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Original Article

Genetic variation of the *PSCA* gene (rs2294008) is not associated with the risk of prostate cancer

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Abstract

Prostate stem cell antigen (*PSCA*) is a cell-membrane glycoprotein consisting of 123 amino acids and highly expressed in the prostate, but there have been few reports on the relationship between rs2294008 of *PSCA* and prostate cancer in the literature. Therefore, we evaluated the association between rs2294008 and the risk of prostate cancer. A total of 240 prostate cancer patients and 306 controls (patients with benign prostatic hyperplasia) were enrolled. Genotype analysis of rs2294008 of *PSCA* was performed using PCR. Logistic regression analysis was performed according to the genotype of *PSCA* rs2294008. We found that CT and TT genotypes were associated with an insignificant risk of prostate cancer compared with the CC genotype ($P = 0.627$ and 0.397, respectively). In addition, there was no significant difference in rs2294008 according to clinicopathological parameters, such as age, Gleason score, prostate-specific antigen (PSA), stage, and metastasis in prostate cancer ($P > 0.05$ for each). Age, Gleason score, PSA, pathologic stage, and metastasis did not modify the association between *PSCA* and the risk of prostate cancer (each $P > 0.05$ for each). Taken together, the genetic polymorphism of *PSCA* rs2294008 was not associated with the risk of prostate cancer. Our results suggest that rs2294008 may not play a role in prostate carcinogenesis.

Keywords: prostatic neoplasms, polymorphism, genetic, risk, prostate, polymorphism, single nucleotide

Introduction

Prostate cancer is the second most common malignant tumor in men in Western countries, and the number of newly diagnosed prostate cancer patients is 129.4 per 100,000 men per year[1]. Among various risk factors for prostate cancer, genetic factors, including race, family, and specific gene variants, might be associated with prostate cancer. Especially, among specific gene variants, mutations in *BRCA1* and *BRCA2*, Hereditary Prostate cancer gene 1 (*HPC1*), and *TMPRSS2-ERG* gene fusion are linked to prostate cancer[2-3].

Recently, several studies have reported that the genetic polymorphisms of several genes at 17q12, 17q24.3, and 8q24 are significantly associated with prostate cancer[4–7]. There is growing interest in the role of genetic polymorphism in the risk for prostate cancer according to genetic polymorphism in patients with prostate cancer.

Prostate stem cell antigen (*PSCA*) is a cell-membrane

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glycoprotein consisting of 123 amino acids and highly expressed in the prostate and bladder[8,9]. Furthermore, *PSCA* is highly expressed in human prostate cancer, but has limited expression in normal tissues, which has become a potential target biomarker in prostate cancer. Interestingly, Joung *et al*.[10] have demonstrated that men with the rs1045531AA genotype of *PSCA* are at high risk of prostate cancer in genotype analysis. However, there have been few reports about the relationship between rs2294008 of *PSCA* and prostate cancer. Therefore, we evaluated the association between rs2294008 and the risk of prostate cancer.

Materials and methods

Study population

This study recruited data from 240 patients who underwent radical prostatectomy or palliative transurethral resection of the prostate (TURP) and were histologically confirmed to have adenocarcinoma of the prostate and 306 benign prostatic hyperplasia (BPH) patients for controls. Gleason scores (GS) were assigned to 12 specimens obtained from core transurethral biopsy, TURP, or radical prostatectomy. Tumor stage was estimated for specimens obtained from radical prostatectomy, and TNM 2002 stage was determined using computed tomography (CT), magnetic resonance imaging, or bone scanning.

Collection and analysis of the samples were approved by the Institutional Review Board of Chungbuk National University, and each subject provided written informed consent (IRB approval number: 2010-12-010).

Blood sampling, DNA extraction, and genotype assays of polymorphism

From each patient, a 5 mL of blood sample of was collected with a 0.1 mL EDTA tube before the operation, frozen in liquid nitrogen, and stored at –80°C. Genomic DNA was extracted from human whole blood for genotyping using a genomic DNA purification kit (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions.

PSCA genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR primers for *PSCA* rs2294008 were 5′-AAACCCGCTG- GTGTTGACT-3′ (sense) and 5′-TCCCTTCCTCCTTCCTTCC-3′ (antisense). DNA (200 ng) was amplified in a total volume of 20 μL, containing 10 pmol of each primer, 0.5 U *Taq* polymerase, 2.5 mmol/L deoxyribonucleotide triphosphate, and 10xPCR buffer. PCR conditions were as follows: initial denaturation step at 95°C for 5 minutes and then 39 cycles of amplification were carried out at 95°C for 45 seconds, 50°C for 45 seconds, and 72°C for 60 seconds, with a final extension step at 72°C

Table 1 **Baseline characteristics of the subjects**

	Control	Prostate cancer
Number	306	240
Age, *n*(%)†	70.12±7.50	68.58±7.12
< 70 years	136 (44.4)	132 (55.0)
⩾70 years	170 (55.6)	108 (45.0)
Gleason score, *n*(%)		
⩽7		120 (50.0)
8		72 (30.0)
⩾9		48 (20.0)
PSA (ng/mL), *n*(%)		
< 20		117 (48.7)
⩾20, < 50		60 (25.0)
⩾50		63 (26.3)
Stage, *n*(%)		
T2		114 (47.5)
T3		80 (33.3)
T4		46 (19.2)
Metastasis, *n*(%)		
non-metastasis		196 (81.7)
metastasis		44 (18.3)

†Prostate cancer compared with controls, $P < 0.05$.

Table 2 **Association between the risk of prostate cancer and the *PSCA* gene rs2294008**

	Control†	Prostate cancer†	OR (95% CI)	*P* value
CC	78 (25.5)	54 (22.5)	1	
CT	163 (53.3)	135 (56.2)	0.882 (0.533-1.462)	0.627
TT	65 (21.2)	51 (21.3)	0.836 (0.552-1.266)	0.397
C	319 (52.1)	243 (50.6)	1	
T	293 (47.9)	237 (49.4)	0.942 (0.741-1.196)	0.632

†Genotype distribution and Hardy-Weinberg equilibrium ($P = 0.623$). OR: odds ratio; CI: confidence interval

Table 3 **Relationship between *PSCA* (rs2294008) and clinicopathological parameters in prostate cancer**

Variables	PSCA			*P* value
	CC (3)	CT (2)	TT (1)	
Age, *n*(%)				0.253
< 70 years	35 (26.5)	71 (53.8)	26 (19.7)	
⩾ 70 years	19 (17.6)	64 (59.3)	25 (23.1)	
Gleason score, *n*(%)				0.633
⩽ 7	26 (21.7)	69 (57.5)	25 (20.8)	
8	20 (27.8)	36 (50.0)	16 (22.2)	
⩾ 9	8 (16.7)	30 (62.5)	10 (20.8)	
PSA (ng/mL), *n*(%)				0.686
< 20	29 (24.8)	64 (54.7)	24 (20.5)	
⩾ 20, < 50	11 (18.3)	33 (55.0)	16 (26.7)	
⩾ 50	14 (22.2)	38 (60.3)	11 (17.5)	
Stage, *n*(%)				0.784
T2	28 (24.5)	63 (55.3)	23 (20.2)	
T3	15 (18.7)	45 (56.3)	20 (25.0)	
T4	11 (23.9)	27 (58.7)	8 (17.4)	
Metastasis, *n*(%)				0.195
non-metastasis	42 (21.4)	108 (55.1)	46 (23.5)	
metastasis	12 (27.3)	27 (61.4)	5 (11.4)	

for 10 minutes. PCR products were digested overnight at 37°C with *Nla III* (New England BioLabs, Beverly, MA, USA) according to the manufacturer's instructions and then separated by electrophoresis on 3% agarose with ethidium bromide staining. PCR products were shown to be digested into 3 types of TT (98 and 158 bp), CT (98, 158, and 256 bp), and CC (256 bp) genotypes.

Statistical analysis

Clinical variables, such as age, GS, PSA, stage, and metastasis, according to genetic polymorphism were compared by the Chi-square test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using logistic regression models. Wild-type genotypes (CC) were considered baseline risk. Using the Hardy–Weinberg equilibrium test, the expected frequency of control genotypes was analyzed. Statistical analyses were performed by using Statistical Package for Social Sciences version 23.0 (IBM, Armonk, NY, USA). All tests were performed using 2-tailed analysis and a P value of < 0.05 was considered statistically significant.

Results

Baseline characteristics

The mean age of 240 prostate cancer patients was 68.58±7.12 years, and that of 306 controls was 70.12±7.50 years. One hundred and fourteen (47.5%) patients had stage T2, 80 (33.3%) had stage T3, and 46 (19.2%) had stage T4 prostate cancer. The baseline characteristics of the patients are summarized in ***Table 1***.

Association between the risk of prostate cancer and *PSCA* (rs2294008)

Table 2 shows genotypes and allele frequencies for rs2294008 in prostate cancer patients and controls. For prostate cancer, the CC, CT, and TT genotypes were observed in 22.5%, 56.2%, and 21.3% of the patients, respectively, and for controls, 25.5%, 53.3%, and 21.2%, respectively. The genotype frequencies for rs2294008 followed the Hardy–Weinberg equilibrium ($P = 0.623$). Compared with the CC genotype, the CT and TT genotypes were associated with insignificant risk of prostate cancer ($P = 0.627$ and 0.397, respectively).

Relationship between *PSCA* and clinicopathologic parameters in prostate cancer

As shown in ***Table 3***, there was no significant difference in rs2294008 according to clinicopathologic parameters, such as age, GS, PSA, stage, and metastasis in prostate cancer ($P > 0.05$ for each).

Table 4 **Stratified analysis of the *PSCA* gene (rs2294008) according to prostate cancer classification**

	PSCA	Prostate cancer	OR (95% CI)	*P* value
Age, *n*(%)				
< 70 years	CC	35 (26.5)	1	
(*n* = 132)	CT	71 (53.8)	1.047 (0.516-2.126)	0.899
	TT	26 (19.7)	0.986 (0.558-1.742)	0.986
⩾70 years	CC	19 (17.6)	1	
(*n* = 108)	CT	64 (59.3)	0.670 (0.319-1.407)	0.290
	TT	25 (23.1)	0.643 (0.343-1.207)	0.169
Gleason score, *n*(%)				
⩽7	CC	26 (21.7)	1	
(*n* = 120)	CT	69 (57.5)	0.867 (0.457-1.644)	0.661
	TT	25 (20.8)	0.787 (0.466-1.332)	0.373
8	CC	20 (27.8)	1	
(*n* = 72)	CT	36 (50.0)	1.042 (0.499-2.173)	0.913
	TT	16 (22.2)	1.161 (0.631-2.136)	0.631
⩾9	CC	8 (16.7)	1	
(*n* = 48)	CT	30 (62.5)	0.667 (0.249-1.787)	0.420
	TT	10 (20.8)	0.557 (0.244-1.272)	0.165
PSA (ng/mL), *n*(%)				
< 20	CC	29 (24.8)	1	
(*n* = 117)	CT	64 (54.7)	1.007 (0.535-1.897)	0.983
	TT	24 (20.5)	0.947 (0.566-1.585)	0.836
⩾20, < 50	CC	11 (18.3)	1	
(*n* = 60)	CT	33 (55.0)	0.573 (0.249-1.321)	0.191
	TT	16 (26.7)	0.697 (0.334-1.451)	0.334
⩾50	CC	14 (22.2)	1	
(*n* = 63)	CT	38 (60.3)	1.061 (0.451-2.495)	0.893
	TT	11 (17.5)	0.770 (0.394-1.504)	0.444
Stage, *n*(%)				
T2	CC	28 (24.5)	1	
(*n* = 114)	CT	63 (55.3)	1.014 (0.534-1.928)	0.965
	TT	23 (20.2)	0.929 (0.552-1.563)	0.781
T3	CC	15 (18.7)	1	
(*n* = 80)	CT	45 (56.3)	0.625 (0.296-1.318)	0.217
	TT	20 (25.0)	0.697 (0.366-1.326)	0.271
T4	CC	11 (23.9)	1	
(*n* = 46)	CT	27 (58.7)	1.146 (0.435-3.018)	0.783
	TT	8 (17.4)	0.851 (0.402-1.805)	0.675
Metastasis, *n*(%)				
non-metastasis	CC	42 (21.4)	1	
(*n* = 196)	CT	108 (55.1)	0.761 (0.447-1.296)	0.314
	TT	46 (23.5)	0.813 (0.520-1.271)	0.363
metastasis	CC	12 (27.3)	1	
(*n* = 44)	CT	27 (61.3)	2.000 (0.670-5.972)	0.214
	TT	5 (11.4)	0.929 (0.447-1.930)	0.843

OR: odds ratio; CI: confidence interval

Stratified analysis of *PSCA* according to prostate cancer classification

The association between *PSCA* and prostate cancer was further evaluated by stratified analysis of age, GS, PSA, pathologic stage, and metastasis. As shown in ***Table 4***, age, GS, PSA, pathologic stage, and metastasis did not modify the association between *PSCA* and the risk of prostate cancer ($P > 0.05$ for each).

Discussion

In this study, we investigated the role of the genetic variation of *PSCA* rs2294008 in prostate cancer susceptibility. It was found that the variation of *PSCA* rs2294008 was not associated with prostate cancer.

PSCA was initially identified and isolated as a tumor antigen highly expressed in prostate cancer[8]. *PSCA* plays a role in signal transduction and cell-growth regulation[11]. *PSCA* is highly expressed in the prostate, bladder, and pancreatic carcinomas, but down-regulated in gastric, esophageal, and head-and-neck carcinomas[9,12–14]. Therefore, the role of *PSCA* in carcinogenesis remains still controversial.

The genetic polymorphism of *PSCA* (rs2294008) is associated with the increased incidence of gastric cancer in the Korean population[15]. Song *et al.*[15] have reported that the CT and TT genotypes are associated with a significantly increased risk of gastric cancer (OR = 1.5 and 1.71, respectively) compared to the CC genotype. However, Lu *et al.*[16] have demonstrated that rs2294008 variant genotypes (CC/CT) are not associated with increased risk of gastric cancer in the Chinese population. Thus, the genetic polymorphism of *PSCA* and its association with gastric cancer risk may differ among races. Similarly, in bladder cancer, Yates *et al.*[17] have suggested that genetic polymorphism on 8q24 is associated with an increased risk of bladder cancer, but not associated with disease aggressiveness. In addition, Fu *et al.*[18] have indicated that rs2294008 is important for bladder cancer susceptibility.

Analysis of 12 genotypes for *PSCA* revealed that the rs1045531 is significantly associated with an increased risk of prostate cancer and that CCCAGGTACGG and CGA haplotype carriers have a significant association with risk of prostate cancer[10]. To the best of our knowledge, there is only one study by Joung *et al.* [(10)] on the association between prostate cancer risk and genetic polymorphisms of *PSCA*.

There are some limitations in the present study. The sample size of our study is relatively small for identifying genetic susceptibility. We investigated only the *PSCA* gene rs2294008 among various *PSCA* genetic polymorphisms. Further large cohort studies are needed to confirm our results. Also, large cohort studies on genetic variants of *PSCA* in Western countries are required to identify the association between the risk of prostate cancer and SNP.

In conclusion, the genetic polymorphism of *PSCA* rs2294008 was not associated with the risk of prostate cancer. Our results suggest that rs2294008 may not play a role in the pathogenesis of prostate cancer. However, since *PSCA* as a transmembrane protein is associated with prostate cancer, additional studies on polymorphisms in the *PSCA* gene in addition to rs2294008 are needed to confirm the association between *PSCA* SNP and prostate cancer.

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