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RESEARCH ARTICLE

Do polymorphisms in *XRCC4* influence prostate cancer susceptibility in North Indian population?

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Abstract

Objective: *XRCC4* play a key role in nonhomologous end-joining repair pathway. Alterations in DNA repair gene have been shown to reduce DNA repair capacity thereby inflicting carcinogenesis.

Methods: In a hospital-based case-control study, 192 prostate cancer (PCa) and 224 healthy controls. They were genotyped for *XRCC4* G-1394T (rs6869366), intron 3 (rs28360071) intron 7 (rs28360317) and intron 7 (rs1805377), polymorphisms using polymerase chain reaction–restriction fragment length polymorphism.

Result: Carriers of GG genotype of rs6869366 were at reduced risk. Del/Del of rs28360071 and 28360317 demonstrated increased risk. The haplotype analysis was observed to be associated with a significant increase in PCa risk. Combined genotype of rs6869366, rs28360071 and rs1805377 have shown significant risk with high Gleason grade.

Conclusion: Our results suggested that the variant genotype of *XRCC4* rs28360071 and rs28360317 and haplotype analysis may be associated with PCa risk.

Introduction

Prostate cancer (PCa) is the most common male malignancy in the Western world, though its incidence varies widely according to race. In the Western world, it is a second leading cause of cancer death in males (Jemal et al., 2008). Literature review discloses Asians with the lowest incidence, and in India it is ranked as the sixth most commonly diagnosed cancer in men (Sinha et al., 2003). In spite of these dismal statistics, the etiology of PCa remains largely unknown. Variation in incidence and mortality rate from PCa around the world suggests that it is multifactorial and polygenic in origin. Polymorphic variations in humans may be responsible for interindividual differences in susceptibility to multifactorial diseases. It is well established that genetic factors also play an important role in pathogenesis of PCa (Lichtenstein et al., 2000).

Several risk factors have been identified, the main factors being age (Sakr et al., 1996), family history (Bratt, 2002) and ethnic origin (Ben-Shlomo et al., 2008). PCa is uncommon in men younger than 50 years, but 80% of men older than 80 years were found to have cancerous cells in their prostate at the time of death (Sakr et al., 1996). Estimates suggest that between 30 and 40% of all early onset cases of PCa (<55 years) are caused by inherited factors (Bratt, 2002).

DNA repair is an important mechanism for maintaining the integrity of DNA. Single nucleotide polymorphisms (SNPs) located in DNA repair genes could be responsible for substantial alterations in DNA repair capacity (DRC). Several studies have demonstrated that polymorphisms in DNA repair genes that are responsible for maintaining genomic integrity are modifiers of tumorigenesis risk (Berwick and Vineis, 2000; Vodicka et al., 2004). Eukaryotic cells have developed two pathways to repair DNA double-strand breaks (DSBs): the homologous recombination and the nonhomologous end-joining (NHEJ) pathways. NHEJ plays a predominant role under most conditions in mammalian cells (West, 2003).

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The DNA repair pathway is essential for maintaining genomic stability of mammalian cells. Deficiencies in the DNA repair system are likely to cause chromosomal aberrations, which in turn lead to cell malfunctioning, cell death and tumorigenesis (van Gent et al., 2001). Many genes play important roles in the repair pathway, for example, the *X-ray cross-complementing group 4* (*XRCC4*; located in the 5q13–q14 region) gene, a key component of NHEJ repair pathway, is found to restore DNA DSB repair and has the ability to support V(D)J recombination (Li et al., 1995). The *XRCC4* gene product interacts directly with Ku70/Ku80 (Mari et al., 2006) and it is hypothesized that *XRCC4* serves as a flexible join between Ku70/Ku80 and its associated protein Ligase4. A number of recognized SNPs of the *XRCC4* gene have been reported to be associated with various cancers, indicating that they might play common and central roles in various cancers. Other studies have shown that *XRCC4* is required for precise end-joining of blunt DNA DSBs in mammalian fibroblasts (van Heemst et al., 2004). The gene targeted mutation studies demonstrate that differentiating lymphocytes and neurons strictly requires 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 (Gao et al., 1998). Thus, it is reasonable that polymorphisms of *XRCC4* gene can be sustained in the genome during long-term process of carcinogenesis. These findings suggest that mutations of the *XRCC4* gene induce serious syndromes from a very early age. As PCa most commonly affects elderly men, these mutations are maybe suitable targets for PCa biomarkers. We therefore focused our study on the SNPs of *XRCC4*, hypothesizing that, though variant genotypes of SNPs may not cause lethal injuries in younger males, they might slightly increase the possibility of genomic instability and lead to PCa carcinogenesis with increasing age.

Since DNA repair gene alterations have been reported to cause a reduction in DRC, we hypothesized that DNA repair gene polymorphisms may be risk factors for PCa. To ensure this proposition, we determined the genotypic frequency for polymorphisms of the *XRCC4* gene at G-1394T (rs6869366), intron 3 (rs28360071), intron 7 (rs28360317) and intron 7 (rs1805377) (Figure 1), using a polymerase chain reaction–based restriction fragment length polymorphism (PCR-RFLP) method. To the best of our knowledge, data in PCa is sparse and only one study has been reported from Taiwanese, this is the first study carried out to evaluate the contribution of *XRCC4* polymorphisms in PCa in India.

Material and methods

Study subjects

Patients were enrolled in Department of Urology (Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh) between January 2007 and June 2009. Symptomatic men for lower urinary tract infection suspected to have PCa based on serum prostate-specific antigen (PSA) of >4 ng/ml and/or abnormal digital rectal examination (DRE) were further subjected to systematic ultrasound-guided needle biopsies. The primary end point was the histologic presence of adenocarcinoma of the prostate in the biopsy specimen. Tumor grade was evaluated in PCa samples by the Gleason scoring system (Gleason and Mellinger, 1974). After histological conformation, 192 cases of adenocarcinoma of prostate were inducted in the study. All the participants in the study were unrelated individuals of similar ethnicity from Lucknow and other adjoining cities of North India. Information on demographic features was obtained through personal interview using a standard clinical proforma.

Age- and ethnicity-matched healthy individuals ($n = 224$) were recruited as controls from men participating in PCa screening programs of the hospital. None of the controls had history for cancer and were frequency matched to cases on age.

The total PSA levels were determined in PCa, and healthy controls using CanAg EIA kits, Goteborg (Sweden). Control individuals with PSA >4.0 ng/ml and/or abnormal DRE were excluded. The study was conducted with prior informed and written consent and with the approval of the Institute research ethics board.

Genotyping

DNA was extracted from peripheral blood lymphocytes by salting out method (Miller et al., 1988). Individual SNP found on *XRCC4* were analyzed by PCR-RFLP. Details of primers and cycle conditions were referred as described earlier (Chiu et al., 2008). 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

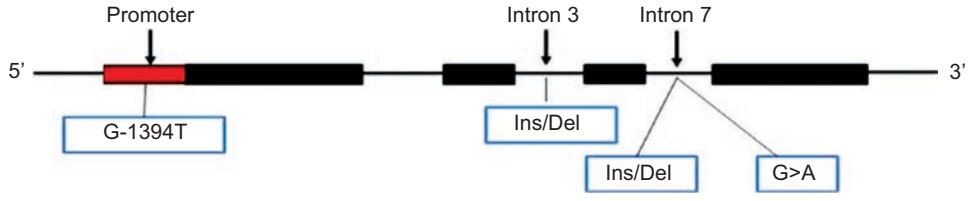

Figure 1. The four single nucleotide polymorphisms selected among the genomic region of *XRCC4*.

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 *TasI* 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. Positive and negative controls were used in each genotyping assay and 5% of randomly selected samples were regenotyped by other lab personal with 100% concordance.

Statistical analysis

Power of the study was calculated using Quanto software version 1.0 (http://hydra.usc.edu/gxe) with input of the following variables: case-control study design, significance level (α) <0.05 (2 sided), model of inheritance = log additive, allele freq, genetic effect for odds ratio (OR) (≥1.6 or ≤0.6). The present study achieved 80% of the statistical power. χ^2 Analysis was used to assess deviation from Hardy–Weinberg equilibrium (HWE) and to compare the genotype = allele = haplotype frequency between patients and controls. ORs were obtained by unconditional logistic regression analysis and adjusted for age and smoking as a continuous variable. PCa patients with different Gleason grades, bone metastasis were identified using the same statistics as mentioned. Bonferroni's correction was applied in case of multiple comparisons using the formula $Pc = p \times n$ (*Pc* represents corrected value where *n* is the number of comparisons performed). All the statistical analyses were conducted using the SPSS software, version 11.5 (SPSS, Chicago, IL). A p value <0.05 was regarded statistically significant.

Table 1. Clinical and demographic details of study subjects.

	Controls (n = 224)	PCa (n = 192)	p Value
Age (years ± SD)	59.1 ± 10.4	62.6 ± 8.9	0.483[b]
Total PSA (mean ± SD) ng/ml	2.3 ± 0.8	221 ± 57.4	<0.0001
Demographic details	*n* (%)	*n* (%)	
Cigarette/bidi smoking[a]			
Nonsmokers	156 (70.0)	125 (65.4)	
Smokers	67 (30.0)	66 (34.6)	0.327
Clinical details	*n* (%)	*n* (%)	
Bone metastasis			
Bone mets (–)	–	91 (47.3)	
Bone mets (+)	–	80 (41.8)	
Bone scan not done		21 (10.9)	
Gleason grade			
<7	–	49 (25.5)	
7	–	57 (29.7)	
>7	–	86 (44.8)	

PCa, prostate cancer; PSA, prostate-specific antigen.
[a]Numbers may not add to the total because of some missing data
[b]Student *t*-test was used to determine the *p* value.

Haplotype analysis

Haplotypes 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 study subjects

A total of 416 individuals (192 PCa and 224 controls) were analyzed in the study. There was no statistical difference between age of the PCa patients (62.6 ± 8.9 years) and healthy controls (59.1 ± 10.4 years) and smoking habits (p = 0.327). As expected, there was high degree of statistical difference between serum PSA of PCa patients (221 ± 57.4 ng/ml) and controls (2.3 ± 0.8 ng/ml). Majority of the patients had high Gleason grade at the time of diagnosis (Gleason score 7 in 29.7% and Gleason score >7 in 44.8%) and 41.8% of patients were diagnosed to have bone metastasis (Table 1).

Association of XRCC4 genotype variants with PCa risk

The observed genotype frequencies of all the polymorphisms were in agreement with HWE in the controls. To evaluate the association between genetic variant with risk of PCa, we compared *XRCC4* genotype frequency distribution in the PCa and control group shown in Table 2. Carriers of the GG genotype of *XRCC4*-1397 (rs6869366) were at reduced risk (p = 0.048; OR = 0.34). In contrast, *XRCC4* intron 3 (rs28360071) variant genotype (Del/Del) demonstrated increased risk with PCa (p = 0.013; OR = 2.99). After combining the heterozygous and homozygous (Ins/Del + Del/Del) in both groups there was still an evident increase risk of 1.5-fold (p = 0.042; OR = 1.56). This effect was also observed in case of alleles (p = 0.002; OR = 1.75). Variant genotype (Del/Del) of *XRCC4* Intron 7 (rs28360317) demonstrated twofold significant risk associated with PCa (p = 0.007; OR = 2.05). Variant allele (Del) also showed marginal significant risk (p = 0.010; OR = 1.49). Our results further showed that *XRCC4* Intron 7 (rs1805377) gene does not influence susceptibility to PCa.

Association with haplotypes

Further, to elucidate the combined influence of these polymorphisms, we constructed *XRCC4* haplotypes of the four polymorphisms examined (rs6869366, rs28360071, rs28360317, rs1805377; Table 3). For statistical advantage, 11 of the haplotypes with a frequency of <5% were excluded from further analysis.

The remaining three haplotypes were subjected for further statistical analysis. Interestingly, haplotypes T/Ins/Del/A ($p < 0.001$; OR = 3.73) showed increased risk for PCa.

Association of XRCC4 gene polymorphism with tumor stage/grade

For genotypic comparison the patients with different Gleason grades were subcategorized into three groups (low grade <7, intermediate grade 7, high grade >7) based on degree of differentiation of cells (Table 4). When the genotype frequencies of *XRCC4* variants were compared with these three Gleason groups, a significant association was observed for the variant allele carrier (TG + GG) of *XRCC4* (rs6869366) ($Pc = 0.016$; OR = 2.91) with high Gleason grade. Combination of heterozygous and homozygous (Ins/Del + Del/Del) of *XRCC4* intron 3 (rs28360071) showed significant association but when Bonferroni correction was applied the significance has been lost (*Pc*, 0.076). Variant allele carrier (AG + GG) of intron 7 (rs1805377) also exhibited significant risk (*Pc*, 0.028; OR, 2.87) with high Gleason grade. We did not find any significant risk associated with *XRCC4* Intron 7 (rs28360317).

Analysis of XRCC4 gene polymorphism with risk for bone metastasis

We also studied *XRCC4* gene variants and their risk associated with bone metastasis. The PCa patients were stratified into two groups, one with positive and the other with negative bone metastasis. When these two groups were analyzed for risk of susceptibility for bone metastasis

Table 2. *XRCC4* gene variants and susceptibility to PCa risk.

Genotype	Controls ($n = 224$) (%)	Cases ($n = 192$) (%)	*p* Value	OR (95%CI)
XRCC4				
rs6869366 (G > T)				
TT	112 (50.0)	117 (60.9)		Reference
TG	98 (43.8)	70 (36.5)	0.045	0.65 (0.43–0.99)
GG	14 (6.3)	5 (2.6)	0.048	0.34 (0.12–0.99)
TG + GG	112 (50.1)	75 (39.1)	0.017	0.61 (0.41–0.91)
T Allele	322 (71.9)	304 (79.2)		Reference
G Allele	126 (28.1)	80 (20.8)	0.015	0.67 (0.48–0.92)
rs28360071 (Ins/Del)				
Ins/Ins	168 (75.0)	124 (64.6)		Reference
Ins/Del	48 (21.4)	49 (25.5)	0.211	1.34 (0.84–2.13)
Del/Del	8 (3.6)	19 (9.9)	0.013	2.99 (1.25–7.12)
Ins/Del + Del/Del	56 (25.0)	68 (35.4)	0.042	1.56 (1.01–2.40)
Ins Allele	384 (85.7)	297 (77.3)		Reference
Del Allele	64 (14.3)	87 (22.7)	0.002	1.75 (1.23–2.51)
rs28360317 (Ins/Del)				
Ins/Ins	76 (33.9)	50 (26.0)		Reference
Ins/Del	90 (40.2)	73 (38.0)	0.158	1.42 (0.87–2.34)
Del/Del	58 (25.9)	69 (35.9)	0.007	2.05 (1.21–3.47)
Ins/Del + Del/Del	148 (66.1)	142 (73.9)	0.075	1.48 (0.96–2.28)
Ins Allele	242 (54.0)	173 (45.1)		Reference
Del Allele	206 (46.0)	211 (54.9)	0.010	1.49 (1.09–1.88)
rs1805377 (A > G)				
AA	149 (66.5)	131 (68.2)		Reference
AG	65 (29.0)	55 (28.6)	0.966	0.99 (0.64–1.53)
GG	10 (4.5)	6 (3.1)	0.456	0.67 (0.23–1.90)
AG + GG	75 (33.5)	61 (31.7)	0.511	0.86 (0.57–1.32)
A Allele	363 (81.0)	317 (82.6)		Reference
G Allele	85 (19.0)	67 (17.4)	0.570	0.90 (0.63–1.28)

CI, confidence intervals; OR, odds ratio adjusted with age and smoking; PCa, prostate cancer.

Table 3. Haplotype analysis of *XRCC4* polymorphism and PCa risk.

Haplotype	Controls $n = 224$(%)	Cases $n = 192$ (%)	*p* Value	OR (95%CI)
T/Ins/Ins/A	233 (52.0)	93 (24.2)	–	Reference
T/Ins/Del/A	65 (14.5)	97 (25.3)	<0.001	3.73 (2.51–5.55)
G/Ins/Del/A	54 (12.1)	33 (8.6)	0.092	1.53 (0.93–2.51)

Table 4. Genotype frequency and OR of the *XRCC4* gene polymorphism in PCa patients with different Gleason grade.

	Gleason <7 (low grade)	Gleason 7 (intermediate grade)	Gleason >7 (high grade)	Between low and intermediate grades		Between low and high grades	
Genotype	*n* = 49(%)	*n* = 57(%)	*n* = 86(%)	Adjusted OR (95%CI)	*p* Value	Adjusted OR (95%CI)	*p* Value
XRCC4							
rs6869366 (G > T)							
TT	29 (63.0)	34 (60.7)	54 (60.0)	Reference		Reference	
TG	16 (34.8)	22 (39.3)	32 (35.6)	1.17 (0.52–2.64)	0.701	1.07 (0.50–2.27)	0.852
GG	1 (2.2)	0 (0)	4 (4.4)	0.00 (0.00)	1.000	2.14 (0.22–20.12)	0.503
TG+GG	17 (37.0)	22 (39.3)	36 (40.0)	1.90 (0.80–4.54)	0.144	2.91 (1.31–6.43)	0.008[a]
rs28360071 (Ins/Del)							
Ins/Ins	31 (67.4)	38 (67.9)	55 (61.1)	Reference		Reference	
Ins/Del	11 (23.9)	13 (23.3)	25 (27.8)	0.96 (0.37–2.45)	0.939	1.28 (0.55–2.95)	0.561
Del/Del	4 (8.7)	5 (8.9)	10 (11.1)	1.02 (0.25–4.12)	0.978	1.40 (0.40–4.87)	0.588
Ins/Del + Del/Del	15 (32.6)	18 (32.2)	35 (38.9)	1.63 (0.68–3.91)	0.271	2.32 (1.04–5.15)	0.038[a]
rs28360317 (Ins/Del)							
Ins/Ins	11 (23.9)	10 (18.0)	29 (32.2)	Reference		Reference	
Ins/Del	20 (43.5)	22 (39.2)	31 (34.4)	1.21 (0.42–3.45)	0.722	0.58 (0.24–1.43)	0.244
Del/Del	15 (32.6)	24 (42.8)	30 (33.4)	1.76 (0.60–5.14)	0.301	0.75 (0.29–1.92)	0.561
Ins/Del + Del/Del	35 (75.1)	46 (82.0)	61 (67.8)	0.83 (0.35–1.95)	0.672	1.47 (0.65–3.33)	0.354
rs1805377 (A > G)							
AA	33 (71.7)	36 (64.3)	62 (68.9)	Reference		Reference	
AG	10 (21.7)	18 (32.1)	27 (30.0)	1.65 (0.66–4.08)	0.279	1.43 (0.62–3.32)	0.397
GG	3 (6.5)	2 (3.6)	1 (1.1)	0.61 (0.09–3.88)	0.602	0.17 (0.01–1.77)	0.141
AG+GG	13 (28.2)	20 (35.7)	28 (31.1)	1.50 (0.58–3.84)	0.394	2.87 (1.23–6.65)	0.014[a]

CI, confidence interval; OR, odd ratio adjusted with age and smoking; PCa, prostate cancer.
[a]Bonferroni corrected *p* value. *Pc* = 0.016, *Pc* = 0.076, *Pc* = 0.028.

Table 5. The genotype frequency and OR of the *XRCC4* gene polymorphism in PCa patients with positive metastasis and negative metastasis status.

Genotype	Metastasis (–) ve*n* = 91(%)	Metastasis (+) ve*n* = 80(%)	Adjusted OR (95%CI)	*p* Value
XRCC4				
rs6869366 (G > T)				
TT	54 (59.3)	46 (57.5)	Reference	
TG	34 (37.4)	32 (40.0)	1.10 (0.59–2.05)	0.754
GG	3 (3.3)	2 (2.5)	0.78 (0.12–4.88)	0.793
TG+GG	37 (40.7)	34 (42.5)	0.79 (0.42–1.51)	0.490
rs28360071(Ins/Del)				
Ins/Ins	54 (59.3)	55 (68.8)	Reference	
Ins/Del	27 (29.7)	16 (20.0)	0.58 (0.28–1.19)	0.142
Del/Del	10 (11.0)	9 (11.3)	0.88 (0.33–2.34)	0.804
Ins/Del + Del/Del	37 (40.7)	25 (31.3)	0.78 (0.40–1.49)	0.456
rs28360317 (Ins/Del)				
Ins/Ins	20 (22.0)	19 (23.8)	Reference	
Ins/Del	39 (42.9)	28 (35.0)	0.75 (0.34–1.67)	0.489
Del/Del	32 (35.2)	33 (41.3)	1.08 (0.49–2.40)	0.840
Ins/Del + Del/Del	71 (78.1)	61 (76.3)	0.62 (0.32–1.22)	0.169
rs1805377 (A > G)				
AA	63 (69.2)	49 (61.3)	Reference	
AG	25 (27.5)	28 (35.0)	1.44 (0.74–2.77)	0.276
GG	3 (3.3)	3 (3.8)	1.28 (0.24–6.65)	0.764
AG + GG	28 (30.8)	31 (38.8)	0.81 (0.41–1.56)	0.534

CI, confidence interval; OR, odds ratio adjusted with age and smoking; PCa, prostate cancer.

with these four polymorphisms, we did not find any significant risk (Table 5).

Interaction of *XRCC4* gene polymorphism with smoking habit in PCa

We evaluated the gene-smoking interaction to study the modulation of PCa risk with respect to *XRCC4* gene polymorphisms. We grouped the PCa patients into two groups, one nonsmoker (never smoked) and the other as smokers (smoking more than 5 years). On analyzing the genotype frequency between these two groups for susceptibility to PCa, none of these polymorphisms demonstrated association (data not shown).

Discussion

Disruption of genomic integrity contributes to malignant transformation and subsequent cancer development. The repair of DNA damage plays a key role in maintaining genomic integrity. 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. The present study has investigated the role of *XRCC4* gene polymorphisms in PCa susceptibility.

The *XRCC4* gene is necessary for DNA ligation in NHEJ pathway, which is responsible for repairing most DSBs (Riha et al., 2006; Yurchenko et al., 2006). SNP G > T (rs6869366) found in promoter region of *XRCC4* may have functional regulatory significance and showed an association with reduced PCa risk. Our result is discordant with previous report, suggesting heterozygous genotype GT to be associated with PCa and lung cancer in Taiwan (Chang et al., 2008; Hsu et al., 2009). However, findings need to be replicated in additional study populations, particularly since homozygote variants were low in our control populations (6.3%), whereas Chang et al. (2008) and Hsu et al. (2009) reported Zero in Taiwan population. Interestingly, we found that the variant genotype Del/Del of *XRCC4* (rs28360071) had a strong significant association with PCa risk. Del allele carrier also showed increased risk of PCa. This is in accordance with a recent study by Chiu et al. (2008) that reported significant risk with oral cancer. Conversely, Chang et al. (2008) did not observe altered risk with PCa.

The second association of interest was with (rs28360317) SNP. We found significant association between the *XRCC4* gene polymorphism and susceptibility to PCa, and Del/Del genotype contributed to a twofold increased risk of PCa. The possible mechanism is that people with the risky genotypes, such as variant, may have lower capacity in their NHEJ for the repair of DNA damage caused by carcinogens. This perhaps suggested the contribution of this polymorphism in the etiology of PCa. On the other hand, Chiu et al. (2008) did not show association with oral cancer and gastric cancer in Taiwan population. It is speculated that these conflicting results may have arisen due to different patient subgroups and/or ethnic groups studied. The *XRCC4* (rs1805377) A > G polymorphism involves a substitution of G > A in the intron 7/exon 8 junction region of *XRCC4*. This SNP potentially abolishes an acceptor splice site at exon 8. Although little is known regarding this SNP in terms of the potential impact on repair capacity of *XRCC4* and the consequent risk of carcinogenesis, significant association was not revealed in our study, which was compatible to the observations reported by Wu et al. (2006) and Chiu et al. (2008) in bladder and oral cancer. The distribution of haplotype was significantly different between the cases and controls, showing increased risk of PCa. This finding demonstrated that combined genotype was associated with PCa.

Moreover, we also analyzed the association of these polymorphisms with clinical characteristics such as tumor grade and bone metastasis. A significant increased risk was observed for variant allele carrier of rs28360071, rs28360317, rs1805377 with high Gleason grade. These findings suggest that these SNPs are involved in progression of PCa. Therefore, our findings might throw some light on understanding the development of the PCa. However none of the polymorphisms were associated with bone metastasis.

The precise mechanism of cancer susceptibility in the elderly is not well understood, but immune function and DNA repair efficiency have been shown to decrease with age, which reduces protection against environmental carcinogens (Perera et al., 2002). Thus, there is emerging belief that the intrinsic fidelity of DNA repair mechanisms, such as DSB, may influence age-associated cancer. Our study has both strengths as well as limitations. High participation rate of eligible cases (98%) and a sample from a homogeneous ethnic background (100% of study participants were from North India only) which reduces the bias due to population stratification. In addition, our study population (both cases and controls) were from all over the state of North India minimizing potential selection bias. Limitations of this study are the small numbers of patients used due to low incident rate of PCa in our country. We also did not consider the analysis of gene–gene interactions. It is possible that the risks observed are the result of interactions but we have not attempted to assess such effects since the estimate of an interaction effect will be unreliable because of small numbers. For this type of analysis, a very large sample size is essential. We also did not adjust for possible differences in lifestyle factors nor account for multiple testing risk due to small number of patients.

In conclusion, these findings suggest that *XRCC4* gene could serve as a biomarker of PCa screening and should be a target of cancer prevention. Though our study achieved sufficient (>80%) power due to low incidence rate of PCa in India, nevertheless, a higher

number of sample size can warrant more modest results. Additional studies of functional DNA repair gene polymorphisms in a large cohort of different ethnicities are required to augment the etiology in the pathogenesis of PCa.

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Declaration of interest

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