

RESEARCH ARTICLE

8q24 rs4242382 Polymorphism is a Risk Factor for Prostate Cancer among Multi-Ethnic Populations: Evidence from Clinical Detection in China and a Meta-analysis

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Abstract

Background: Evidence supporting an association between the 8q24 rs4242382-A polymorphism and prostate cancer (PCa) risk has been reported in North American and Europe populations, though data from Asian populations remain limited. We therefore investigated this association by clinical detection in China, and meta-analysis in Asian, Caucasian and African-American populations. **Materials and Methods:** Blood samples and clinical information were collected from ethnically Chinese men from Northern China with histologically-confirmed PCa (n=335) and from age-matched normal controls (n=347). The 8q24 (rs4242382) gene polymorphism was genotyped by polymerase chain reaction-high-resolution melting analysis. We initially analyzed the associations between the risk allele and PCa and clinical covariates. A meta-analysis was then performed using genotyping data from a total of 1,793 PCa cases and 1,864 controls from our study and previously published studies in American and European populations, to determine the association between PCa and risk genotype. **Results:** The incidence of the risk allele was higher in PCa cases than controls (0.222 vs 0.140, $P=7.3 \times 10^{-5}$), suggesting that the 8q24 rs4242382-A polymorphism was associated with PCa risk in Chinese men. The genotypes in subjects were in accordance with a dominant genetic model (OR_{adj}=2.03, 95% CI: 1.42-2.91, P_{adj}= 1.1×10^{-4}). Presence of the risk allele rs4242382-A at 8q24 was also associated with clinical covariates including age at diagnosis ≥ 65 years, prostate specific antigen >10 ng/ml, Gleason score <8, tumor stage and aggressive PCa, compared with the non-risk genotype ($P=4.6 \times 10^{-5}$ - 3.0×10^{-2}). Meta-analysis confirmed the association between 8q24 rs4242382-A polymorphism and PCa risk (OR=1.62, 95% CI: 1.39-1.88, $P=1.0 \times 10^{-5}$) across Asian, Caucasian and African American populations. **Conclusions:** The replicated data suggest that the 8q24 rs4242382-A variation might be associated with increased PCa susceptibility in Asian, Caucasian and African American populations. These results imply that this polymorphism may be a useful risk biomarker for PCa in multi-ethnic populations.

Keywords: 8q24 rs4242382-A - prostate cancer - susceptibility - meta-analysis - multi-ethnic populations

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Introduction

The prevalence of prostate cancer (PCa) and the frequencies of risk alleles differ across populations, and it is therefore important for understanding the effects of these genetic polymorphisms in people of different ethnic backgrounds. To date, 25 genome-wide association studies (GWASs) have revealed about 180 single nucleotide

polymorphisms (SNPs) associated with PCa risk in various populations worldwide, in addition to SNPs identified in other studies.

Chromosome 8q24 is an established risk locus for many common epithelial cancers and 11 of the 25 GWASs identified a significant association between chromosome 8q24 regions and PCa risk. Numerous genetic studies have confirmed associations between 8q24 variants and

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susceptibility to PCa in European and African populations. (Gudmundsson, 2007; Murphy, 2012) Asian studies, however, have been restricted to one Japanese GWAS, one Chinese replication study (Liu et al., 2011) and several small-scale studies in Japanese, Taiwanese and southern Chinese populations (Terada, 2008; M et al., 2009; Liu, 2009; M et al., 2010; Takata, 2010; Liu, 2010). To date, GWASs have revealed 16 loci on chromosomal band 8q24 associated with PCa risk in various populations worldwide (Amundadottir et al., 2006; Yeager et al., 2007; Christopher et al., 2007; Schumacher et al., 2007; Robbins, 2007; Gudmundsson, 2007; Terada, 2008; Gilles Thomas et al., 2008; Salinas, 2008; Tan, 2008; Eeles, 2008; Eeles et al., 2009; Al Olama, 2009; Gudmundsson, 2009; Liu, 2009; M et al., 2009; 2010; Takata, 2010; Liu, 2010; Liu et al., 2011; Murphy, 2012). Enhancer elements located in the 8q24 regions associated with susceptibility to PCa have been reported to increase Myc promoter activity (Sotelo et al., 2010). Among these genetic variations, the 8q24 rs4242382-A polymorphism (Ahn et al., 2011) was significantly associated with PCa risk in North American and European populations, though data for Asian populations are still rare. We hypothesize the SNP rs4242382 in Chinese population may behave differently from in other ethnicity and region. If not, it may be because of undergoing similar mechanism of regulation of nearby genes.

To determine whether the risk loci also affect PCa risk in Asian men, where PCa risk is much lower but is rising steadily, we evaluated their relationship with PCa risk in a population-based study conducted in North China, where air pollution is heavy. We also explored the relationship between PCa clinical covariates and risk variants in northern Chinese men to clarify the susceptible genetic polymorphism as a potential marker. We therefore investigated the association between the rs4242382-A variant at 8q24 and PCa in Chinese men, and compared our data with previous results from other populations by meta-analysis, to clarify the significance of the locus in multi-ethnic populations.

Materials and Methods

Study population

A total of 335 men with PCa and 347 matched normal controls from Northern China were enrolled in the study. All cases were diagnosed with histologically confirmed PCa at the Department of Urology, Beijing Hospital, Ministry of Health; Tianjin Urology Institute, Second Hospital of Tianjin Medical University; General Hospital of Ningxia Medical University; and First Hospital of China Medical University between January 1, 2000 and December 1, 2012. A few cases were outpatients who attended after undergoing surgery for PCa elsewhere. Clinical data, including age at diagnosis, Gleason score, tumor stage and serum prostate specific antigen (PSA) levels, were obtained by medical record review. Patients with PSA >20 ng/ml, Gleason score \geq 8, and/or pathological stage III or higher were defined as having aggressive PCa. Controls were local residents participating in routine physical examinations and were frequency-

matched to cancer cases by age. Men with PSA <4.0 ng/ml, negative digital rectal examination, and no family history of PCa were included in the control group.

This study was approved by the ethics committees at the four participating hospitals and informed consent was obtained from all study subjects.

A meta-analysis of the association between PCa risk and genotype was performed on data from 1793 PCa cases and 1864 controls derived from the current study population and from populations in previously published studies in American and European populations.

SNP genotyping

Only subjects with sufficient DNA available were included in the SNP rs4242382 (8q24) genotyping study. In total, 335 PCa cases and 347 controls were included. All subjects were men of unrelated Northern Han Chinese ancestry. We created a Consolidated Standards of Reporting Trials diagram to demonstrate the flow of participants through each stage of the study.

Experimental methods

DNA was extracted from peripheral blood samples (0.5 ml) using a whole genome DNA extraction kit (Biochain Science-Technology, Beijing, People's Republic of China). The concentration and purity of the extracted DNA were assayed by NanoDrop 2000c. According to the measured results, DNA samples were diluted to working solutions of 20 ng/ μ l.

Polymerase chain reaction (PCR) was performed using a Bio-Rad C1000TM Thermal Cycler under conditions including initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, annealing for 30 s, extension at 72°C for 6 s and completion at 72°C for 7 min. After two cycles at 94°C for 30 s and at 25°C for 2 min, PCR products were transferred into high-resolution melt (HRM)-specific 96-well plates, genotyped automatically and verified manually using a LightScanner[®] TMHR-I96.

Five samples randomly selected from individuals of each genotype were sequenced for verification. The PCR procedure involved initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, annealing for 30 s, extension at 72°C for 15 s and completion at 72°C for 7 min. PCR products were subjected to electrophoresis on 8% polyacrylamide gels, visualized with a gel imaging system, and sequenced by Beijing Tianyi Huiyuan Bioscience and Technology Inc.

Statistical analysis

Pearson's χ^2 test was used to test the Hardy-Weinberg equilibrium (HWE) of the SNP rs4242382 (8q24) in control subjects. Risk allele (1) versus non-risk allele (2) was evaluated by odds ratio (OR) and 95% confidence intervals (95%CI). ORs and 95%CIs were calculated in dominant (11+12 vs 22) and recessive models (11 vs 12+22) to compare genotype frequencies between PCa cases and controls using Pearson's χ^2 or Fisher's exact test. The risk locus confirmed in our study was evaluated for its associations with clinical covariates in cases and controls. Statistical analysis was performed using SPSS version 17.0 with *P* values <0.05 considered significant. Pooled

ORs were used as the metric of choice. Heterogeneity between studies was assessed by the χ -based Q statistic and confirmed as significant if $p < 0.10$. Pooled ORs were calculated by the fixed-effects model (Mantel-Haenszel) with no heterogeneity among studies. I^2 was also calculated to represent the percentage of total variation across studies, with values $< 25\%$ considered 'low', about 50% considered 'moderate' and $> 75\%$ considered 'high'. Larger values indicated increasing heterogeneity. The meta-analysis was carried out using Review Manager 5.2.

Results

Baseline clinical characteristics

Table 1 shows the demographic characteristics of study participants. The mean ages (\pm SD) of cases and controls were 66.9 ± 10.86 years (range 35-88) and 72.0 ± 9.14 years (range 39-90), respectively. PSA levels in cases and controls were 58.00 ± 143.10 ng/ml (range 0.05-1,329) and 1.04 ± 0.63 ng/ml (range 0-10.93), respectively. Gleason scores ranged from 4 to 7 in 69.8% of patients and from 8 to 10 in 30.2%. Tumor stages were I, II, III and IV in 8.3%, 50.5%, 33.0% and 8.3% of patients, respectively. PCa was

Table 1. Demographic Characteristics of Study Subjects

Characteristic	Cases	Controls	P
Number of subjects	335	347	
Age (years) (mean [SD])	66.9 (10.86)	72.0 (9.14)	1.85×10^{-6}
Range (years)	35-88	39-90	
Body mass index (BMI, kg/m ²)	128	-	
Underweight (<18.5)	2	-	
Normal (18.5-22.9)	43	-	
Overweight (23-27.5)	72	-	
Obese (>27.5)	10	-	
PSA ng/ml (mean [SD])	58.00 (143.10)	1.04 (0.63)	
Range	0-1329	0-10.93	
<10	88	-	
10-20	37	-	
>20	111	-	
Gleason score	149	-	
<8	104	-	
≥ 8	45	-	
Tumor stage	109	-	
I	9	-	
II	55	-	
III	36	-	
IV	9	-	
Aggressiveness	140	-	
Non-aggressive PCa	37	-	
Aggressive PCa	103	-	

*Aggressive PCa: PSA >20 ng/ml, and/or clinical stage \geq III, and/or a Gleason score ≥ 8

considered aggressive in 73.6% of patients. Missing data were not included when calculating the proportions. None of the 347 controls had a family history of PCa.

PCa-associated allele in a Chinese population

The rs4242382 A/G locus is located at chromosome 8q24, in an intergenic region. Its minor allele frequency is 0.16 in the Beijing Chinese Han population. Analysis of its allelic frequency in 335 PCa cases and 347 controls showed that the 8q24 rs4242382 risk allele (A) was significantly associated with PCa risk (OR=1.76, 95%CI: 1.33-2.42, $P=7.3 \times 10^{-5}$) (Table 2).

PCa-associated genotype in a Chinese population

8q24 rs4242382 genotyping was carried out in 682 subjects. The 8q24 rs4242382 genotype frequency in controls conformed to the HWE ($P > 0.05$). Genotype distribution analysis showed that rs4242382 was associated with PCa risk ($P=3.1 \times 10^{-4}$). There was a 2.03-fold increase in PCa risk in the dominant model (95%CI: 1.42-2.91, $P_{adj}=1.1 \times 10^{-4}$) (Table 2), but no association in the recessive model ($P_{adj}=0.058$). Sequence results were consistent with the genotypes of variants identified by HRM curves (Figure 1).

PCa covariate-associated genotype in a Chinese population

The 8q24 rs4242382-A polymorphism was associated with ages at diagnosis 65-74 years (OR=2.13, 95%CI:1.47-3.29) and > 75 years (OR=1.70, 95%CI:1.15-2.65), PSA 10-20 ng/ml (OR=1.99, 95%CI:1.15-3.69) and > 20 ng/ml (OR=1.66, 95%CI:1.12-2.58), Gleason score < 8 (OR=1.80, 95%CI:1.22-2.80), disease stage $< III$ (OR=1.96, 95%CI:1.24-3.35) and stage $\geq III$ (OR=1.81, 95%CI:1.05-3.30), aggressive PCa (OR=2.13, 95%CI:1.21-4.06) and non-aggressive PCa (OR=1.64, 95%CI:1.09-2.56) (Table 3).

PCa-associated genotype meta-analysis in different populations

We compared our data with data from published populations of different ethnicities by meta-analysis. Six previous articles reported an association between 8q24 rs4242382-A and PCa risk, but only two of them provided genotype data that met our inclusion criterion for meta-analysis. Table 4 shows that 8q24 rs4242382-A was significantly associated with PCa in Caucasian and African Americans, Caucasians (Serbia), and East Asians (Chinese). 8q24 rs4242382-A was significantly associated

Table 2. Association between rs4242382 Alleles and Genotypes and Risk of PCa

Groups		Cases(%)	Controls(%)	OR ^a	Unadjusted 95%CI ^b	P	Age-adjusted		
							OR	95%CI	P
Genotypes	total	335	347			3.1×10^{-4}			
	AA	14(4.2)	6(1.7)	1.90 ^c	1.37-3.05	9.5×10^{-5}	2.03	1.42-2.91	1.1×10^{-4}
	AG	121(36.1)	85(24.5)						
	GG	200(59.7)	256(73.8)						
Alleles	total	670	694	1.76	1.33-2.42	7.3×10^{-5}			
	A	149(22.2)	97(14.0)						
	G	521(77.8)	597(86.0)						

^aOR=odds ratio; ^bCI=confidence interval; ^c dominant model: AA+AG vs GG

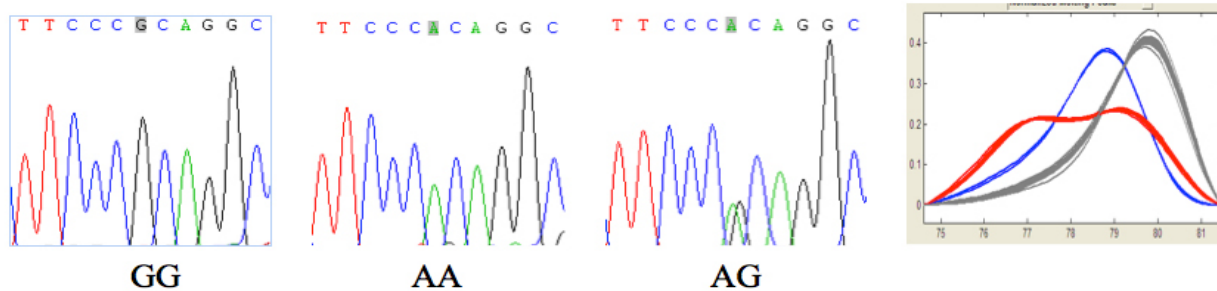


Figure 1. Sequencing Results Consistent with HRM Curves

Table 3. Genotypic Distributions of rs4242382 According to Clinical Covariates and Odds Ratios of Risk Allele Relative to Non-Risk Allele

Groups	Total	AA	AG	GG	A	G	Case-control P	OR(95%CI)	Case-only P
Controls	347	6	85	256	97	597		1.00 (Ref)	
PCa cases	335	14	121	200	149	521			
Age at diagnosis	258	14	93	151	121	395			
<65	41	0	18	23	18	64	0.055	1.73(0.98-3.21)	0.577
65-74	113	7	44	62	58	168	4.6×10 ⁻⁵	2.13(1.47-3.29)	
≥75	104	7	31	66	45	163	0.008	1.70(1.15-2.65)	
PSA (ng/ml)	226	10	78	138	98	354			
<10	79	4	25	50	33	125	0.125	1.43(0.90-2.33)	0.803
10-20	41	1	18	22	20	62	0.013	1.99(1.15-3.69)	
>20	106	5	35	66	45	167	0.011	1.66(1.12-2.58)	
Gleason	146	6	53	87	65	227			
<8	104	5	37	62	47	161	0.003	1.80(1.22-2.80)	0.828
≥8	42	1	16	25	18	66	0.069	1.68(0.96-3.09)	
Tumor stage	106	4	42	60	50	162			
<III	62	3	24	35	30	94	0.004	1.96(1.24-3.35)	0.804
≥III	44	1	18	25	20	68	0.03	1.81(1.05-3.30)	
Aggressiveness	137	5	51	81	61	213			
Aggressive PCa	37	3	13	21	19	55	0.008	2.13(1.21-4.06)	0.409
Non-aggressive PCa	100	2	38	60	42	158	0.016	1.64(1.09-2.56)	

with PCa in the three populations combined (OR=1.62, 95%CI: 1.39-1.88, $P=1.0 \times 10^{-5}$). Heterogeneity testing showed that the distribution of the three populations was homogeneous ($\chi^2=1.84$, $df=2$, $p=0.40$; $I^2=0\%$). The forest plot is shown in Figure 2.

Discussion

PCa-risk variants have been identified in different populations. Ethnic and regional differences, as well as other complex factors such as gene-environment or gene-gene interactions, may contribute to the observed heterogeneity in the results. Crawford established age, African ancestry, and a positive family history of disease as risk factors for PCa, (Crawford, 2003) and twin studies and epidemiologic observations have suggested the existence of a substantial genetic contribution to disease risk (Steinberg GD, 1990). People living in different area are influenced by variant environment, and Chinese bear heavier pollution, i.e. global satellite-derived PM2.5 in Eastern Asia, averaged over 2001-2006, is the highest (van Donkelaar et al., 2010). The prevalence of PCa and the allele frequencies differ among populations and area, and it is important to understand the effects of ethnicity and region on these markers. Not all PCa risk loci identified

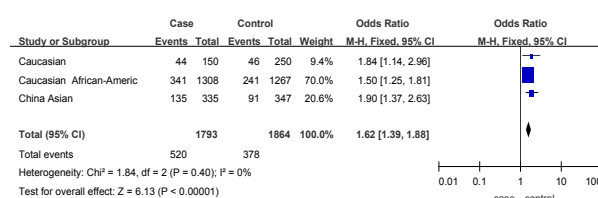


Figure 2. Meta-analysis of Association between PCa Risk and 8q24 rs4242382-A Polymorphism

in western populations were found to be also sensitive in Chinese. (Zhang et al., 2012; Wang et al., 2013; Zhang et al., 2014)

Among the genomic alterations associated with the development and progression of PCa, those affecting the 8q24 region are of particular interest. The region at 8q24 is known to harbor several risk variants associated with inherited susceptibility to PCa, with some variants linked to a more aggressive phenotype (Yeager et al., 2007; Cussenot et al., 2008; Jia et al., 2009; M et al., 2009). Risk alleles have been identified for some of the most frequent human carcinomas, including those of the prostate, colon and breast (Ghoussaini et al., 2008; Ahn et al., 2011). Many PCa risk variants at 8q24 have been identified and investigated in European and American populations, but few similar studies have been performed

Table 4. Combined Results from three Populations

First Author	Journal	Publication Year	Study population	Genotyping method	No. of cases	No. of controls	Cases			Controls			OR (95%CI)	P
							GG	GA	AA	GG	GA	AA		
Liesel M	Clin Cancer Res.	2009.5	Caucasian and African American	SNPlex	1308	1267	967	315	26	1026	228	13	1.39(1.18-1.66)	1.3×10 ⁻⁴
Ana S	Pathol. Oncol. Res.	2013.2	Caucasian (Serbia)	genotyping PCR-RFLP	150	100	106	42	2	80	20	0	1.58(0.86-2.91)	0.11
Zhao CX		2013	East Asian (Chinese) total	HRM-PCR & sequencing	335	347	200	121	14	256	85	6	1.98(1.14-3.43)	0.014
					1793	1864	1273	478	42	1406	338	20	1.76(1.33-2.42)	7.3×10 ⁻⁵

in Asian populations.

8q24 is often called a 'desert' because of its paucity of genes. The closest cancer-associated gene, the proto-oncogene *Myc*, which is a well known target of the WNT signaling pathway, is located about 263 kb telomeric to rs1447295 in region 1 and 624 kb telomeric to rs16901979 in region 2. Multiple enhancer elements are present within the genetically identified cancer-associated regions on chromosome 8q24, and these are able to regulate the transcription of *Myc*. Sotelo et al. demonstrated the presence of enhancer elements within the cancer-associated regions on chromosome 8q24, and showed that these elements were able to regulate *Myc* promoter activity in a reporter assay (Gudmundsson, 2009).

Several studies have reported strong associations between cancers with high Gleason scores and rs1447295 (Amundadottir et al., 2006; Suuriniemi et al., 2007; Wang et al., 2007; Zheng et al., 2007; Cussenot et al., 2008), which is in high linkage disequilibrium with rs4242382 ($r^2=1.0$ in HapMap CEU samples) (Yeager et al., 2008). rs4242382 is more closely adjacent to *Myc* than rs1447295, with a distance of 230 kb. Jia and colleagues reported that rs11986220, which is strongly linked to rs4242382 at 8q24, localized with embedded regulatory enhancers, potentially influencing binding activity of FoxA1 and androgen responsiveness (Zheng et al., 2007). The mechanisms responsible for this association are unclear and may involve a cis-regulatory enhancer element for *Myc*, or another locus on a distinct chromosome.

rs16901966, rs1447295, rs11986220 and rs10090154 at 8q24 (region 1, region 2) have been shown to be associated with PCa in Northern Chinese men (Liu et al., 2012). In the current population-based study of PCa, we systematically evaluated the associations between the 8q24 rs4242382-A gene variation and PCa and PCa clinical phenotypes in Northern Chinese men and in populations of other descents. The 8q24 rs4242382-A polymorphism was associated with PCa in Asian Chinese according to different genetic models. Furthermore, the results of our meta-analysis suggested that 8q24 rs4242382-A was significantly positively associated with PCa in Asian, Caucasian and African American populations, as well as in the three populations combined, although there were plenty of genetic differences among the three populations being reported. We therefore conclude that the 8q24 rs4242382-A polymorphism might represent a suitable multi-ethnic biomarker for PCa.

In the meta-analysis, the study by Ana included 100 normal controls and 150 patients with benign prostatic hyperplasia (BPH) patients as additional controls, with *P* values of 0.11 and 0.014, respectively. This study analyzed three models, which used 100 normal controls, 150 BPH controls, and all 250 men combined as controls, respectively. There were significant results for each model, and no heterogeneity in any model, and we therefore used the combined set of 250 controls in our meta-analysis.

We investigated associations between risk genotypes and several clinical PCa covariates. Men older than 65 years who carried the A allele of rs4242382 were at increased risk of PCa relative to controls, confirming that PCa is an age-related disease. Our study also indicated that the risk allele 8q24 rs4242382-A was associated with higher PSA level, higher disease stage, aggressive PCa and non-aggressive PCa. However, different studies have failed to produce consistent results. (Amundadottir et al., 2006; Gudmundsson, 2007; Terada, 2008; Liu M, 2009; Liu, 2010; Takata, 2010; Zeegers; 2011, Liu et al., 2011) This may be because genetic background and environmental factors such as food, lifestyle, and air pollution can modify the risk factors in different populations.

Further studies with larger sample sizes in Asian and other ethnic populations are needed to confirm the current findings and to evaluate additional genetic variants, especially those with modest effects. The identification of variants associated with PCa will improve our understanding of the disease etiology, and may have potential implications for its early detection, diagnosis, and treatment in clinical practice.

In conclusion, we detected a significant association between the 8q24 rs4242382 SNP and the risk of PCa in Chinese men. Meta-analysis suggested that the variation might also be associated with increased PCa susceptibility across Asian, Caucasian and African American populations. Our results therefore demonstrated that the 8q24 rs4242382-A polymorphism may be a suitable risk biomarker for PCa in multi-ethnic populations.

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