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ORIGINAL ARTICLE

Study of Genetic Variants of 8q21 and 8q24 Associated with Prostate Cancer in Jing-Jin Residents in Northern China

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SUMMARY

Background: **To identify the genetic risk of six genetic variants at 8q21 and 8q24 (including rs1512268, A; rs12543663, C; rs10086908, C; rs1016343, T; rs13252298, A, and rs6983561, C) associated with prostate cancer in Beijing and Tianjin (Jing-jin) area residents in northern China.**

Methods: **574 subjects were enrolled. Blood samples and clinical information were collected from histologically confirmed prostate cancer cases (n = 286) and clinically evaluated matched normal controls (n = 288) from Chinese men in northern China. Six SNPs at 8q21 and 8q24 were genotyped by high-resolution melt and sequencing in subjects. We compared statistical differences between the prevalence of risk genotypes with prostate cancer in cases and controls and analyzed the association between clinical covariates and risk loci in case groups to infer their relationship with aggressive prostate cancer.**

Results: **Three genotypes of rs10086908, CC (OR = 2.48; 95% CI = 1.02 - 5.98, p = 0.037) rs1016343, TT (OR = 1.64, 95% CI = 1.07 - 2.53, p = 0.023); and rs6983561, CC (OR = 1.91; 95% CI = 1.09 - 3.63, p = 0.044) at 8q24 were identified to be associated with prostate cancer risk in Jing-jin Chinese. The D' values of both two-locus haplotypes (T-A: rs1016343 vs. rs13252298; T-C: rs1016343 vs. rs6983561) were 0.907 and 0.859, respectively, the three-locus haplotype, only TAC constituted by the loci (rs1016343, T; rs13252298, A; rs6983561, C) was also associated with prostate cancer (p = 0.033), revealing rs1016343 vs. rs6983561 with significant differences between cases and controls. According to clinical covariates and odds ratios of risk genotypes relative to non-risk genotypes, rs6983561, CC was associated with age (OR = 2.5; 95% CI = 1.02 - 6.13, p = 0.039), and tumor aggressiveness (OR = 1.15; 95% CI = 1.06 - 1.23, p = 0.013).**

Conclusions: **The loci including rs10086908, rs1016343, and rs6983561 at 8q24 could be associated with prostate cancer in Jing-jin residents in northern China. Our results suggest that these loci could influence susceptibility to prostate cancer in the northern Chinese population.**

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KEY WORDS

8q21, 8q24, Chinese, prostate cancer, association

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INTRODUCTION

Prostate cancer (PCa) is the most common noncutaneous tumor in developed countries and the emerging countries, including China. In addition, along with lifestyle changes and the process of westernization, the morbidity from PCa has increased dramatically in China in recent years, and is becoming a great health burden [1,2].

To date, genome-wide association studies (GWASs) have indicated that chromosome 8q24 is a region of interest for further exploration of health disparity markers relative to PCa incidence and outcomes. Most association studies of 8q24 common variants with PCa have been performed in European and African populations, due to the higher incidence of the disease in western countries compared to Asia [3-5]. To clarify whether these risk loci also affect PCa risk in Asian men, i.e., Chinese, we evaluated the genetic variants at 8q24 and the NKX3-1 gene variation at 8q21 association with PCa risk in a population-based study conducted in Beijing and Tianjin (Jing-jin) area, China.

MATERIALS AND METHODS

Study Population

The study population consisted of 574 unrelated Northern Han Chinese men. All were permanent residents of the Jing-jin area. Using a case-control design, 286 patients with PCa and 288 healthy controls were enrolled in the study from January 1, 2000 to October 1, 2011. All cases were diagnosed with histologically confirmed PCa at the Department of Urology, Beijing Hospital. The controls were age-matched and had normal PSA levels (< 4.0 ng/mL) and negative results from digital rectal examination (DRE); none of them had a family history of PCa. This study was approved by the ethics committee of the two participating hospitals, and informed consent was obtained from all study participants.

Selection of SNPs for Genotyping

We selected six SNPs on 8q21 and 8q24 for genotyping that were found to be significantly associated with PCa risk in previous GWASs of European and Japanese populations [6-8].

These included one SNP at 8q21 (rs1512268, A) and five in regions mapping to 8q24 (rs12543663, C; rs10086908, C; rs1016343, T; rs13252298, A; and rs6983561, C).

Genomic DNA was extracted from blood samples, and the selected loci genotyped. Briefly, polymerase chain reaction-high resolution melting curves (PCR-HRM) of small amplicons were carried out in a final reaction volume of 10 μL including an extra 0.8 μL of 1 × LC-green PLUS fluorescent dye and 0.02 μL of each pair of low and high temperature calibrators (10 pmol/μL) compared with standard PCR (Polymerase Chain Reaction). After PCR amplification, the PCR products were transferred into matching 96-well plates to be genotyped automatically and verified manually using the Lightscanner TMHR-I 96 (Idaho Technology, Inc., Salt Lake City, UT, USA). To validate the accuracy of genotyping, 5 samples were randomly selected from the 3 different verified genotypes of each risk variant to be sequenced (Beijing Genomics Institute [BGI], Beijing, PRC).

All primers used for both PCR-HRM and sequencing of PCR products were designed using Oligo (version 6.0; Molecular Biology Insights, Inc., Cascade, CO, USA).

Statistical Analysis

Pearson's χ^2 test was used to test control sample genotypes of each SNP separately for departures from Hardy-Weinberg equilibrium (HWE). The ORs were performed for a homozygote comparison model (AA versus CC), a dominant (AA+AC versus CC), a recessive model (AA+CC versus AC), and an over dominant model (AA+CC versus AC). ORs and 95% CIs were calculated to compare the genotype frequencies between PCa cases and controls. Statistical analyses were performed using the Statistical Package for the Social Sciences software package (version 16.0; SPSS, Inc., Chicago, IL, USA), and $p < 0.05$ was the cut off value for significance.

Table 1. Clinical characteristics of patients with prostate cancer.

Phenotypes	n (%)
PSA (ng/mL):	215
< 10	102 (47.4%)
10 - 20	39 (18.1%)
> 20	74 (34.4%)
Gleason score:	143
< 8	101 (70.6%)
≥ 8	42 (29.4%)
Tumor stage:	135
I	9 (6.7%)
II	69 (51.1%)
III	44 (32.6%)
IV	13 (9.6%)
Aggressive	154
No	106 (68.8%)
Yes	48 (31.2%)

Table 2. Association of six SNPs with prostate cancer risk, as assessed in a comparison of genotype frequencies between patients with prostate cancer and normal controls

SNP		Cases (%)	Controls (%)	OR (95% CI)	p	HWE (p)
rs1512268	Codominant					0.18
	GG	118 (41.4)	131 (45.6)	1 (reference)		
	AG	139 (48.8)	133 (46.4)	1.16 (0.81 - 1.66)	0.39	
	AA	28 (9.8)	23 (8.0)	1.35 (0.71 - 2.58)	0.32	
	Dominant					
	GG	118 (41.4)	131 (45.6)	1 (reference)		
	AA + AG	167 (58.6)	156 (54.4)	1.18 (0.95 - 1.85)	0.08	
	Recessive					
	GG + AG	257 (90.2)	264 (92.0)	1 (reference)		
	AA	28 (9.8)	23 (8.0)	1.25 (0.702 - 2.23)	0.45	
	Overdominant					
	GG + AA	146 (51.2)	154 (53.6)	1 (reference)		
	AG	139 (48.8)	133 (46.4)	1.1 (0.79 - 1.53)	0.561	
rs12543663	Codominant					0.13
	AA	231 (81.3)	242 (84.6)	1 (reference)		
	AC	47 (16.6)	40 (14.0)	1.23 (0.78 - 1.95)	0.37	
	CC	6 (2.1)	4 (1.4)	1.57 (0.44 - 5.64)	0.49	
	Dominant					
	AA	231 (81.3)	242 (84.6)	1 (reference)		
	CC + AC	53 (18.7)	44 (15.4)	1.26 (0.81 - 1.96)	0.298	
	Recessive					
	AA + AC	278 (97.9)	282 (98.6)	1 (reference)		
	CC	6 (2.1)	4 (1.4)	1.52 (0.43 - 5.45)	0.516	
	Overdominant					
	AA + CC	237 (83.4)	246 (86.0)	1 (reference)		
	AC	47 (16.6)	40 (14.0)	1.22 (0.78 - 1.93)	0.395	
rs10086908	Codominant					0.55
	TT	171 (65.5)	138 (63.9)	1 (reference)		
	TC	70 (26.8)	71 (32.9)	0.79 (0.53 - 1.19)	0.261	
	CC	20 (7.7)	7 (3.2)	2.3 (0.95 - 5.61)	0.059	
	Dominant					
	TT	171 (65.5)	138 (63.9)	1 (reference)		
	CC + TC	90 (34.5)	78 (36.1)	0.93 (0.64 - 1.36)	0.71	
	Recessive					
	TT +TC	241 (92.3)	209 (96.8)	1 (reference)		
	CC	20 (7.7)	7 (3.2)	2.48 (1.02-5.98)	0.037	
	Overdominant					
	TT + CC	190 (73.2)	145 (67.1)	1 (reference)		
	TC	70 (26.8)	71 (32.9)	0.75 (0.50-1.12)	0.16	
rs1016343	Codominant					0.97
	CC	103 (36.3)	108 (38.0)	1 (reference)		
	TC	118 (41.5)	134 (47.2)	0.92 (0.63 - 1.35)	0.66	
	TT	63 (22.2)	42 (14.8)	1.57 (0.95 - 2.60)	0.067	

	Dominant					
	CC	**103 (36.3)**	**108 (38.0)**	**1 (reference)**		
	TT + TC	**181 (63.7)**	**176 (62.0)**	**1.08 (0.65 - 1.32)**	**0.66**	
	Recessive					
	CC+ TC	**221 (77.8)**	**242 (85.2)**	**1 (reference)**		
	TT	**63 (22.2)**	**42 (14.8)**	**1.64 (1.07 - 2.53)**	**0.023**	
	Overdominant					
	CC + TT	**166 (58.5)**	**150 (52.8)**	**1 (reference)**		
	TC	**118 (41.5)**	**134 (47.2)**	**0.79 (0.57 - 1.11)**	**0.177**	
rs13252298	**Codominant**					**0.78**
	GG	**27 (9.7)**	**21 (7.8)**	**1 (reference)**		
	AG	**103 (37.2)**	**111 (41.6)**	**0.72 (0.38 - 1.36)**	**0.31**	
	AA	**147 (53.1)**	**135 (50.6)**	**0.85 (0.46 - 1.57)**	**0.59**	
	Dominant					
	GG	**27 (9.7)**	**21 (7.8)**	**1 (reference)**		
	AA + AG	**250 (90.3)**	**246 (92.2)**	**0.79 (0.44 - 1.44)**	**0.44**	
	Recessive					
	GG + AG	**130 (46.9)**	**133 (49.4)**	**1 (reference)**		
	AA	**147 (53.1)**	**135 (50.6)**	**1.12 (0.79 - 1.56)**	**0.53**	
	Overdominant					
	GG + AA	**174 (62.8)**	**156 (58.4)**	**1 (reference)**		
	AG	**103 (37.2)**	**111 (41.6)**	**0.83 (0.59 - 1.17)**	**0.295**	
rs6983561	**Codominant**					**0.68**
	AA	**139 (50.4)**	**156 (55.1)**	**1 (reference)**		
	AC	**108 (39.1)**	**110 (38.9)**	**1.10 (0.76 - 1.59)**	**0.58**	
	CC	**29 (10.5)**	**17 (6.0)**	**1.91 (1.09 - 3.63)**	**0.044**	
	Dominant					
	AA	**139 (50.4)**	**156 (55.1)**	**1 (reference)**		
	CC + AC	**137 (49.6)**	**127 (44.9)**	**1.22 (0.58 - 1.17)**	**0.25**	
	Recessive					
	AA + AC	**247 (89.5)**	**266 (94.0)**	**1 (reference)**		
	CC	**29 (10.5)**	**17 (6.0)**	**3.09 (1.64 - 5.8)**	**< 10-4**	
	Overdominant					
	AA + CC	**168 (60.9)**	**173 (61.1)**	**1 (reference)**		
	AC	**108 (39.1)**	**110 (38.9)**	**1.01 (0.72 - 1.42)**	**0.95**	

RESULTS

Subjects' data and clinical baselines

The mean ages in cases with PCa and control individuals were 72.17 ± 7.72 years vs. 70.47 ± 7.60 years, respectively. Total serum PSA mean levels in cases with PCa and those in control individuals were 46.42 ± 143.11 and 1.20 ± 1.01 ng/mL, respectively, and no significant difference between both of them ($p = 0.129$). The clinical baseline characteristics of the 286 PCa cases are shown in Table 1. Gleason scores were 2 - 7 in 101/143 (70.6%) of patients, while 42/143 (29.4%) had scores of 8 - 10. Tumors were classified as stage I in 9/135 (6.7%) of patients, while 69/135 (51.1%) tumors were stage II, 44/135 (32.6%) stage III, and 13/135 (9.6%) stage IV. PCa was classified as aggressive in 48/154(31.2%) of the patients.

Hardy-Weinberg equilibrium (HWE) in the control population was analyzed in candidate loci including the five genetic variants at 8q24 and a variation in NKX3-1 (rs1512268, A) gene at 8q21 and all was in accord with HWE ($p \geq 0.05$) (Table. 2).

Table 3. Main Results of the Pooled Data in the Meta-Analysis.

Ethnic groups	No Case/Control	CC / AA		AC / AA		(AC+CC) / AA		First Author
		OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	
Chinese	286/288	1.91 (1.09 - 3.63)	0.044	1.1 (0.76 - 1.59)	0.58	1.22 (0.58 - 1.17)	0.25	Hui J
Taiwanese	324/336	1.92 (1.1 - 3.34)	0.02	1.45 (1.05 - 2.00)	0.024	1.52 (1.12 - 2.07)	0.007	Chen M [5]
Combined	610/624	3.86 (2.01 - 7.41)	0.009	1.17 (0.92 - 1.49)	0.19	1.26 (1.0 - 1.58)	0.049	
West African	308/469	1.89 (1.23 - 2.91)	0.003	1.37 (0.91 - 2.06)	0.13	1.56 (1.06 - 2.3)	0.023	Murphy AB [1]
African American	127/345	2.38 (1.33 - 4.26)	0.003	2.48 (1.45 - 4.25)	0.001	2.44 (1.48 - 4.02)	0.0001	Wang Y [4]
African American	149/85	4.12 (1.4 - 12.1)	0.008	3.8 (2.0 - 7.4)	$> 10^{-5}$	4.01 (2.02 - 7.97)	0.0001	Salinas CA [6]
Combined	584/899	2.1 (1.53 - 2.87)	$< 10^{-6}$	2.12 (1.59 - 2.81)	$< 10^{-7}$	2.11 (1.61 - 2.77)	$< 10^{-8}$	
Caucasians	1308/1266	2.57 (0.49 - 13.28)	0.242	1.78 (1.33 - 2.38)	$< 10^{-5}$	1.80 (1.35 - 2.39)	$< 10^{-5}$	Salinas CA [6]

Table 4. Distribution of risk genotype rs6983561 related to clinical covariates in patients with prostate cancer.

rs6983561					Case-only
Clinical covariates	total	CC	AC+AA	OR (95% CI)	p
Age at diagnosis	276	29	249		
≥ 75	109	7	102	2.5 (1.02 - 6.13)	0.039
< 75	143	21	122		
PSA (ng/mL)	207	19	188		
> 20	72	9	63	1.05 (0.44 - 2.48)	0.91
≤ 20	135	17	125		
Gleason	135	15	120		
≥ 8	40	6	34	1.69 (0.56 - 5.1)	0.351
< 8	95	9	86		
Tumor stage	127	13	114		
< III	72	4	68	3.33 (0.97 - 11.45)	0.046
≥ III	55	9	46		
Tumor aggressiveness	146	13	133		
Aggressive prostate cancer	102	13	89	1.15 (1.06 - 1.23)	0.013
Non-aggressive prostate cancer	44	0	44		

Genotype associated with PCa risk

Analysis of genotype frequency comparisons between cases with PCa and control individuals showed that three of the six loci i.e., the rs1512268 at 8q21; rs12543663 and rs13252298 at 8q24 were negatively associated with PCa risk in the Jing-jin Chinese population (Table 2). Analysis of genotype frequencies of the other three loci at 8q24 indicated that rs10086908, TT was associated with PCa risk in the recessive model ($p = 0.037$); rs1016343, TT was associated with PCa risk in the recessive model ($p = 0.023$); rs6983561, CC was associated with disease risk in the codominant model ($p = 0.044$) and recessive model ($p = 0.044$) and recessive model ($p < 10^{-4}$) (Table 2).

Table 5. D' and r^2 values between SNPs at 8q24.

SNP	D'	r^2
rs12543663 rs10086908	0.194	0.016
rs12543663 rs1016343	0.083	0.001
rs12543663 rs13252298	0.044	0.0
rs12543663 rs6983561	0.315	0.004
rs10086908 rs1016343	0.072	0.002
rs10086908 rs13252298	0.001	0.0
rs10086908 rs6983561	0.056	0.0
rs1016343 rs13252298	0.907	0.229
rs1016343 rs6983561	0.859	0.417
rs13252298 rs6983561	0.645	0.063

Table 6. Association analysis of haplotypes consisting of three loci at 8q24 determined to be in LD in our population using Haploview.

Haplotypes	Case (freq)	Control (freq)	Chi-squared	p-value	OR (95% CI)
CAA	0.300	0.324	0.692	0.405	0.895 (0.689 - 1.163)
CGA	0.260	0.265	0.027	0.868	0.977 (0.742 - 1.287)
TAA	0.134	0.163	1.678	0.195	0.798 (0.568 - 1.123)
TAC	0.274	0.219	4.543	0.033	1.359 (1.024 - 1.803)

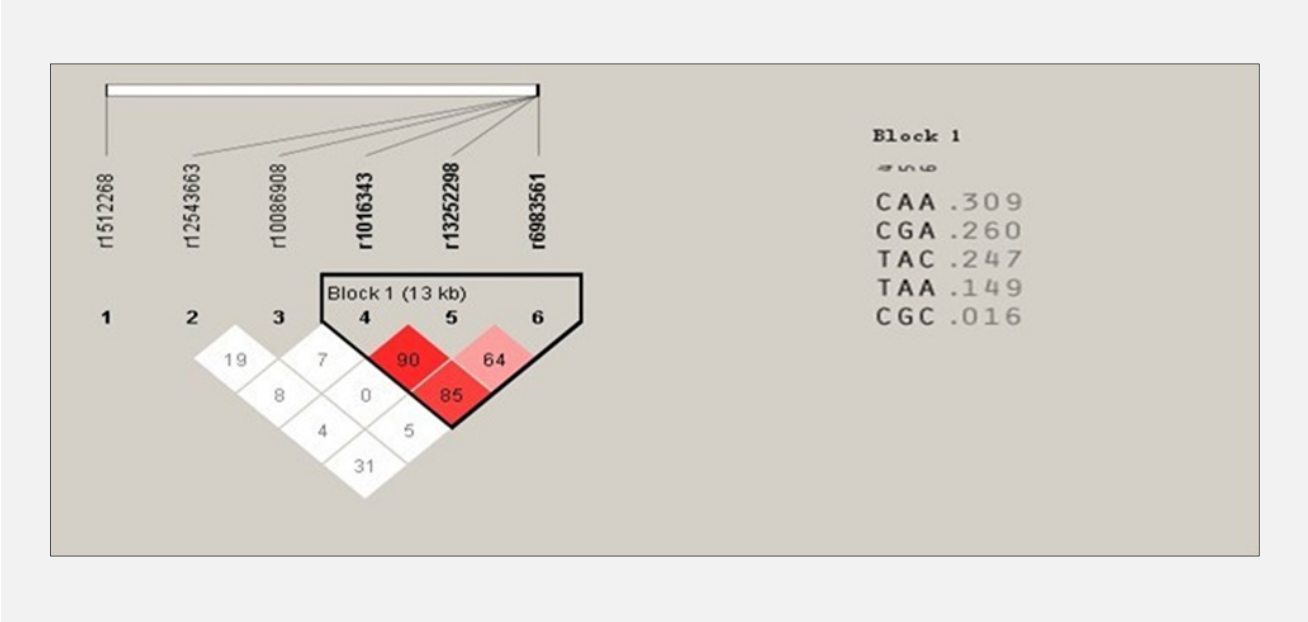

Figure 1. Haplotype blocks and LD tests of 6 risk loci.

Table 3 lists the main results by meta-analysis. For the overall data including three groups (Asian, African, and Caucasians) in the homozygote comparison, results of the subgroup analysis on PCa showed a significant association of rs6983561 polymorphism with PCa risk, indicating that individuals carrying risk genotype "CX" had an increased carcinoma risk compared with homozygous AA.

PCa-associated genotype and clinical covariates

We analyzed the association of the genotype of rs6983561 at 8q24 with the clinical covariates, including ages at diagnosis, serum PSA levels, Gleason scores, tumor stages and aggression through stratification comparison in cases with PCa. The results showed that the cases with genotype CC of rs6983561 were associated with age at diagnosis (OR = 2.5; 95% CI = 1.02 - 6.13, p = 0.039) and tumor aggressiveness (OR = 1.15; 95% CI = 1.06 - 1.23, p = 0.013). However, the risk genotype was not significantly associated with PSA levels, Gleason scores, and tumor stages in cases with PCa (p > 0.05) (Table 4).

Genetic linkage disequilibrium analysis

Five variants (rs12543663, C; rs10086908, C; rs1016343, T and rs13252298, A, and rs6983561, C) at 8q24 were analyzed for linkage disequilibrium (LD) with Haploview software. A very strong LD existed between rs1016343, T and rs13252298, A (D' = 0.907) and between rs1016343 and rs6983561 (D'= 0.859). The other pair of SNPs showed weak LD (Table 5).

Haplotype associated with PCa risk

One haplotype block covering 13 kb and containing 3 variants (rs1016343, T; rs13252298, A; and rs6983561, C) at 8q24 was identified in our Jing-jin Chinese population (Figure 1). Association analysis showed that the T-A-C haplotype (rs1016343, rs13252298, rs6983561) was strongly associated with PCa (OR 1.359, 95% CI 1.024 - 1.803, p = 0.033) in the Jing-jin Chinese population (Table 6).

DISCUSSION

Although many PCa risk variants have been identified, most initial scans and replication studies have only been observed and performed in European and American populations. Similar studies in Asian, especially northern Chinese populations, are still scarce. The fact that the prevalence of PCa and the allele frequencies differs across populations means that it is important to understand the effect of these genetic molecules on risk of PCa in people of different ethnicities. Therefore, in this study we re-examined the associations of six identified variants at 8q21 and 8q24, previously associated with PCa risk, in a northern Chinese population.

We identified that one risk locus, rs6983561 at 8q24, was associated with PCa in Chinese men in Jing-jin areas in the recessive model (OR = 3.09; 95% CI = 1.64 - 5.8, $p < 10^{-4}$). Benford and coworkers (2010) observed a 1.70-fold increase in PCa risk among African descent carriers of rs6983561, C [9], but only 1.20-fold risk increase in the "C" carriers in Chinese population. In fact, the individuals with genotype of rs6983561, CC have a higher PCa risk than those with the other two types (AC vs. AC+CC) among Asians (OR: 3.86 vs. 1.17 vs. 1.26) and Caucasians (OR: 2.57 vs. 1.78 vs. 1.80) by meta-analysis across ethnic groups (Table 3). Besides that, the risk genotype of rs6983561, CC associated with the clinical covariates related to PCa showed that it was markedly associated with age at diagnosis (OR = 2.5; 95% CI = 1.02 - 6.13, p = 0.039) and tumor aggressiveness (OR = 1.15; 95% CI = 1.06 - 1.23, p = 0.013) in cases with PCa in the Jing-jin population. These results suggested that some PCa risk variants identified in populations of European descent are also relevant to Chinese men, a population with low-risk of mostly clinical prostate cancer [10-13].

Haiman et al. initially identified Block 1 by a genome-wide linkage scan using samples from African Americans and confirmed that rs10086908 is a PCa susceptibility locus [14]. Our study also revealed that one haplotype (TAC at Block 1) at chromosome 8q24 conferred a 1.35-fold increase in the probability of prostate cancer development in northern Chinese men in Jing-jin areas. A haplotype block covering three SNPs rs10086908, C; rs1016343, T and rs6983561, C with 13 kb intervals would be necessary to illustrate that the restructuring or mutations could occur among the variants.

Our study also showed that the homozygous genotypes of rs10086908 (CC: OR = 2.48; 95% CI = 1.02 - 5.98, p = 0.037) and rs1016343 (TT: OR = 1.64, 95% CI = 1.07 - 2.53, p = 0.023) at 8q24 Region 2 could be susceptible to prostate cancer in northern Chinese men in Jing-jin areas. In a previous study, the strongest associations were found for chromosome 8q24 Region 2 (rs1016343: OR = 2.07, 95% CI = 1.35 - 3.20, $p = 9.4 \times 10^{-4}$) in Caucasian men [15].

A recent GWAS identified rs1512268 on chromosome 8q21 as a marker associated with PCa susceptibility [16] and associated with common variants at known PCa risk regions in African Americans. A study by Akamatsu and Takata determined that the GWAS identified that a region on 8q21, which tagged common variables in NKX3-1, was involved in regulation of downstream genes relevant to PCa susceptibility. In addition, they were able to confirm this association in a PCa GWAS of a Japanese population [8,17]. By contrast, our findings indicate that NKX3-1 had no association with PCa in the population of northern Chinese men in our study (p > 0.05).

We conclude that rs6983561 at chromosome 8q24 may be associated with PCa in northern Jing-jin Chinese men. The results of this study of PCa risk loci at 8q24 differed from those of other international research and there are various possible explanations for this, such as genetic background, environmental factors i.e., food,

lifestyle, etc. in Chinese, leading to this population having different genetic risk factors compared to Europeans, Africans and the others. So, our findings suggest that the genetic locus at 8q24 could contribute to susceptibility to PCa among the northern Chinese population in Jing-jin area, China.

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Declaration of Interest:
None

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Supplementary text

A total of 11 publications were preliminarily eligible, among which 2 were excluded because they were review articles. Then, three articles were discarded because they were not case-control studies. Afterwards, two studies written in Japanese were excluded because its data were the same as those of one included study containing larger sample sizes. Lastly, four case-control studies [10-13] regarding the association between rs6983561 polymorphism and prostate cancer risk were selected. All the included studies were written in English. We established a database according to the extracted information from each article. The relevant information was listed in Supplementary Table 1.

Supplementary Table1. Distribution of rs6983561 genotype among prostate cancer cases and controls included in the meta-analysis.

First Author	Publication Year	Case				Control				HWE (control)
		N	AA	CC	AC	N	AA	CC	AC	p
Murphy AB [1]	2012	308	45	118	145	469	99	137	233	> 0.05
Wang Y [4]	2011	127	25	40	62	345	104	70	104	> 0.05
Chen M [5]	2010	324	135	37	152	336	175	25	136	> 0.05
Salinas CA [6]	2008	1457	1146	22	241	1351	1188	8	118	> 0.05