RESEARCH ARTICLE

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Association of rs2294008 (*PSCA*) Gene polymorphism in Urothelial Bladder Cancer of South Indian Population

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Abstract

Background: Urothelial Bladder cancer (UBC) is a significant health problem worldwide. Prostate stem cell antigen (PSCA) gene has been reported earlier in Genome Wide Association Study (GWAS) for the risk of UBC. It is highly expressed in urothelial bladder tumours and considered to be involved in the cell proliferation inhibition and/or cell-death induction activity. The study aims to investigate *PSCA* gene as a possible marker for the diagnosis and prognosis of urothelial bladder cancer. **Patients and Methods:** We genotyped *PSCA* rs2294008C/T gene by Real Time Taqman® probes to evaluate the risk of UBC in histologically confirmed bladder tumour patients 107 and healthy controls 105 (age and gender matched) in a hospital-based setting from South Indian population. Statistical analysis was carried out using (SPSS ver 22.0 Armonk NY, USA). **Results:** The heterozygous CT genotype showed increased significance risk to UBC with *PSCA* rs2294008 C/T having 2.77 folds risk ($p<0.0001$). The variant allele T was also significantly associated with UBC risk ($p<0.0001$; OR=3.349) for *PSCA* rs2294008C/T. A significant UBC risk was noted when risk was evaluated with tumor-grade-stage level for *PSCA* rs2294008C/T with heterozygous CT genotype for high grade tumours ($p<0.001$; OR=1.984). The smoking factor was significantly modulated with the risk of UBC in patients with heterozygous CT genotype ($p<0.0001$) for *PSCA* rs2294008C/T gene polymorphism. Urothelial Bladder cancer patients receiving BCG treatment showed no significant association with genotype of *PSCA*. **Conclusions:** The present study has unveiled a complex intervention of *PSCA* rs2294008C/T conferring a higher risk of UBC risk among smokers in South Indian population, providing evidence that it may contribute to bladder carcinogenesis regardless of ethnicity. These findings suggest that the *PSCA* rs2294008C/T polymorphism of *PSCA* gene could be served as a biomarker for genetic susceptibility to bladder cancer in Indian populations.

Keywords: Urothelial bladder cancer- polymorphism- prostate stem cell antigen- south Indian population

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Introduction

Urothelial carcinoma, also known as bladder cancer, is the ninth most common cancer worldwide. In 2022, more than 614,000 people were diagnosed with bladder cancer, and more than 220,000 died from the disease as per GLOBOCAN 2020. In India, it accounts for 5.6% to 10% of all cancers in men and 1.8% in women. The crude rate incidence is about one in 174 men and one in 561 women.

Roughly 75% of UBC patients are non-muscle-invasive bladder cancer (NMIBC), and the majority of cases constitute Muscle invasive bladder cancer (MIBC) [1]. Because of its high recurrence rate and slow natural history, NMIBC is very common. The most common signs of UBC patients are haematuria or lower urinary tract symptoms, while some could be silent or have unexpected findings [2].

External risk factors are linked to the majority of UBC cases. Up to 50% of patients with urothelial carcinoma have a family history of cancer, and 4.3% of UBC patients had a first-degree relative with UBC [1]. The following risk factors in UBC have been found to have sufficient evidence, according to the International Agency for Research on Cancer (IARC): tobacco use, occupational exposures (such as painting, firefighting, rubber and aluminium production, and exposure to different dyes like magenta and auramine or dye intermediates like 4-aminobiphenyl), environmental exposures (such as gamma or X-ray radiation, and arsenic), medications (such as cyclophosphamide and chlornaphazine), opium intake, and Schistosoma infection [3-5].

More than 150 areas have been linked to different types of cancer using genome-wide association studies (GWAS)

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that focus on the etiology and pathophysiology of cancer. The findings effectively broaden our understanding of the mechanisms behind carcinogenesis [6]. Prostate stem cell antigen *PSCA*, is found on chromosome 8q24.2 [7, 8]. This glycoprotein, a 123-amino acid cell membrane, is a member of the LY-6/Thy-1 family of cell surface antigens [9].

When compared to normal tissues, prostate cancer cell lines were found to overexpress *PSCA*, a cell surface marker [10]. *PSCA* is down-regulated in esophageal cancer, gastric cancer, and gallbladder carcinoma and up-regulated in several solid tumors, including pancreatic, bladder, renal cell carcinoma, and ovarian mucinous [11, 12]. The rs2294008 polymorphism in *PSCA* is a major risk factor for enhanced gastric cancer susceptibility in the Caucasian population [13], while it decreases gastric cancer risk in the Asian population [14], according to two recent GWAS based on people with various ethnic origins. Variations in the transcriptional activity of an upstream *PSCA* fragment may be significantly influenced by the rs2294008 polymorphism in the first exon of the *PSCA* gene [15]. The evidence supporting the *PSCA* rs2294008 polymorphism's function as a marker of genetic origin for cancer risk is still ambiguous, even though mounting data have shown a correlation between the polymorphism and cancer risk [16, 17]. There is a dearth of data on the Indian populace. Thus, in this study, we investigate the link between a subset of South Indians and the *PSCA* rs2294008 polymorphism and urothelial bladder cancer risk.

Materials and Methods

Study subjects

The study was approved by Institutional Ethics Committee (KAHER/EC/21-22/003. A total of 107 patients with histological-confirmed UBC (mean age 59.8 years; 84 males and 23 females) attending the division of urologic-oncologic clinic at a single tertiary care centre of South India were enrolled for the study upon obtaining informed consent from all the subjects. Patients with a history of other cancer, metastasis from another origin, and previous radiotherapies were excluded. Healthy and genetically unrelated individuals visiting the hospital for routine check-ups or health awareness camps were enrolled as the normal healthy control (n = 105). All the controls were age and sex-matched with similar ethnicity and had no evidence of malignancy or chronic disease. The mean age of the controls was 58.9 years. A questionnaire was designed for the study participants to collect data on clinical demographic characteristics, smoking history, occupation history, and other lifestyle factors. 4mL of peripheral blood was collected in a EDTA vial, coded and stored for further analysis at -80 C.

Clinical Demographic data collection

The demographic details were obtained by using a questionnaire each individual in cases and controls. Individuals who smoked once a day for more than 5 years were defined as smokers, similar to alcohol and tobacco consumption. The individuals who had never smoked or been exposed to other habits in their lifetime were regarded as non-smokers

Clinical data collection

The demographic and clinical characteristics of the patients are presented in Table 1. The clinical information about tumor size, number, stage and tumor grade, intra-vesical therapy and dates of recurrence, chemotherapy, radical cystectomy, and pathological findings at cystectomy were recorded. The tumor stages were classified per the American Joint Committee on Cancer's TNM staging system [18]. Of the 107 total patients enrolled in the study, 39 (36.4%) patients had non-muscle-invasive bladder cancer whereas the rest 68 (63.6) had muscle-invasive bladder cancer. A blood sample was collected in EDTA from all subjects for genotyping at the time of enrolment and stored at -80oC.

Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by spin column method [19]. For genotyping of *PSCA* gene polymorphisms, predesigned TaqMan assays were performed with CFX96 Real-Time System Thermal Cycler (BIO-RAD). Positive and negative controls were used in each genotyping assay, and 10% of the samples were randomly selected and run in duplicates with 100% concordance. The results reproduced had no variations.

Statistical analysis

The power of the study was calculated using Quanto software, version 1.0 (available from: http://hydra.usc.edu/gxe), with the input of the following variables: case-control study design, significance level (α), model of inheritance was log additive, and the genetic effect for odds ratio (OR) was 1.50 or greater. The goodness-of-fit chi-square test was used to analyze any deviation from the Hardy-Weinberg equilibrium in controls. A binary logistic regression model was used to estimate the risk as the OR at the 95% CI. The statistical analysis was done using the Statistical Package for Social Sciences software, version 22.0 (SPSS) (version 22.0. Armonk, Chicago, USA), and $p<0.05$ was considered statistically significant.

Results

The clinical demographic data and tumor characteristics of the subjects are summarized in (Table 1). This study population included 107 cases and 105 controls. Bladder cancer patients were similar in age and gender proportion to controls. For Grade-wise distribution 41 patients had low-grade (38.3%) and 66 patients had high-grade (61.7%) tumours. For Occupational exposure, 53.2% of patients were exposed to chemicals and hazardous substances in Bladder cancer patients when compared to 28.6% in controls. Around 37.4% of patients had Urological infections in the lower urinary tract in Bladder cancer patients. The genotype frequencies of rs2294008 among bladder cancer patients and controls and their associations with the risk of bladder cancer is presented in (Table 2).

The frequencies of the CC, CT, and TT genotypes of

Table 1. General Characteristics of Subjects

Variables		Bladder Cancer (107)	Controls (105)
Gender	Male	84 (78.51)	80 (76.2)
	Female	23 (21.49)	25 (23.80)
Age	<55	31 (28.9)	32 (30.4)
	55-64	24 (22.5)	32 (30.4)
	>65	52 (48.6)	41 (39.2)
Grade	Low	41 (38.3)	-
	High	66 (61.7)	-
Tumour Stage	Superficial	39 (36.4)	-
	Invasive	68 (63.6)	-
Occupational Exposure	Yes	57 (53.2)	30 (28.6)
	No	50 (46.7)	75 (71.4)
History of Urological Infection	Yes	40 (37.4)	16 (15.3)
	No	67 (62.6)	89 (84.7)

rs2294008 polymorphism were 26.2, 42.9, and 30.9% in bladder cancer patients and 51.5, 30.4, and 18.1% in controls, respectively. Genotype frequencies in controls were in Hardy-Weinberg equilibrium (p<0.053). Compared with the CC genotype, CT and TT genotype was associated with an increased risk of bladder cancer (adjusted odds ratio [OR] CT=2.77, 95% confidence interval [CI] =1.459 - 3.247; OR TT = 3.349, 95% CI = 1.610-6.922). Moreover, the combined CT/ TT was associated with a significantly increased risk of bladder cancer (OR CT/TT=2.949, 95% confidence interval [CI] = 1.656 – 5.252) (Table 2).

Shows the associations between rs2294008 and bladder cancer were further evaluated by stratified analysis of tumor grade, and tumor stage as shown in (Table 3). The outcome of the rs2294008 CT/TT was higher in High-grade tumors (OR CT/TT = 1.984, 95% CI = 0.892-4.413) than in Low-grade tumors (OR CT/TT = 0.452, 95% CI = 0.213 - 0.961). Similarly, for stage-wise distribution superficial showed fewer effective values (T0-T1: OR CT/TT = 0.3367, 95% CI = 0.157-0.721) than in invasive (T2-T4: OR CT/TT=2.658, 95%CI=1.131- 6.246) bladder cancer.

The effect of the rs2294008 CT/TT was relatively higher in tobacco exposure (OR CT/TT= 2.374, 95% CI = 1.075 – 5.243) than in non-smoking (OR CT/TT = 0.236, 95% CI = 0.101 – 0.551) with significant association (p=0.0001*, p<0.001) (Figure 1), For smoking exposure

Table 2. Genotype Frequencies of PSCA rs2294008 among Bladder Cancer and Controls

Genotype	Bladder Cancer (107)	Controls (105)	Odds Ratio
CC	28 (26.2)	54 (51.5)	Reference
CT	46 (42.9)	32 (30.4)	2.772 (1.459-3.247)
TT	33 (30.9)	19 (18.1)	3.349 (1.610 - 6.922)
CT+TT	78 (73.8)	51 (48.5)	2.949 (1.656 - 5.252)
C ALLELE	79 (46.8)	124 (82.4)	Reference
T ALLELE	89 (53.2)	54 (17.6)	2.586 (1.665 - 4.018)

(OR CT/TT= 2.008, 95% CI = 0.948 – 4.251) than in non-smoking (OR CT/TT= 0.537, 95% CI = 0.243- 1.101) with significant association (p=0.0001*, p<0.001)and finally for Alcohol exposure (OR CT/TT= 1.643, 95% CI = 0.752 – 3.589) than in non-alcohol (OR CT/TT = 0.583, 95% CI = 0.278 – 1.224) with no significant association (p=0.0199*, p<0.001). Allelic discrimination for *PSCA* single nucleotide polymorphism in a set of samples was noted on qRT-PCR, FAM dye represents T nucleotide and VIC dye represents C nucleotide (Figure 1).

Discussion

Urothelial Bladder cancer still remains a major worldwide health concern, and finding the genetic elements impacting its onset and progression will take time. Studies involving bladder cancer patients have looked into the *PSCA* (Prostate Stem Cell Antigen) gene, more especially the rs2294008 polymorphism. The *PSCA* gene contains a single nucleotide polymorphism (SNP) called rs2294008; genetic changes in this area have been linked to an increased risk of bladder cancer. In the 8q24.3 region, the rs2294008 (C/T) SNP is the most researched variant linked to bladder cancer susceptibility to date. This missense variant alters a nucleotide in the start codon and is found in *PSCA* exon 1. Wu et al. [6]. first reported the GWAS link between the SNP rs2294008 found in the *PSCA* gene and bladder cancer susceptibility, which was later confirmed by Rothman et al. [20]. In the combined set of 10,196 cases and 44,705 controls, rs2294008 was associated with a per-allele OR = 1.13 (95% CI = 1.09 – 1.17, P=4.4× 10^{-11}).

Remarkably, a different GWAS study published on bladder cancer found a correlation between the rs2294008 T allele and Caucasian susceptibility to bladder cancer validated this SNP [6, 20]. While not achieving statistical significance, rs2294008 displayed a similar association

Table 3. Subgroup Analysis of the Association between *PSCA* rs2294008 and Risk of Bladder Cancer

	Odds Ratio		p-value
	CC	CT/TT	
Tumour Grade			
Low	1 (Reference)	0.452 (0.213 - 0.961)	<0.0001
High	1 (Reference)	1.984 (0.892 - 4.413)	
Tumour Stage		Odds Ratio	
Superficial (T0-T1)	1 (Reference)	0.3367 (0.157 - 0.721)	<0.0001
Invasive (T2-T4)	1 (Reference)	2.658 (1.131 - 6.246)	

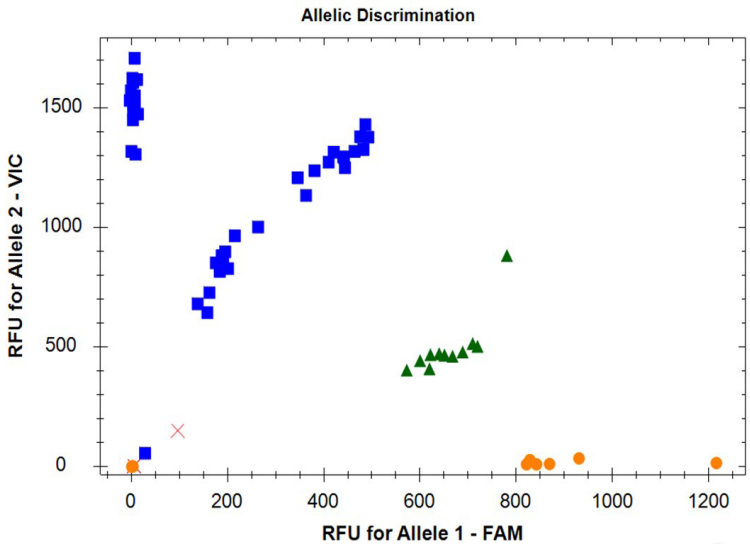

Figure 1. Allelic Discrimination Plot for Ssingle Nucleotide Polymorphism, VIC Dye Represents C Nucleotide FAM dye Represents T nucleotide.

with the known risk alleles in Caucasians in a study conducted on a Chinese population [21]. An increased risk of bladder cancer was linked to the rs2294008 CT genotype but not the TT genotype, according to a different Chinese study [22]. A statistically significant increase in the risk of bladder cancer was found in the genotype was comparable to that observed in Caucasians (OR CT/TT = 1.38, 95% CI = 1.09 - 1.75 in Chinese; OR CT/TT = 1.33, 95%CI =1.22-1.45 in Caucasians) [6, 22]. Another chinese study conducted on three distinct ethnic groups: Han, Dai, and Bai. They found that the Han population, a prominent ethnic group in China, had a T allele frequency of 0.265 in the control group and was higher in the case group (OR 1.34; 95% CI 1.17–1.69). The association analysis revealed that the T genotype was associated with an increased risk of higher pathological grade and invasive bladder cancer. The findings were comparable with another GWAS in the Chinese population [22]. T allele risk and connection with invasive bladder cancer can also be seen in Dai and Bai populations. Dai people had a much greater frequency of T allele (0.406) than Han people. The T allele risk for invasive tumours was reported in all three populations. In contrast, the T allele risk on tumor grade was not identified in the two minor ethnicities [23]. rs2294008 was recently discovered as a susceptibility allele for diffuse-type gastric cancer in Japan [11]. The T allele is more common in Europeans (MAF = 0.46) and Koreans (MAF = 0.46) compared to Chinese (MAF = 0.26) and Africans (MAF = 0.25), according to HapMap data. However, it is a prominent allele in Japanese (frequency = 0.62). The genetic background of this SNP and why only Japanese have a distinct minor allele remains unknown.

India is a multi-ethnic country with population from various genetic pools. It is interesting to study the genetics of different populations and compare. Therefore, here in our study we focused on UBC cases from southern part of country and compare the results with Northern population published by Singh V. et al. [24]. In north Indian population, a notable increase in bladder cancer risk was linked to the heterozygous CT genotype of *PSCA* rs2294008C/T, presenting a 1.86-fold risk (p=0.004; OR=1.86). The T variant allele was strongly correlated with bladder cancer risk (p=0.027; OR=1.38) for *PSCA* rs2294008C/T. A notable bladder cancer risk was identified while assessing risk in relation to tumor grade and stage for *PSCA* rs2294008C/T with the heterozygous CT genotype (p=0.045; OR=1.02). Smoking substantially influenced the bladder cancer risk in individuals with the heterozygous CT genotype (p=0.025) for the *PSCA* rs2294008C/T gene polymorphism [24].

Whereas, in the present study involving South Indian population, heterozygous CT genotype showed increased significance risk to UBC with *PSCA* rs2294008 C/T having 2.77 folds risk (p<0.0001). The variant allele T was also significantly associated with UBC risk (p<0.0001; OR=3.349) for *PSCA* rs2294008C/T. Additionally, our findings demonstrated that the combined CT/TT genotype had a greater influence on tobacco exposure among smokers (OR CT/TT = 2.374, 95% CI = 1.075 – 5.243) compared to non-smokers (OR CT/TT = 0.236, 95% CI = 0.101 – 0.551). We conducted a stratified analysis based on tumor grade and stage and discovered that there was significant heterogeneity in the effect of the combined CT/TT genotype between superficial bladder cancer (T0-T1: OR CT/TT = 0.3367, 95% CI = 0.157-0.721) and invasive bladder cancer (OR CT/TT = 0.452, 95% CI = 0.213 - 0.961).

Since numerous tobacco carcinogens change gene expression and damage DNA, smoking is a major risk factor for bladder cancer [25, 26]. Current smokers had an almost threefold higher risk than non-smokers, according to a meta-analysis combining the epidemiological study of urinary tract cancer risk and cigarette smoking [27]. The risk of bladder cancer was shown to be 2.2 and 3.4 fold greater in smokers with the rs2294008 CC and CT/TT genotypes, respectively, than in non-smokers with the

CC genotype, according to a Chinese study's findings [22]

Similarly, Wang et al. [22] found that invasive bladder cancer was more likely to be affected by this genotype. In contrast, contradictory findings were found in a second study conducted by Lee et al. [28]. One explanation for the discrepancy, though it is still unclear why, could be that the subgroup analysis lacked sufficient power due to the very small sample size. Another explanation could be the differences in the epidemiological traits, etiology, and pathophysiology of superficial and invasive bladder tumors [29]. Thus, more research with bigger sample sizes is required to validate these results. A unique translation initiation codon is created upstream of the conventional start location by the risk allele T of rs2294008, which results in the production of a protein with nine extra amino acids. Reduced promoter transcriptional activity is a result of the *PSCA* protein's elongation. According to a functional investigation, carriers of the risk allele T of rs2294008 exhibit greater expression of *PSCA* mRNA in bladder cancer samples as compared to normal tissues [30].

In Conclusions, the present study demonstrates a population size of single tertiary care centre which included (107 cases and 105 controls) reduced the statistical power for identifying interaction effects, which was one of its limitations. In summary, our findings indicate that the *PSCA* gene polymorphism rs2294008 is linked to a higher risk of bladder cancer in the South Indian population, implying that rs2294008 plays a role in the development of urothelial bladder cancer.

Author Contribution Statement

All authors contributed equally in this study.

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