

RESEARCH ARTICLE

Genetic Variation in PSCA is Associated with Bladder Cancer Susceptibility in a Korean Population

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Abstract

Background: Genetic factors play important roles in the pathogenesis of human cancer. A recent genome wide association study (GWAS) identified an association between the rs2294008 polymorphism of the prostate stem cell antigen (PSCA) gene and bladder cancer risk in Caucasians. The aim of this study was to determine whether the rs2294008 polymorphism is similarly associated with bladder cancer susceptibility in a Korean population. **Materials and Methods:** We conducted a case-control study of 411 bladder cancer patients and 1,700 controls. **Results:** The frequencies of the CC, CT, and TT genotypes of the rs2294008 polymorphism were 16.9, 54.0, and 28.8% in bladder cancer patients and 24.4, 48.1, and 27.5% in controls, respectively. We found that the combined CT/TT genotypes were associated with a significantly increased risk of bladder cancer (OR CT/TT =1.58, 95% CI= 1.15-2.17), compared with the CC genotype. Smoking habits, tumor grade and tumor stage did not modify the association between rs2294008 and the risk of bladder cancer. **Conclusions:** Our study showed that the rs2294008 polymorphism in the PSCA gene is associated with the risk of bladder cancer in a Korean population, providing evidence that it may contribute to bladder carcinogenesis regardless of ethnicity.

Keywords: PSCA - bladder cancer - SNP - ethnicity

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Introduction

Bladder cancer is the most common malignancy of the urinary tract with an estimated 386,300 new cases and 150,200 deaths worldwide in 2008 (Jemal et al., 2011). Although bladder cancer is more frequent in Europe, it is the 14th common cancer in Korea, with an age-standardized incidence rate of 8.9 per 100,000 among males and 1.5 per 100,000 among females in 2010 (The Korea Central Cancer Registry, 2012). Cigarette smoking, occupational exposure to aromatic amines and environmental exposures (e.g., arsenic in drinking water) have been linked to the risk of bladder cancer (Kiriluk et al., 2012). However, not all people exposed to these risk factors developed bladder cancer, which suggests that genetic factors may also play an important role in bladder carcinogenesis.

In the past, many inherited genetic variants were reported to be associated with bladder cancer risk using a candidate-gene approach, such as GSTM1 and NAT2, related to environmental toxicant metabolism. However, candidate gene studies lacked sufficient power to identify

the genetic loci for clarification of the mechanisms of bladder cancer (Dudek et al., 2013). Recently, genome-wide association studies (GWAS) have permitted the identification of novel susceptibility loci in the human genome associated with many complex diseases, including cancer (Dudek et al., 2013). To date, three GWAS for bladder cancer risk have been conducted, and eight single nucleotide polymorphisms (SNPs) were identified near the following genes: MYC (8q24), TP63 (3q28), PSCA (8q24), FGFR3/TACC3 (4p16), CBX6/APOBEC3A (22q13), CCNE1 (19q12), UGT1A (2q37), and SLC14A1 (18q12) (Kiemeny et al., 2008; Wu et al., 2009; Kiemeny et al., 2010; Rothman et al., 2010; Rafnar et al., 2011).

Notably, rs2294008 (C/T) on 8q24.3 within the prostate stem cell antigen (PSCA) gene was confirmed to have functional consequences and was shown to modify bladder cancer risk by influencing gene expression in an allele-specific manner. Although rs2294008 has been established as the bladder cancer susceptibility region by epidemiologic and functional studies (Wu et al., 2009; Rothman et al., 2010; Wang et al., 2010; 2014 Fu et al.,

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2012), the effect of rs2294008 might vary according to ethnic background. Most studies have been conducted in Western populations and evidence from Asian populations is limited (Wang et al., 2010; 2014); thus, this association requires validation in other ethnic populations. The aim of this study was to determine whether the rs2294008 polymorphism is associated with bladder cancer susceptibility in a Korean population.

Materials and Methods

Subjects

The study population consists of 411 patients with newly diagnosed bladder cancer and 1700 controls. All enrolled patients were pathologically confirmed by Chonnam National University Hwasun Hospital between February 2004 and April 2011. Cases with secondary or recurrent tumors were excluded. Control subjects recruited in present study have been described a previous report (Song et al., 2011). The control group (n = 1700) consisted of participants in the Thyroid Disease Prevalence Study conducted from July 2004 to January 2006 in Yeonggwang and Muan Counties of Jeollanam-do Province and in Namwon City of Jeollabuk-do, Korea.

Genotyping

Genotyping was performed high resolution melting (HRM) analysis, using a Rotor-Gene 6000™ (Corbett Research, Sydney, Australia). PCR primers were as follows: forward primer, 5'-aagtcacctgaggcctctc-3' and reverse primer, 5'-ctcaccctgctcccttc-3'. The reaction mixture for HRM included 200 nM PCR primer, 1 μM SYTO 9 fluorescent dye (Invitrogen, Carlsbad, CA, USA), 0.5 U f-Taq polymerase (Solgent, Daejeon, Korea), and 40 ng genomic DNA in 10 μl reaction volumes. The Cycling conditions included an initial 5 min hold at 95°C, followed by 40 cycles at 95°C for 5 sec, 69°C for 30 sec, and 72°C for 10 sec and melting increasing from 83°C to 93°C at 0.1°C per second. In 96 subjects, the results of HRM analyses were compared with those from sequencing (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems) and the resulting concordance rate was 100%.

Statistical analysis

Chi-square test was used to evaluate the differences in demographic variables and genotype distribution of rs2294008 polymorphisms between cases and controls. Association between rs2294008 genotypes and risk of bladder cancer were evaluated by adjusted odds ratios (OR) and 95% confidence intervals (CI), using logistic regression models. Subjects with the wild type genotypes (CC) were considered to be baseline risk. The expected frequency of control genotypes was checked by the Hardy-Weinberg equilibrium test. All tests were conducted at the P=0.05 level of significance, were done using SPSS (Chicago, IL, USA) for windows version 16.0.

Results

The demographic and tumor characteristics of the subjects are summarized in Table 1. This study population

included 411 cases and 1700 controls. Bladder cancer patients were older and had higher proportion of men than controls. We adjusted for age and sex in the subsequent multivariate logistic regression analysis.

Table 2 shows genotype frequencies of rs2294008 among bladder cancer patients and controls and their associations with risk of bladder cancer. The frequencies of the CC, CT, and TT genotype of rs2294008 polymorphism were 17.0, 54.0, and 29.0% in bladder cancer patients and 24.4, 48.1, and 27.5% in controls, respectively. Genotype frequencies in controls were in Hardy-Weinberg equilibrium (p=0.13).

Compared with the CC genotype, CT genotype was associated with increased risk of bladder cancer, but not TT genotype ratio (adjusted odds ratio [OR] CT=1.69, 95% confidence interval [CI]=1.20-2.39; OR TT=1.35, 95%CI=0.93-1.98). The combined CT/TT were associated with a significantly increased risk of bladder cancer (OR CT/TT=1.56, 95%CI=1.15-2.17) (Table 2).

The associations between rs2294008 and bladder cancer were further evaluated by stratified analysis of smoking, tumor grade, and tumor stage. As shown in Table 3, the effect of the rs2294008 CT/TT was larger in smoking (OR CT/TT=1.80, 95%CI=1.14-2.84) than in non-smoking (OR CT/TT=1.52, 95%CI=0.80-2.86), in superficial (T0-T1: OR CT/TT=1.90, 95%CI=1.23-2.93) than in invasive (T2-T4: OR CT/TT=1.32, 95%CI=0.84-2.05) bladder cancer, but there were no heterogeneity

Table 1. General Characteristics of Subjects

	Control	Bladder cancer
No.	1700	411
Age (years)*	52.2 (19.9-74.9)	67.0 (13.0-93.1)
≤ 60	1117 (65.7)	82 (20.0)
> 60	583 (34.3)	329 (80.0)
Sex*		
Male	821 (48.3)	348 (84.7)
Female	879 (51.7)	63 (15.3)
Smoking habit		
Never	1000 (58.8)	207 (50.3)
Ever	655 (38.5)	193 (47.0)
Unknown	45 (2.6)	11 (2.7)
Grade		
Low		155 (37.7)
High		237 (57.7)
Unknown		19 (4.6)
Tumor stage		
Superficial (T0-T1)		217 (52.8)
Invasive (T2-T4)		180 (43.8)
Unknown		14 (3.4)

*Bladder cancer compared with controls, p<0.01

Table 2. Genotype Frequencies of the PSCA rs2294008 among Controls and Bladder Cancer and their Associations with Risk of Bladder Cancer

Genotype	Control (n=1700)	Case (n=411)	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
CC	414 (24.4)	70 (17.0)	1 (Reference)	1 (Reference)
CT	818 (48.1)	222 (54.0)	1.61 (1.20-2.15)	1.69 (1.20-2.39)
TT	468 (27.5)	119 (29.0)	1.50 (1.09-2.08)	1.35 (0.93-1.98)
CT/TT	1286 (75.6)	341 (82.8)	1.57 (1.19-2.08)	1.56 (1.13-2.16)

^aOR^a, odd ratios adjusted by age, sex and smoking habit; CI, confidence interval

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

	Adjusted OR ^a		p*
	CC	CT/TT	
Smoking habit**			0.66
Non-smoking	1 (Reference)	1.52 (0.80-2.86)	
Smoking	1 (Reference)	1.80 (1.14-2.84)	
Tumor grade			0.92
Low	1 (Reference)	1.55 (0.97-2.48)	
High	1 (Reference)	1.49 (0.99-2.25)	
Tumor stage			0.22
Superficial (T0-T1)	1 (Reference)	1.90 (1.23-2.93)	
Invasive (T2-T4)	1 (Reference)	1.32 (0.84-2.05)	

ORa, odds ratio adjusted for age, sex and smoking habit; CI, confidence interval; *P-value for interaction; **Only men were included in the analysis because of the low prevalence of smoking in women

according to smoking habit and tumor stage. Tumor grade also did not modify the association between the rs2294008 and the risk of bladder cancer.

Discussion

In the present study, we investigated the rs2294008 polymorphism in the PSCA gene as a risk factor for bladder cancer in a Korean population. We found that the rs2294008 CT, but not the TT genotype was associated with increased risk of bladder cancer. The combined CT/TT genotype as a dominant genetic model was associated with elevated risk of bladder cancer. In subgroup analyses, we observed no significant interactions between rs2294008 and smoking habits, tumor grade or tumor site.

Recently, rs2294008 was associated with diffuse-type gastric cancer in the GWAS by Sakamoto et al. (2008). The association between the rs2294008 T allele and increased risk of gastric cancer were validated in several studies (Wang et al., 2012), including our study in a Korean population (Song et al., 2011). Interestingly, another published GWAS related to bladder cancer further identified an association between the rs2294008 T allele and bladder cancer susceptibility in Caucasians (Wu et al., 2009); this SNP was subsequently confirmed by Rothman et al. (2010). A study in a Chinese population also found that rs2294008 showed the same association with the reported risk alleles in Caucasians, albeit not reaching statistical significance (Ma et al., 2013). Another Chinese study found that the rs2294008 CT but not TT genotype was associated with an elevated risk of bladder cancer (Wang et al., 2010). In the dominant genetic model, the combined rs2294008 CT/TT genotypes conferred a statistically significant increase in the risk of bladder cancer, and the effect of the combined CT/TT genotype was similar to that 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) (Wu et al., 2009; Wang et al., 2010). These results are consistent with those of the present study.

Tobacco smoking has been established a major risk factor for bladder cancer because many carcinogens present in tobacco alter gene expression and damage DNA (Wallerand et al., 2005; Volanis et al., 2010). A meta-analysis summarizing the epidemiology of urinary tract

cancer risk and cigarette smoking observed that current smokers have an approximately threefold higher risk than non-smokers (Zeegers et al., 2000). In a Chinese study, the authors showed that smokers with rs2294008 CC and CT/TT genotypes had 2.2 and 3.4 fold, respectively, higher risk of developing bladder cancer as did non-smokers with the CC genotype (Wang et al., 2010). Our results also showed that the effect of the combined CT/TT genotype was more predominant in smoking than in non-smoking males. But these two studies did not find heterogeneity between the rs2294008 polymorphism and the risk of bladder cancer according to smoking habits.

We also performed a stratified analysis for tumor grade and tumor stage and found that the effect of the combined CT/TT genotype was larger in superficial than in invasive bladder cancer, although there was no heterogeneity. In contrast, Wang et al. observed a reverse trend in that the effect of this genotype was more prominent in invasive bladder cancer (Wang et al., 2010). Although the reason for the discrepancy remains unclear, one possible explanation was the relatively small sample size, which lacked sufficient power in the subgroup analysis. Another reason might be that superficial and invasive bladder cancers differ in their epidemiological characteristics, etiology, and pathogenesis (Wu, 2005). Therefore, additional studies with larger sample sizes are needed to further confirm these findings.

The PSCA gene encodes the glycosylphosphatidylinositol (GPI)-anchored cell surface protein belonging to the Thy-1/Ly-6 family. Although the function of PSCA has not been clarified in normal or tumor tissue, the members of the Thy-1/Ly-6 family have been implicated in carcinogenesis (Saeki et al., 2010). rs2294008 (C/T) is a missense variant located in exon 1 of the PSCA gene. The risk allele T of rs2294008 creates a novel translation initiation codon upstream of the regular start site, leading to expression of a protein with nine additional amino acids. This extension of the PSCA protein contributes to decreased promoter transcriptional activity. A functional study reported increased PSCA mRNA expression in bladder cancer samples compared with normal tissues, with enhanced expression present among carriers of the risk allele T of rs2294008 (Fu et al., 2012).

A limitation of our study was the relatively small sample size (411 cases and 1,700 controls), which limited the statistical power for detection of interaction effects.

In conclusion, our results provide evidence supporting the association of the rs2294008 polymorphism in the PSCA gene with the risk of bladder cancer in a Korean population, suggesting that rs2294008 contributes to bladder carcinogenesis.

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