RESEARCH ARTICLE

Association of 8 Loci on Chromosome 8q24 with Prostate Carcinoma Risk in Northern Chinese Men

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Abstract

Multiple genetic studies have confirmed association of 8q24 variants with susceptibility to prostate cancer (PCa). As PCa risk SNPs may also influence disease outcome, we studied here eight 8q24 risk alleles, and evaluated their role in PCa clinical covariates in northern Chinese men. Blood samples and clinical information were collected from ethnically Chinese men from Northern China with histologically-confirmed PCa (n=289) and from age-matched normal controls (n=288). Eight 8q24 SNPs were genotyped by polymerase chain reaction-high-resolution melting analysis in 577 subjects. We examined the prevalence distribution of 8q24 risk alleles and analyzed the associations between the risk allele and PCa and clinical covariates to infer their impact on aggressive PCa. Three of the eight SNPs were associated with PCa risk in northern Chinese men, including rs16901966 (OR 1.31, 95% CI 1.01-1.70, *p*=0.042), rs1447295 (OR 1.47, 95% CI 1.09-1.98, *p*=0.011) and rs10090154 (OR 1.55, 95% CI 1.14-2.12, *p*=0.005). Haplotype analysis based association with the risk alleles revealed significant differences between cases and controls (OR 1.43, 95%CI 0.99-2.06, *p*=0.049). The risk alleles rs16901966, rs1447295 and rs10090154 were associated with age at diagnosis and tumor stage as compared with controls, while rs16901966 was associated with aggressive PCa (OR 1.43, 95% CI 1.01-2.03, *p*=0.042). The evidence for 8q24 SNPs with PCa risk in northern Chinese men showed rs16901966, rs1447295 and rs10090154 at 8q24 (region 1, region 2) to be strongly associated with PCa and clinical covariates. The three SNPs at 8q24 could be PCa susceptible genetic markers in northern Chinese men.

Keywords: Prostate cancer - association - northern Chinese - 8q24

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Introduction

Chromosome 8q24 is an established risk locus for many common epithelial cancers and 11 of the 25 genome-wide association studies (GWASs) identified significant associations between chromosome 8q24 regions and prostate cancer (PCa) risk. Multiple genetic studies have confirmed the associations of 8q24 variants with susceptibility to PCa in European and African populations (Gudmundsson et al., 2007; Adam et al., 2012). Asian studies have been restricted to a Japanese GWAS, one Chinese replication study (Liu et al., 2010) and several small scale studies in Japanese, Taiwanese and southern Chinese populations (Terada et al., 2008; Chen et al., 2009; Liu et al., 2009; Chen et al., 2010; Takata et al., 2010; Zheng et al., 2010).

To date, GWAS have revealed 16 loci on chromosomal band 8q24 associated with PCa risk in various populations worldwide (Amundadottir et al., 2006; Gudmundsson et al., 2007; Haiman et al., 2007; Robbins et al., 2007; Schumacher et al., 2007; Yeager et al., 2007; Eeles et al., 2008; Salinas et al., 2008; Tan et al., 2008; Terada et al., 2008; Terada et al., 2008; Thomas et al., 2008; Al Olama et al., 2009; Chen et al., 2009; Eeles et al., 2009; Gudmundsson et al., 2009; Liu et al., 2009; Chen et al., 2010; Liu et al., 2010; Takata et al., 2010; Zheng et al., 2010; Adam et al., 2012), in 3 noted PCa risk regions on 8q24 [represented by single nucleotide polymorphisms (SNPs) rs1447295 (region 1), rs16901979 (region 2), and rs6983267 (region 3)]. We hypothesize the SNPs at 8q24 in Chinese population may behave differently from in other ethnicity and region.

To determine whether these risk loci also affect PCa risk in Asian men, where PCa risk is much lower but is rising steadily, we evaluated previously reported PCa risk loci on 8q24 in northern Chinese men. Using a case-control design, 8 genetic mutations were genotyped individually for their association with PCa. Simultaneously, based

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on our results we evaluated the association between haplotypes that contain risk alleles and PCa. We also explored the relationship between PCa clinical covariates and risk variants in northern Chinese men to clarify the susceptible genetic polymorphism as potential markers.

Materials and Methods

Study Population

A total of 289 men with PCa and 288 matched normal controls from Northern China were enrolled in the study. Subjects were permanent residents of Beijing and Tianjin. All cases were diagnosed with histologically confirmed PCa at the Department of Urology, Beijing Hospital, Ministry of Health or Tianjin Urology Institute, Second People's Hospital of Tianjin Medical University between January 1, 2000 and December 1, 2010. A few cases were outpatients after PCa operations were done elsewhere. PCa associated clinical data, including age at diagnosis, Gleason score, tumor stage and serum prostate specific antigen (PSA) levels, were obtained from a medical record review. PCa in patients with PSA greater than 20ng/ml, Gleason score 8 or higher, and/or pathological stage III or higher was defined as aggressive.

Age-matched controls were local residents participating in routine physical examination for this research in the two area. Those with PSA less than 4.0 ng/ml, negative digital rectal examination and no family history of PCa were included in the control group.

Only subjects with sufficient DNA available were included in the study for genotyping. All subjects were men of unrelated Northern Han Chinese ancestry. We created a Consolidated Standards of Reporting Trials diagram of the flow of participants through each stage of the study. This study was approved by the ethics committee at the two participating hospitals and informed consent was obtained from all study subjects.

SNP Selection for Evaluation and Genotyping

We selected 8 SNPs on 8q24 that were significantly associated with PCa risk in 6 previous GWASs of European and American populations ($p<10\text{-}8$) (Gudmundsson et al., 2007; Yeager et al., 2007; Eeles et al., 2008; Al Olama et al., 2009; Eeles et al., 2009; Gudmundsson et al., 2009), and in a multiethnic cohort study of a functional locus (Jia et al., 2009). These loci are included in three independent regions on 8q24. They are region 2-rs16901966, region 3-rs16902094, rs445114, rs620861 and rs6983267, and region 1-rs1447295 rs10090154 and rs7837688.

Experimental methods

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

Polymerase chain reaction (PCR) was performed using a PTC-225 Tetrad® DNA Thermal Cycler under certain 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-I 96.

Five samples randomly selected from individuals of different genotypes 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 Biosience & Technology Inc.

Statistical Analysis

Student's t test was used to determine the age difference between cases and controls. Pearson's χ^2 test was used to test the Hardy-Weinberg equilibrium (HWE) for each SNP separately among control subjects. Each risk allele (1) versus each non-risk allele (2) was evaluated by odds ratio (OR) and 95% confidence interval (95% CI). ORs and 95% CIs in models of a dominant mode (11+12 vs. 22) and of a recessive mode (11 vs. 12+22) were calculated to compare genotype frequencies between PCa cases and controls using Pearson's χ^2 or Fisher's exact test. Three risk loci confirmed in our study were evaluated for an association with clinical covariates in controls and cases. Statistical analysis was done using SPSS®, version 17.0 with <0.05 considered significant. The pair-wise linkage

Table 1. Selected Demographic Characteristics of Study Subjects

Characteristics	Cases	Control	*p*
Number of subjects	289	288	–
Age (years) (mean [SD])	72.2 (5.88)	70.5 (6.46)	0.0097
Range (years)	39–93	59–89	–
Body mass index (BMI; kg/m^2)	131	121	
Underweight (<18.5)	4	2	
Normal (18.5-22.9)	36	34	
Overweight (23-27.5)	78	56	
Obese (>27.5)	13	29	
PSA ng/mL (mean [SD])	20.19 (26.14)	1.04 (0.63)	5.1×10^{-5}
Range	0.05–1338	0–11.193	–
<10	102	–	–
10–20	39	–	–
>20	74	–	–
Gleason score	143	–	–
<8	101	–	–
≥8	42	–	–
Tumour stage	134	–	–
I	9	–	–
II	68	–	–
III	44	–	–
IV	13	–	–
Aggressiveness	154	–	–
Nonaggressive PCa	48	–	–
Aggressive PCa	106	–	–

Aggressive PCa, PSA>20 ng/ml, and/or clinical stage≥III, and/or Gleason score≥8

Table 2. Association Analysis Between the Alleles of 8 Candidate PCa Risk SNPs and PCa in Northern Chinese Men

refSNP ID	Chr pos	Alleles *(1/2)	RAF case	RAF control	Genotypes case			Genotypes control			Allelic OR		Additive model (df=2)	Genotypic OR Dominant model (11+12 vs 22)		Genotypic OR Recessive model (11 vs 12+22)	
			1	1	22	12	11	22	12	11	OR (95%CI)	*p*	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
rs16901966	128179433	G/A	0.297	0.244	142	118	26	159	113	13	1.31(1.01-1.70)	**0.042**	0.067	1.28(0.91-1.80)	0.142	2.09(1.01-4.40)	**0.032**
rs16902094	128389527	G/A	0.286	0.265	140	117	21	155	112	20	1.11(0.85-1.44)	0.426	0.686	1.16(0.82-1.63)	0.386	1.09(0.55-2.15)	0.789
rs445114	128392362	T/C	0.546	0.542	61	137	87	58	145	82	1.01(0.80-1.28)	0.905	0.798	0.94(0.61-1.43)	0.757	1.09(0.75-1.58)	0.647
rs620861	128404854	C/T	0.559	0.562	58	136	92	54	133	88	0.99(0.79-1.26)	0.936	0.976	0.96(0.62-1.48)	0.849	1.01(0.70-1.46)	0.966
rs6983267	128482486	G/T	0.463	0.424	77	149	56	94	137	51	1.17(0.93-1.48)	0.187	0.297	1.33(0.91-1.94)	0.120	1.12(0.72-1.75)	0.591
rs1447295	128554219	A/C	0.224	0.164	161	108	8	197	86	4	1.47(1.09-1.98)	**0.011**	**0.026**	1.58(1.10-2.26)	**0.009**	-	-
rs10090154	128601318	T/C	0.208	0.145	168	106	5	203	73	4	1.55(1.14-2.12)	**0.006**	**0.009**	1.74(1.20-2.53)	**0.002**	-	-
rs7837688	128608542	T/G	0.203	0.163	171	103	5	194	84	4	1.30(0.96-1.77)	0.088	0.176	1.39(0.97-2.00)	0.062	1.27(0.29-5.68)	0.725

*Risk alleles are listed first (1) in the allele column; *P*<0.05 are in bold

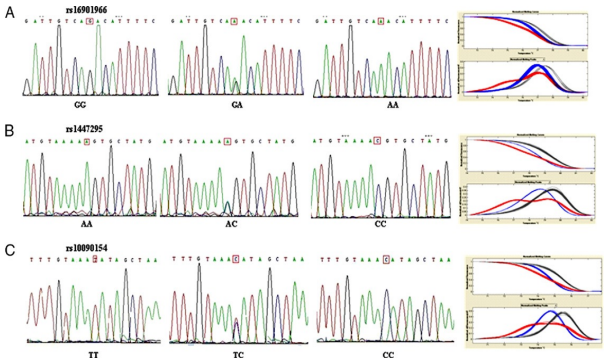

Figure 1. Sequencing Results from Samples Genotyped Using HRM

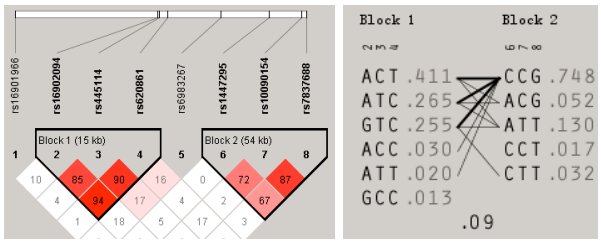

Figure 2. Haplotype Blocks and LD Pattern of 8 SNPs at 8q24. Each box represents LD (range 0 to 1) between pairs of SNPs indicating strong (red) LD (D'=1) and low (white) LD (D'=0). The numbers in each squares represent D' value

disequilibrium (LD) was estimated in control subjects using Haploview 4.2. Haplotype blocks were inferred using the default option of the Gabriel method (Jia et al., 2009).

Results

Table 1 shows the demographic characteristics of study participants. There was significant difference in age (mean±SD) between cases and controls (72.2±5.88 years, range 39 - 93 vs. 70.5±6.46, range 59 - 89, p =0.0097). Mean PSA in cases and controls was 20.19 ±26.14 ng/ml (range 0.05 - 1,338) and 1.04±0.63 ng/ml (range 0 - 11.193), respectively ($p=5.1\times10^{-5}$). Gleason score was 4 to 7 in 70.6% of patients and 8 to 10 in 29.4%. Tumor stage was I to IV in 6.7%, 50.7%, 32.8% and 9.7% of patients, respectively. PCa was considered aggressive in 68.8% of patients. Missing data were not included when calculating the proportion. None of the 288 controls had a family history positive for PCa.

Analysis of allelic frequency of PCa cases and controls showed that 3 of the 8 loci were associated with PCa,

Table 3. Haplotype-based Association Analysis of the 3 Risk Loci at 8q24 Associated with PCa

Haplotypes	Case (freq)	Control (freq)	Chi-squared	*p*-value	OR (95% CI)
A A T	0.144	0.101	3.87	**0.049**	1.43(0.99-2.06)
A C C	0.439	0.557	5.37	**0.020**	**0.79(0.64-0.97)**
G A T	0.010	0.023	2.56	0.110	0.46(0.15-1.31)
G C C	0.225	0.252	0.90	0.402	0.89(0.68-1.17)

three risk loci composed by the risk alleles of rs16901966, rs1447295 and rs10090154; $p < 0.05$ in our study are in bold; All haplotypes with a frequency of < 0.05were removed from the analysis; OR, odds ratio, CI, confidence interval

Table 4. Genotypic Distribution of rs16901966 According to Clinical Covariates and Odds Ratios of Risk Alleles Relative to Non-risk Alleles

rs16901966					Case-control		Case-only
Groups	total	GG	AG	AA	*P*	OR	*P*
Controls	288	13	113	159		1.00 (Ref)	
PCa cases							
Age at diagnosis	289	26	118	142			
<65	37	5	18	14	0.013	1.89(1.14-3.13)	0.023
65–74	110	15	46	49	0.004	1.64(1.17-2.29)	
≥75	111	6	42	63	0.986	1.00(0.69-1.43)	
PSA (ng/ml)	212	19	85	108			
<10	99	8	41	50	0.221	1.25(0.87-1.8)	0.505
10–20	39	3	13	23	0.996	1.00(0.58-1.73)	
>20	74	8	31	35	0.068	1.44(0.97-2.14)	
Gleason	141	16	53	72			
<8	99	10	39	50	0.134	1.32(0.92-1.89)	0.847
≥8	42	6	14	22	0.196	1.39(0.84-2.29)	
Tumour stage	134	14	51	69			
<III	77	6	24	47	0.795	0.95(0.62-1.44)	0.011
≥III	57	8	27	22	0.003	1.88(1.23-2.87)	
Aggressiveness	153	15	57	81			
Nonaggressive PCa	47	2	16	29	0.3	0.84(0.49-1.42)	0.065
Aggressive PCa	106	13	41	52	0.042	1.43(1.01-2.03)	

including rs16901966, rs1447295 and rs10090154. The genotype frequency of all 8 SNPs among controls did not deviate from the Hardy-Weinberg equilibrium (p>0.05). Genotype distribution showed that rs16901966 was associated with PCa risk in a 2.09-fold in the recessive model (p=0.032) while rs1447295 and rs10090154 were associated with PCa in the dominant model (Table 2). Sequence results were consistent with the genotypes of variants identified by HRM curves (Figure 1).

Two haplotype blocks identified by a total of 8 SNPs were inferred in our combined case and control groups, including block 1, identified by rs16902094, rs445114 and rs620861, covering 15 kb; and block 2, identified by rs1447295, rs10090154 and rs7837688, covering 54 kb

at 8q24 (Figure 2). In the haplotype based association analysis between the case and control groups, the AAT haplotype of the haplotypes identified by the 3 risk loci confirmed in our study showed significant differences (OR= 1.43, 95% CI: 0.99-2.06, *p*=0.049). Meanwhile, the corresponding non-risk haplotype ACC has significant differences between the case and control groups, with high frequency (0.439 and 0.557 respectively) (Table 3).

Compared with controls, rs16901966 -G was associated with age at diagnosis less than 75 years (OR= 1.89 to 1.64), stage III or higher (OR= 1.88) and aggressive PCa (OR=1.43) (Table 4). The risk allele A of rs1447295 was associated with age at diagnosis 65 years or greater (OR= 1.54), PSA less than 10 ng/ml (OR= 1.61), Gleason score 8 or greater (OR= 1.81) and disease stage less than III (OR= 1.73). Also, rs10090154 -T was associated with age at diagnosis 65 to 74 years (OR= 1.58), PSA less than 10 ng/ml (OR= 1.69) and disease stage less than III (OR= 1.90).

Discussion

Although many PCa risk variants have now been identified, most initial scans and replication studies were observed and performed in European as well as American populations. Similar studies in Asian are still few, especially Chinese, whose PCa risk frequence was rising with the aging progress. Ethnic and regional differences as well as other complex factors, such as gene-environment or gene-gene interactions, may contribute to the heterogeneity observed. The prevalence of prostate cancer and the allele frequencies differ across populations, it is important to understand the effect of these markers in other people of other ethnicities. Therefore, it is important for us to examine the associations of 8 previously identified risk SNPs on 8q24 with PCa risk in northern Chinese men.

In this population-based study of PCa in northern Chinese men, we systematically evaluated 8 reported PCa risk loci on 8q24 identified through GWAS in populations of other descents. Our study confirmed an association between 3 risk loci on 8q24 and PCa, and the joint effect of rs16901966 -A, rs1447295 -A and rs10090154 -T on PCa risk compared with controls in a sample of northern Chinese men. These results suggest that PCa risk variants identified in populations of some descent are also relevant for northern Chinese men, a low-risk population with mostly clinically relevant cancers.

To date, different studies did not produce consistent results. Amundadottir et al. initially identified region 1 of 8q24 by a genome-wide linkage scan with samples of European ancestry from Iceland, Sweden and the United States, and confirmed that rs1447295 -A was a PCa susceptibility locus (Amundadottir et al., 2006). But Gudmundsson et al. reported that allele A of rs1447295 was not significantly associated with PCa in some groups of European and black American ancestry (Gudmundsson et al., 2007). Another study did not replicate the association with the SNP rs1447295 in the Dutch population (Zeegers et al., 2011). In a Japanese population rs6983267 was not associated with PCa in one study (Liu et al., 2009) but it was associated with PCa risk in others (Terada et al., 2008; Liu et al., 2010). In the south China population rs6983267 was not associated with PCa (Zheng et al., 2010). Genetic background and environment (pollution, food, lifestyle, etc.) factors have effects on risk factor modification in different populations. Due to variations of allele frequencies, testing these risk alleles across populations is an important step to confirm the universality of associations. It may guide deep sequencing and fine mapping of these regions, thus identifying some functional and even some causal loci.

Consequently we concluded that rs16901966 -G in region 2, and rs1447295 -A and rs10090154 -T in region 1 were associated with PCa in northern Chinese men. These results were consistent with those from four previous studies of 8q24 in Asian populations (Haiman et al., 2007; Tan et al., 2008; Terada et al., 2008; Zheng et al., 2010), which found significant associations with regions 1 and 2 while results for region 3 were mixed, including the study in southern Chinese men but differ from findings in other studies. Taken together, these results suggest that risk regions 1 and 2 of 8q24 may be more important than region 3 of 8q24 in relation to PCa risk in Asian populations. Ethnic and regional differences as well as other complex factors, such as gene-environment or gene-gene interactions, may contribute to the heterogeneity observed. Larger studies are needed to confirm these findings.

We also investigated associations between risk alleles and several PCa clinical covariates. Three risk alleles validated in our study were associated with 2 or more PCa related variables. Only rs16901966 -G of the 3 risk SNPs was associated with aggressive PCa. To our knowledge the relationship between rs16901966 and PCa aggressiveness was not previously reported but rs6983561, located in region 2, showed strong LD ($r^2 \geq 0.8$) with rs16901966 in phase II of the International HapMap Project data on Utah residents with ancestry from northern and western Europe. We noted that rs16901966 was associated with disease stage III or greater, similar to rs6983561 -C in Taiwanese men (Chen et al., 2010). Our study also indicated that men younger than 75 years who carried the risk allele rs16901966 -G were at increased risk for PCa with time relative to controls. Thus, the risk loci of rs16901966 may be associated with early onset PCa.

An aim of association studies is to define the full spectrum of risk alleles in the population as well as further localize the causal alleles. The alleles of rs6983267 are known to differentially bind transcription regulating elements such as TCF7L2/TCF4 (Ahmadiyeh et al., 2010; Wright et al., 2010). The downstream activation of corresponding snpRNAs have also been involved in the phenotypic orientation of prostate cancer (Glinskii et al., 2011). An in vivo functional study demonstrated that the cancer associated variant rs6983267 lies in a prostate enhancer sequence, of which expression mimics that of a nearby proto-oncogene Myc (Wasserman et al., 2010). It was also reported that the rs6983267 risk allele is expressed approximately twofold more with Myc than the non-risk allele (Wright et al., 2010). The increased metastatic risk of inflammatory tumors might be promoted by a deregulation of the Myc. We observed no association

between rs6983267 in region 3 and PCa. Futher studies are needed to find strong linkage of the PCa risk alleles on 8q24 and nearby functional gene, and in vitro functional studies test the relationship of 8q24 and clinical covariates. The genetic variants do affect regulated expression of a target gene that could help explain cancer predisposition.

Limitations of the study should be noted. 1) Our sample size was relatively small for a case-control association study, which may have affected the stability of results or the number of risk loci showing significant differences between the case and control groups. 2) Data on clinically related variables were missing on some patients. Sample size must be increased to replicate these risk loci.

We hope future functional study of these loci and analysis of gene-gene interaction would improve the power to detect a difference between cases and controls, and among different levels of aggressiveness.

In summary, we detect significant association of the 8q24 SNPs with the risk of PCa in northern Chinese men. Joint effects among SNPs are likely to have a considerable effect on disease risk and severity. Rs16901966, rs1447295 and rs10090154 on 8q24 could be the PCa susceptible genetic markers in northern Chinese men. Larger studies in the Chinese population are needed to confirm these findings and further evaluate additional genetic variants, especially those with a modest effect. Identification of variants associated with PCa may improve our understanding of the disease etiology and have potential implications for the early detection, diagnosis, and treatment of PCa.

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