A common genetic variant of 5p15.33 is associated with risk for prostate cancer in the Chinese population

Q. Ren[1,3]*, B. Xu[2,3]*, S.Q. Chen[2,3]*, Y. Yang[2,3], C.Y. Wang[2,3], Y.D. Wang[2,3], X.H. Wang[2,3], L.X. Hua[4] and M. Chen[2,3]

[1]Department of Anesthesiology,
Affiliated Zhongda Hospital of Southeast University, Nanjing, China
[2]Department of Urology, Affiliated Zhongda Hospital of Southeast University, Nanjing, China
[3]Surgical Research Center, Southeast University Medical School, Nanjing, China
[4]Department of Urology, First Affiliated Hospital of Nanjing Medical University, Nanjing, China

*These authors contributed equally to this study.
Corresponding authors: M. Chen
E-mail: mingchenseu@gmail.com

ABSTRACT. Recent evidence has suggested that single-nucleotide polymorphisms (SNPs) located at 5p15.33 contribute to susceptibilities for several cancer types, including prostate cancer. To determine whether SNP rs402710 in this region plays a role in prostate cancer, we analyzed these associations in a Chinese population; 251 prostate cancer patients and 273 control subjects were included in this case-control study. Genotypes were determined by PCR-RFLP. We found that subjects carrying the CC homozygote had a decreased risk for prostate cancer compared to those carrying TT/TC genotypes (odds ratio (OR) = 0.69, 95% confidence interval (CI) = 0.48-0.98, P = 0.038). Compared with the TT homozygote, subjects carrying the

CC homozygote also had a decreased risk for prostate cancer (OR = 0.71, 95%CI = 0.51-0.99, P = 0.043). We conclude that rs402710 polymorphisms in the 5p15.33 region are associated with prostate cancer risk in the Chinese population. Further investigations with large cohorts and done worldwide are warranted to determine whether our findings are detected in other populations.

Key words: Prostate cancer; Polymorphism; 5p15

INTRODUCTION

Prostate cancer (PCa) is one of the most common malignancies in men in Western countries (Jemal, 2011). In the Chinese, the incidence of PCa has increased in recent years (McCracken et al., 2007). Although the underlying etiology of PCa and the mechanisms by which it progresses are unclear, environmental factors and genetic predisposition likely contribute to risk. Besides established risk factors, such as age and a positive family history (Crawford, 2003), considerable evidence suggests that genetic factors are associated with PCa susceptibility (Dennis et al., 2002; Schaid, 2004; Mandal et al., 2010). Furthermore, twin studies have indicated that the contribution of genetic factors to the development of PCa is larger than that in other common human cancers (Lichtenstein et al., 2000).

Recently, genome-wide association studies (GWASs) have identified genetic variants associated with risk for cancers of the prostate, breast, colon and rectum, lung, urinary bladder, and skin (Kiemeney et al., 2008; Amundadottir et al., 2009; Rafnar et al., 2009). In prostate cancer, Gudmundsson et al. (2007) found 2 variants in the 8q24 region that contribute significantly to the risk of PCa in 4 populations of European descent. In the same year, Yeager et al. (2007) showed that the presence of at least two independent loci within 8q24 could contribute to prostate cancer in men of European ancestry. In addition to these variants in the 8q24 region, other variants in the 17q, 11q13, 5p15, and other regions have been found to be associated with PCa risk. For instance, Rafnar et al. (2009) found that rs401681[C] on chromosome 5p15.33 is associated with many cancers, including PCa. Another variant, rs402710 T/C in the 5p15.33, has also been extensively studied.

Both rs401681 and rs402710 reside on chromosome 5p15.33, and they are in an area of high linkage disequilibrium (LD; $r^2 = 0.66$ in HapMap for the population of European descent and $r^2 = 0.89$ for the Chinese). The LD block, in which rs402710 resides, contains two known genes: the human telomerase reverse transcriptase (TERT) gene and the cleft lip and palate transmembrane 1-like (CLPTM1L, alias CRR9) gene. Furthermore, rs402710 is in a region of high LD that includes the promoter regions of TERT and the entire coding region of the CLPTM1L gene (intron 16; Yang et al., 2010). Previous studies have found that the rs402710 polymorphism is a potentially new susceptibility locus for lung cancer (McKay et al., 2008). However, few studies have investigated the association between single-nucleotide polymorphisms (SNPs) of rs402710 and PCa risk.

In this study, we hypothesized that, just as in lung cancer risk, rs402710 is important in PCa risk. To confirm this hypothesis, we performed a case-control study in which we explored the association between rs402710 T>C polymorphisms and the risk of PCa in a Chinese population. Moreover, we investigated the association between this polymorphism and Gleason score, clinical stage, and prostate-specific antigen (PSA) level in PCa cases.

PATIENTS AND METHODS

Study subjects

Both patients and controls were enrolled at the Affiliated Zhongda Hospital of Southeast University and the First Affiliated Hospital of Nanjing Medical University in Nanjing, China. All patients (N = 251) were diagnosed between January 2007 and December 2010 and were pathologically proven to have prostate adenocarcinoma after transrectal systematic ultrasound-guided needle biopsies in the hospital. The control group (N = 273) comprised age-matched, healthy checkup examinees without cancer history, recruited during the same period as that used to recruit the patient group. Control subjects with an abnormal PSA level (≥4 ng/mL) or with a history of cancer or abnormal findings on digital rectal examination were excluded from the study.

The study protocol was approved by Affiliated Zhongda Hospital of Southeast University and the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital). After informed consent was obtained, a 2-mL peripheral blood sample was collected and each subject was asked to complete a questionnaire that included questions about age, race, tobacco and alcohol use, and family history of cancer. Smoking more than five cigarettes per day for more than 5 years was defined as smoking. Pack-years of smoking [(cigarettes per day / 20) × (years smoking)] were calculated to indicate the cumulative smoking dose. A drinking habit was defined as drinking at least 3 times per week for more than 10 years. Family history of cancer was defined as cancer in first-degree relatives (parents, siblings, or children).

Tumor pathologic grade was evaluated in PCa samples using the Gleason scoring system (Gleason and Mellinger, 1974). Disease stage was determined from pathologic findings, pelvic computed tomography, magnetic resonance imaging, and radionuclide bone scans. The tumor stage was determined using tumor-node-metastasis (TNM) classification and graded according to World Health Organization guidelines.

Genotyping

Polymorphism was analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Each PCR analysis was performed in a total volume of 10 μL, which contained 5 ng genomic DNA, 2.5 pmol of each primer, 4 μL ddH$_2$O, and 5 μL PCR Mix (Nanjing Bioedify Biotechnology Co.). PCR was subjected to 35 thermal cycles at 95°C for 30 s, 59.4°C for 40 s, and 72°C for 45 s using a PTC 200 Thermal Cycler (Bio-Rad, Inc.). Primers were 5'-ACATTTGCTTTCAGTGGCTCA-3' (sense) and 5'-CCGTTGGCTTGGTTAGGTT-3' (antisense). For RFLP analysis, PCR products were digested overnight with each restriction enzyme at 37°C, and the resulting fragments were separated on a 3.0% agarose gel and subsequently stained with ethidium bromide. Two researchers performed RFLP and gel reading. In addition, 10% of the samples were retested and the results were consistent.

Statistical analyses

Hardy-Weinberg equilibrium was calculated using control subjects by comparing expected genotype frequencies to observed genotype frequencies with the chi-square test. Distribution of demographic characteristics and substance genotypes were assessed by calculat-

ing the odds ratio (OR) and 95% confidence interval (95%CI), which were obtained through unconditional logistic regression analysis and adjusted for age as a continuous variable. A P value of <0.05 was considered to be statistically significant, and all statistical tests were two sided. Patients with PCa and various Gleason grades or clinical stages were identified using the same statistics as mentioned. All statistical analyses were performed with the Statistics Analysis System Software (Version 9.1.3; SAS Institute, Inc., Cary, NC, USA).

RESULTS

Characteristics of the study population

The distribution of the demographic characteristics of the 251 PCa patients and 273 cancer-free control subjects are shown in Table 1. No significant difference was found between ages of the patients and healthy controls (grouping relative to age 70 years; P = 0.546). In addition, the differences in distributions of cigarette smoking and alcohol consumption between the patients and control subjects were not statistically significant (P = 0.351 and 0.127, respectively).

Table 1. Demographic characteristics of prostate cancer cases and controls.

Characteristics	Cases (N = 251)		Controls (N = 273)		P value*
	N	%	N	%	
Age (years)					0.546
≤70	112	44.62	129	47.25	
>70	139	55.38	144	52.75	
Body mass index (kg/m^2)					0.016
≤23	82	32.67	117	42.86	
>23	169	67.33	156	57.14	
Cigarette smoking (pack-years)					0.351
0	100	39.84	121	44.32	
≤20	56	22.31	65	23.81	
>20	95	37.85	87	31.87	
Alcohol drinking					0.127
Never	139	55.38	133	48.72	
Ever	112	44.62	140	51.28	
Family history of cancers					0.018
No	189	75.30	230	84.25	
Yes	62	24.70	43	15.75	

*Two-sided χ^2 test for the distributions or allele frequencies between the cases and controls.

A higher number of subjects in the PCa group had a family history of cancer and large body mass index (BMI >23 kg/m^2) compared to controls (24.70 *vs* 15.75%, P = 0.018 and 67.33 *vs* 57.14%, P = 0.016, respectively; see Table 1). Consistent with previous research, a history of cancer and large BMI are considered risk factors for PCa. Therefore, further adjustments for these variables were made in the logistic regression model to control possible confounding of the main effects of the studied polymorphisms. Additionally, the risk factors were used in the later stratifications and gene-environment interaction analysis.

Genotype distributions of the rs402710 T>C polymorphism and risk of PCa

Table 2 shows the genotypic frequencies of SNPs in PCa patients and controls. The

genotypic distributions in the controls fit Hardy-Weinberg equilibrium (chi-square = 1.569, P = 0.210). As shown, we found significant associations between rs402710 in 5p15.33 and PCa risk. After adjusting for age, BMI, cigarette smoking, alcohol consumption, and family history of cancer, we found that the CC homozygote (OR = 0.71, 95%CI = 0.51-0.99) had a decreased risk of PCa (P = 0.043) compared with that in the TT homozygote. In addition, compared with the frequency of the CT/TT carrier, the frequency of the CC carrier was lower in patients than in controls (OR = 0.69, 95%CI = 0.48-0.98, P = 0.038). These data indicated that the CC homozygote might have a protective effect on PCa incidence.

Table 2. Genotypes in patients with prostate cancer (PCa) and controls.

Genotype	No. PCa (%)	No. Control (%)§	P*	Adjusted OR (95%CI)¶
Participants	251	273		
rs402710				
TT	26 (10.36)	17 (6.23)		1.00 (reference)
CT	118 (47.01)	118 (43.22)	0.306	0.70 (0.35-1.39)
CC	107 (42.63)	138 (50.55)	0.043	0.71 (0.51-0.99)
TT/CT	144 (57.37)	135 (49.45)	0.038	1.00 (reference)
CC	107 (42.63)	138 (50.55)		0.69 (0.48-0.98)

§The genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium (χ^2 = 1.569, P = 0.210). *Two-sided χ^2 test for the distrib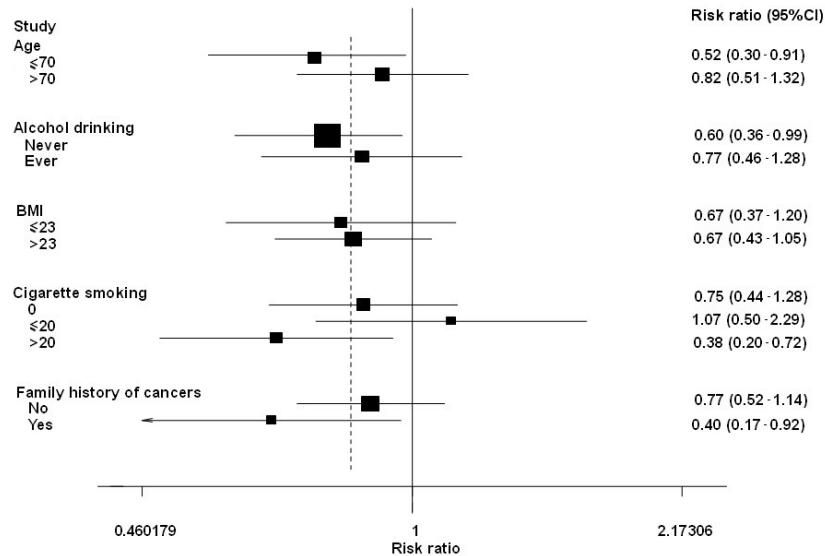utions or allele frequencies between the cases and controls. ¶Odds ratios (ORs) were obtained from a logistic regression model with adjusting for age, BMI, cigarette smoking, alcohol drinking, family history of cancers. 95%CI = 95% confidence interval.

Stratification analyses of the rs402710 T>C polymorphism and risk of PCa

The associations between genotypes and PCa risk, stratified by age, BMI, smoking and alcohol consumption status, and family history of cancer were further examined, and the results are given in Figure 1. As shown, compared with that in CT/TT carriers, the decreased risk of PCa associated with the CC homozygote was more pronounced in subgroups of subjects with a family history of cancer (OR = 0.40, 95%CI = 0.17-0.92, P = 0.032). Furthermore, in the subgroup of subjects who never consumed alcohol, were aged ≤70 years old, and had a cigarette smoking index of >20, also displayed a decreased PCa risk associated with the CC homozygote (OR = 0.60, 95%CI = 0.36-0.99, P = 0.047; OR = 0.52, 95%CI = 0.30-0.91, P = 0.021; OR = 0.38, 95%CI = 0.20-0.72, P = 0.003, respectively).

Stratification analysis of the rs402710 T>C polymorphism in PCa patients by clinicopathological characteristics

The association of rs402710 with PCa clinicopathological characteristics was further examined in subgroups according to selected variables. Clinical stages were stratified by disease stage (localized: $T_{1-2}N_0M_0$; advanced: $T_{3-4}N_xM_x$, $T_xN_1M_x$, or $T_xN_xM_1$), pathologic grade (Gleason score <7, 7, and >7), and serum PSA level (≤20 or >20 ng/mL). As shown in Figure 2, compared with CT/TT genotypes, the CC homozygote was significantly associated with reduced PCa risk in subjects with Gleason scores >7 (OR = 0.49, 95%CI = 0.29-0.82, P = 0.006) and PSA ≤20 ng/mL (OR = 0.62, 95%CI = 0.38-0.996, P = 0.048).

Figure 1. Forest plots represent rs402710 polymorphism and clinicopathological characteristics in patients with prostate cancer. Odds ratios were obtained from a logistic regression model with adjusting for age, body mass index (BMI), cigarette smoking, alcohol drinking, family history of cancers. 95%CI = 95% confidence interval.
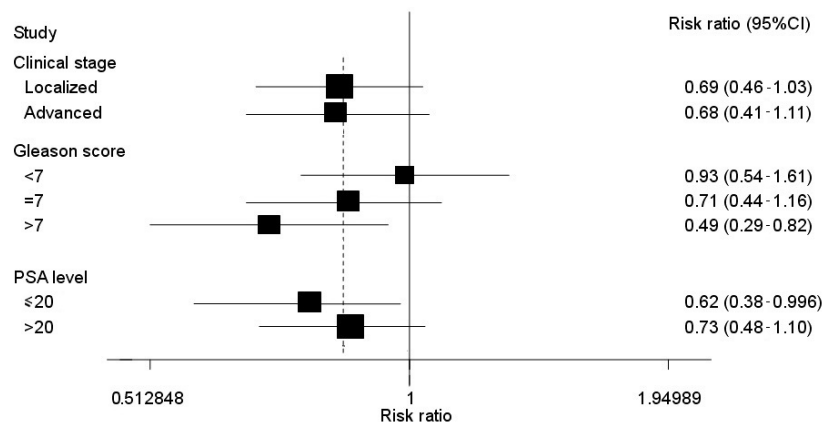
Figure 2. Forest plots represent the association and stratification analysis between rs402710 and risk of prostate cancer. Odds ratios were obtained from a logistic regression model with adjusting for age, BMI, cigarette smoking, alcohol drinking, family history of cancers. 95%CI = 95% confidence interval; PSA = prostate-specific antigen.

DISCUSSION

PCa is a complex disease, and its etiology is still unknown. Aside from studies of the most considered risk factors, such as family history of cancer, age, cigarette smoking, and others, gene polymorphisms have been most studied (Rodriguez et al., 1997; Jemal et al., 2009). In addition, the interplay between multiple genes and SNPs appears to influence the incidence of PCa (Dennis et

al., 2002; Mandal et al., 2010). Recently, GWASs have reported an association between chromosome loci and various cancer susceptibilities, including PCa. Some candidate SNPs at multiple loci have been linked to prostate cancer risk, whereas others are being validated.

The region of chromosome 5p15.33 has been identified in GWASs of several cancers, including brain tumors, lung cancer, basal cell carcinoma, and PCa. Previous research had reported that two SNPs at 5p15.33 (rs402710 and rs401681), which are in strong LD regions, are associated with lung cancer risk (Truong et al., 2010). Because SNP rs401681 has been found to be associated with PCa risk, the association of SNP rs402710 with PCa risk is not difficult to comprehend.

Although Rafnar et al. (2009) have found that rs401681[C] and rs2736098[A] on 5q15.33 were associated with PCa risk, few studies have explored the association between rs402710[C] and PCa risk detail. In the present study, we examined this association through a case-control study of 251 PCa cancer cases and 273 cancer-free controls. Overall, our data suggested that the CC homozygote in rs402710 might have a protective effect on PCa incidence. Furthermore, decreased PCa risk associated with the CC homozygote was also observed in the subgroup of subjects with a family history of cancer who never consumed alcohol, were aged ≤70 years, and had a cigarette smoking index of >20.

Because rs402710 resides in a region of high LD that includes the promoter regions of TERT and the entire coding region of the CLPTM1L gene, the protective effect may be associated with the functions of TERT and CLPTM1L, as with SNP rs401681. TERT is the reverse transcriptase component of telomerase, making it essential for telomerase enzyme production, which is responsible for telomere regeneration. Telomerase stabilizes telomere structures in established tumors, but also contributes to cancer development at the early stages of tumorigenesis. Previous studies have also found that the re-expression of telomerase is a key factor in cancer cell biology, enabling malignant cells to proliferate indefinitely. CLPTM1L is a predicted transmembrane protein expressed in a range of normal and malignant tissues including those in the lung, ovary, and cervix. Furthermore, expression of CLPTM1L had been shown to sensitize ovarian cancer cells to cisplatin-induced apoptosis. Because promoters determine the transcriptional activity of genes, genetic variants in the promoter region may influence the functions of CLPTM1L. Therefore, genetic variants of rs402710 in this high LD and their possible interactions with other variants could play a role in PCa risk. Further studies of the potential effects of genetic variants of rs402710 at the 5p15.33 locus on the functions and expression of TERT and CLPTM1L in PCa are warranted, especially those that include subjects with different ethnic backgrounds.

In summary, our study provided evidence that the rs402710 polymorphism in the 5p15.33 region is associated with PCa risk in Chinese subjects. Compared with TT or CT/TT carriers, the CC homozygote had a decreased risk of PCa. In the subgroup of subjects with a family history of cancer who never consumed alcohol, were aged ≤70 years, and had a cigarette smoking index of >20, a decreased PCa risk associated with the CC homozygote was also observed. The results implied that SNP rs402710 T>C might influence PCa susceptibility and that the CC homozygote had a protective effect. In the future, studies of large cohorts that include worldwide participants are warranted to validate our results.

ACKNOWLEDGMENTS

REFERENCES

Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, et al. (2009). Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. *Nat. Genet.* 41: 986-990.

Crawford ED (2003). Epidemiology of prostate cancer. *Urology* 62: 3-12.

Dennis LK, Lynch CF and Torner JC (2002). Epidemiologic association between prostatitis and prostate cancer. *Urology* 60: 78-83.

Gleason DF and Mellinger GT (1974). Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J. Urol.* 111: 58-64.

Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, et al. (2007). Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat. Genet.* 39: 631-637.

Jemal A, Siegel R, Ward E, Hao Y, et al. (2009). Cancer statistics, 2009. *CA Cancer J. Clin.* 59: 225-249.

Jemal A, Bray F, Center MM, Ferlay J, et al. (2011). Global cancer statistics. *CA Cancer J. Clin.* 61: 69-90.

Kiemeney LA, Thorlacius S, Sulem P, Geller F, et al. (2008). Sequence variant on 8q24 confers susceptibility to urinary bladder cancer. *Nat. Genet.* 40: 1307-1312.

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, et al. (2000). Environmental and heritable factors in the causation of cancer - analyses of cohorts of twins from Sweden, Denmark, and Finland. *N. Engl. J. Med.* 343: 78-85.

Mandal RK, Kapoor R and Mittal RD (2010). Polymorphic variants of DNA repair gene XRCC3 and XRCC7 and risk of prostate cancer: a study from North Indian population. *DNA Cell Biol.* 29: 669-674.

McCracken M, Olsen M, Chen MS Jr, Jemal A, et al. (2007). Cancer incidence, mortality, and associated risk factors among Asian Americans of Chinese, Filipino, Vietnamese, Korean, and Japanese ethnicities. *CA Cancer J. Clin.* 57: 190-205.

McKay JD, Hung RJ, Gaborieau V, Boffetta P, et al. (2008). Lung cancer susceptibility locus at 5p15.33. *Nat. Genet.* 40: 1404-1406.

Rafnar T, Sulem P, Stacey SN, Geller F, et al. (2009). Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. *Nat. Genet.* 41: 221-227.

Rodriguez C, Calle EE, Miracle-McMahill HL, Tatham LM, et al. (1997). Family history and risk of fatal prostate cancer. *Epidemiology* 8: 653-657.

Schaid DJ (2004). The complex genetic epidemiology of prostate cancer. *Hum. Mol. Genet.* 13 (Spec No. 1): R103-R121.

Truong T, Hung RJ, Amos CI, Wu X, et al. (2010). Replication of lung cancer susceptibility loci at chromosomes 15q25, 5p15, and 6p21: a pooled analysis from the International Lung Cancer Consortium. *J. Natl. Cancer Inst.* 102: 959-971.

Yang P, Li Y, Jiang R, Cunningham JM, et al. (2010). A rigorous and comprehensive validation: common genetic variations and lung cancer. *Cancer Epidemiol. Biomark. Prev.* 19: 240-244.

Yeager M, Orr N, Hayes RB, Jacobs KB, et al. (2007). Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat. Genet.* 39: 645-649.