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Common Variants at 8q24 Confer Susceptibility to Urothelial Bladder Cancer in the Pakistani Population

Syeda Hafiza Benish Ali,[1] Muhammad Younis,[2,3] Kashif Sardar Bangash,[4] Abdur Rauf,[5] Khursheed Anwar,[4] Raja Khurram,[1] Muhammad Athar Khawaja,[6] Abid Ali Qureshi,[7] Saeed Akhter,[6] Maleeha Azam,[1] Lambertus A. Kiemeney[8] and Raheel Qamar[1,9]

[1]*Department of Biosciences, Faculty of Science, COMSATS Institute of Information Technology, Islamabad-45600, Pakistan;*
[2]*King Edward Medical University, Lahore-54000, Pakistan*
[3]*Department of Urology, Mayo Hospital, Lahore-54000, Pakistan*
[4]*Pakistan Atomic Energy Commission General Hospital, Islamabad-44000, Pakistan*
[5]*Nishtar Medical College & Hospital, Multan-60000, Pakistan*
[6]*Department of Urology, Shifa International Hospital, Islamabad-44000, Pakistan*
[7]*Department of Radiology, The Children's Hospital & The Institute of Child Health, Lahore-54000*
[8]*Department for Health Evidence and Department of Urology, Radboud University Medical Centre, PO Box 9101, 6500 HB, Nijmegen, the Netherlands*
[9]*Al-Nafees Medical College and Hospital, Isra University, Islamabad, Pakistan*

Abstract.- 8q24 has recently received much attention after genome-wide studies identified the association of common sequence variants in the 8q24.21 gene desert as well as a 5′UTR polymorphism of the *PSCA* gene with individual susceptibility to urinary bladder cancer (UBC). The objective of the present study was to determine the association of selected single nucleotide polymorphisms (SNPs) at the 8q24 locus (rs9642880 and rs6983267 at 8q24.21 and rs2294008 near the prostate stem cell antigen [*PSCA]*) with urothelial bladder cancer (UBC) among the Pakistani population. For this purpose, genotyping of selected SNPs was performed by simple allele-discriminating polymerase chain reaction (PCR) in 200 UBC cases and 200 healthy controls. All three variants were found to be associated with a significantly increased UBC risk ($p<0.05$) after adjusting for age, gender and smoking. rs9642880 was found to be associated slightly stronger with low grade as compared to high grade UBC, while rs2294008 was associated with increased risk of high grade and invasive stages of the disease, and rs6983267 exhibited a strong positive association with UBC risk irrespective of tumor stage and grade. In conclusion, the current study shows a significant involvement of the 8q24 locus in UBC etiology in Pakistani patients.

Keywords: Urothelial bladder cancer, 8q24 locus, genotyping, single nucleotide polymorphism

INTRODUCTION

Genome-wide association studies (GWAS) have identified the 8q24.21 locus to be a significant modulator of bladder cancer risk (Kiemeney *et al*., 2008). This chromosomal region is a gene desert with the *MYC* proto-oncogene as the only well-annotated gene residing closest to the cancer-associated linkage disequilibrium (LD) blocks of this region (Jia *et al*., 2009). Moreover, this region and *MYC* have been found to be amplified in bladder and other cancers (Sauter *et al*., 1995; van Duin *et al*., 2005; Ribeiro *et al*., 2006; Kang *et al*., 2007) highlighting their putative carcinogenic roles.

Functional attributes of the common cancer risk-modifying variants in this region are not yet clear. It has been postulated on the basis of *in vitro* studies that SNPs in this region interact with enhancer elements of *MYC* and thus epigenetically control the expression of this proto-oncogene (Jia *et al*., 2009; Wasserman *et al*., 2010). Two of the single nucleotide polymorphisms (SNPs) of this region associated with carcinogenesis *i.e.*, rs9642880 and rs6983267 were genotyped in the present study. Previous investigations by different researchers have shown an association of rs9642880 with bladder cancer (Kiemeney *et al*., 2008; Wang *et al*., 2009; Cortessis *et al*., 2010), while a non-

* Corresponding author: raheelqamar@hotmail.com
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association of rs6983267 (Kiemeney *et al.*, 2008; Cortessis *et al.*, 2010). However, rs6983267 was found to be significantly associated with reduced risk of UBC in ever-smokers (Park *et al.*, 2008).

Prostate stem cell antigen (PSCA) is a member of Thy-1/Ly-6 family of glycosylphosphatidylinositol-anchored cell surface antigens (Wu *et al.*, 2009; Lochhead *et al.*, 2011) identified in a prostate cancer study (Reiter *et al.*, 1998). Despite its name, PSCA is neither expressed exclusively in prostate nor is it a stem cell antigen; it is also expressed in other tissues including bladder urothelium (Lochhead *et al.*, 2011; Bahrenberg *et al.*, 2000; Sakamoto *et al.*, 2008). PSCA monoclonal antibodies impart negative effects on tumor growth and its metastatic potential in animal models (Saffran *et al.*, 2001) suggests a possible involvement of this antigen in cell proliferation and migration (Wu *et al.*, 2009). In addition, the levels of PSCA are altered in solid tumors including an up-regulation in bladder cancer (Elsamman *et al.*, 2006) and a down-regulation in gastric and oesophageal cancers (Bahrenberg *et al.*, 2000; Sakamoto *et al.*, 2008). rs2294008 (C>T) is a missense change in the 5′ untranslated region (UTR) (Fu *et al.*, 2012) of *PSCA* gene, whose variant allele (T) was identified to be associated with UBC risk by Wu *et al.* (2009).

As there was a distinct possibility that in the Pakistani population the etiology of UBC as well as the haplotype structure of the locus could be different from those of the previous studies mainly performed among Caucasians, the present study was conducted to find out the role of these polymorphisms in UBC among Pakistani patients. To the best of our knowledge, this is the first report of a genetic association study of UBC in the Pakistani population.

MATERIALS AND METHODS

This was a case-control genetic association study in which cases suffering from UBC were recruited from different hospitals of the Punjab province, while controls were ethnicity-matched, malignancy-free, unrelated individuals collected at random from the general population. Patients went through standard diagnostic protocols including cystoscopy and trans-urethral resection of bladder tumor. Tumor specimen collected during either of these protocols were analyzed by histopathologists for identification of tumor grade and stage. Tumors were classified as low grade or high grade, in addition on the basis of muscle-invasiveness, tumors were either categorized as non-muscle-invasive bladder cancer (NMIBC) or muscle-invasive bladder cancer (MIBC). Demographic information of participants was recorded through a questionnaire-based interview. The study conformed to the tenets of the Helsinki declaration and was approved by the Ethics Committee of the COMSATS Institute of Information Technology, Islamabad. Genomic DNA was extracted by a standard phenol/chloroform method from peripheral leucocytes collected by venipuncture, after obtaining informed written consent from the participants.

Genotyping was performed by simple allele-discriminating polymerase chain reaction (PCR) using the following primers:

rs9642880

Internal control forward (ICF):
5′-ACT CCA ggT TAC CCA AAg TAC TgC-3′;

G-allele forward
5′-Agg CTg gAg TTA ggA gAA CCT g-3′;

T-allele forward
5′-Agg CTg gAg TTA ggA gAA CCT T-3′;

Common reverse (CR)
5′-TgA ATT CTT gAC CAA ACA gTg C-3′);

rs6983267

ICF:
5′-CAA ATg ATT ACA AgC TTC TTT TCC Tg-3′

T-allele forward:
5′-CCT TTg AgC TCA gCA gAT gAA AAT-3′;

G-allele forward:
5′-CCT TTg AgC TCA gCA gAT gAA AAg-3′;
CR:
5′-TTg gCT ggC ACT gTC TgT ATA CA-3′) and

rs2294008

ICF:
5′-ATA ATC Tgg gTC TTg Agg ACg TTT C-3′

C-allele forward
5′-CAC AgC CCA CCA gTg ACC TC-3′;

T-allele forward
5′-CAC AgC CCA CCA gTg ACC TT-3′;
CR:
5′-Tgg TCg TgC CAA gAg CTT C-3′).

Two independent PCR reactions were performed for every sample, each containing an ICF primer, one of the allele-specific primers and the common reverse (CR) primer. The internal control and allele-specific reaction products were electrophoretically separated on 2% agarose gel stained with ethidium bromide. Presence or absence of the allele-specific PCR product was used to classify the genotype of the samples (Fig.1). 10% of the samples were randomly selected for sequencing, results of which were found to be in agreement with the initial genotyping results. Chi-square tests of independence and multivariate logistic regression analyses were performed using StatCalc and the online statistical analysis tool SNPStats (Sole *et al*., 2006), respectively.

RESULTS

Of 200 UBC cases, 157 (78.5%) were men and 43 (21.5%) were women, while 155 (77.5%) of the total 200 controls were men and 45 (22.5%) were women. The average age of UBC cases (mean±SD = 55.5±13.24years) was statistically not different (t = 1.03, p = 0.3) from that of the controls (mean±SD = 54.3±9.9years). There were 92 (46%) smokers in the UBC group and 72 (36%) in the controls. Among UBC cases, 117 (58.5%) were low grade and 83 (41.5%) were high grade tumors, while 124 (62%) were non-muscle-invasive bladder cancer (NMIBC) and 76 (38%) were muscle-invasive bladder cancer (MIBC).

Overall analysis

Logistic regression analysis was used to find out association of studied polymorphisms with UBC susceptibility under co-dominant model for genotypes and log-additive model (LAM) for alleleotypes. rs9642880 (Odds Ratio $[OR]_{TT}$ = 1.9, 95% Confidence Interval [CI] = 1.1-3.4; OR_{LAM} = 1.4, 95%CI = 1.1-1.8), rs6983267 (OR_{TG} = 2.8,

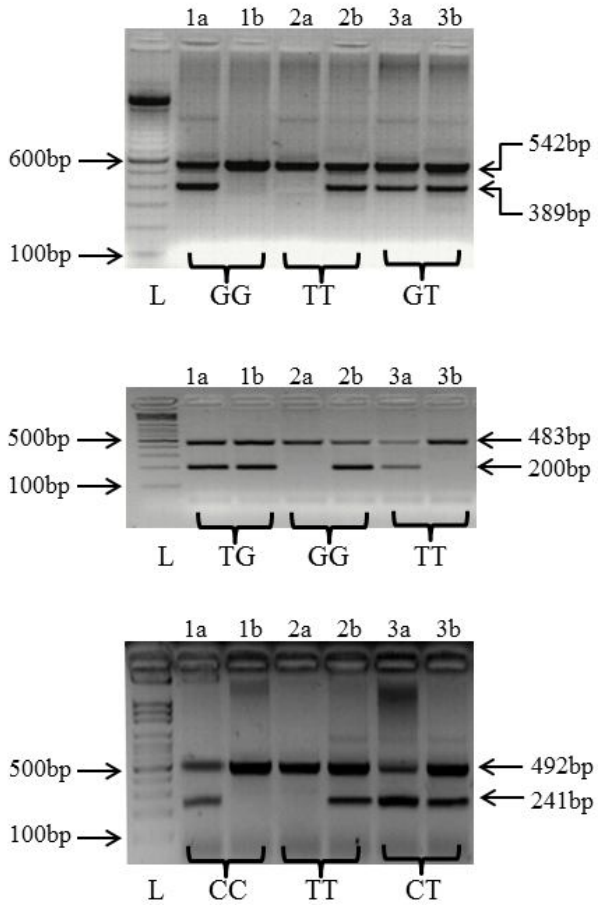

Fig. 1. Results of PCRs for rs9642880, rs6983267 and rs2294008 SNPs. All samples were run on 2% agarose gel, Lane L contains the 100bp ladder. For each sample, two PCRs were performed each with a common reverse primer, an internal control (IC) primer and one of the allele-specific primers in alternate reactions labeled as a and b. Top figure shows PCR results of rs9642880; IC fragment: 542bp; allele-specific fragment (ASF): 389bp; GG genotype: ASF visible only in G allele-specific reaction; TT genotype: ASF visible only in T allele-specific reaction; GT genotype: ASF visible in both reactions. Middle figure shows PCR results of rs6983267; IC fragment: 483bp; ASF: 200bp; TG genotype: ASF visible in both reactions; GG genotype: ASF visible only in G allele-specific reaction; TT genotype: ASF visible only in T allele-specific reaction. Bottom figure shows PCR results of rs2294008; IC fragment: 492bp; ASF: 241bp; CC genotype: ASF visible only in C allele-specific reaction; TT genotype: ASF visible only in T allele-containing reaction; CT genotype: ASF visible in both reactions.

95%CI = 1.3-6.2; OR_{GG} = 6.9, 95%CI = 2.7-17.5; OR_{LAM} = 2.6, 95%CI = 1.7-4.03) and *PSCA* rs2294008 (OR_{CT} = 1.7, 95%CI = 1.08-2.6; OR_{TT} = 5.03, 95%CI = 1.3-20; OR_{LAM} = 1.8, 95%CI = 1.2-2.7) were found to be associated with significantly enhanced risk of UBC (Table I).

Gene-smoking interaction

For the analysis of gene-smoking interaction, smoker UBC cases were compared with smoker controls and non-smoker cases with non-smoker controls. rs9642880 enhanced the risk for UBC development only among the smokers (OR_{TT} = 2.5, 95%CI = 1.1-5.8; OR_{LAM} = 1.6, 95%CI = 1.1-2.5), rs6983267 was found to increase UBC risk in smokers (OR_{GG} = 12.9, 95%CI = 2.2-77; OR_{LAM} = 3.89, 95%CI = 1.8-8.7) as well as non-smokers (OR_{TG} = 2.6, 95%CI = 1.03-6.3; OR_{GG} = 4.96, 95%CI = 1.6-15; OR_{LAM} = 2.2, 95%CI = 1.3-3.8) while *PSCA* rs2294008 exhibited a positive association with UBC risk in non-smokers only (OR_{CT} = 1.9, 95%CI = 1.06-3.5; OR_{LAM} = 1.94, 95%CI = 1.2-3.3). The gene-smoking interaction effect was non-significant for all of the three polymorphisms (Table I).

Association with tumor characteristics

Tumors were classified either as low grade or high grade and NMIBC (non-invasive) or MIBC (invasive) and each one of these categories were independently analyzed against the control group (Table II). rs9642880 was shown to be positively related to low grade (OR_{TT} = 2.3, 95%CI = 1.2-4.4; OR_{LAM} = 1.5, 95%CI = 1.1-2.1), non-invasive (OR_{LAM} = 1.4, 95%CI = 1.02-1.9) and invasive (OR_{TT} = 2.2, 95%CI = 1.01-4.9) tumor. rs6983267 correlated significantly with an elevated risk of low grade (OR_{TG} = 2.9, 95%CI = 1.1-7.7; OR_{GG} = 8.1, 95%CI = 2.6-24.8; OR_{LAM} = 2.8, 95%CI = 1.7-4.7), high grade (OR_{GG} = 5.7, 95%CI = 1.6-20; OR_{LAM} = 2.3, 95%CI = 1.3-4), non-invasive (OR_{TG} = 2.9, 95%CI = 1.1-7.9; OR_{GG} = 7.9, 95%CI = 2.6-24.5; OR_{LAM} = 2.8, 95%CI = 1.7-4.7) and invasive (OR_{GG} = 6.4, 95%CI = 1.8-22.8; OR_{LAM} = 2.5, 95%CI = 1.4-4.4) tumor growths. rs2294008 SNP of *PSCA* was found to confer an enhanced risk of high grade (OR_{CT} = 2.3, 95%CI = 1.2-4.2; OR_{TT} = 6.9, 95%CI = 1.4-34.6; OR_{LAM} = 2.37, 95%CI = 1.4-4.2) and invasive disease (OR_{CT} = 2.9, 95%CI = 1.4-5.7; OR_{TT} = 6.6, 95%CI = 1.2-38.4; OR_{LAM} = 2.8, 95%CI = 1.5-5.1).

DISCUSSION

In the present study, both the studied SNPs from the 8q24.21 region, *i.e.* rs9642880 and rs6983267, were found to be associated with increased risk of UBC after adjusting for age, gender and smoking. Results of rs9642880 are consistent with previous findings (Kiemeney *et al*., 2008; Wang *et al*., 2009; Cortessis *et al*., 2010) while the results of rs6983267 are in contradiction with other studies where it was not found to be associated with UBC (Kiemeney *et al*., 2008; Cortessis *et al*., 2010). Moreover, we found the genotype-smoking interaction of rs9642880 to be non-significant in our study, which is more or less contradictory to a report from non-Hispanic White population of Los Angeles, where the risk conferred by the T allele was confined to non-smokers and former smokers (Cortessis *et al*., 2010). In the present study, the genotype-smoking interaction was non-significant for rs6983267 as well. However, Park *et al*. (2008) have reported an inverse relation between this SNP and smoking, since in their study it was shown to be associated with a low risk of bladder cancer among ever-smokers. Therefore, the role of these variants with respect to smoking needs further revision.

The 8q24 chromosome is a highly interesting cancer-implicated locus, amplifications within this gene desert have been associated with several cancer types, *e.g.*, prostate cancer (van Duin *et al*., 2005; Ribeiro *et al*., 2006) and lung cancer (Kang *et al*., 2007). Absence of well-characterized genes in the region raises the possibility of some epigenetic phenomenon. This locus, containing a number of risk variants is 500kb long and the closest annotated gene to this region is *MYC*, residing ~200kb telomeric from the nearest LD block of this locus (Jia *et al*., 2009). *MYC* is a proto-oncogene, found to be amplified in different carcinomas (Sauter *et al*., 1995; van Duin *et al*., 2005; Ribeiro *et al*., 2006). It is also involved in regulating cell proliferation, differentiation and apoptosis in mammalian cells (DePinho *et al*., 1991; Adhikary *et al*., 2005). In

Table I.- **Overall and smoking-based comparison of the genotype and allele frequencies among the cases of urothelial bladder carcinoma and healthy controls. Logistic regression analysis has been performed under co-dominant model for genotypes and log-additive model for allelotypes adjusting by smoking, age and gender in overall comparison and by age and gender in smoking-based analysis. Statistically significant values (p≤0.05) are presented in bold format.**

Variation	Geno-type	Overall				Smokers				Non-smokers			
		Cases n(%)	Controls n(%)	χ^2 (p)	OR (95%CI)	Cases n(%)	Controls n(%)	χ^2 (p)	OR (95%CI)	Cases n(%)	Controls n(%)	χ^2 (p)	OR (95%CI)
8q24	GG	33(16.5)	48(24)	**6.03 (0.05)**	Ref.	16(17.4)	20(27.8)	5.7	Ref.	17(15.7)	28(21.9)	1.6	Ref.
rs9642880	GT	84(42)	90(45)		1.4 (0.8-2.4)	33(35.9)	31(43)	(0.06)	1.3(0.6-3)	51(47.2)	59(46.1)	(0.4)	1.4 (0.7-2.9)
	TT	83(41.5)	62(31)		**1.9(1.1-3.4)**	43(46.7)	21(29.2)		**2.5(1.1-5.8)**	40(37)	41(32)		1.6 (0.8-3.3)
	G	150(37.5)	186(46.5)	**6.7 (0.01)**	**1.4(1.1-1.8)**	65(35.3)	71(49.3)	**6.5 (0.01)**	**1.6(1.1-2.5)**	85(39.4)	115(45)	1.5	1.2 (0.9-1.8)
	T	250(62.5)	214(53.5)			119(64.7)	73(50.7)			131(60.6)	141(55)	(0.2)	
					Interaction p-value	0.49							
rs6983267	TT	9(4.5)	28(14)	**20.5 (0.0)**	Ref.	2(2.2)	7(9.7)	**11 (0.0)**	Ref.	7(6.5)	21(16.4)	**8.5 (0.01)**	Ref.
	TG	146(73)	153(76.5)		**2.8(1.3-6.2)**	65(70.7)	58(80.6)		3.9(0.8-19.7)	81(75)	95(74.2)		**2.6 (1.03-6.3)**
	GG	45(22.5)	19(9.5)		**6.9(2.7-17.5)**	25(27.2)	7(9.7)		**12.9(2.2-77)**	20(18.5)	12(9.4)		**4.96 (1.6-15)**
	T	164(41)	209(52)	**10.2 (0.0)**	**2.6(1.7-4.03)**	69(37.5)	72(50)	**5.2 (0.02)**	**3.89(1.8-8.7)**	95(44)	137(53.5)	**4.3 (0.03)**	**2.2 (1.3-3.8)**
	G	236(59)	191(48)			115(62.5)	72(50)			121(56)	119(46.5)		
					Interaction p-value	0.59							
PSCA	CC	49(24.5)	71(35.5)	**8.0 (0.02)**	Ref.	26(28.3)	26(36)	3.3	Ref.	23(21.3)	45(35.2)	**6.5 (0.04)**	Ref.
rs2294008	CT	142(71)	126(63)		**1.7(1.08-2.6)**	63(68.5)	46(64)	(0.2)	1.4(0.7-2.7)	79(73.1)	80(62.5)		**1.9 (1.06-3.5)**
	TT	9(4.5)	3(1.5)		**5.03(1.3-20)**	3(3.3)	0(0)		NA	6(5.6)	3(2.3)		3.9 (0.9-17.3)
	C	240(60)	268(67)	**4.2 (0.04)**	**1.8(1.2-2.7)**	115(62.5)	98(68)	1.1	1.6(0.8-3.01)	125(58)	170(66.4)	3.6	**1.94 (1.2-3.3)**
	T	160(40)	132(33)			69(37.5)	46(32)	(0.3)		91(42)	86(33.6)	(0.06)	
					Interaction p-value	0.36							

OR (95%CI): Odds ratio (95% Confidence Interval)
Ref.: Referent genotype
Interaction p-value for genotype-smoking interaction

Table II.- **Comparison of the genotype and allele frequencies among the cases of urothelial bladder carcinoma and healthy controls with respect to tumor characteristics. Logistic regression analysis has been performed under co-dominant model for genotypes and log-additive model for allelotypes adjusting by smoking, age and gender. Statistically significant values (p≤0.05) are presented in bold format.**

Variation	Geno-type	Controls n(%)	Low grade			High grade			NMIBC			MIBC		
			Cases n(%)	χ^2 (p)	OR (95%CI)	Cases n(%)	χ^2 (p)	OR (95%CI)	Cases n(%)	χ^2 (p)	OR (95%CI)	Cases n(%)	χ^2 (p)	OR (95%CI)
8q24	GG	48(24)	18(15.4)	5.7	Ref.	15(18.1)	2.4	Ref.	22(17.7)	4.4	Ref.	11 (14.5)	3.9	Ref.
rs9642880	GT	90(45)	49(41.9)	(0.06)	1.5 (0.8-2.9)	35(42.2)	(0.3)	1.3(0.6-2.6)	50(40.3)	(0.1)	1.3(0.7-2.4)	34 (44.7)	(0.14)	1.8 (0.8-3.8)
	TT	62(31)	50(42.7)		**2.3 (1.2-4.4)**	33(39.8)		1.7(0.8-3.4)	52(41.9)		1.9(1-3.6)	31 (40.8)		**2.2 (1.01-4.9)**
	G	186(46.5)	85(36)	**6.3 (0.01)**	**1.5 (1.1-2.1)**	65(39)	2.6	1.3(0.9-1.8)	94(38)	**4.6 (0.03)**	**1.4(1.02-1.9)**	56 (37)	**4.2 (0.04)**	1.4 (0.99-2.1)
	T	214(53.5)	149(64)			101(61)	(0.1)		154(62)			96 (63)		
rs6983267	TT	28(14)	5(4.3)	**18.4 (0.0)**	Ref.	4(4.8)	**8.9 (0.01)**	Ref.	5(4)	**17.6 (0.0)**	Ref.	4 (5.3)	**9.5 (0.0)**	Ref.
	TG	153(76.5)	83(70.9)		**2.9 (1.1-7.7)**	63(75.9)		2.7(0.9-8.2)	90(72.6)		**2.9(1.1-7.9)**	56 (73.7)		2.8 (0.9-8.4)
	GG	19(9.5)	29(24.8)		**8.1 (2.6-24.8)**	16(19.3)		**5.7(1.6-20)**	29(23.4)		**7.9(2.6-24.5)**	16 (21.1)		**6.4 (1.8-22.8)**
	T	209(52)	93(40)	**9.3 (0.0)**	**2.8 (1.7-4.7)**	71(43)	**4.2 (0.04)**	**2.3(1.3-4)**	100(40)	**8.7 (0.0)**	**2.8(1.7-4.7)**	64 (42)	**4.5 (0.03)**	**2.5 (1.4-4.4)**
	G	191(48)	141(60)			95(57)			148(60)			88 (58)		
PSCA	CC	71(35.5)	33(28.2)	3.7	Ref.	16(19.3)	**9.1 (0.0)**	Ref.	37(29.8)	3.9	Ref.	12 (15.8)	**11.1 (0.01)**	Ref.
rs2294008	CT	126(63)	79(67.5)	(0.2)	1.4 (0.8-2.3)	63(75.9)		**2.3(1.2-4.2)**	81(65.3)	(0.14)	1.3(0.8-2.1)	61 (80.2)		**2.9 (1.4-5.7)**
	TT	3(1.5)	5(4.3)		4.3 (0.9-19)	4(4.8)		**6.9(1.4-34.6)**	6(4.8)		4.2(0.98-18)	3 (3.9)		**6.6 (1.2-38.4)**
	C	268(67)	145(62)	1.7	1.5 (0.9-2.4)	95(57)	**4.9 (0.03)**	**2.37(1.4-4.2)**	155(62.5)	1.4	1.4(0.9-2.3)	85 (56)	**5.9 (0.02)**	**2.8 (1.5-5.1)**
	T	132(33)	89(38)	(0.2)		71(43)			93(37.5)	(0.2)		67 (44)		

OR (95%CI): Odds ratio (95% Confidence Interval)
Ref.: Referent genotype

addition, Sauter *et al*. (1995) have shown the over-expression of c-MYC to be associated with low grade and early-stage bladder cancer, while a low level copy number gain to be more frequent in advanced stage tumors. In the present study, the rs9642880 variant was found to be significantly associated with low grade UBC only and associated at the same level of significance with non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). On the other hand, rs6983267 was associated with elevated risk of UBC irrespective of the grade or stage of the tumor. In contrast, Wang *et al*. (2009) have reported a positive association of rs9642880 with low-risk UBC. Therefore, the role of these variants in disease prognosis should be considered with caution, particularly in the Pakistani population.

A number of studies have evaluated an association between rs9642880 and *MYC* expression: Kiemeney *et al*. (2008) did not find an association between the T allele of rs9642880 and *MYC* expression in whole blood and adipose tissue; Wang *et al*. (2009) found a significant difference in *MYC* expression levels in bladder tissues carrying the T allele versus those that did not. These contradictory results suggest the possibility of an organ-specific mechanism of rs9642880 in *MYC* expression. While a definite conclusion about the role of 8q24 in carcinogenesis is lacking, current evidence of Jia *et al.* (2009) and Wasserman *et al.* (2010) suggests the presence of cis-regulatory elements, which interact with enhancers of the *MYC* proto-oncogene (Jia *et al*., 2009; Wasserman *et al*., 2010).

In the analysis of the *PSCA* polymorphism (rs2294008), the TT and CT genotype individuals were found to be at a much greater risk of UBC than those carrying the CC genotype, which is comparable to previous reports in different populations (Wu *et al*., 2009 ; Wang *et al*., 2010; Fu *et al*., 2012). The gene-smoking interaction was found to be non-significant in present study which is consistent with the observations of Wang *et al*. (2010), as well as with Wu *et al*. (2009) who have reported a significant association of this SNP with UBC risk irrespective of smoking behavior. In addition, the CT and TT genotypes exerted a strong risk of high grade and invasive stages in agreement with Wang *et al*. (2010). On the basis of this association, PSCA may be evaluated in future studies as a new candidate for bladder cancer prognosis since the variant form of this antigen may possibly lead to aggressive forms of bladder cancer.

The replacement of T by the C allele at the rs2294008 locus, shifts the translation initiation site up into the 5′UTR resulting in a longer form of the protein, which is subsequently cleaved during post-translational processing, resulting in the same-sized product for both the alleles (Fu *et al*., 2012). However, Wu *et al*. (2009) postulated that this change in the length and sequence of the N-terminus may affect protein folding, intracellular processing and/or intracellular transport. Fu *et al.* (2012) have observed a higher PSCA mRNA expression in bladder neoplastic tissue as compared to neighboring normal cells. Moreover, they observed that the T allele carrying individuals had a higher expression in normal as well as cancerous bladder tissue. This observation is in contrast to that of Wang *et al*. (2010) who found a reduced mRNA expression in normal tissues adjacent to tumor cells in the presence of CT/TT genotypes as compared to CC genotypes. Wu *et al*. (2009) evaluated mRNA expression in UBC cell lines and found the highest expression in a TT genotype cell line followed by intermediate expression by the CT genotype in three cell lines and the lowest in three cell lines of CC genotype as well as two of the TT cell lines. Results of these studies are contradictory to each other as well as to the finding that the substitution of C by T greatly reduces upstream transcriptional *PSCA* promoter activity as suggested by an *in vitro* reporter assay by Wu *et al*. (2009) on bladder cells and in a gastric cancer study by Sakamoto *et al*. (2008). Thus, although, the T allele is considered to be a risk factor for UBC onset, the functional relevance of this association remains elusive.

We observed the genotype distribution of rs6983267 and the *PSCA* polymorphism to deviate from Hardy Weinberg Equilibrium (HWE) among the control group. Our quality control criteria, including sequencing of 10% of the genotypes and 10% random re-typing, excluded the foremost possibility of a genotyping error. Further, the log-additive model, which does not assume the genotype frequencies to be in HWE (Sasieni, 1997), also

supported the association and non-association results, during the present analysis. However, it must be pointed out that the disequilibrium may arise because of population heterogeneity, consanguinity and non-random mating (Riaz and Iqbal, 2012), which may have resulted in biased results.

In conclusion, we report that the selected variants from 8q24 are associated with an elevated risk of urothelial carcinoma of the bladder in the Pakistani population. Despite limited power due to small sample size in the sub-group analyses, there is an indication of a somewhat stronger association of rs9642880 with low grade tumor and rs2294008 with high grade and invasive tumor. This *PSCA* SNP (rs2294008) may therefore be a new prognostic marker in UBC progression, but further studies are required to validate this finding.

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Conflict of interest statement

There is no conflict of interest of any of the authors of this manuscript, and there was no financial relationship of any author with the grant funding agencies. In addition we had full control of the primary data and if required these can be made available to the journal for review purposes.

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