RESEARCH ARTICLE

rs10505474 and rs7837328 at 8q24 Cumulatively Confer Risk of Prostate Cancer in Northern Han Chinese

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Abstract

Aims: Genome-wide association studies (GWAS) have identified several risk variants for prostate cancer (pCa) mainly in Europeans, which need to be further verified in other racial groups. We selected six previously identified variants as candidates and to define the association with PCa in Northern Han Chinese. Methods: 749 subjects from Beijing and Tianjin in Northern China were included. Six variants (rs10505474, rs7837328, rs4242384, rs7813, rs486907 and rs1058205) were genotyped by high resolution melting (HRM) assays. The individual and cumulative contribution for of the risk of PCa and clinical covariates were analyzed. Results: Among the six candidate variants, onlyrs10505474, and rs7837328, both locating at 8q24 region, were associated with PCa in our population.rs10505474 (A) was associated with PCa ($OR_{recessive}$= 1.56, p=0.006); and rs7837328 (A) was associated with PCa ($OR_{dominant}$= 1.38, p=0.042/$OR_{recessive}$=1.99, p=0.003). Moreover, we observed a cumulative effects between them (p_{trend}=2.58×10^{-5}). The joint population attributable risk showed the two variants might account for 71.85% of PCa risk. In addition, we found the homozygotes of rs10505474 (A) and rs7837328 (A) were associated with PCa clinical covariants (age at onset, tumor stage, respectively) (p_{age}=0.046, $P_{tumorstage}$ =0.048). Conclusion: rs10505474 (A) and rs7387328 (A) at 8q24 are associated with PCa and cumulatively confer risk, suggesting the two variations could determine susceptibility to PCa in the Northern Chinese Han population.

Keywords: Prostate cancer - genotype - rs10505474 - rs7837328 - Northern Chinese

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Introduction

Prostate cancer (pCa) is the most commonly diagnosed noncutaneous cancer in men in the United States. Although the incidence of PCa in Asia is lower than Oceania, Europe, and North America (Matshela et al., 2013), it is also increasing rapidly (Wang et al., 2012). Many lines of studies have attempted to identify high-penetrance genetic variants that confer the increased risk of developing PCa. However, causal genetic variants underlying susceptibility remains unknown due to the genetic complexity of PCa (Ishak et al., 2011). Particularly, the effect of these variants associated with the risk of PCa identified in Caucasians is largely unknown in the Chinese populations (Wang et al., 2012).

Recently, the genome-wide association studies (GWAS) have identified several risk variants. The variants locating at 8q24 region (rs7837328, rs10505474 and rs4242384) and rs7813, rs486907 and rs1058205 were reported strongly associated with PCa risk in Europeans, Americans and southern Asians), (Terada et al., 2008; Chen et al., 2009; Liu et al., 2009; Takata et al., 2010; Chen et al., 2010; Zheng et al., 2010; Liu et al., 2012.). However, consider the great difference of genetic background, environments and lifestyle (Liu et al., 2012) between southern and northern Chinese, whether those variations also confer the PCa risk in Northern Chinese men remains unknown. It is essential to identify the genetic contribution of these variants to PCa in northern Chinese.

Materials and Methods

Study subjects

A total of 405 histopathologically confirmed PCa patients and 344 healthy male controls from Genetic Resource Database Project (GRDP) without PCa history

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Table 1. Distribution and Compare of Alleles and Genotype of Six Variations

SNPs	Alleles	Allele frequency (1/2)			Genotype number (11/12/22)						P**	P***
	1/2	PCa	Control	P*	PCa			Control				
rs7837328	A/G	0.416/0.584	0.342/0.658	0.004	56	210	122	56	173	115	0.008	0.500
rs10505474	A/G	0.436/0.564	0.404/0.596	0.215	103	237	53	119	160	55	0.003	0.921
rs4242384	A/C	0.788/0.212	0.807/0.193	0.480	171	159	49	138	147	39	0.086	0.988
rs7813	C/T	0.340/0.660	0.347/0.653	0.800	10	118	220	11	50	9	0.636	0.065
rs486907	A/G	0.201/0.799	0.244/0.756	0.064	16	102	215	25	102	181	0.177	0.079
rs1058205	C/T	0.415/0.585	0.414/0.586	0.978	60	172	117	32	168	139	0.565	0.063

*Compared of allele frequency; **Compared of Genotype frequency using additive model; ***Hardy-Weinberg test in controls

Table 2. Association Analysis by Dominant and Recessive Model Between PCa and Controls

SNPs	Risk allele*	Dominant Model			Recession Model		
		Genotype	OR(95%CI)	*P*	Genotype	OR(95%CI)	*P*
rs7837328	A	AG+AA/GG	1.38(1.01-1.90)	0.038	AA/AG+GG	1.93(1.19-3.12)	0.003
rs10505474	A	AG+AA/GG	1.56(1.12-2.17)	0.006	AA/AG+GG	0.79(0.51-1.21)	0.26
rs4742384	C	AC+CC/AA	1.31(0.88-1.96)	0.17	CC/AA+AC	0.57(0.22-1.47)	0.20
rs7813	T	TT+CT/CC	0.90(0.56-1.45)	0.66	TT/CT+CC	1.10(0.81-1.50)	0.52
rs486907	G	AG+GG/AA	1.68(0.84-3.39)	0.12	GG/AA+AG	1.27(0.91-1.77)	0.15
rs1058205	C	CC+CT/TT	1.09(0.79-1.51)	0.57	CC/CT+TT	0.87(0.57-1.32)	0.49

*Assumes the risk allele from reported by other literatures

were included. All the subjects were Han nationality residents from north of China (Beijing and Tianjin). The study protocol had been approved by the Ethics Committee of Beijing Hospital, Ministry of Health, China. All participants had signed the informed consent. The PCa patients were sub-grouped according to prostate specific antigen (pSA) levels, international tumor-node-metastasis (TNM) staging system. Aggressive PCa with PSA>20 ng/ml, clinical stage ≥II or Gleason score≥8 (Gaj et al., 2012) and pathologic grade were recorded.

Genotype analysis

Genomic DNA was extracted from blood samples. High resolution melting assays (HRM) were performed using the PTC-225 Thermal Cycler and the Light scanner TMHR-I 96 (Idaho Technology, Inc., Salt Lake City, Utah, USA). All primers used for HRM and sequencing of Polymerase Chain Reaction (pCR) products were designed using Oligo (version 7.0). All the assays were controlled by genotype known samples confirmed by Sanger sequencing (Beijing Genomics Institute, Beijing, China).

Statistical analysis

A Student's t test was used to determine the significant difference in age between groups. Pearson's χ^2 test was used to evaluate the Hardy-Weinberg equilibrium for each variation separately among controls using a cut off of $p>0.05$. The power value estimationfor sample size was determined by genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). Linkage disequilibrium (LD) was evaluated using the haploview software (version 4.2) (Barrett et al., 2005) by determining D' and r^2 values. The odd ratio (OR) and 95% confidence interval (CI) were estimated for each risk allele *vs* each non risk allele. ORs and *95%CIs* in models of a dominant mode (AG+AA *vs* GG) and of a recessive mode (AA *vs* AG+GG) were calculated to compare genotype frequencies between PCa patients and controls using Pearson's chi-square or Fisher's exact test. The association of the significant risk variants for PCa patients with pathological characteristics including PSA levels (>10 and ≤10) and Gleason score (>7 and ≤7) and tumor stage (>I and ≤II), and aggressiveness were statistically analyzed by using SPSS® (version 17.0) with <0.05 considered significant. Two risk variations were evaluated population attributable risk percent (pAR %) after adjustment for other covariates with the use of the following equation: PAR%=100×p (odds ratio-1)÷[*p* (odds ratio-1)+1] (Zheng et al., 2008). The cumulative effects of the significant risk variations were assessed by summarizing the three single nucleotide polymorphisms (SNPs), counting the number of risk alleles in each subject, and then modeling the summary variable categorically (0 as reference, 1, 2 and ≥3).

Results

No difference was observed in mean age between PCa patients and controls (69.41±10.11 yrs *vs* 66.87±10.86 yrs). Among the PCa patients, the PSA level was 49.09±119.99ng/ml. There were 328 PCa patients with Gleason scores≥ 7, 154 PCa patients with TNM stage >II and 66.49% of PCa patients had aggressive tumors.

All the variants were in Hardy-Weinberg equilibrium ($p>0.05$). Each of the allele and genotype frequencies was compared between PCa patients and controls (Table 1). No significant association was observed except for rs7837328 and rs10505474.The rs7837328 (A) was more frequent in PCa (41.6%) than controls (34.2%) (P_{allele}=0.004; $P_{additive}$=0.008). rs7837328 (A) was associated with a 1.93-fold and 1.38-fold increase of PCa risk by the recessive model (p=0.003) and dominant model (p=0.038), respectively. And, rs10505474 (A) was associated with a 1.56-fold increase of PCa risk by dominant model (p=0.006). The other 4 variations (rs4242384, rs7813,

Table 3. Cumulative Effects of Risk Alleles of rs7837328, rs10505474 to Risk of PCa

	Case	Control	OR(95%CI)	χ^2	*P*	P_{Trend}
0 (reference)	224	232	-	-	-	
1	80	111	0.75(0.52-1.06)	2.83	0.09	
2	316	236	1.39(1.07-1.79)	6.63	0.01	
≥3	221	145	1.58(1.18-2.11)	10.37	0.001	2.578×10^{-5}

rs486907 and rs105820) showed no significant association with PCa (Table 2).

As to the clinical variables, we observed the age at onset was associated with rs10505474 (A) (p=0.046), and the tumor stages was associated with rs7837328 (A) (p=0.048). No association of these two variants was observed to be associated with serum levels of PSA, Gleason scores, or aggressiveness.

The cumulative effects test of risk alleles (rs7837328, rs10505474) showed an increasing risk to PCa in a frequency-dependent manner ($P_{trend}=2.58\times10^{5}$). Subjects with more than 3 risk alleles had the most significant susceptibility to PCa (p=0.0013, OR=1.58), compared with those who had no risk allele (Table 3). We further assessed the PAR ofrs7837328 and rs10505474 individually and jointly, and found rs7837328 (A) and rs10505474 (A) could account for 24.01% and 62.96% of the total prevalence rate of PCa, respectively.

Linkage disequilibrium test indicated the three variations (rs7837328, rs10505474 and rs4242384) at 8q24 region was only in slight linkage disequilibrium (Table 5). Haplotype analysis indicated that A-A-A seemed to be more frequent in PCa patients than the controls (p<0.001).

Discussion

The present study firstly demonstrated two genetic variants at 8q24, rs7837328 and rs10505474, were also associated with PCa in northern Han Chinese. However, we failed to find any association of rs442384, rs7813, rs486907 and rs1058205, although they were previously identified to be associated with PCa in European men (Agalliu et al., 2010; Parikh et al., 2010; Liu et al., 2012). The finding provided a helpful basis for seeking genetic etiology of prostate cancer specific to northern Han Chinese, considering the increasing incident rate of PCa. Although many studies, including GWAS, have reported many common alleles associated with PCa (Wang et al., 2012), there are obvious difference between ethnic populations. It is essential to understand the effects of these genetic molecules on risk of PCa in different ethnicities (Zhang et al., 2012; Zhao et al., 2013; Wang et al., 2013).

Although the susceptibility of PCa risk has been identified mostly in European and American populations, similar studies in Asian, particularly Chinese, are still limited. In southern Chinese, rs7837328was reported not associated with PCa (Zheng et al., 2010). Interestingly, in northern Chinese Han population, we observed that rs7837328 (A) was associated with PCa, both of alleles (p=0.004) and genotypes (p=0.008). The difference could be explained by the variances in genetic, life style and there complex interaction between the north and south of China. In other ethnics, rs7837328 (A) was reported significant in Hispanics (Beuten et al., 2009), Caucasians, but not in African American (Beuten et al., 2009).

We also found that rs10505474 (A) was associated with PCa by dominant model (p=0.006), in accordance with the results from a GWAS study (Murabito et al., 2007). At 8q24, another variant rs4242384 was reported to be strongly associated with the risk of PCa in European ancestry (OR=1.56, $p=3.0\times10^{-16}$) by GWAS (http://gds.nih.gov/) in 2011. A similar result was also shown in European (OR= 1.88, $p=2.8\times10^{-17}$) (Eeles et al., 2008). However, our results did not show a significant association between rs4242384and the risk of PCa. We further assessed the cumulative effects of rs7837328 and rs10505474 on the risk of PCa, and found that each variant had a strong cumulative association with the risk of PCa. The risk variant alleles conferred an increasing risk to PCa in a dose-dependent manner. The results suggested it might be more effective using the combined risk alleles to assess the risk of PCa, although it has to be verified in a prospective study. Besides the PCa risk, we also found some supportive evidence about the association between7837328 (A), rs10505474 (A) and clinical variables (age at onset, tumor stages).

In summary, this study suggested thatrs10505474 and rs7387328 at 8q24 were cumulatively associated with PCa in northern Han Chinese. They were also associated with age at onset and tumor stages. However, since the subjects were only from Beijing and Tianjin city residents, different population stratification and larger sample size should be considered in the future validation and application.

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