

RESEARCH ARTICLE

Susceptibility Loci Associations with Prostate Cancer Risk in Northern Chinese Men

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Abstract

Background: KLK3 gene products, like human prostate-specific antigen (PSA), are important biomarkers in the clinical diagnosis of prostate cancer (PCa). G protein-coupled receptor RFX6, C2orf43 and FOXP4 signaling plays important roles in the development of PCa. However, associations of these genes with PCa in northern Chinese men remain to be detailed. This study aimed to investigate their impact on occurrence and level of malignancy. **Methods:** All subjects were from Beijing and Tianjin, including 266 cases with prostate cancer and 288 normal individuals as controls. We evaluated associations between clinical covariates (age at diagnosis, prostate specific antigen, Gleason score, tumor stage and aggressive) and 6 candidate PCa risk loci, genotyped by PCR- high resolution melting curve and sequencing methods. **Results:** Case-control analysis of allelic frequency of PCa associated with PCa showed that one of the 6 candidate risk loci, rs339331 in the RFX6 gene, was associated with reduced risk of prostate cancer (odds ratio (OR) = 0.73, 95% confidence interval (CI) = 0.57-0.94, $P = 0.013$) in northern Chinese men. In addition, subjects with CX (CC+TC) genotypes had a decreased risk for prostate cancer compared to those carrying the TT homozygote (OR = 0.64, 95% CI = 0.45-0.90, $P = 0.008$). The TT genotype of 13q22 (rs9600079, T) was associated with tumor stage ($P = 0.044$, OR = 2.34, 95% CI = 0.94-5.87). Other SNPs were not significantly associated with clinical covariates in prostate cancer ($P > 0.05$). **Conclusions.** rs339331 in the RFX6 gene may be associated with prostate cancer as a susceptibility locus in northern Chinese men.

Keywords: prostate cancer - association - northern Chinese men - genetic variants

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Introduction

Prostate cancer (PCa) is the most commonly diagnosed noncutaneous cancer in the United States men with over 200,000 new cases and 30,000 deaths estimated in 2010 (Jemal et al., 2010). Amundadottir et al. (2006) evidenced a greater estimated Population attributable risk (PAR) (16%) that may contribute to higher incidence of PCa in African American men than in men of European ancestry (Amundadottir et al., 2006). The prevalence of PCa in Chinese population in mainland of China was low before, but with population ageing, the prevalence is increasing by years recently (Sun et al., 2004). PCa incidence was in the third place of malignant tumors in Chinese population and it has become a important risk to expectancy life of Chinese men over the age of 50 years old (Sun et al., 2004).

Recent genome-wide association studies (GWAS) have identified multiple single nucleotide polymorphisms (SNPs) associated with PCa risk (Duggan et al., 2007; Gudmundsson et al., 2007; Yeager et al., 2007; Gudmundsson et al., 2008; Thomas et al., 2008; Gudmundsson et al., 2009; Yeager et al., 2009; Schumacher et al., 2011). Confirmation of these risk loci among European descent has been reported extensively. There have been the studies gradually in other racial/ethnic groups (Waters et al., 2009; Takata et al., 2010; Batra et al., 2011; Jianfeng et al., 2012; Lindström et al., 2012; Nobata et al., 2012; Ren et al., 2012). Takata R, et al. (2010) carried out a GWAS and replication study in Japanese men with prostate cancer and their study confirmed the association of nine SNPs at $p < 1.0 \times 10^{-7}$ and ten SNPs at $p < 0.05$ in Japanese population. In addition, they

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reported five new loci for prostate cancer susceptibility, at 5p15 (lambda-corrected probability $p(\text{GC}) = 3.9 \times 10^{-18}$), GPRC6A/RFX6 ($p(\text{GC}) = 1.6 \times 10^{-12}$), 13q22 ($P(\text{GC}) = 2.8 \times 10^{-9}$), C2orf43 ($P(\text{GC}) = 7.5 \times 10^{-8}$) and FOXP4 ($P(\text{GC}) = 7.6 \times 10^{-8}$). These findings contributed to our understanding of the genetic basis of prostate carcinogenesis and also emphasized the genetic heterogeneity of prostate cancer susceptibility among different ethnic populations.

To determine whether those variants were also associated with prostate cancer risk in the northern Chinese population, we evaluated 6 prostate cancer susceptibility loci in a population based case-control study. All subjects were from Beijing and Tianjin, including 266 prostate cancer cases and 288 population controls. We also explored the relationship between PCa clinical covariates and the genetic risk variants to infer their impact on occurrence and aggression of prostate cancer in northern Chinese men.

Materials and Methods

Study Population

Permanent residents of Beijing and Tianjin involved in this study. The 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. PCa related clinical data, including age at diagnosis, serum PSA, Gleason score and tumor stage, were acquired from a medical record review. PSA more than 20 ng/mL, Gleason score 8 or higher, and/or pathological stage III or higher with PCa patients were defined as aggressive. Local residents participating in routine physical examination were defined as controls with age matched for this research in the Beijing and Tianjin. Those residents with PSA less than 4.0 ng/mL, negative digital rectal examination and no family history of PCa were also included in the control group. The study were a total of 266 PCa cases and 288 controls. All participants were men of unrelated Northern Han Chinese ancestry. We conducted a Consolidated Standards of Reporting Trials diagram of the flow of participants through each stage of the study. The ethics committee at the 2 participating hospitals approved this study and all subjects signed informed consent. None of the 288 controls had the family history with tumor.

SNP Selection for Genotyping

6 SNPs with PCa risk in previous GWASs of European and American populations were selected randomly. These loci included rs13385191, rs12653946, rs1983891, rs339331, rs9600079 and rs2735839. Whole blood genomic DNA extraction kit (Biochain (Beijing) Science-Technology Beijing, People's Republic of China) was used to extract blood genomic DNA. PCR-high resolution melting curves of small amplicons were done according to a previous study. PCR was performed using a PTC-225 Tetrad[®] DNA Thermal Cycler under certain conditions, including initial denaturation at 95 °C for 3 minutes, followed by 45 cycles at 95°C for 30 seconds, annealing for 30 seconds, extension at 72°C for 4 seconds and

completion at 72°C for 2 minutes. After 2 cycles at 94°C for 30 seconds and at 24°C for 2 minutes. PCR products were genotyped automatically and verified manually using a LightScanner[®] TMHR-I 96. Six samples were randomly selected from the 3 verified genotypes of each risk variant, amplified by PCR and sequenced elsewhere to confirm genotyping results. The PCR procedure involved initial denaturation at 95°C for 3 minutes, followed by 35 cycles at 95°C for 30 seconds, annealing for 30 seconds, extension at 72°C for 30 seconds and completion at 72°C for 2 minutes.

Statistical Analysis

Pearson's chi-square test was used to test the Hardy-Weinberg equilibrium for each SNP separately among controls using a cut off of $p > 0.05$. The OR and 95% CI were estimated for each risk allele vs each nonrisk allele. OR and 95% CI in models were calculated to compare genotype frequencies between PCa case and control using Pearson's chi-square or Fisher's exact test. Statistical analysis was done using SPSS[®], version 16.0 with < 0.05 considered significant.

Results

Subjective clinical data

The mean ages in case with PCa and control individuals were 72.17 ± 7.72 years vs 70.47 ± 7.60 years separately. Total serum PSA mean levels in case with PCa and these 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 Table 1 lists the clinical baseline characteristics of the 266 cases, including Gleason score, tumor stage and aggressive status of patients etc.

PCa-associated allele

The genotype frequency of all SNPs among controls did not deviate from the Hardy-Weinberg equilibrium ($p > 0.05$). Comparing the allelic frequency between cases with PCa and controls showed that only RFX6 gene(rs339331,C) was associated with PCa (OR= 0.73, 95% CI =0.57-0.94, $P = 0.013$) by genetic statistical analysis of the 6 loci.

Table 1. Clinic Baseline Characteristics of Patients with PCa

	n (%)
PSA (ng/ml):	216
Less than 10	102 (47.2)
10-20	39 (18.1)
Greater than 20	75 (34.7)
Gleason score:	143
Less than 8	101 (70.6)
8 or Greater	42 (29.4)
Tumor stage:	134
I	9 (6.7)
II	68 (50.7)
III	44 (32.8)
IV	13 (9.7)
Aggressive	154
Yes	48 (31.2)
No	106 (68.8)

Table 2. Prostate Cancer Risk Associated with Individual SNPs

SNP	Cases, n=266(%)	Controls, n=288(%)	OR (95%CI)	p
Rs2735839				
Allelic frequency				
G	330(57.9)	340(60.7)		
A	240(42.1)	220(39.3)	0.89(0.70-1.14)	0.335
Genotypic frequency				
GG	102(35.8)	98(35.0)		0.081
GA	126(44.2)	144(51.4)		
AA	57(20.0)	38(13.6)		
GG + GA	228(80.0)	242(86.4)	0.63(0.39-1.01)	0.041
Rs13385191				
Allelic frequency				
G	272(47.2)	255(44.4)		
A	304(52.8)	319(55.6)	1.12(0.88-1.42)	0.341
Genotypic frequency				
GG	68(23.6)	53(18.5)		0.293
GA	136(47.2)	149(51.9)		
AA	84(29.2)	85(29.6)		
GG + GA	204(70.8)	202(70.4)	1.02(0.70-1.49)	0.906
Rs12653946				
Allelic frequency				
C	329(56.9)	347(60.2)		
T	249(43.1)	229(39.8)	0.87(0.69-1.10)	0.252
Genotypic frequency				
CC	98(33.9)	101(35.1)		0.21
CT	133(46.0)	145(50.3)		
TT	58(20.1)	42(14.6)		
CC + CT	231(79.9)	246(85.4)	0.68(0.43-1.07)	0.082
Rs1983891				
Allelic frequency				
T	206(35.6)	174(30.3)		
C	372(64.4)	400(69.7)	0.79(0.61-1.01)	0.054
Genotypic frequency				
TT	37(12.8)	129(10.1)		0.146
TC	132(45.7)	116(40.4)		
CC	120(41.5)	142(49.5)		
TT + TC	169(58.5)	245(50.5)	0.82(0.59-1.13)	0.202
Rs339331				
Allelic frequency				
C	179(31.0)	215(38.0)	0.73(0.57-0.94)	0.013
T	399(69.0)	351(62.0)		
Genotypic frequency				
CC	31(10.7)	39(13.8)		0.029
CT	117(40.5)	137(48.4)		
TT	141(48.8)	107(37.8)		
CC+CT	148(51.2)	176(62.2)	0.64(0.45-0.90)	0.008
Rs9600079				
Allelic frequency				
G	310(53.6)	325(56.4)	0.89(0.71-1.13)	0.341
T	268(46.4)	251(43.6)		
Genotypic frequency				
GG	89(30.8)	96(33.3)		0.636
GT	132(45.7)	133(46.2)		
TT	68(23.5)	59(20.5)		
GG + GT	221(76.5)	229(79.5)	0.84(0.55-1.27)	0.378

PCa-associated genotypes

We found subjects carrying (CC+CT) genotypes had a decreased risk for prostate cancer compared to those carrying the TT homozygote (OR =0.64, 95% CI = 0.45-0.90, $P = 0.008$) in RFX6 gene (rs339331, C) (Table 2).

Analysis of PCa-associated risk factors

We analyzed the association of the 6 SNPs with the clinical covariants (phenotypes) prostate cancer through case-control comparison, including the PSA levels,

Table 3. Association of the Genotypes of rs9600079 with the Clinical Covariants of Patients with Prostate Cancer

rs9600079	TT	GT +GG	OR (95%CI)	P
PSA				
≥10	30	84	1.92(0.93-4.00)	0.057
<10	16	86		
age				
≥75	27	84	1.19(0.63-2.21)	0.569
<75	32	118		
Gleason score				
≥8	13	29	2.07(0.83-5.12)	0.083
<8	18	83		
Tumor stage				
>2	17	40	2.34(0.94-5.87)	0.044
≤2	12	66		
Aggressive				
Yes	7	41	0.53(0.19-1.41)	0.165
No	26	80		

ages at diagnosis, Gleason scores, Tumor stages and aggressive in patients with PCa by stratification analysis. The result showed that only the genotypes TT of in 13q22 (rs9600079, T) was associated with tumor stage ($P=0.044$, OR=2.34, 95% CI=0.94-5.87). For the other SNPs we not observed significantly associated with any clinical covariants in cases with prostate cancer ($P > 0.05$) (Table 3).

Discussion

The products of KLK3 gene are as the important biomarkers i.e. human prostate-specific antigen (PSA) using in the clinical diagnosis of prostate cancer (PCa). The G protein-coupled receptor RFX6, C2orf43 and FOXP4 signaling play the important role in the development of PCa. We studied six loci involving rs2735839(G) located in KLK3, rs13385191(G) of C2orf43, rs1983891(T) in FOXP4, rs339331(C) in RFX6 gene, rs12653946(C) in 5p15 and rs9600079(T) in 13q22 to investigate the association of these genetic variations with PCa patients in Northern Chinese, to infer their impact on occurrence and aggression of prostate cancer in northern Chinese men.

Kallikrein-related peptidase 3 (Previous names: kallikrein 3, (prostate specific antigen)) is one of the fifteen kallikrein subfamily members located in a cluster on chromosome 19. Its protein product is a protease present in seminal plasma. Serum level of this protein, called PSA in the clinical setting, is useful in the diagnosis and monitoring of prostatic carcinoma. Waters et al. (2009) produced the Multiethnic Cohort (African Americans, European Americans, Latinos, Japanese, Americans, and Native Hawaiians). They found KLK2/3, rs2735839 (OR, 1.06; 95% CI, 0.97-1.16; $P < 0.05$) variant was positively associated with PCa risk, with statistically significant associations from ethnic-pooled analyses. While our populational study haven't found statistically significant associations with PCa risk in KLK3 gene.

The function of Forkhead box P4 (FOXP4) is transcriptional repressor that represses lung-specific expression. Regulatory factor X, 6 (RFX6), transcription factor required to direct islet cell differentiation during

endocrine pancreas development. Specifically required for the differentiation of 4 of the 5 islet cell types and for the production of insulin. Not required for pancreatic PP (polypeptide-producing) cells differentiation. Acts downstream of NEUROG3 and regulates the transcription factors involved in beta-cell maturation and function, thereby restricting the expression of the beta-cell differentiation and specification genes, and thus the beta-cell fate choice. Activates transcription by forming a heterodimer with RFX3 and binding to the X-box in the promoter of target genes. Lindström et al. (2012) found SNPs (rs13385191, rs12653946, rs1983891, and rs339331) were significantly associated with prostate cancer risk (P values ranging from 0.01 to 1.1×10^{-5}). Allele frequencies and OR were overall lower in population of European descent than these in the discovery Asian population.

Four SNPs associated with prostate cancer risk in an Asian population are also associated with prostate cancer risk in men of European descent. Batra et al. (2011) undertook a replication study in 1,357 prostate cancer patients and 1,403 healthy Australian males of European descent. The rs12653946 SNP at 5p15 was found to be significantly associated with prostate cancer risk (OR = 1.20, 95% confidence interval: 1.07, 1.34; $P = 0.002$) in the Japanese population. Takata et al. (2010) found five loci for prostate cancer susceptibility, at 5p15 (lambda-corrected probability $P(\text{GC}) = 3.9 \times 10^{-18}$), GPRC6A/RFX6 ($P(\text{GC}) = 1.6 \times 10^{-12}$), 13q22 ($P(\text{GC}) = 2.8 \times 10^{-9}$), C2orf43 ($P(\text{GC}) = 7.5 \times 10^{-8}$) and FOXP4 ($P(\text{GC}) = 7.6 \times 10^{-8}$) in the Japanese population. After, Wang et al. (2012) found that three genetic variants were associated with prostate cancer risk ($P = 4.33 \times 10^{-8}$ for rs12653946 at 5p15, 4.43×10^{-5} for rs339331 at 6q22 and 8.42×10^{-4} for rs9600079 at 13q22, respectively) in Japanese men. These results provide further evidence that the risk loci identified in Japanese men also to be possible to contribute to prostate cancer susceptibility in Chinese men.

In conclusion, our results shows that the RFX6 gene (rs339331, C) variant is associated with PCa risk, the 13q22 (rs9600079, T) variant in is related to advanced tumor stage in northern Chinese men. However, the other loci in this study were failed to be replicated, probably are not risk alleles associated with PCa in northern Chinese men, which maybe harbor genetic differences from the other populations.

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References

- Amundadottir LT, Sulem P, Gudmundsson J, et al (2006). A common variant associated with prostate cancer in European and African populations. *Nat Genet*, **38**, 652-8.
- Batra J, Lose F et al (2011). A replication study examining novel common single nucleotide polymorphisms identified through a prostate cancer genome-wide association study in a Japanese population. *Am J Epidemiol*, **174**, 1391-5.
- Duggan D, Zheng SL, Knowlton M, et al (2007). Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP. *J Natl Cancer Inst*, **99**, 1836-44.
- Gudmundsson J, Sulem P, Gudbjartsson DF, et al (2009). Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. *Nat Genet*, **41**, 1122-6.
- Gudmundsson J, Sulem P, Rafnar T, et al (2008). Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nat Genet*, **40**, 281-3.
- Gudmundsson J, Sulem P, Steinthorsdottir V, et al (2007). Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet*, **39**, 977-83.
- Jemal A, Siegel R, Xu J, Ward E (2010). Cancer statistics, 2010. *CA Cancer J Clin*, **60**, 277-300.
- Lindström S, Schumacher FR, Campa D et al (2012). Replication of five prostate cancer loci identified in an Asian population—results from the NCI Breast and Prostate Cancer Cohort Consortium (BPC3). *Cancer Epidemiol Biomarkers Prev*, **21**, 212-6.
- Nobata S, Hishida A et al (2012). Association between KLK3 rs2735839 G/A Polymorphism and Serum PSA Levels in Japanese Men. *Urol Int*, **89**, 39-44.
- Ren Q, Xu B, Chen SQ et al (2012). A common genetic variant of 5p15.33 is associated with risk for prostate cancer in the Chinese population. *Genet Mol Res*, **11**, 1349-56.
- Schumacher FR, Berndt SI, Siddiq A, et al (2011). Genome-wide association study identifies new prostate cancer susceptibility loci. *Hum Mol Genet*, **20**, 3867-75.
- Sun Y (2004). our country the current research status of prostate cancer. *Chin J Urol*, **25**, 77-80.
- Takata R, Akamatsu S et al (2010). Genome-wide association study identifies five new susceptibility loci for prostate cancer in the Japanese population. *Nat Genet*, **42**, 751-4.
- Thomas G, Jacobs KB, Yeager M, et al (2008). Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet*, **40**, 310-5.
- Wang M, Liu F, Hsing AW, et al (2012). Replication and cumulative effects of GWAS-identified genetic variations for prostate cancer in Asians: a case-control study in the China PCa consortium. *Carcinogenesis*, **33**, 356-60.
- Waters KM, Le Marchand L, Kolonel LN, et al (2009). Generalizability of associations from prostate cancer genome-wide association studies in multiple populations. *Cancer Epidemiol Biomarkers Prev*, **18**, 1285-9.
- Xu J, Zengnan Mo, Dingwei Ye, et al (2012). Genome-wide association study in Chinese men identifies two new prostate cancer risk loci at 9q31.2 and 19q13.4. *Nat Genet*, **44**, 1231-5.
- Yeager M, Chatterjee N, Ciampa J, et al (2009). Identification of a new prostate cancer susceptibility locus on chromosome 8q24. *Nat Genet*, **41**, 1055-7.
- Yeager M, Orr N, Hayes RB, et al (2007). Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet*, **39**, 645-9.