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Prostate stem cell antigen rs2294008 (C>T) polymorphism and bladder cancer risk: a meta-analysis based on cases and controls

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ABSTRACT. Several published articles have evaluated the association between the prostate stem cell antigen (PSCA) rs2294008 (C>T) polymorphism and bladder cancer risk, but the results remain inconclusive. In order to derive a more precise estimation of the association, we performed a meta-analysis of four case-control studies that included 9617 cases and 16,323 controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of the association. Our meta-analysis showed that, overall, the rs2294008 (C>T) polymorphism was associated with bladder cancer susceptibility (OR = 1.29, 95%CI = 1.20-1.40 for TT *vs* CC; OR = 1.24, 95%CI = 1.16-1.31 for CT *vs* CC; OR = 1.25, 95%CI = 1.18-1.33 for TT/CT *vs* CC; OR = 1.13, 95%CI = 1.06-1.20 for TT *vs* CT/CC). In the stratified analyses, the risk remained significant for studies of European populations, Asian populations, population-based studies, and hospital-based studies.

In conclusion, the results suggest that the PSCA rs2294008 (C>T) polymorphism is a risk factor for bladder cancer development.

Key words: PSCA; rs2294008 (C>T) polymorphism; Bladder cancer; Meta-analysis

INTRODUCTION

Bladder cancer is the most common malignancy of the urinary tract. It is the 7th most common cancer in men and the 17th in women, and the global world mortality rate is 4 per 100,000 men and 1.1 per 100,000 women (van Rhijn et al., 2009; Babjuk et al., 2011). Although several researchers have concluded that bladder cancer results from alterations in multiple environmental factors as well as genetic alterations, such as genetic polymorphisms (García-Closas et al., 2005), the precise molecular mechanisms of bladder cancer are still not entirely clear.

Among the known genetic alterations, the prostate stem cell antigen (PSCA) gene is up-regulated in bladder cancer and is thought to abet tumor progression (Saeki et al., 2010). Studies have shown that the PSCA gene maps to chromosome 8q24.2 and consists of 3 exons and 2 introns (Reiter et al., 1998). The PSCA gene encodes a glycosylphosphatidylinositol (GPI)-anchored membrane protein with an unknown biological function, and is also up-regulated in other tumors including prostate cancer, renal cell carcinoma, hydatidiform moles, and ovarian mucinous tumors (Reiter et al., 1998). As with other GPI-anchored proteins, it is thought that PSCA locates in a lipid raft (a special microdomain enriched in glycosphingolipids, cholesterol, and other lipidated proteins) on the outer surface of the cell membrane, and plays an important role in subcellular signal transduction (Sharom and Radeva, 2004). In addition, the most extensively studied single nucleotide polymorphism (SNP) in PSCA is rs2294008 (C>T). To date, several studies have reported a role of the PSCA rs2294008 (C>T) polymorphism in bladder cancer risk (Wu et al., 2009; Wang et al., 2010; Fu et al., 2012; Ma et al., 2013; Kohaar et al., 2013; Gakis and Stenzl, 2013), but the results remain inconclusive, partially because of the relatively small sample sizes in each of the published studies. Hence, we performed a meta-analysis on all eligible case-control studies involving 9617 cases and 16,323 controls to estimate the bladder cancer risk as well as to quantify the potential between-study heterogeneity.

MATERIAL AND METHODS

Publication search

The meta-analysis was performed as described previously (Gu et al., 2010). PubMed was searched (updated to May 21, 2013) using the search terms “PSCA” or “rs2294008”, “genetic variant” or “polymorphism”, “bladder cancer” or “carcinoma of bladder”. The search was limited to English language papers. We also manually searched the reference lists of original studies or reviewed articles on this topic to identify additional studies, and all of the searched studies were retrieved. For overlapping and republished studies, we selected the most recently published articles with the largest number of subjects. Studies included in our meta-analysis had to meet the following inclusion criteria: a) use of a case-control design and more than 50 samples, b) evaluation of the PSCA rs2294008 (C>T) polymorphism and bladder

cancer risk, and c) available genotype frequency data for both patient and control populations. As a result, four eligible case-control studies were included in the meta-analysis.

Data extraction

Information was carefully extracted from all eligible publications independently by two of the authors. Agreement was reached after discussion in cases of conflicting evaluations. The following data were extracted from each study: the first author's name, year of publication, country of origin, ethnicity, source of control groups (mixed-, population-, or hospital-based controls), genotyping method, and number of genotyped cases and controls. Subjects of different ethnic descent were categorized as Asian and European. For studies including subjects of different ethnic groups, data were extracted separately for each ethnic group whenever possible.

Statistical analysis

Crude odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of the association between the PSCA rs2294008 (C>T) polymorphism and bladder cancer risk. The ORs were pooled for the co-dominant model (TT *vs* CC, CT *vs* CC), dominant model (TT/CT *vs* CC), and recessive model (TT *vs* CT/CC). Between-study heterogeneity was evaluated by the *Q*-test (Handoll, 2006). The fixed-effect model (Mantel-Haenszel method) was used to calculate the summary OR estimate of each study when the P value of the *Q*-test was >0.05 (Mantel and Haenszel, 1959). Otherwise, the random-effect model (DerSimonian and Laird method) was selected (DerSimonian and Laird, 1986). Stratified analyses were also performed by ethnicity and source of controls. Sensitivity analyses were performed to assess the stability of the results, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual dataset to the pooled OR. In addition, both a funnel plot and the Egger test were used to assess the potential publication bias (Egger et al., 1997). All statistical analyses were performed in the Statistical Analysis System software (version 11.0; StataCorp. LP; College Station, TX, USA), and all tests were two-sided.

RESULTS

Characteristics of studies

A total of 4 eligible studies that met the inclusion criteria involving 9617 cases and 16,323 controls were included in this meta-analysis (Wu et al., 2009; Wang et al., 2010; Fu et al., 2012; Ma et al., 2013). The main characteristics of these studies are shown in Table 1. These eligible publications included studies from China, Finland, USA, Spain, England, and other European (multi-country) populations. All studies were case-control studies. Bladder cancers were confirmed histologically or pathologically. In addition, controls were matched by gender and age in most studies, 6 of which were population-based, 2 were hospital-based, and 1 was mixed.

Main results

The main results of this meta-analysis and of the heterogeneity tests are summarized

in Table 2. Significant associations were observed between the rs2294008 (C>T) polymorphism and risk of bladder cancer in the overall population. In addition, significant effects were observed in each model (OR = 1.29, 95%CI = 1.20-1.40, $P_{heterogeneity}$ = 0.594 for TT *vs* CC; OR = 1.24, 95%CI = 1.16-1.31, $P_{heterogeneity}$ = 0.677 for CT *vs* CC; OR = 1.25, 95%CI = 1.18-1.33, $P_{heterogeneity}$ = 0.659 for TT/CT *vs* CC; OR = 1.13, 95%CI = 1.06-1.20, $P_{heterogeneity}$ = 0.611 for TT *vs* CT/CC). Furthermore, in the stratified analysis, there were significantly increased risks among studies with population-based controls (OR = 1.27, 95%CI = 1.10-1.45, $P_{heterogeneity}$ = 0.907 for TT *vs* CC; OR = 1.27, 95%CI = 1.14-1.43, $P_{heterogeneity}$ = 0.798 for CT *vs* CC; OR = 1.27, 95%CI = 1.15-1.42, $P_{heterogeneity}$ = 0.913 for TT/CT *vs* CC) and hospital-based controls (OR = 1.17, 95%CI = 1.01-1.36, $P_{heterogeneity}$ = 0.106 for CT *vs* CC; OR = 1.16, 95%CI = 1.00-1.34, $P_{heterogeneity}$ = 0.079 for TT/CT *vs* CC). In the subgroup analysis by ethnicity, statistically significant increased risks were found among Europeans (OR = 1.29, 95%CI = 1.20-1.39, $P_{heterogeneity}$ = 0.392 for TT *vs* CC; OR = 1.22, 95%CI = 1.15-1.30, $P_{heterogeneity}$ = 0.680 for CT *vs* CC; OR = 1.24, 95%CI = 1.17-1.32, $P_{heterogeneity}$ = 0.567 for TT/CC *vs* CC; OR = 1.13, 95%CI = 1.06-1.21, $P_{heterogeneity}$ = 0.420 for TT *vs* CT/CC) and Asians (OR = 1.40, 95%CI = 1.15-1.70, $P_{heterogeneity}$ = 0.764 for CT *vs* CC; OR = 1.38, 95%CI = 1.14-1.66, $P_{heterogeneity}$ = 0.875 for TT/CT *vs* CC).

Table 1. Characteristics of literatures included in the meta-analysis.

Author	Year	Country	Ethnicity	Sounce of controls	Genotyping method	Cases			Controls		
						CC	CT	TT	CC	CT	TT
Wu	2009	Multi-country	European	Mixed	Multi-method	1288	2613	1137	2842	4668	1853
Wang	2010	China	Asian	Hospital	PCR-RFLP	272	259	50	316	220	44
Fu	2012	Finland	European	Population	Illumina Human Hap 610-Quad	71	227	103	163	370	171
		USA	European	Population	Illumina Human Hap 610-Quad	162	365	158	209	363	154
		USA	European	Population	Illumina Human Hap 610-Quad	165	325	139	223	369	167
		USA	European	Population	Illumina Human Hap 610-Quad	170	364	157	543	910	406
		Spain	European	Hospital	Human 1M-Duo	315	572	216	308	529	210
		England	European	Population	TaqMan	80	154	80	92	169	62
Ma	2013	China	Asian	Population	MALDI-TOF	84	80	11	543	355	64

Table 2. Meta-analysis of the PSCA rs2294008 (C>T) polymorphism on bladder cancer.

Variables	N[a]	TT *vs* CC		CT *vs* CC		TT/CT *vs* CC (dominant)		TT *vs* CT/CC (recessive)	
		OR (95%CI)	P[b]	OR (95%CI)	P[b]	OR (95%CI)	P[b]	OR (95%CI)	P[b]
Total	9	1.29 (1.20-1.40)	0.594	1.24 (1.16-1.31)	0.677	1.25 (1.18-1.33)	0.659	1.13 (1.06-1.20)	0.611
Ethnicities									
European	7	1.29 (1.20-1.39)	0.392	1.22 (1.15-1.30)	0.680	1.24 (1.17-1.32)	0.567	1.13 (1.06-1.21)	0.420
Asian	2	1.26 (0.87-1.81)	0.676	1.40 (1.15-1.70)	0.764	1.38 (1.14-1.66)	0.875	1.08 (0.76-1.54)	0.621
Source of controls									
Population-based	6	1.27 (1.10-1.45)	0.907	1.27 (1.14-1.43)	0.798	1.27 (1.15-1.42)	0.913	1.09 (0.97-1.22)	0.728
Hospital-based	2	1.07 (0.87-1.33)	0.287	1.17 (1.01-1.36)	0.106	1.16 (1.00-1.34)	0.079	1.00 (0.83-1.21)	0.489

*Random-effects model was used when P value for heterogeneity test <0.05; otherwise, fixed-effect model was used. [a]Number of comparisons; [b]P value of the *Q*-test for heterogeneity test.

Sensitivity analysis

A single study involved in the meta-analysis was deleted each time to evaluate the influence of individual datasets to the pooled ORs, and no single study was found to influence

the pooled ORs qualitatively as indicated by sensitivity analyses (data not shown), suggesting that the results of this meta-analysis are stable.

Publication bias

Both Begg's funnel plot and the Egger test were performed to assess the publication bias of the literature. The shape of the funnel plots did not reveal any evidence of obvious asymmetry under all compared models. Then, the Egger test was used to provide statistical evidence of funnel plot symmetry. The results still did not show any evidence of publication bias (P > 0.05 for TT/CT *vs* CC; Figure 1).

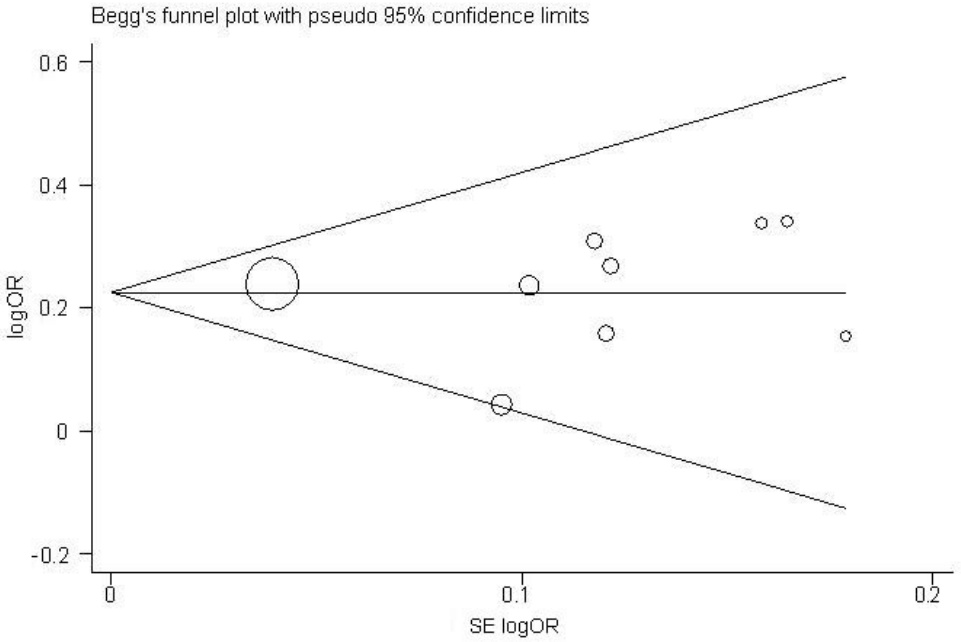

Figure 1. Begg's funnel plot with pseudo 95% confidence limits of publication bias test for the PSCA rs2294008 (C>T) polymorphism (TT/CT *vs* CC). Each point represents a separate study for the indicated association. Log[OR] = natural logarithm of odds ratio. Horizontal line = mean effect size.

DISCUSSION

The present meta-analysis, including 9617 cases and 16,323 controls from four case-control studies, explored the association between the PSCA rs2294008 (C>T) polymorphism and bladder cancer risk. The results indicated that variant genotypes of the PSCA rs2294008 (C>T) polymorphism were associated with an increased risk of bladder cancer. Given the important roles of PSCA in the regulation of cell proliferation, it is biologically plausible that genetic variations of the PSCA rs2294008 (C>T) polymorphisms may modulate the risk of bladder cancer.

Previous studies have shown that PSCA was initially identified as a prostate-specific cell-surface antigen (Argani et al., 2001), and that it is highly expressed in prostate cancer and other non-prostatic malignancies, such as gastric cancer, pancreatic cancer, and bladder cancer (Elsamman et al., 2006; Grubbs et al., 2006). Studies have also reported the differential expression of PSCA in these tumors, and significant discrepancies among its genetic polymorphisms (Elsamman et al., 2006; Feng et al., 2008). Among these variants, rs2294008 (C>T) is the most widely studied. Recently, many studies were conducted to investigate the associations between the PSCA rs2294008 (C>T) polymorphism and bladder cancer risk across different countries. However, the results remain inconclusive. In this meta-analysis, our results suggest that the PSCA rs2294008 (C>T) polymorphism is significantly associated with increased risk of bladder cancer in both the overall population and in subgroups.

In the subgroup analysis by ethnicity, there was a significant association detected in both Europeans and Asians, indicating that the PSCA rs2294008 (C>T) polymorphism is significantly associated with increased risk of bladder cancer in different ethnicities, and that differences in the genetic backgrounds and environment do not play a role in the association (Hirschhorn et al., 2002). In addition, a similar result was observed in the stratified analysis by population-based and hospital-based controls, suggesting that the different source of controls did not influence the association.

To some extent, some limitations may have affected the objectivity of the conclusions and should be considered when interpreting the results. First, publication bias may have occurred even though the statistical analysis of the data did not show this effect, because the eligible studies included in the meta-analysis were only those that have been published. Second, the overall outcomes were based on individual unadjusted ORs, while a more precise evaluation should be adjusted by other potentially suspected factors (i.e., age, smoking, and environmental factors) if such information is widely available. Third, the PSCA gene contains many more polymorphisms than the rs2294008 (C>T) polymorphism evaluated in this meta-analysis. Given the limited evidence available on other PSCA polymorphisms, this meta-analysis was restricted to the most extensively studied rs2294008 (C>T) polymorphism. Fourth, meta-analysis remains a retrospective form of research that is subject to methodological deficiencies. In spite of these limitations, the meta-analysis also had some advantages. First, although the number of studies in the meta-analysis was relatively small, the number of total cases and controls was substantial, which significantly increased the statistical power of the analysis. Second, in this meta-analysis, the quality of case-control studies was satisfactory according to our selection criteria. Third, the results may be unbiased as no publication biases were detected.

In conclusion, this meta-analysis suggests that the PSCA rs2294008 (C>T) polymorphism may contribute to genetic susceptibility of bladder cancer. The PSCA rs2294008 (C>T) polymorphism may be an independent risk factor for bladder cancer. Further studies with more detailed individual data including a wider spectrum of subjects should be carried out to investigate the association between PSCA polymorphisms and bladder cancer risk in combination with other potential bladder cancer risks.

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