JBUON 2015; 20(5): 1223-1228
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Association of THADA, FOXP4, GPRC6A/RFX6 genes and 8q24 risk alleles with prostate cancer in Northern Chinese men

Xing-hui Li[1,2], Yong Xu[3], Kuo Yang[3], Jian-jian Shi[3], Xiao Zhang[2] , Fang Yang[2], Huiping-Yuan[2], Xiaoquan-Zhu[2], Yu-hong Zhang[1], Jian-ye Wang[2], Ze Yang[2]

[1]School of Public Health, Ningxia Medical University, Yinchuan; [2]The Key Laboratory of Geriatrics, Beijing Hospital and Beijing Institute of Geriatrics, Chinese Ministry of Health, Beijing; [3]Department of Urology, The Second Hospital of Tianjin Medical University, Tianjin, China

Summary

Purpose: *Prostate cancer (PCa) is one of the most common malignancies in males, and multiple genetic studies have confirmed association with susceptibility to PCa. However, the risk conferred in men living in China is unkown. We selected 6 previously identified variants as candidates to define their association with PCa in Chinese men.*

Methods: *We genotyped 6 single nucleotide polymorphisms (SNPs) (rs1465618, rs1983891, rs339331, rs16901966, rs1447295 and rs10090154) using high resolution melting (HRM) analysis and assessed their association with PCa risk in a case-control study of 481 patients and 480 controls in a Chinese population. In addition, the individual and cumulative contribution for the risk of PCa and clinical covariates were analysed.*

Results: *We found that 5 of the 6 genetic variants were associated with PCa risk. The T allele of rs339331 and the G allele of rs16901966 showed a significant association with PCa susceptibility: OR (95%CI)= 0.78 (0.64-0.94), p<0.009 and OR (95%CI)= 0.66 (0.54-0.81), p<0.0001, as well as A allele of rs1447295 (OR [95%CI]=1.46 (1.17-1.84), p<0.001) and T allele of rs10090154 (OR [95%CI]= 0.58 (0.46-0.74), p<0.0001). rs339331(T) was associated with a 0.71-fold and 1.42-fold increase of PCa risk by dominant model (p=0.007) and recessive model (p=0.007). rs16901966 (G) was associated with a 0.51-fold and 1.98-fold increase of PCa risk by dominant model (p=0.006) and recessive model (p=0.0058). rs10090154 (T) was associated with a 1.89-fold and 0.53-fold increase of PCa risk by dominant model (p=0.000006) and recessive model (p=0.000006). And, rs1983891(C) was associated with a 0.77-fold increase of PCa risk by recessive model (p=0.045). rs1447295 was associated with a 1.57-fold increase of PCa risk by dominant model (p=0.008). rs1465618 showed no significant association with PCa. The cumulative effects test of risk alleles (rs rs1983891, rs339331, rs16901966, rs1447295 and rs10090154) showed an increasing risk to PCa in a frequency-dependent manner (p_{trend}=0.001), and men with more than 3 risk alleles had the most significant susceptibility to PCa (OR=1.99, p=0.001), compared with those who had one risk allele (OR=1.17, p=0.486).*

Conclusion: *Our results provide further support for association of the THADA, FOXP4, GPRC6A/RFX6 and 8q24 genes with Pca in Asian populations. Further work is still required to determine the functional variations and finally clarify the underlying biological mechanisms.*

Key words: *clinical phenotype, Northern Chinese, prostate cancer, single nucleotide polymorphism*

Introduction

PCa is one of the most common malignancies in males worldwide with increasing trend [1], and the second leading cause of cancer-related deaths in most Western countries [2]. With lifestyle transition, PCa has become more and more prevalent in Asia and has become an important risk to the expectancy of life of the elderly, while seriously affecting the quality of life, although Chinese pop-

Correspondence to: Ze Yang, PhD. The Key Laboratory of Geriatrics, Beijing Hospital and Beijing Institute of Geriatrics, Chinese Ministry of Health, Beijing, China. Tel: +86 010 58115043, Fax: + 86 010 65132969, E-mail: yang-ze@sina.com
Received: 11/05/2015; Accepted: 28/05/2015

ulations have the lowest incidence and mortality rate of PCa in the world.

It is known that age, race, and family history are major PCa risk factors [3-5]. The idea that genetics play an important role in the susceptibility to almost all human cancers is largely accepted. Two twin studies showed that approximately 42% of PCa risk can be explained by heritable genetic factors [6,7]. Genome-wide association studies have identified that more than 50 loci were significantly associated with a risk of PCa in various racial populations, and 5 new loci were identified in Japanese population. Some studies have reported that THADA, FOXP4, GPRC6A/RFX6 and 8q24 variants were associated with aggressive PCa [8-10], while other studies have not corroborated these findings. However, the associations of the SNP rs1465618 in THADA, rs1983891 in FOXP4, rs339331 in GPRC6A/RFX6, rs16901966, rs1447295 and rs10090154 in 8q24 with PCa have not been studied in Chinese populations before [11] to highlight the complex genetic effect on prostate carcinogenesis. Investigation in diverse populations of the variants may enhance the understanding of the genetic mechanisms of PCa.

Herein, we present the results of a case-control study of these 6 loci for potential association with PCa in northern Chinese population. In addition to studying more advanced disease, we provide the simultaneous replication of associations between 6 independent regions and PCa, which might provide evidence of associations of THADA, FOXP4, GPRC6A/RFX6 and 8q24 variants with PCa risk among different ethnic populations. Furthermore, we evaluated the potential effect of these genetic variants on age, prostate-specific antigen (PSA), Gleason score and tumor stage.

Ethics statement

Written informed consent was obtained from all participants and the study was approved by the institutional review board of Beijing Hospital.

Study sample

We performed a population-based case-control study conducted between January 2010 and December 2014, which included 961 Chinese men consisting of 481 PCa patients and 480 healthy geography- and age-matched controls. All cases and controls were men of unrelated Northern Han Chinese ancestry. Consecutive patients were recruited who were permanent residents of Beijing and Tianjin in the Jingjin area. All patients were independently and consistently diagnosed at the Department of Urology, Beijing Hospital, Ministry of Healthy or Tianjin Urology Institute, Second People's Hospital of Tianjin Medical University, based on medical records and pathological evaluation of prostate biopsy. Each PCa patient filled in a structured questionnaire covering age at diagnosis, serum PSA, Gleason score, and tumor stage. Eligible controls were men age-matched, residing within the study area, with PSA <4.0 ng/ml, negative digital rectal examination, and without personal history of PCa or other cancer.

SNP selection for genotyping

Six primary variants were selected, which were previously associated with PCa risk in genome-wide association studies (GWAS) of European and American populations. These loci included rs1465618, rs1983891, rs339331, rs16901966, rs1447295 and rs10090154. Blood genomic DNA was extracted from peripheral blood using a whole blood genomic DNA extraction kit (Biochain, Science-Technology, Beijing, China); polymerase chain reaction (PCR) was performed using a PTC-225 Tetrad DNA Thermal Cycler (Bio-Rad, Hercles, CA, USA). The PCR procedure involved initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, annealing for 30 s, extension at 72°C for 15 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 genotypes of each risk variant using a LightScanner® TMHR-I 96. Six samples randomly selected from individuals of each genotype were sequenced for verification (sequenced by Beijing Tianyi Huiyuan Bioscience and Technology Inc.)

Statistics

Hardy-Weinberg equilibrium (HWE) was assessed for SNP in case and control groups using Pearson's x^2 test. OR and 95%CI in dominant and recession models were calculated to compare genotype frequencies between PCa cases and controls using Pearson's x^2 or Fisher's exact test. Statistical analysis was done using SPSS, version 18.0 software. Significance was set at $p \leq 0.05$ (all p values were two-sided).

Results

The mean age was 72.6±9.14 years for pa-

tients and 70.0±10.81 years for controls (p>0.05). The median PSA level of the cases was 46.73 ng/ml (range 0.09-1334) . The patient characteristics are presented in Table 1.

The allele and genotype frequencies of rs1465618, rs1983891, rs339331, rs16901966, rs1447295 and rs1009054 were determined in patients with PCa and controls. The genotype frequency distributions agreed with the HWE (p>0.05). Each of the allele and genotype frequencies was compared between PCa patients and controls (Table 2). The T allele of rs339331 and the G allele of rs16901966 showed a significant association with PCa susceptibility (OR [95%CI]= 0.78 (0.64-0.94), p<0.009 and OR [95%CI]= 0.66 (0.54-0.81), p< 0.0001, as well as A allele of rs1447295 (OR [95%CI]= 1.46(1.17-1.84), p <0.001) and T allele of rs10090154 (OR [95%CI]= 0.58 (0.46-0.74), p<0.0001), which produced similar positive associations in Caucasians.

The distributions of the genotypes of the 6 SNPs in the cases and controls were also evaluated. rs339331(T) was associated with a 0.71-fold and 1.42-fold increase of PCa risk by dominant model (p=0.007) and recessive model (p=0.007). rs16901966 (G) was associated with a 0.51-fold and 1.98-fold increase of PCa risk by dominant model (p=0.006) and recessive model (p=0.0058). rs10090154 (T) was associated with a 1.89-fold and 0.53-fold increase of PCa risk by dominant model (p=0.000006) and recessive model (p=0.000006). And, rs1983891(C) was associated with a 0.77-fold increase of PCa risk by recessive model (p=0.045). rs1447295 was associated with a 1.57-fold increase of PCa risk by dominant model (p=0.008). rs1465618 showed no significant association with PCa (Table 3).

Table 1. Patient and tumor characteristics

Characteristics	*N*	%
Age (years)		
<65	58	12.1
≥65	395	82.1
Missing data	28	5.8
PSA (ng/ml)		
<10	225	46.8
10-20	52	10.8
>20	112	23.3
Missing data	91	18.9
Gleason score		
<7	173	36
≥7	105	21.8
Missing data	203	42.2
Tumor stage		
T1	15	31.2
T2	76	15.8
T3	46	9.6
T4	13	2.7
Missing data	330	68.6

The association between the 6 risk variants (rs1465618, rs1983891, rs339331, rs16901966, rs1447295 and rs10090154) and PCa stratified by age, PSA, Gleason score and tumor stage was further evaluated among cases. As shown in Table 4, none of the 6 genetic variants was significantly associated with the PSA and tumor stage; only rs339331 was associated with age (p=0.002) and rs1983891 was associated with Gleason score (p=0.00001).

The cumulative effects test of risk alleles (rs1983891, rs339331, rs16901966, rs1447295 and rs10090154) showed an increasing risk to PCa in a frequency-dependent manner (p_{trend}=0.001). Subjects with more than 3 risk alleles had the most significant susceptibility to PCa (OR=1.99, p=0.001), compared with those who had one risk allele (OR=1.17, p=0.486; Table 5).

Discussion

Despite numerous studies addressing genetic susceptibility to PCa, it is still unclear how its genome polymorphisms can affect the risk of PCa. The present case-control study investigated the relationship between variants and the risk of PCa in northern Chinese men. In this study we found that two significant variants (rs1983891 and rs339331) had risk estimates in the same region as that reported for Japanese men [12].

The SNP rs1465618 in THADA at 2p21 has been identified as being associated with PCa risk in Europeans; however, it is not clear whether this SNP is connected to PCa risk in multiple populations. In our study, no significant association was detected for rs1465618, which differed from the Zhu et al. study among 1000 samples of several Asian ethnic groups [13].

rs339331 is located in the chromosome 6q22 and was found to be significantly associated with PCa risk in our Chinese population, and includes two genes, RFX6 and GPRC6A [14,15]. RFX6 is a member of the regulatory factor X family of transcription factors, which has been shown to be primarily expressed in many tissues and organs, including the testis. GPRC6A is highly expressed in testis Leydig cells, which had altered circulating testosterone and estrogen levels in knockout mice [16,18].

Table 2. Distribution and comparison of alleles and genotype of 6 variations

SNP ID	Location	Allele	Allele frequency(1/2)			p*	Genotype number(11/12/22)						p**
		1/2	Case	Control	OR (95%CI)		Case (freq)			Control(freq)			
rs1465618	43326810	A/G	0.732/0.277	0.718/0.282	1.02 (0.84-1.25)	0.830	254(0.534)	180 (0.378)	42 (0.088)	249 (0.518)	193 (0.401)	39 (0.081)	0.740
rs1983891	41568689	C/T	0.657/0.343	0.699/0.301	0.82 (0.68-0.99)	0.046	206(0.428)	220 (0.457)	55 (0.114)	236 (0.493)	198 (0.413)	45 (0.094)	0.120
rs339331	116888889	T/C	0.703/0.297	0.647/0.353	0.78 (0.64-0.94)	0.009	242(0.503)	192 (0.399)	47 (0.098)	198 (0.417)	219 (0.461)	58 (0.122)	0.02
rs16901966	127098007	G/A	0.699/0.301	0.778/0.222	0.66 (0.54-0.81)	0.0001	49(0.103)	190 (0.397)	239 (0.500)	26 (0.055)	160 (0.335)	291 (0.610)	$6.3*10^{-3}$
rs1447295	127472793	A/C	0.772/0.228	0.832/0.168	1.46 (1.17-1.84)	0.001	16(0.034)	182 (0.388)	271 (0.578)	9 (0.019)	143 (0.299)	327 (0.683)	$2.76*10^{-3}$
rs10090154	127519892	T/C	0.788/0.212	0.864/0.136	0.58 (0.46-0.74)	0.0001	14(0.241)	172 (0.465)	285 (0.605)	7 (0.015)	113 (0.241)	348 (0.744)	$3*10^{-5}$

*Comparison of allele frequency;**Comparison of genotype frequency using additive model

Table 3. Association analysis by dominant and recessive model between PCa cases and controls

SNPs	Risk allele*	Dominant model			Recession model		
		Genotype	OR (95%CI)	p	Genotype	OR (95%CI)	p
rs1465618	A	AA+AG/GG	1.65 (1.13-3.23)	0.008	AA/AG+GG	1.21 (1.00-1.99)	0.051
rs1983891	C	CC+CT/TT	0.80 (0.53-1.68)	0.3	CC/CT+TT	0.77 (0.60-1.78)	0.045
rs339331	T	TT+TC/CC	0.71 (0.55-1.10)	0.007	TT/TC+CC	1.42 (1.10-2.19)	0.007
rs16901966	G	GG+GA/AA	0.51 (0.31-1.17)	0.006	GG/GA+AA	1.98 (1.21-2.86)	0.0058
rs1447295	A	AA+AC/CC	1.57 (1.21-2.36)	0.008	AA/AC+CC	1.88 (0.82-1.7)	0.127
rs10090154	T	TT+TC/CC	1.89 (1.43-2.83)	$6*10^{-6}$	TT/TC+CC	0.53 (0.40-0.87)	$6*10^{-6}$

*assumes the risk allele from other reported studies

Table 4. Association of the 6 genetic variants with pathologic characteristics of PCa among cases

	rs1465618				rs1983891				rs339331				rs16901966				rs1447295				rs10090154			
	AA	AG	GG	p	CC	CT	TT	p	TT	TC	CC	p	GG	AG	AA	p	AA	AC	CC	p	TT	TC	CC	p
Age (years)				0.76				0.841				0.002				0.128				0.65				0.883
<65	26	21	8		17	23	16		29	21	6		8	27	21		2	18	32		1	18	35	
≥65	196	152	44		105	174	116		123	159	113		39	152	201		13	154	220		12	141	236	
PSA (ng/ml)				0.16				0.346				0.18				0.852				0.85				0.927
<10	114	81	27		53	94	76		64	94	65		21	90	109		7	87	125		6	81	133	
10-20	31	19	3		17	23	13		22	20	11		5	18	30		3	19	31		2	19	32	
>20	46	48	17		35	48	29		45	40	27		13	42	57		4	38	67		3	35	71	
Gleason score				0.17				1.49×10^{-5}				0.15				0.485				0.09				0.312
<7	71	51	12		45	63	27		46	52	37		11	49	73		2	51	78		2	46	84	
≥7	73	49	24		25	55	66		35	61	50		17	58	71		10	54	82		7	51	87	
Tumor stage				0.24				0.333				0.19				0.422				0.34				0.425
T1	8	10	3		9	8	4		7	10	4		1	7	13		2	11	10		1	7	14	
T2	47	27	7		28	32	21		36	33	12		8	31	41		2	37	41		2	38	42	
T3	23	17	6		21	20	6		23	14	10		8	20	19		2	15	27		1	13	31	
T4	4	5	4		7	2	4		2	9	1		1	8	4		1	2	9		1	3	9	

Table 5. Cumulative effect of the 5 risk loci on the risk of PCa

	Cases		*Controls*		*OR (95%CI)*	*p*	p_{trend}
	N	%	*N*	%			
0	56	11.8	84	17.9			
1	85	17.8	109	23.3	1.17 (0.74-1.85)	0.486	
2	144	30.3	131	28	1.65 (1.06-2.56)	0.017	
≥3	191	40.1	144	30.8	1.99 (1.30-3.03)	0.001	0.001

rs1983891 is located on chromosome region 6p21 region in intron2 of FOXP4 (forkhead box P4). Mapping analysis of this region identified a 72kb Pca susceptibility region [19]. FOXP4 was first reported as a novel forkhead transcription factor, which is expressed in both thymocytes and peripheral CD(4+) and CD(8+) T-cells and is necessary for normal T-cell cytokine recall responses to antigen following pathogenic infection. However, the function of FOXP4 was unkown and further studies will be required to clarify their functional associations with prostate carcinogenesis [20,21].

Three SNPs discussed in this paper are located in 8q24 chromosomal region, which contains 3 independent blocks along a 600-kb segment associated with PCa susceptibility [22,23]. Region 8q24 is described as a "gene desert" [24], and the allelic and genotypic analyses in this study showed that SNPs in the region 2 (rs16901966) and region 1 (rs1447295 and rs10090154) of 8q24 were significantly associated with PCa, which was also confirmed in all of Caucasian population studies, however, differed from findings in some groups of European and black American ancestry reported by Cropp et al. [25].

In conclusion, our results provided further confirmation for a significant association of SNPs rs1465618, rs1983891, rs339331, rs16901966, rs1447295 and rs10090154 with susceptibility to PCa in northern Chinese men. One limitation of our study was that the number of our samples was relatively small; consequently, studies with larger populations were needed in the future.

Acknowledgements

We thank all the nurses in the Department of Urology, Beijing Hospital, for collecting specimens and clinical information. Jianjian Shi and Kuo Yang of the Tianjin Institute of Urology in the second Hospital of Tianjin Medical University for dealing with patient information and experimental data. This research was supported by the Natural Science Foundation of China (81370445, 81472408), the National Department of Public Benefit Research Foundation by Ministry of Health of the P. R. China (201302008) and the twelfth 5-year National Program from the Ministry of Scientific Technology (2012BAI10B01).

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