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ORIGINAL ARTICLE

Association of FOXP4 Gene with Prostate Cancer and the Cumulative Effects of rs4714476 and 8q24 in Chinese Men

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SUMMARY

Background: **The tumor suppressor forkhead box P4 (FOXP4) plays important roles in oncogenesis, and the *FOXP4* variant rs1983891 is associated with prostate cancer (PCa) in several studies. However, association studies conducted in Northern and Southern Chinese have provided conflicting results. Therefore, here we performed fine mapping of *FOXP4* to identify the association with PCa and the potential application in Chinese men.**
Methods: **We examined 11 variants spaced approximately 55 kb apart spanning *FOXP4* using high-resolution melting-curve analysis and sequencing methods in 286 PCa patients and 630 controls, and the association between these variants and PCa risk was evaluated. Additionally, we evaluated the cumulative effect of rs4714476 and 2 variants in 8q24 (rs16901966, rs10090154) confirmed in our previous study.**
Results: **Of 11 SNPs, only rs4714476-C at the 5′ near gene of *FOXP4* was associated with increased age-adjusted PCa risk (p = 0.012, OR = 1.32, 95% CI = 1.06 - 1.63) and aggressive PCa (p = 0.026). The CG haplotype covering rs4714476-C demonstrated significant differences between PCa cases and controls (p = 0.009). The cumulative effect analysis showed men who carried any combination of 1, 2, or 3 risk genotypes had a gradually increased PCa risk (age-adjusted OR is from 1.244 to 3.312).**
Conclusions: **These data suggest that rs4714476 at the 5′ near gene of *FOXP4* potentially contributes to the susceptibility of PCa in Chinese men. The cumulative effect of rs4714476 at *FOXP4* and 8q24 could increase PCa risk.**
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KEY WORDS

prostate cancer (PCa), FOXP4, 8q24, association, variants

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LIST OF ABBREVIATIONS

PCa - prostate cancer
SNP - single nucleotide polymorphism
GWAS - genome-wide association study
FOXP4 - forkhead box P4
LD - linkage disequilibrium
PCR-HRM - polymerase chain reaction-high resolution melting curves
HWE - Hardy-Weinberg equilibrium
PSA - prostate specific antigen

INTRODUCTION

The most recent cancer statistics show that prostate cancer (PCa) is the most common carcinoma of all newly diagnosed cancers and the second leading cause of death from malignancy in the United States [1]. Although the incidence of PCa is much lower in China compared with other western countries, the incidence of PCa is slowly rising in the Chinese men because of the influence of increased aging of the population [2,3]. PCa is a multifactorial and complex disease, family and twin studies suggest that genetic background is an important, immutable factor that contributes to the pathogenesis of PCa [4,5].

Forkhead-box (FOX) family members are evolutionarily conserved DNA-binding proteins involved in the transcriptional regulation of cell growth and differentiation. FOX proteins are divided into 15 classes from FoxA to FoxO and have different functions and can act as either tumour suppressors or oncogenes [6]. Several family members of FOX proteins are associated with PCa: FOXA1 is overexpressed in metastatic PCa and mutations of *FOXA1* are also detected in PCa [7,8]; however, as tumor suppressors, *FOXO1* and *FOXF1* mRNA levels are downregulated in PCa [9,10]; *FOXP1* is fused to the *ETV1* gene in PCa as a result of chromosomal translocations [11]; and in a recent study, the association between FoxP3 single nucleotide polymorphism (SNP) and non-small cell lung cancer was confirmed in Chinese Han population [12]. So, evaluating the association between FOX family can be useful for etiological studies of PCa, as well as biomarkers for predicting of PCa.

A genome-wide association study (GWAS) of Japanese identified that rs1983891 in *FOXP4* on chromosome 6p21 was associated with PCa [13]. The result has been confirmed in men of European descent and in Chinese [14,15], but failed to be replicated in other studies [16,17]. Because of genetic heterogeneity among different ethnicities, further fine mapping of *FOXP4* is required in broad and local geographic regions.

To further explore the association between *FOXP4* and PCa, we evaluated the association of 11 *FOXP4* SNPs with PCa in Chinese men. We explored the relationship between the identified PCa risk locus and PCa clinical covariates. Further, the linkage disequilibrium (LD) structure of *FOXP4* was assessed to apply a haplotype-based association analysis to the data. Additionally, two confirmed variants at 8q24 in our previous study were genotyped in a larger population and the cumulative effects of the PCa risk variants were evaluated.

MATERIALS AND METHODS

Study Population

The case-control study comprised 286 PCa patients and 630 geographically matched healthy controls. Of the total population, 265 case subjects and 288 control subjects were the same as our previous study and the detailed inclusion criteria of cases and control can refer to our description [18]. Additionally, we increased the samples especially the controls to make a ratio of cases to controls is about 1:2. This study was initiated following approval from the ethics committee of the two participating hospitals, and informed consent was obtained from all participants.

Selection of SNPs for Genotyping

Nine tagSNPs at *FOXP4* gene were chosen by using Haploview V4.1 software (Broad Institute, Cambridge, MA, USA). The starting and ending positions were both extended 5 kbp and entered from chr6 41617kb to chr6 41683kb according to NCBI database that *FOXP4* gene starts chr6 41,622,142 and ends chr6 41,678,099, spanning 66 kbp. Supplementary Table 1 is the information of selected tag SNPs and the representative SNPs. In addition, mutations near promoter regions would have important regulatory role, one SNP rs4714476 located at 5′ near gene of *FOXP4* in NCBI database was selected. Figure 1 shows the location of 11 SNPs including rs1983891 at *FOXP4* gene.

Blood genomic DNA was extracted and SNPs were genotyped by the small amplicons method of polymerase chain reaction-high resolution melting curves (PCR-HRM) as previously described [18,19]. To validate the accuracy of genotyping, some samples (about 10%) were randomly selected for duplicate analysis, and 5 samples were randomly selected from the three different verified genotypes of each risk variant to be sequenced (Beijing Genomics Institute [BGI], Beijing, PRC) to confirm the genotyping results. Supplementary Table 2 lists the information of all used primers.

Analysis of cumulative effects

We previously reported that rs16901966 and rs10090154 (represent region 2 and region 1 in 8q24, respectively) were associated with PCa risk in northern Chinese men [18]. We genotyped and reanalyzed these two loci in present enlarged samples. Linkage disequilibrium analysis showed that rs16901966 and rs10090154 were in the independent region in 8q24 in northern Chinese men, and therefore we determined the cumulative risk of the identified PCa risk variant (rs4714476) at *FOXP4* and 8q24 (rs16901966 and

rs10090154). Combined with genotyping data, samples that carried none, one or more of the risk genotypes were called by value 0, 1, 2, or 3. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to compare the frequency of risk genotypes between PCa cases and controls.

Statistical analysis

Pearson's χ^2 was used to test the Hardy-Weinberg equilibrium (HWE) for each SNP separately among control subjects. Unadjusted and age-adjusted ORs and 95% CIs were estimated for each risk allele versus each non-risk allele. In models of a dominant mode and recessive mode, ORs and 95% CIs were calculated to compare the genotype frequencies between PCa cases and controls. Logistic regression analysis was used to estimate all ORs and 95% CIs, including cumulative effects. The risk loci identified in our study were evaluated for an association with clinical covariates in controls and cases. 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 considered significant. Haploview V 4.1 was used to perform Haplotype analysis, LD testing, and haplotype based association studies.

RESULTS

Demographic characteristics of study subjects

The mean ± SD age of cases and controls were 72.3 ± 7.5 years (range 46 to 93) and 70.5 ± 7.9 years (range 58 to 94), respectively ($p = 0.002$). Mean total serum prostate specific antigen (PSA) levels in cases and controls were 34.51 ± 106.41 (range 0.15 to 1338) and 1.45 ± 1.66 (range 0 to 4) ng/mL, respectively ($p = 0.000$). Gleason scores of 2 to 7 were seen in 101/146 (69.2%) of patients, and 45/146 (30.8%) had scores of 8 to 10. Tumors were classified as stage I to IV in 9/138 (6.5%), (69/138) 50%, 46/138 (33.3%), 14/138 (10.1%), and 115/163 (70.6%) of patients. Patients with aggressive PCa accounted for 115/163 (70.6%). Supplementary Table 3 lists the demographic characteristics of study subjects.

SNPs based case-control study

The distribution of risk alleles and genotypes of SNPs in PCa cases and control subjects and the test results for HWE are shown in Supplementary Table 4. The genotype frequencies in control subjects showed that all SNPs did not deviate from HWE using a threshold of $p > 0.05$. Table 1 displays frequencies of these alleles in PCa cases and controls and the results of the case-control association analysis. Analysis of the allelic frequencies showed that the C allele of rs4714476 at 5' near gene of *FOXP4* was associated with an increase in PCa risk (OR 1.30, 95% CI 1.05 - 1.61, $p = 0.014$; age-adjusted OR 1.32, 95% CI 1.06 - 1.63, $p = 0.012$). Other SNPs were not found to contribute to increased or decreased risk for PCa before or after controlling for age. The genotypic frequencies analysis in three genetic models showed that rs4714476 was associated with PCa risk in the additive model ($p = 0.04$) and in the dominant model (age-adjusted $p = 0.012$; OR, 1.46; 95% CI, 1.09 - 1.97) (Supplementary Table 5). Sequencing results were consistent with the genotypes of variants identified by HRM (Figure 2). The association analysis of rs4714476 and clinical related phenotypes suggested that the risk allele of rs4714476 was more frequent in patients with age at diagnosis of 65 - 74 years ($p = 0.017$; OR, 1.43; 95% CI, 1.06 - 1.93), in patients with PSA levels > 20 ng/ml ($p = 0.005$; OR, 1.65; 95% CI, 1.16 - 2.33), in patients of all Gleason score ≥ 8 ($p = 0.001$; OR, 1.78; 95% CI, 1.14 - 2.8), in patients with tumour stage < III ($p = 0.005$; OR, 1.65; 95% CI, 1.16 - 2.33), and in patients with aggressive PCa ($p = 0.026$; OR, 1.41; 95% CI, 1.04 - 1.91) compared with controls (Table 2).

LD structure and haplotype analysis

We identified evidence for three extended LD blocks when evaluated by D′ and R^2 (Figure 3): block 1, which covers 5′ near gene of *FOXP4*, composed by rs4714476 and rs7764874 (approximately span 6 kb), block 2, which encompasses rs9349197, rs1983891, and rs4714485 (approximately span 13 kb), and block 3, including rs9367108, rs3800285, and rs9357364 (approximately span 8 kb). Table 3 shows the haplotype based association analysis between the case and control groups. Of the total nine haplotypes, the CG haplotype (constituted by rs4714476 -C and rs7764874-G in block 1) showed significant differences between PCa cases and controls ($p = 0.009$).

Cumulative effects of rs4714476 and 8q24

Based on the results above, only rs4714476 at the 5′near gene of *FOXP4* was associated with PCa in Chinese men. The cumulative effect of rs4714476 and 8q24 indicated that compared to men who did not have any of these risk variants, men who carried any combination of 1, 2, or 3 risk genotypes have a gradually increased PCa risk and the dramatic OR value (age-adjusted) was 3.31 ($p = 1.097 \times 10^{-4}$) (Table 4).

Table 1. Association analysis between alleles of 11 SNPs and PCa in northern Chinese men.

SNP ID	Position	Function	Allele	Risk allele	RAF		Unajusted allelic OR		Age-adjusted allelic OR	
					Case	Control	OR (95% CI)	p	OR (95% CI)	p
rs 4714476	41621717	5' near gene	T/C	C	0.375	0.316	1.30 (1.05 - 1.61)	**0.014**	1.32 (1.06 - 1.63)	**0.012**
rs 7764874	41628394	intron	G/A	A	0.207	0.194	1.08 (0.84 - 1.39)	0.531	1.13 (0.87 - 1.45)	0.360
rs 9349197	41631043	intron	A/G	G	0.166	0.131	1.32 (1.00 - 1.74)	0.053	1.29 (0.97 - 1.71)	0.081
rs 1983891	41644405	intron	C/T	T	0.357	0.313	1.22 (0.99 - 1.50)	0.065	0.81 (0.65 - 1.00)	0.050
rs 4714485	41644564	intron	T/G	G	0.337	0.313	1.11 (0.90 - 1.38)	0.324	1.12 (0.90 - 1.39)	0.309
rs 7739759	41648786	intron	C/T	C	0.855	0.851	1.03 (0.77 - 1.36)	0.863	0.98 (0.73 - 1.30)	0.873
rs 1475365	41658149	intron	C/A	A	0.191	0.16	1.23 (0.95 - 1.60)	0.114	1.20 (0.92 - 1.56)	0.182
rs 9367108	41660706	intron	C/T	C	0.898	0.891	1.09 (0.78 - 1.51)	0.626	1.07 (0.76 - 1.48)	0.711
rs 3800285	41666420	intron	A/G	G	0.309	0.289	1.10 (0.88 - 1.37)	0.391	1.09 (0.87 - 1.36)	0.450
rs 9357364	41669157	intron	G/A	G	0.877	0.868	1.09 (0.80 - 1.47)	0.596	1.05 (0.78 - 1.43)	0.742
rs 932797	41670286	intron	T/C	T	0.661	0.631	1.14 (0.91 - 1.42)	0.242	1.13 (0.90 - 1.41)	0.301

RAF - risk allele frequency, p < 0.05 are in bold.

Table 2. Genotypic distribution of rs4714476 according to clinical covariates and odds ratios of risk alleles relative to non-risk alleles.

Groups	Frequency of genotype			p	OR (95% CI)
	CC	TC	TT		
Controls	0.093	0.445	0.462		1.00 (Ref)
PCa cases					
Age at diagnosis	0.123	0.508	0.369		
< 65	0.139	0.389	0.472	0.754	1.08 (0.65 - 1.8)
65 - 74	0.130	0.537	0.333	**0.017**	1.43 (1.06 - 1.93)
≥ 75	0.111	0.519	0.370	0.113	1.28 (0.94 - 1.73)
PSA (ng/mL)	0.124	0.512	0.364		
< 10	0.102	0.480	0.418	0.465	1.13 (0.82 - 1.55)
10 - 20	0.105	0.553	0.342	0.232	1.34 (0.83 - 2.16)
> 20	0.164	0.534	0.301	**0.005**	1.65 (1.16 - 2.33)
Gleason	0.158	0.489	0.353		
< 8	0.133	0.500	0.367	0.063	1.34 (0.98 - 1.84)
≥ 8	0.220	0.463	0.317	**0.011**	1.78 (1.14 - 2.8)
Tumour stage	0.171	0.481	0.349		
< III	0.192	0.479	0.329	**0.005**	1.65 (1.16 - 2.33)
≥ III	0.143	0.482	0.375	0.139	1.35 (0.91 - 2.02)
Aggressiveness	0.120	0.520	0.360		
Nonaggressive PCa	0.087	0.522	0.391	0.522	1.16 (0.74 - 1.81)
Aggressive PCa	0.135	0.519	0.346	**0.026**	1.41 (1.04 - 1.91)

P < 0.05 are in bold.

Table 3. Haplotype block and haplotype-based association analysis of 11 SNPs at FOXP4 gene with PCa.

Haplotypes	Case (freq)	Control (freq)	Chi-squared	p-value	OR (95% CI)
Block 1					
C A	0.203	0.189	0.442	0.506	1.091 (0.845 - 1.408)
C G	0.173	0.126	6.846	0.009	1.454 (1.097 - 1.927)
T G	0.619	0.681	6.188	0.013	0.763 (0.616 - 0.945)
Block 2					
A C T	0.646	0.675	1.331	0.249	0.880 (0.707 - 1.094)
A T G	0.170	0.179	0.160	0.689	0.946 (0.723 - 1.240)
G T G	0.167	0.131	3.880	0.049	1.328 (1.001 - 1.762)
Block 3					
C A G	0.686	0.709	0.579	0.447	0.915 (0.729 - 1.150)
C G G	0.185	0.158	2.174	0.140	1.223 (0.936 - 1.598)
T G A	0.097	0.109	0.478	0.489	0.888 (0.633 - 1.245)

Block 1 - contained the risk alleles of rs4714476 and rs7764874.
Block 2 - contained the risk alleles of rs9349197, rs1983891 and rs4714485.
Block 3 - contained the risk alleles of rs9367108, rs3800285 and rs9357364.

Table 4. Cumulative effects of rs4714476 and 8q24 on PCa risk.

No. of risk genotypes *	Case	Control	Unadjusted OR			Age-adjusted OR		
			OR	95% CI	p-value	OR	95% CI	p-value
0	34 (0.125)	111 (0.188)	1.00 (Ref)			1.00 (Ref)		
1	94 (0.344)	255 (0.433)	1.203	0.766 - 1.890	0.421	1.244	0.785 - 1.971	0.352
2	108 (0.396)	186 (0.316)	1.896	1.207 - 2.977	0.005	1.937	1.221 - 3.071	0.005
3	37 (0.136)	37 (0.063)	3.265	1.799 - 5.925	9.993×10^{-5}	3.312	1.805 - 6.077	1.097×10^{-4}

*** - Risk genotypes were defined as the homozygote and heterozygote genotypes of risk allele (rs4714476 CC, TC, rs16901966 GG, AG, and rs10090154 TT, TC).**

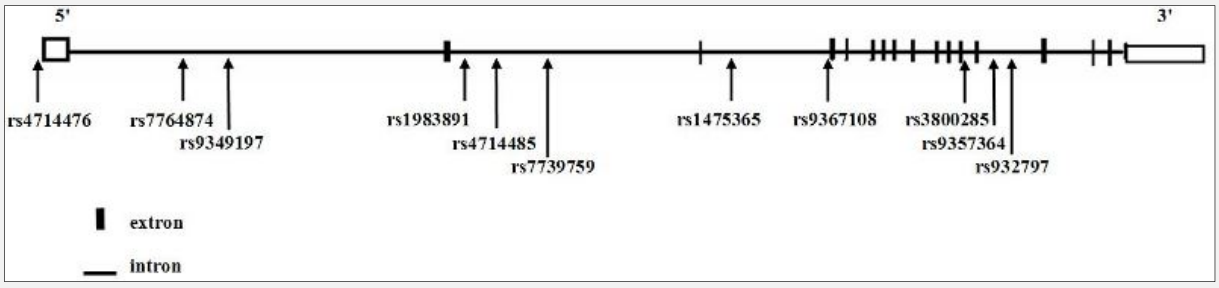

Figure 1. Location of 11 SNPs selected at FOXP4 gene.

Rs4714476 is at the 5′near gene of *FOXP4*; rs7764874 and rs9349197 are at intron 1 of *FOXP4*; rs1983891, rs4714485 and rs7739759 are at intron 2 of *FOXP4*; rs1475365 and rs9367108 are at intron 3 of *FOXP4*; rs3800285 is at intron 12 of *FOXP4*; rs9357364 and rs932797 are at intron 13 of *FOXP4*.

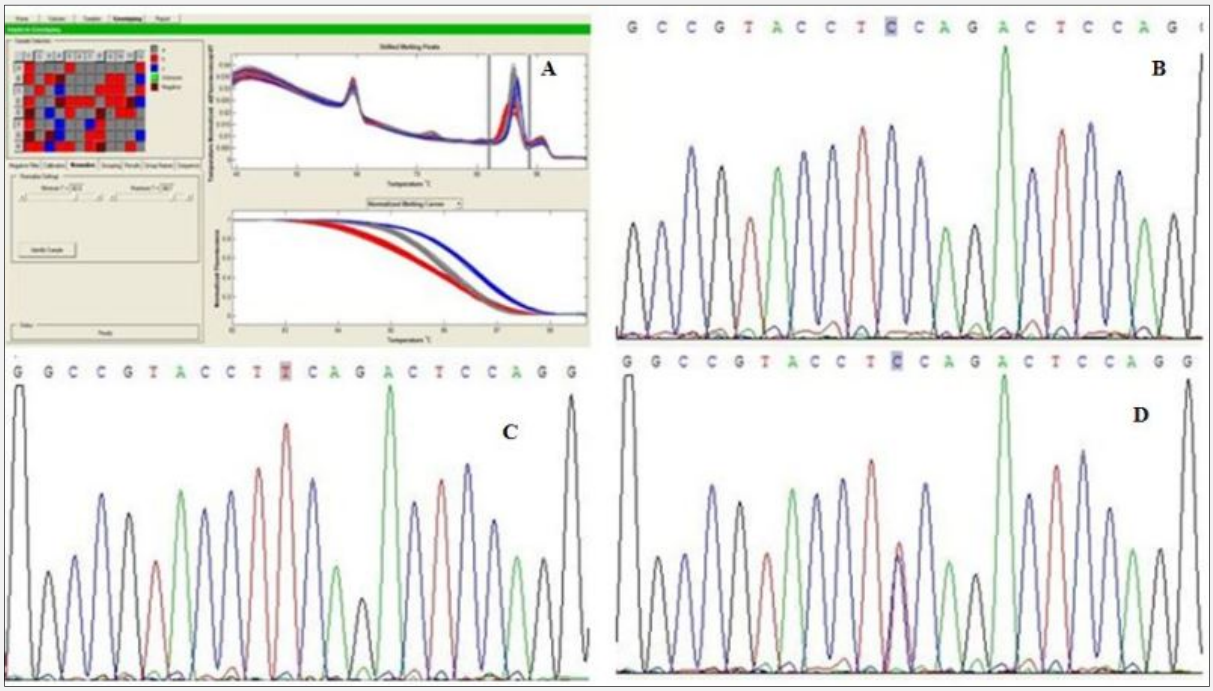

Figure 2. Sequencing and genotyping of rs4714476.

A displays the normalized melting curves and genotyping results using LightScanner Call IT software. Blue curves represent AA or TT genotypes; gray curves represent CC or GG genotypes; and red curves represent heterozygous types. B, C and D show the sequencings of homozygotes (CC, TT) and heterozygotes (CT) for each risk allele.

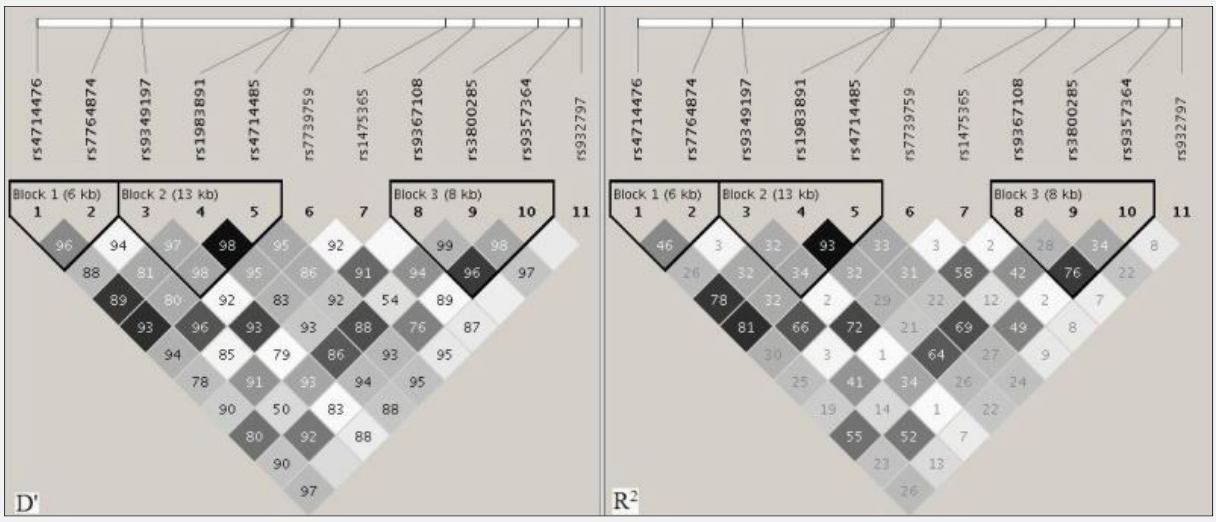

Figure 3. LD pattern of 11 SNPs at FOXP4 gene generated by Haploview V.4.1.

Each box represents LD (range from 0 to 1) between pairs of SNPs. Black corresponds to strong LD (D' = 1), and white corresponds to low D' (D' = 0). The number in the square represents the D' value. Three extended LD blocks were identified when evaluated by D′and R^2.

DISCUSSION

We performed a case-control study using 11 *FOXP4* SNPs to verify their association with PCa and to identify the *FOXP4* PCa susceptibility region. We identified that the novel variant rs4714476, which resides near the 5′ end of *FOXP4*, was associated with PCa and PCa clinical covariates. A haplotype comprising rs4714476 and rs7764874, covering a 6 kb region, associated significantly with PCa. The cumulative effect of rs4714476, rs16901966, and rs10090154 would make the OR value of PCa risk increase to 3.31.

The SNP rs1983891 in intron 2 of *FOXP4* on chromosome 6p21 was first identified as a PCa susceptible locus in a GWAS on a Japanese population ($p = 7.6 \times 10^{-8}$, OR = 1.23) [13]. The association was not detected in some Chinese men (p = 0.87, OR = 0.99; p = 0.054, OR = 1.27) [16,17]. However, rs1983891 was confirmed as a PCa risk locus ($p = 6.22 \times 10^{-5}$, OR = 1.34) in northern Chinese men, and a fine-mapping study provided further evidence for a significant association of rs1983891 with susceptibility to PCa [15]. In subjects of European ancestry whose genetic background differs significantly from Japanese, rs1983891 showed a strong association with PCa ($p = 2.5 \times 10^{-4}$, OR = 1.09) [14]. Our previous study showed that rs1983891 was not associated with PCa (p = 0.054) [17]. In the present study, we did not detect PCa susceptibility in carriers of the rs1983891 risk allele when we enlarged the sample size. However, rs4714476, which resides more than 22 kb away and is located the 5′ near gene of *FOXP4*, was identified here as a PCa-risk locus. In our allelic association study, the risk of patients with PCa who carry rs4714476-C is increased by 32% (p = 0.012) after adjusting for age. Moreover, rs4714476 is associated with PCa in additive and dominant models (p = 0.04, p = 0.012, respectively). Consistent with this result, in a subsequent haplotype-based association study, the frequency of the CG haplotype in block 1 comprising rs4714476-C and rs7764874-G was higher in patients with PCa compared with controls (p = 0.009). However, block 2 encompassing rs1983891 only exhibited a critical association with PCa (p = 0.049).

This discrepancy between our data and those of previous studies may be explained as follows: certain disease-susceptibility loci are specific for certain ethnicities, or there are inherent differences in LD structures and risk allele frequencies between Chinese and other populations. We analyzed SNP data at the same region of the human genome (from chr6 41617 kb to chr6 41683 kb) in eastern and western populations using HaploviewV4.1 software, and LD patterns showed obvious differences (Supplementary Figure 1). For the inconsistent results between our study and the previous study in Chinese population [15], we consider that maybe rs4714476 has a higher statistically significant difference than other variants. This would explain the result of the study in southern Chinese men [16]. Our result needs to be confirmed in the future in a larger group of Chinese men.

To our knowledge, the functional features of *FOXP4* polymorphisms in patients with PCa are unknown. FOXP4 is evolutionarily conserved and is expressed in the heart, brain, lungs, liver, kidneys, and testes in adults. Teufe et al. evaluated the expression pattern of FOXP4 in wild-type mouse tissues and human neoplastic tissues and found that FOXP4 expression was significantly reduced in patients with kidney tumors [20]. Frohme et al. also demonstrated that FOXP4 was downregulated in patients with carcinomas of the larynx [21]. The role of FOXP4 in prostate tumorigenesis has not been determined. However, a recent study indicates that the expression of FOXP4 in both thymocytes and peripheral CD4+ and CD8+ T cells plays a role in effector T cell-cytokine responses in chronic infection [22]. If tumors including PCa induce biological changes at higher frequencies in regulatory T cells, this may enable tumor cells to escape immunosurveillance. Moreover, expression of genes in regulatory T cells in patients with metastatic castration-resistant PCa differs compared with healthy donors [23]. Moreover, the expression of the androgen-induced secretory protein anterior gradient 2 (AGR2) is markedly elevated in PCa tissues or PCa cells [24-26], and AGR2 may be useful as a urinary-sediment marker for detecting PCa [27]. Li et al. demonstrated that FOXP4 directly targets and represses expression of AGR2 in the secretory epithelium of the lung [28]. Further research will be required to determine whether FOXP4 contributes to the pathogenesis of PCa through AGR2.

PCa is a complex polygenic disease. In our previous study, two regions located in 8q24 (region 2 was represented by rs16901966, region 1 was represented by rs10090154 in our study) were associated with PCa risk in northern Chinese men [18]. It is known that the contribution of a single variant in polygenic disease is very small (OR value < 2). However, the risk of PCa in men with 6 or more risk alleles was higher than that in men with two or fewer risk alleles (OR = 6.22) [29]. In southern Chinese population, men who carried 5 - 6 risk alleles had a 2.26-fold increased risk of PCa compared with those who carried 0 - 2 risk alleles [16]. Our data indicated men who carry three risk genotypes (rs4714476, rs16901966, and rs10090154) would make the risk of PCa increase 2.31-fold (age adjusted OR, 3.312). It has been verified that some variants at 8q24 are PCa susceptible loci in different populations, so confirming this cumulative effect to evaluate the PCa risk may be useful for the future translation medicine. Taken together, the studies described above suggest a potential correlation between FOXP4 function and the generation or progression of PCa. We evaluated here the associations between rs4714476 and several clinical covariates of PCa. The results indicate that men aged 65 to 74 years who carried the risk allele C of rs4714476 were at increased risk for PCa as a function of age compared with controls (OR = 1.43). Thus, the risk of PCa in these carriers would increase as follows: PSA levels

> 20 ng/mL, 65% ($p = 0.005$); all patients with Gleason scores ≥ 8, 78% ($p = 0.011$); and patients with tumor stage < III ($p = 0.005$), 65%. Further, the rs4714476-C allele is associated with an increase in aggressive PCa by a factor of 1.41 ($p = 0.026$). These results will be useful for guiding further efforts to determine the functional variants around rs4714476 or within the LD block.

Our present study has several limitations. The sample size was relatively small for a case-control association study, which limited its statistical power and the follow-up confirmation study. Second, some of the analyses were limited, because the data for clinically related variables were missing for some outpatients after PCa surgery performed at several other hospitals. Therefore, populations for which detailed clinical information is available from multiple centers in China as well as data for PCa related to environmental exposure should be examined to confirm the association between these SNPs and PCa. It remains to be determined in a larger sample size and wide Chinese population in a future study.

CONCLUSION

Here we report the identification of a SNP rs4714476 at the 5′ near gene of FOXP4 and a 6 kb region covering rs4714476 which are associated with PCa in Chinese men. The cumulative effect of rs4714476 at FOXP4 and 8q24 could increase PCa risk. These results can be useful for genetic and etiological studies of PCa and will supply useful data for translation medicine.

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Statement:
All study participants provided informed consent, and the study design was approved by the appropriate ethics review boards.

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Declaration of Interest:
The authors declare no conflicts of interest.

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Supplementary Table 1. The selected tag SNPs from FOXP4 gene using HaploviewV4.1 and the representative SNPs.

Test	Alleles captured
rs4714485	rs4714485, rs1886816, rs9381080, rs4714487, rs6917029, rs1983891, rs93074
rs9349197	rs1983892, rs10947980, rs4714484, rs9381079, rs9349197
rs3800285	rs6920495, rs9381084, rs9369296, rs745548, rs3800285, rs912883
rs9367108	rs9369291, rs9367108, rs9394812
rs3747744	rs3747744, rs2148342, rs2395779
rs9357364	rs9357364, rs913075
rs2395778	rs2395778
rs4714475	rs4714475
rs932797	rs932797
rs7739759	rs7739759
rs3747746	rs3747746
rs1475365	rs4714494, rs1475365
rs11758591	rs11758591
rs16894825	rs16894825
rs9394816	rs9394816
rs7764874	rs7764874
rs9394811	rs9394811

The final selected SNPs are in bold, SNPs not in the gene are in italic, SNPs with MAF < 0.05 are in bold italic.

Supplementary Table 2. PCR primers of SNPs and annealing temperature for HRM.

SNP ID		Primer (5'-3')	Tm (°C)	Length (bp)
rs4714476	F:	ACCGTGTGGGATCAGGACTTG	54	61
	R:	TTGCAAACCCCGGCCAT		
rs7764874	F:	AAGGGGTTTTCGGCACTC	54	51
	R:	AGCCCAGCTGCAGTTCTT		
rs9349197	F:	TCTTTCTACCACAGAGGTTAG	54	71
	R:	TTCATCTTGAAACAGGC		
rs1983891	F:	GGTCCCATTCTCCTCGTA	54	70
	R:	ACAAGGACCAACAGACAG		
rs4714485	F:	ATAAACAGGGCGAGTCAGAAGTC	54	64
	R:	TCAGCACTGTTTGTTTCAGCC		
rs7739759	F:	CCCAAGCTCTTCGACTT	51	69
	R:	GGCAGGGACGTGATAAT		
rs1475365	F:	CCCAAATCCCAGTTAGCC	54	46
	R:	GAAATCACCAAGCCTCAC		
rs9367108	F:	GTTTACACCTCACCAAGGG	60	60
	R:	TCTGGCCTGAGCAGTCTCT		
rs3800285	F:	TCAAGCCCATGATCTTCA	60	53
	R:	GCTTGAGTGTGAATACTATGTG		
rs9357364	F:	TCCCAAGGCCCGTGTTGT	63	52
	R:	CTGGCAGGGGCTTCGTAAAC		
rs932797	F:	AACGAAGATGATGAAAC	51	47
	R:	AACTAAATGCTATCCAG		

Supplementary Table 3. Demographic characteristics of study subjects.

Characteristics	Cases	Control	p
Number of subjects	286	630	–
Age, years (mean [SD])	72.3 (7.48)	70.5 (7.90)	0.002
Range	46 - 93	58 - 94	–
PSA ng/mL (mean [SD])	34.51 (106.41)	1.45 (1.66)	0.000
Range	0.15 - 1338	0 - 4	–
< 10	102	–	–
10 - 20	40	–	–
> 20	78	–	–
Gleason score	146	–	–
< 8	101	–	–
≥ 8	45	–	–
Tumour stage	138	–	–
I	9	–	–
II	69	–	–
III	46	–	–
IV	14	–	–
Aggressiveness	163	–	–
Nonaggressive PCa	48	–	–
Aggressive PCa	115	–	–
Positive family history of PCa	8	–	–

PCa - prostate cancer. * - Aggressive PCa: PSA > 20 ng/mL, clinical stage ≥ III, or Gleason score ≥ 8. The total number of cases does not add up to 286 in all categories because of missing data.

Supplementary Table 4. Distribution of the risk alleles and genotypes (n/fre) of 11 SNPs in PCa cases and control subjects, and the test result for HWE.

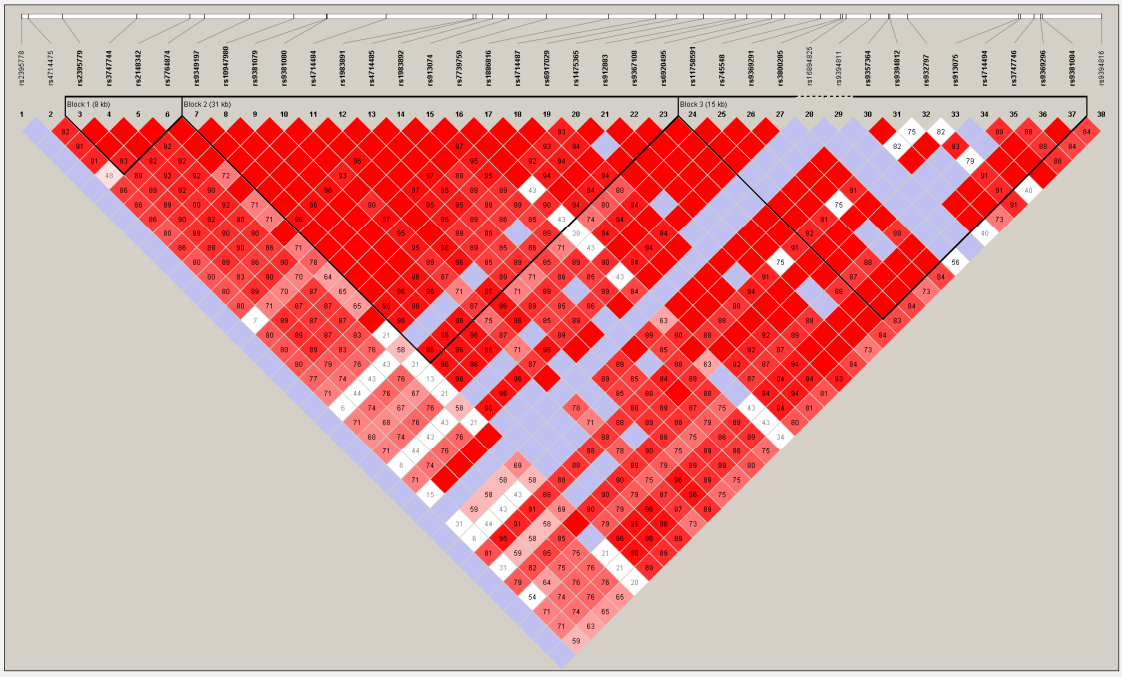
		Our study									Hap-map
SNPs ID	Alleles	Allele (n/MAF)			Genotype (n/freq)						MAF
	* (1/2)	Case	Control	HWE	Case			Control			
				(p)	11	12	22	11	12	22	
rs4714476	T/**C**	208 (0.375)	380 (0.316)	0.454	104 (0.375)	138 (0.498)	35 (0.126)	278 (0.462)	268 (0.445)	56 (0.093)	0.375
rs7764874	G/**A**	114 (0.207)	234 (0.194)	0.140	170 (0.616)	98 (0.355)	8 (0.029)	387 (0.641)	200 (0.331)	17 (0.028)	0.189
rs9349197	A/**G**	92 (0.166)	162 (0.131)	0.059	193 (0.697)	76 (0.274))	8 (0.029)	470 (0.763)	130 (0.211)	16 (0.026)	0.189
rs1983891	**T**/C	205 (0.355)	180 (0.314)	0.626	36 (0.125)	133 (0.460)	120 (0.415)	30 (0.105)	120 (0.418)	137 (0.477)	0.410
rs4714485	T/**G**	184 (0.337)	374 (0.313)	0.624	118 (0.432)	126 (0.462)	29 (0.106)	279 (0.467)	262 (0.439)	56 (0.094)	0.415
rs7739759	C/**T**	82 (0.145)	180 (0.149)	0.906	205 (0.727)	72 (0.255)	5 (0.018)	439 (0.724)	154 (0.254)	13 (0.021)	0.110
rs1475365	C/**A**	108 (0.191)	197 (0.160)	0.946	184 (0.652)	88 (0.312)	10 (0.035)	434 (0.706)	165 (0.268)	16 (0.026)	0.178
rs9367108	C/**T**	57 (0.102)	134 (0.109)	0.579	225 (0.804)	53 (0.189)	2 (0.007)	484 (0.791)	122 (0.199)	6 (0.010)	0.100
rs3800285	A/**G**	172 (0.309)	360 (0.289)	0.684	133 (0.478)	118 (0.424)	27 (0.097)	312 (0.502)	260 (0.418)	50 (0.080)	0.311
rs9357364	G/**A**	69 (0.123)	158 (0.132)	0.871	212 (0.757)	67 (0.239)	1 (0.004)	449 (0.752)	138 (0.231)	10 (0.017)	0.133
rs932797	T/**C**	168 (0.339)	422 (0.369)	0.354	117 (0.472)	94 (0.379)	37 (0.149)	233 (0.407)	256 (0.448)	83 (0.145)	0.356

*** - 1 represents wildtype allele, 2 represents mutation allele. The minor allele of HCB from Hapmap database is in bold.**

Supplementary Table 5. Association analysis between the different genetic models of SNPs and PCa in northern Chinese men.

		Unadjusted genotypic OR				Age-adjusted genotypic OR			
	Additive model	Dominant model		Recessive model		Dominant model		Recessive model	
SNP ID	(df = 2)	(11 + 12 vs. 22)		(11 vs. 12 + 22)		(11 + 12 vs. 22)		(11 vs. 12 + 22)	
	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
rs4714476	**0.04**	1.42 (1.06 - 1.9)	**0.019**	1.42 (0.9 - 2.22)	0.129	1.46 (1.09 - 1.97)	**0.012**	1.40 (0.89- 2.20)	0.151
rs7764874	0.78	-	-	1.03 (0.44- 2.42)	0.945	-	-	1.07 (0.46 - 2.52)	0.876
rs9349197	0.10	-	-	1.12 (0.47 - 2.64)	0.804	-	-	1.02 (0.42 - 2.48)	0.962
rs1983891	0.17	1.30 (0.98 - 1.73)	0.069	1.47 (0.95 - 2.27)	0.088	1.34 (1.01 - 1.79)	**0.046**	1.43 (0.91 - 2.23)	0.117
rs4714485	0.60	1.15 (0.86 - 1.54)	0.335	1.15 (0.72 - 1.84)	0.567	1.18 (0.88 - 1.58)	0.278	1.12 (0.69 - 1.81)	0.659
rs7739759	0.94	-	-	1.01 (0.74 - 1.39)	0.937	-	-	0.96 (0.7 - 1.32)	0.797
rs1475365	0.26	-	-	1.38 (0.62 - 3.08)	0.430	-	-	1.33 (0.59 - 3.04)	0.494
rs9367108	0.87	-	-	1.08 (0.76 - 1.54)	0.662	-	-	1.07 (0.75 - 1.53)	0.722
rs3800285	0.65	1.10 (0.83 - 1.46)	0.52	1.23 (0.75 - 2.01)	0.408	1.08 (0.81 - 1.44)	0.585	1.22 (0.74 - 2.01)	0.441
rs9357364	0.26	-	-	1.03 (0.74 - 1.43)	0.871	-	-	1.00 (0.71 - 1.39)	0.974
rs932797	0.16	0.97 (0.64 - 1.47)	0.879	1.3 (0.96 - 1.75)	0.087	0.95 (0.62 - 1.46)	0.825	1.28 (0.94 - 1.73)	0.115

- The genetic models were not analyzed because one of the genotype frequencies was less than 0.05. P < 0.05 are in bold.



LD patterns of FOXP4 in CHB+JTP populations.

LD patterns of FOXP4 in CEU population.

Supplementary Figure 1. Different LD patterns of FOXP4 in different populations.

JPT - Japanese in Tokyo, Japan.
CHB - Han Chinese in Beijing, China.
CEU - CEPH (Utah residents with ancestry from northern and western Europe).