0)	104(53.4)	Reference	
-/+	94(37.6)	65(33.3)	0.88 (0.58-1.34)	0.566
+/+	16(6.4)	26(13.3)	2.57 (1.23-5.39)	0.012
(-/+) +(+/+)	110(44.0)	91(46.6)	1.36 (0.91-2.04)	0.123
Allele -	374(74.8)	273(70.0)	Reference	
Allele +	126(25.2)	117(30.0)	1.27(0.94-1.71)	0.111
XPC Exon 15				
AA	127(50.8)	94(48.2)	Reference	
AC	104(41.6)	73(37.4)	0.92 (0.61-1.40)	0.724
CC	19(7.6)	28(14.4)	1.99(1.00-3.95)	0.047
AC+CC	123(49.2)	101(51.8)	1.35(0.91-2.00)	0.134
Allele A	358(71.6)	261(66.9)	Reference	
Allele C	142(28.4)	129(33.1)	1.24(0.93-1.66)	0.133

Odds ratio adjusted with age and smoking

Table 6: Genotype frequency distribution of XPC gene polymorphisms and association in controls and BC cases

	Controls n (%)	Patients n (%)	OR (95%CI)	*P* value
XPC Intron 9				
-/-	136(54.4)	108(50.9)	Reference	
-/+	103(41.2)	81(38.3)	1.04(0.70-1.54)	0.841
+/+	11(4.4)	23(10.8)	2.34(1.07-5.09)	0.032
(-/+) +(+/+)	114(45.6)	104(49.1)	1.15(0.79-1.67)	0.445
Allelel -	375(75.0)	297(70.0)	Reference	
Allele +	125(25.0)	127(30.0)	1.28(0.96-1.71)	0.092
XPC Exon 15				
AA	118(47.2)	100(47.2)	Reference	
AC	116(46.4)	87(41.1)	0.87(0.59-1.30)	0.517
CC	16(6.4)	25(11.7)	1.72(0.85-3.48)	0.130
AC+CC	132(52.8)	134(53.6)	0.99(0.66-1.40)	0.854
Allele A	352(70.4)	287(67.7)	Reference	
Allele C	148(29.6)	137(32.3)	1.13(0.85-1.50)	0.374

Odds ratio adjusted with age and smoking

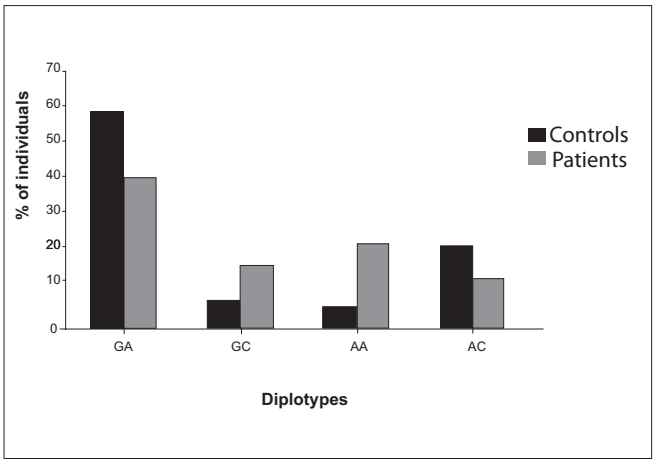

Figure 1: Association of XPD diplotypes with PCa susceptibility GC-pc,0.004; OR, 3.44(2.15-5.51), AA-pc,0.004; OR, 4.96(3.08-7.98)

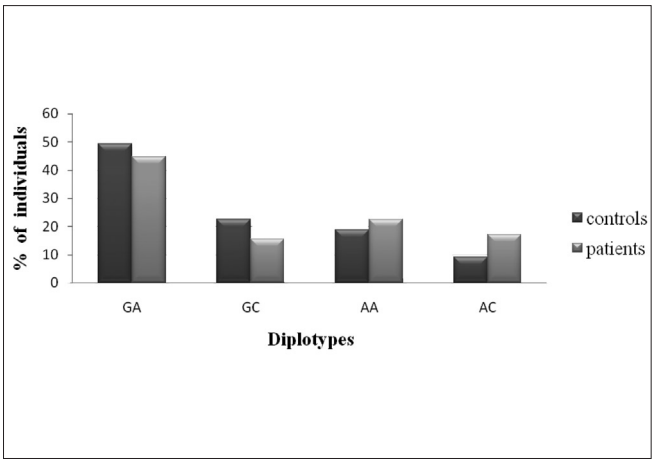

Figure 2: Association of XPD diplotypes with BC susceptibility AC-pc, 0.004; OR, 2.06 (1.36-3.12)

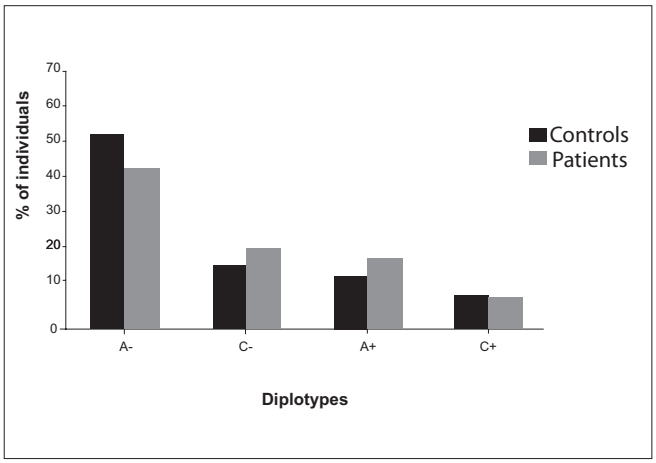

Figure 3: Association of XPC (Exon 15 and Intron 9) diplotypes with PCa susceptibility A+, pc, 0.044; OR, 1.60(1.11-2.30)

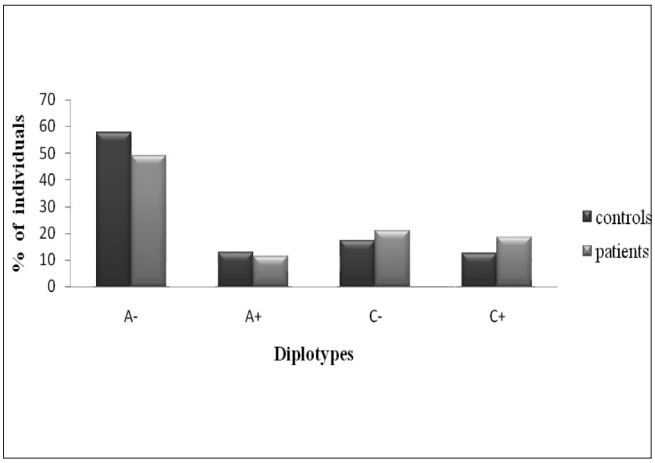

Figure 4: Diplotypes analysis of XPC (Exon 15 and Intron 9) diplotypes with BC susceptibility C+, pc, 0.008; OR, 1.79(1.23-2.62)

diplotype containing wild type were considered as reference. The two diplotype GC and AA (XPC exon 10, 23) showed 3.44 and 4.96 folds increase risk for PCa (Bonferroni corrected *P* value (P_c) - 0.004). Whereas in BC, only one diplotype AC demonstrated 2.06 folds significant risk (P_c- 0.004). Regarding XPC diplotypes, A+ (exon 15 and Intron 9) diplotype shown association with PCa (P_c-0.044). In BC diplotype, C+ revealed 1.8 fold increased risk (P_c-0.008).

Analysis of XPD and XPC genes polymorphisms with risk for tumor grades in PCa and BC

For genotypic comparison, we have divided the PCa patients with different Gleason grades into three groups (low grade <7, intermediate grade 7, and high grade >7) based on the degree of differentiation between the cells. Similarly in BC, we stratified the patients into three categories according to stage/grade (TaG1 (low risk NMIBT), TaG2,3 + T1G1–3 (high risk NMIBT), and T2+ (muscle invasive). The OGG1 GG genotype showed an increased risk in the TaG2–3 + T1G1–3 tumor stage (*P*, 0.027). Heterozygous (-/+) and variant (+/+) genotype of XPC intron 9 shows association with high grade of tumor in PCa (*P* 0.042, 0.004). Variant allele containing genotype (-/+/+/+) also showed increase risk with high gleason grade of PCa (*P*, 0.003). Similarly variant homozygous genotype (TT) of XPC exon 15 demonstrated 5.29 folds significant risk with high gleason grade of PCa (*P*, 0.012). Among BC, XPD exon

10 variant genotype AA showed 4.53 folds significant increased risk in the muscle invasive stage (*P*, 0.042). XPC exon 15 heterozygous AC genotype was observed to be associated significantly with elevated risk with $TaG_{2,3}$+$T1G_{1-3}$ stage of BC (*P*, 0.048). Overall statistically no significant association was observed in case of XPD exon 23 genotype with any of the grade of PCa and BC [Tables 7 and 8].

Analysis of XPD and XPC genes polymorphism with risk for bone metastasis with PCa

We also studied XPD and XPC gene variants and their risk associated with bone metastasis. The PCa patients were divided into two groups; one with bone metastasis and the other with non-metastasis. When these two groups were analyzed for risk of susceptibility for bone metastasis only variant genotype (+/+) of XPC intron 9 demonstrated increased risk with PCa bone metastasis (*P,* 0.011) [Table 9]. We did not find any significant risk with other SNPs of these two genes.

Interaction of XPD and XPC genes polymorphisms with smoking habit in PCa and BC

We evaluated the gene-smoking interaction to study the modulation of PCa risk with respect to these genes polymorphisms. We divided the PCa patients into two groups; one non smoker (never smoked) and the other as smokers (smoking more than 5 years). On analyzing these polymorphisms, we did not observe any association with PCa risk (data not shown). Among BC, we stratified the patients into non-users of tobacco and users of tobacco. Tobacco use of an individual was measured as former (smokers and smokeless) tobacco users and current (smokers and smokeless) tobacco users. The non-users were considered as reference and risk of BC was calculated for tobacco users. Since the numbers in the heterozygous and homozygous genotype group were low, we merged the two groups as the variant allele carrier. We observed that XPC intron 9 was associated with increased risk in former tobacco users (*P*, 0.05) [Figure 5].

Table 7: Influence of XPC gene polymorphisms with tumour grade of PCa patients

	Gleason <7 Low grade	Gleason 7 (Intermediate Grade)	Gleason >7 (High grade)	Between Low and Intermediate Grades		Between Low and High Grades	
	n(%)	n(%)	n(%)	OR (95%CI)	*P* value	OR (95%CI)	*P* value
XPC Intron 9							
-/-	35(64.8)	35(61.4)	32(38.1)	Reference		Reference	
-/+	17(31.5)	14(24.6)	34(40.5)	0.82(0.35-1.92)	0.654	2.18(1.02-4.65)	0.042
+/+	2(3.7)	8(14.0)	18(21.4)	4.00(0.79-20.18)	0.093	9.84(2.11-45.80)	0.004
(-/+) +(+/+)	19(35.2)	22(38.6)	52(61.9)	1.15(0.53-2.50)	0.710	2.99(1.47-6.09)	0.003
XPC Exon 15							
CC	26(48.1)	32(56.1)	36(42.9)				
CT	25(46.3)	22(38.6)	26(31.0)	0.71(0.33-1.54)	0.394	0.75(0.35-1.58)	0.452
TT	3(5.6)	3(5.3)	22(26.1)	0.81(0.15-4.36)	0.809	5.29(1.43-19.58)	0.012
CT+TT	28(51.9)	25(43.9)	48(57.1)	0.72(0.34-1.53)	0.400	1.23(0.62-2.46)	0.542

Odds ratio adjusted with age and smoking

Table 8: Influence of XPD and XPC gene polymorphisms with tumor stage/grade categories of BC patients

Stage/Grade Category	Genotypes			*P*-value (a-b)	OR (95% CI)	*P*-value (a-c)	OR (95% CI)
	(a)	(b)	(c)				
	n (%)	n (%)	n (%)				
XPD Exon10	GG	GA	AA				
TaG1	13(37.1)	19 (54.3)	3 (8.6)	1.00	-----	--------	
TaG_{2-3},$T1G_{1-3}$	51(45.5)	45(40.2)	16(14.3)	0.216	0.59(0.26-1.35)	0.751	1.24(0.31-5.00)
T2+	14(21.5)	36(55.4)	15(23.1)	0.247	1.74(0.67-4.50)	0.042	4.53(1.05-19.4)
XPC Exon 15	AA	CA	CC				
TaG1	22(62.8)	10(28.6)	3 (8.6)	1.00	-----	--------	
TaG_{2-3},$T1G_{1-3}$	49(43.8)	51(45.5)	12(10.7)	0.048	2.28(0.89-5.13)	0.128	2.10(0.80-5.47)
T2+	29(44.6)	26(40)	10(15.4)	0.228	1.99(0.49-6.11)	0.172	2.72(0.64-11.4)

Odds ratio adjusted with age and smoking

Table 9: Frequency distribution of XPC gene polymorphisms with risk of bone metastasis in PCa patients

	Metastasis (-) ve n(%)	Metastasis (+) ve n(%)	OR (95%CI)	*P* value
XPC Intron 9				
-/-	50(54.9)	33(41.3)	Reference	
-/+	33(36.3)	29(36.3)	1.33(0.68-2.58)	0.399
+/+	8(8.8)	18(22.4)	3.40(1.32-8.74)	0.011
(-/+) +(+/+)	41(45.1)	37(58.7)	1.73(0.94-3.18)	0.075

Odd ratio adjusted with age and smoking

Discussion

The NER pathway has particular importance, because this system removes complex bulky adducts. Several polymorphisms in NER genes were found to alter DNA repair, including variants in XPD and XPC. We therefore assessed whether polymorphisms in XPD exon 10, 23 and XPC Intron 9 and exon 15 had a clinically relevant impact on PCa and BC susceptibility in North Indian population. Our results showed *XPD* exon 10 G>A to be associated with increased risk of PCa and BC. Same result obtained from Rybicki *et al*., 2004 and Bau *et al*., 2007 in PCa.[16,19] Wu *et al*., 2006 also reported that only carriers of the variant A allele of *XPD*, Asp312Asn showed evidence of association with BC.[20] Concerning the XPD exon 10 (G>A) Asp312Asn polymorphism, most of the reported data indicate a higher level of DNA adducts in Asn individuals than in Asp individuals, which is interpreted as lower repair efficiency for the XPD Asn allele.[21] Further, the other polymorphic site of XPD exon 23 did not show any association with PCa and BC. The XPC gene product contributes to the global genome repair (GGR) pathway, is a member of the NER pathway, and is tightly associated with one of the two human homologues of Saccharomyces cerevisiae RAD23 protein (HR23B). Interestingly, we found significant association with variant genotype of XPC intron 9 of PCa and BC. This polymorphism has been shown to be associated with increased risks of skin,[22] and breast cancer.[23] Qiao *et al*., 2002 reported that, individuals with the PAT Ins/Ins polymorphism had a virtual reduction in NER capacity of 23.4% compared with the PAT -/- individuals, and has been recently proposed as a useful biomarker to identify individuals at increased risk for developing cancer.[11]

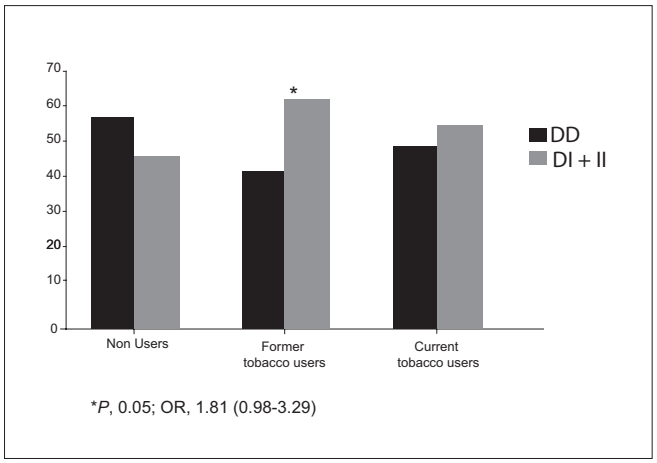

Figure 5: Influence of XPC Intron 9 gene polymorphisms with BC susceptibility in tobacco users

Regarding association with XPC Exon 15 A>C SNP with PCa. Our observation is compatible with the findings from Gangwar *et al*., 2010 in cervical cancer, Blankenburg *et al*., 2005 in melanoma.[22,24] The lack of associations was found in XPC (A>C) CC gene polymorphism with BC in our study; this finding was consistent with the results of a previous study in BC.[25]

Moreover, we examined the diplotype of XPD exon 10, 23 and XPC Intron 9 and 15 in PCa and BC. Interestingly the frequency of G-C and A-A (XPD exon 10 and 23) was significantly increased in PCa and one diplotype AC showed risk with BC. Diplotype (A+) and (C+) of XPC demonstrated statistically increased risk with PCa and BC. The finding demonstrated that the combined genotype was associated with PCa and BC. When we analyzed these genes polymorphisms with clinical grades of the both the disease, we found that variant allele of XPC intron 9 and exon 15 were significantly higher in high Gleason grade of PCa. Similarly XPD exon 10 variant allele is associated with muscle invasive stage and XPC exon 15 heterozygous AC genotype was observed to be increase risk with $TaG_{2,3}$+$T1G_{1-3}$ stage of BC. XPC Intron 9 variants genotype revealed increased risk with bone metastasis with PCa. This clearly suggested that these gene variants may be associated with initiation of as well as progression of PCa and BC.

We also analyzed the interaction of XPD and XPC genotypes with tobacco exposure to investigate the modulation of risk in a case-only analysis. Case-only approaches are believed to be better than case-control

studies; Tobacco carcinogen exposure has a strong association with BC. In the present study, only XPC intron 9 elevated risks was observed in former tobacco users of BC patients. None of the others SNPs shows significant association with PCa and BC.

In summary, we found increased risk of PCa and BC in individuals with two copies of the XPD exon 10A allele and XPC intron 9 + allele. We also observed that XPC exon 15 A>C genotype was associated with PCa risk. The combined genotype of XPD exons 10 and 23 and XPC intron 9 and exon 15 was found to be associated with PCa and BC risk. Though our study achieved sufficient (>80%) power due to the low incidence rate of PCa and BC in India nevertheless a higher number of sample size can warrant more modest results. These results suggest that genetic variants in NER repair pathways may be involved in PCa and BC etiology.

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