RESEARCH ARTICLE

Association of Six Susceptibility Loci with Prostate Cancer in Northern Chinese Men

Yu-Rong Zhang[1,4], Yong Xu[5], Kuo Yang[5], Ming Liu[1&], Dong Wei[3], Yao-Guang Zhang[3], Xiao-Hong Shi[1], Jian-Ye Wang[3], Fan Yang[1], Xin Wang[3], Si-Ying Liang[1], Cheng-Xiao Zhao[2], Fei Wang[3], Xin Chen[3], Liang Sun[1], Xiao-Quan Zhu[1], Ling Zhu[6], Yi-Ge Yang[1], Lei Tang[1], Hai-Yan Jiao[4], Zheng-Hao Huo[4], Ze Yang[1*]

Abstract

Background/Aim: Six prostate cancer (PCa) susceptibility loci were identified in a genome-wide association study (GWAS) in populations of European decent. However, the associations of these 6 single-nucleotide polymorphisms (SNPs) with PCa has remained tobe clarified in men in Northern China. This study aimed to explore the loci associated with PCa risk in a Northern Chinese population. Methods: Blood samples and clinical information of 289 PCa patients and 288 controls from Beijing and Tianjin were collected. All risk SNPs were genotyped using polymerase chain reaction (PCR)-high resolution melting curve technology and gene sequencing. Associations between PCa and clinical covariates (age at diagnosis, prostate-specific antigen [PSA], Gleason score, tumor stage, and level of aggressiveness) and frequencies of alleles and genotypes of these SNPs were analyzed using genetic statistics. Results: Among the candidate SNPs, 11p15 (rs7127900, A) was associated with PCa risk (P = 0.02, odds ratio [OR] = 1.64, 95% confidence interval [CI] = 1.09–2.46). Genotypes showed differences between cases and controls on 11p15 (rs7127900, A), 11q13 (rs7931342, T), and HNF1B (rs4430796, A) (P = 0.03, P = 0.01, and P = 0.04, respectively). The genotype TG on 11q13 (rs7931342, T) was positively associated with an increased Gleason score (P = 0.04, OR = 2.15, 95% CI = 1.02–4.55). Patients carrying TG on 17q24 (rs1859962, G) were negatively associated with an increased body mass index (BMI) (P = 0.03, OR = 0.44, 95% CI = 0.21–0.92) while those with AG on HNF1B (rs4430796, A) were more likely to have PSA increase (P = 0.002). Conclusion: Our study suggests that 11p15 (rs7127900, A) could be a susceptibility locus associated with PCa in Northern Chinese. Genotype TG on 11q13 (rs7931342, T) could be related to an increased Gleason score, AG on HNF1B (rs4430796, A) could be associated with PSA increase, and TG on 17q24 (rs1859962, G) could be negatively associated with an increased BMI in Chinese men with PCa.

Keywords: Association - susceptibility loci - prostate cancer - Northern Chinese

Asian Pacific J Cancer Prev, **13 (12)**, 6273-6276

Introduction

Prostate cancer (PCa) is one of the most common cancers affecting men worldwide. It has different morbidity rates in different countries. Although the American population has a higher morbidity than the Chinese population, the prevalence of PCa in China requires attention (Jamal et al., 2007; Zhang et al., 2011).

Risk factors for PCa increase with age, ethnic background, and familial history of PCa (Chung et al., 2011). A genome-wide association study (GWAS) found at least 35 loci related to PCa (Sun et al., 2008; Thomas et al., 2008). Testing of these risk alleles across populations is important (Waters et al., 2009). However, the association of these 6 single-nucleotide polymorphisms (SNPs) with PCa was still unknown in Northern Chinese men. Study of the PCa risk loci distribution in Northern Chinese populations could increase clinical understanding of the PCa genetic etiology. Therefore, this study aimed to exam the 6 risk loci (MSMB, rs10993994, T; 11p15, rs7127900, A; 11q13, rs7931342, T; HNF1B, rs4430796, A; 17q24, rs11859962, G; KLK2, rs2735839, A) associated with PCa risk in Northern Chinese populations.

*[1]The Key Laboratory of Geriatrics, Beijing Hospital & Beijing Institute of Geriatrics, Ministry of Health, [2]Peking Union Medical College and Chinese Academy of Medical Sciences, Graduate School, [3]Department of Urology and Beijing Hospital, Chinese Ministry of Health, [6]Physical Examination Centre, Beijing Hospital, Ministry of Health, Beijing, [4]Basic Medical Academy of Ningxia Medical University, Yinchuan, [5]Department of Urology, the Second Hospital of Tianjin Medical University, Tianjin, China [&]Equal contributors *For correspondence: yangze016@yahoo.com.cn*

Materials and Methods

Study Population

To 2010 August, data were collected on 289 PCa patients (214 with nonaggressive PCa, Gleason score < 8, and disease stage < III; 75 with aggressive PCa, Gleason score ≥ 8, and/or disease stage ≥ III) and 288 controls among men of Chinese ancestry. The investigators conducted a case-control study. The mean age of the patients with PCa was 72.3 ± 7.48 years, and the mean age of the total control population was 70.5 ± 7.9 years. Patients were recruited at Beijing Hospital and Tianjin Urology Research Institute during the same period.

All PCa patients were diagnosed by histopathology. Blood samples and clinical information (family history of PCa, diagnostic age, body mass index [BMI], Gleason score, prostate-specific antigen [PSA] level, and neoplasm staging) were collected. Standards of controls were male, no family history of PCa, negative digital rectal examination, and PSA level < 4 ng/mL. Each tumor was graded using the Gleason score system and staged using the tumor-node-metastasis system. This study was approved by the Ethics Committee of Beijing Hospital and Tianjin Urology Research Institute, and informed consent was obtained from all study participants.

Reagents

Kits for extracting DNA were purchased from Bio Chain Company in Beijing. Taq DNA polymerase and deoxyribonucleotide triphosphates were purchased from Beijing Dingguo biotechnology Co. Ltd. LC-green PLUS saturated fluorescent dye was obtained from American Idaho Company. Synthetic primers were purchased from Shanghai Shenggong Biotechnology Co. Ltd.

SNP Selection for Genotyping

Six SNPs were selected from European decent risk loci (MSMB, rs10993994, T; 11p15, rs7127900, A; 11q13, rs7931342, T; HNF1B, rs4430796, A; 17q24, rs11859962, G; and KLK2, rs2735839, A). Blood genomic DNA was extracted using a whole blood genomic DNA extraction kit (Biochain Science-Technology, Beijing, China). Polymerase chain reaction (PCR) amplification and high-resolution melting curve analysis of small amplicons were performed according to a study by Liew et al. (Liew et al., 2004). PCR was performed in a PTC-225 Tetrad® DNA Thermal Cycler under the following conditions: initial denaturation at 95°C for 5 min; 35 cycles at 95°C for 30 s, annealing for 30 s, and extension at 72°C for 6 s; and completion at 72°C for 7 min, followed by 2 cycles at 94°C for 30 s and at 24°C for 2 min. The automatically and manually verified PCR products were genotyped using the Light Scanner® TMHR-I 96. The PCR procedure included denaturation at 95°C for 5 minutes; 35 cycles at 95°C for 30 seconds, annealing for 30 seconds, and extension at 72°C for 45 seconds; and completion at 72°C for 7 minutes (Liu et al., 2012).

Statistical Analysis

Fisher's exact test was used to evaluate Hardy-Weinberg equilibrium (HWE) for each SNP among cases and controls. Hardy-Weinberg balance testing reveals the representative of the sample group. Allele frequency and genotype differences between cases and controls were tested for each SNP using the chi-square test with 1 degree of freedom (Jielin et al., 2008). Odds ratios (ORs) and 95% confidence intervals (CIs) of the effect of each variant on PCa risk were computed using SHEsis and SPSS version 11.5 ($P < 0.05$).

Results

No significant difference was noted in mean age between cases and controls (72.3 ± 7.48 years versus 70.5 ± 7.9 years). There were 49 patients with a BMI value of 25 or greater and 85 patients with a BMI value of less than 25. Seventy-four patients had a PSA value of 20 or greater and 102 patients had a value of less than 10; a PSA value between 10 and 20 was reported in 39 patients. Forty-two patients had a Gleason score of 8 or greater and 101 patients had a score of less than 8. Tumor stage ≥ III was reported in 57 patients and stage < III was reported in 78 patients (Table 1).

Allele frequencies were tested between cases and control subjects; 11p15 (rs7127900, A) showed a significant difference ($P = 0.02$, OR = 1.64, 95% CI = 1.09–2.46). Genotype results also provided information of PCa risk with sequence variants. 11p15 (rs7127900, A), 11q13 (rs7931342, T), and HNF1B (rs4430796, A) showed a difference in genotype frequencies between cases and control subjects ($P = 0.03$, $P = 0.01$, and $P = 0.04$, respectively) (Table 2).

We assessed associations of these risk variants with phenotypes of PCa among cases using the best-fitting genetic model. Patients with genotype TG on 11q13 (rs7931342, T) were positively associated with an increased Gleason score ($P = 0.04$, OR = 2.15, 95% CI = 1.02–4.55). Patients carrying TG on 17q24 (rs1859962, G) were negatively associated with an increased BMI ($P = 0.03$, OR = 0.44, 95% CI = 0.21–0.92). Patients with AG on HNF1B (rs4430796, A) were associated with PSA

Table 1. Phenotypes of Patients with PCa

	Number of patients
BMI	134
25 or greater	49
Less than 25	85
Age	261
75 or greater	111
65–75	113
Less than 65	37
PSA (ng/ml)	215
20 or greater	74
10–20	39
Less than 10	102
Gleason score	143
8 or greater	42
Less than 8	101
Tumor stage	135
I	10
II	68
III	44
IV	13

Table 2. Allele and Genotype Distributions

SNPs	Gene			Case = 289	Control = 288	P	OR	95%CI
rs10993994	MSMB	allele	C	256 (0.48)	226 (0.51)	0.33	0.88	0.69–1.13
			T	280 (0.52)	218 (0.49)			
		genotype	CC	63 (0.28)	61 (0.28)	0.59		
			CT	130 (0.49)	104 (0.47)			
			TT	75 (0.28)	57 (0.26)			
		HEW (P)			0.35			
rs7127900	11p15	allele	A	84 (0.15)	84 (0.10)	0.02	1.64	1.09–2.46
			G	464 (0.85)	353 (0.90)			
		genotype	AA	5 (0.02)	3 (0.02)	0.03		
			AG	74 (0.27)	33 (0.17)			
			GG	195 (0.71)	160 (0.82)			
		HEW (P)			0.4			
rs7931342	11q13	allele	G	149 (0.26)	162 (0.28)	0.39	0.89	0.69–1.16
			T	423 (0.74)	410 (0.72)			
		genotype	GG	27 (0.09)	17 (0.06)	0.01		
			GT	95 (0.33)	128 (0.45)			
			TT	164 (0.57)	141 (0.49)			
		HEW (P)			0.08			
rs4430796	HNF1B	allele	A	298 (0.76)	227 (0.71)	0.1	1.33	0.95–1.86
			G	92 (0.24)	93 (0.29)			
		genotype	AA	119 (0.61)	77 (0.48)	0.04		
			AG	60 (0.31)	73 (0.46)			
			GG	16 (0.08)	10 (0.06)			
		HEW (P)			0.18			
rs1859962	17q24	allele	G	242 (0.45)	217 (0.41)	0.15	1.2	0.94–1.52
			T	292 (0.55)	313 (0.59)			
		genotype	GG	54 (0.20)	41 (0.16)	0.31		
			GT	134 (0.50)	135 (0.51)			
			TT	79 (0.30)	89 (0.34)			
		HEW (P)			0.38			
rs2735839	KLK2/KLK3	allele	A	240 (0.42)	220 (0.39)	0.33	1.12	0.89–1.43
			G	330 (0.58)	340 (0.61)			
		genotype	AA	57 (0.20)	38 (0.14)	0.08		
			AG	126 (0.44)	144 (0.51)			
			GG	102 (0.36)	98 (0.35)			
		HEW (P)			0.19			

increase (P = 0.002). Other variants showed no significant relationship with PCa in terms of age grading, PSA level, and Gleason score (P > 0.05).

Discussion

In this study, we showed that 11p15 (rs7127900, A) (P = 0.02, OR = 1.64, 95% CI = 1.09–2.46) was associated with PCa risk in Northern Chinese populations. We also showed that patients with genotype TG on 11q13 (rs7931342, T) were positively associated with an increased Gleason score (P = 0.04, OR = 2.15, 95% CI = 1.02–4.55). AG on HNF1B (rs4430796, A) was related to PSA increase (P = 0.002). Patients carrying TG on 17q24 (rs1859962, G) were negatively associated with an increased BMI (P = 0.03, OR = 0.44, 95% CI = 0.21–0.92). These data were in accordance with results previously reported in the GWAS with regard to loci related to PCa (Sun et al., 2008; Thomas et al., 2008). Because these PCa risk-associated loci (i.e., 11p15 [rs7127900, A], 11q13 [rs7931342, T], and 17q24 [rs1859962, G]) are located in inter-genic regions, the functions related to PCa are still not clear.

HNF1B is located on chromosome 17q12. It is a transcription factor that encodes 3 isoforms: transcriptional activators A and B and transcriptional repressor C. Two risk alleles for PCa have been detected in the HNF1B non-coding sequence (Gudmundsson et al., 2007; Waters et al., 2009; Setiawan et al., 2012). Jielin et al. (Jielin et al., 2008) first found PCa risk-associated SNPs at 17q12 (HNF1B) and 17q24.3. Furthermore, they showed that 2 17q SNPs had a risk genotype AA that plays a part in PCa. However, they did not find the association with clinical characteristics in their research. They believed that the SNPs at 17q12 and 17q24.3 were related to risk for more and less aggressive tumors; these SNPs likely influenced aspects of PCa initiation rather than progression (Sun et al., 2008). In our study, patients carrying TG on 17q24 (rs1859962, G) were negatively associated with an increased BMI (P = 0.03, OR = 0.44, 95% CI = 0.21–0.92). AG on HNF1B (rs4430796, A) was related to PSA increase (P = 0.002). In another study, researchers concluded from GWAS that HNF1B (rs4430796, A) was related to endometrial cancer risk in women of European background (Spurdle et al., 2011). HNF1B had also been reported to be associated with maturity-onset diabetes of the young subtype 5 (MODY5), renal cysts, pancreatic atrophy, and uterine abnormalities caused by incomplete Mullerian duct fusion and Mullerian duct aplasia. Interestingly, diabetes and PCa had a common biotic link; the former was associated with a decreased

risk of the latter. Further, HNF1B was related to both these diseases (Pierce et al., 2008; Elliott et al., 2010; Stevens et al., 2010). Perhaps, the other factors also played certain modifier role, i.e. Cancer incidence in Middle East immigrants in California was 2.4 times higher than rates in home countries (Nasseri et al., 2007) .In summary, HNF1B plays a role in several genetic diseases. Further research on this topic is warranted.

In summary: Our study suggests that 11p15 (rs7127900, A) could be the susceptible gene associated with PCa risk in Northern Chinese populations. The genotype TG on 11q13 (rs7931342, T) could be related to an increased Gleason score, AG on HNF1B (rs4430796, A) is possibly associated with PSA increase, and TG on 17q24 (rs1859962, G) is possibly associated with an increased BMI in Chinese men with PCa.

Acknowledgements

We thank all nurses from the Department of Urology in Beijing Hospital for collecting specimens and clinical information; YC Qu and Y Liu of the Tianjin Institute of Urology in the Second Hospital of Tianjin Medical University for collecting blood samples; YY Zhao from the Physical Examination Centre and MY He from the clinical laboratory in Beijing Hospital for collecting blood samples from healthy controls; and L Zhou, J Huang, LM Pu, and J Ren from the Institute of Geriatrics and Beijing Hospital for dealing with the patients' information and experimental data. This study was funded by the National Natural Science Foundation Project of China (30972709, 81061120527 and 81241082). The major funding from Beijing Hospital (BJ-2010-30) was awarded to Z Yang, and funding from the key project of clinical disciplines at the subordinate hospital, Ministry of Health (10120101), was awarded to JY Wang. We thank all of the patients and research workers for their participation.

References

Chung CC, Ciampa J, Yeager M, et al (2011). Fine mapping of a region of chromosome 11q13 reveals multiple independent loci associated with risk of prostate cancer. *Hum Mol Genet*, **20**, 2869-78.

Elliott KS, Zeggini E, McCarthy MI, et al (2010). Evaluation of association of HNF1B variants with diverse cancers: Collaborative analysis of data from 19 genome-wide association studies. *PLoS One*, **5**, e10858.

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.

Jamal A, Siegel R, Ward E, et al (2007). Cancer statistics, 2007. *CA Cancer J Clin*, **57**, 43-66.

Liew M, Pryor R, Palais R, et al (2004). Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. *Clin Chem*, **50**, 1156-64.

Liu M, Wang JY, Xu Y, et al (2012). Risk loci on chromosome 8q24 are associated with prostate cancer in northern Chinese men. *J Urol*, **187**, 315-21.

Nasseri K, Mills PK, Allan M (2007). Cancer Incidence in the Middle Eastern Population of California, 1988–2004. *Asian Pac J Cancer Prev*, **8**, 405-11.

Pierce BL, Plymate S, Ostrander EA, et al (2008). Diabetes mellitus and prostate cancer risk. *Prostate*, **68**, 1126-32.

Setiawan VW, Haessler J, Schumacher F, et al (2012). HNF1B and endometrial cancer risk: results from the PAGE study. *PLoS One*, **7**, e30390.

Spurdle AB, Thompson DJ, Ahmed S, et al (2011). Genome-wide association study identifies a common variant associated with risk of endometrial cancer. *Nat Genet*, **43**, 451-4.

Stevens VL, Ahn J, Sun J, et al (2010). HNF1B and JAZF1 genes, diabetes, and prostate cancer risk. *Prostate*, **70**, 601-7.

Sun J, Purcell L, Gao Z, et al (2008). Association between sequence variants at 17q12 and 17q24.3 and prostate cancer risk in European and African Americans. *Prostate*, **68**, 691-7.

Sun J, Zheng SL, Wiklund F, et al (2008). Evidence for two independent prostate cancer risk-associated loci in the HNF1B gene at 17q12. *Nat Genet*, **40**, 1153-5.

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.

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.

Zhang YF, Guan YB, Yang B, et al (2011). Prognostic value of Her-2/neu and clinicopathologic factors for evaluating progression and disease-specific death in Chinese men with prostate cancer. *Chin Med J (Engl)*, **124**, 4345-9.