Polymorphisms of DNA repair-related genes with susceptibility and prognosis of prostate cancer

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ABSTRACT. We aimed to investigate the association between genetic variants of the DNA repair genes *XPG*, *CSB*, *XPC*, *CCNH*, and *MMS19L* in the nucleotide excision repair (NER) pathway and risk of prostate cancer in a population in China. This study included 229 patients with newly diagnosed and histopathologically confirmed primary prostate cancer and 238 healthy controls. Genotyping of *XPG*, *CSB*, *XPC*, *CCNH*, and *MMS19L* were performed on a 384-well plate on the MassARRAY platform. Associations between the polymorphisms of the six genes and risk of prostate cancer were analyzed using conditional logistical regression. We found that the variant genotype TT of the *XPG* rs2296147 polymorphism was moderately significantly associated with a higher risk of prostate cancer compared to the wild-type genotype CC [odds ratio (OR) = 1.79, 95% confidence interval (CI) = 1.01-3.25], and individuals carrying the GG genotype of the *CSB* rs2228526 polymorphism were associated with an increased risk of prostate cancer (OR = 1.95, 95%CI = 1.02-3.74). The combination genotype of the *XPG* T allele and the *CSB* G allele was associated with a moderately higher risk of prostate cancer risk (OR = 1.84, 95%CI = 1.06-3.20). In conclusion, we found that polymorphisms in *XPG* rs2296147 and *CSB* rs2228526 were significantly associated with prostate

cancer susceptibility in the Chinese population analyzed. Our results support the hypothesis that naturally occurring genetic variation of DNA repair genes increases susceptibility to prostate cancer.

Key words: *XPG*; *CSB*; DNA repair-related genes; Prostate cancer

INTRODUCTION

Prostate cancer is the second most commonly diagnosed solid tumor and the fifth most common cancer worldwide, accounting for 10% of all male cancer-related deaths (Globocan, 2008). The etiology of prostate cancer is unknown, and it is a multi-factorial disease that is caused by both genetic and environmental factors (Lichtenstein et al., 2000). The risk of prostate cancer increases with age, which reflects the accumulation of DNA damage due to oxidative stress, inflammation, and environmental carcinogens as well as a reduced DNA damage repair capacity (Bostwick et al., 2000; Pathak et al., 2005; Khandrika et al., 2009).

DNA repair mechanisms are important pathways for the removal of oxidative DNA compounds or DNA adducts from damaged genomic sites. There are a number of DNA repair pathways responsible for repairing various types of DNA damage, including base excision repair (BER), nucleotide excision repair (NER), double-strand break repair, and homologous recombination repair (HRR). BER, NER, and HRR constitute the main defenses against lesions generated by ionizing radiation, alkylating agents, and reactive oxygen species (Smith et al., 2003). Variations in DNA repair genes may affect the capacity of encoded DNA repair enzymes to effectively remove DNA adducts or lesions, and subsequently enhance the risk of cancer (Goode et al., 2002; Hu et al., 2002, 2004).

Previous studies have examined the association between single-nucleotide polymorphisms (SNPs) in DNA repair genes and the risk of prostate cancer in various populations (Hyytinen et al., 1999; Hooker et al., 2008; Berhane et al., 2012). However, the results of these studies are inconsistent due to different populations, case selections, and sample sizes. Therefore, we aimed to investigate the association between genetic variants of *XPG*, *CSB*, *XPC*, *CCNH*, and *MMS19L* in the NER pathway and risk of prostate cancer in a population in China.

MATERIAL AND METHODS

Characteristics of study subjects

The subjects were recruited from an ongoing multicenter case-control study being conducted in China. This study included 247 patients with newly diagnosed and histopathologically confirmed primary prostate cancer from the Shanghai Pudong New Area Zhoupu Hospital and the Shanghai East Hospital Affiliated to Tongji University between January 2008 and May 2012. A total of 229 prostate cancer patients agreed to participate with a participation rate of 92.7%. A total of 264 cancer-free controls, who sought health examinations at the Shanghai Pudong New Area Zhoupu Hospital and the Shanghai East Hospital Affilated to Tongji University, were selected during the same time period, and 238 individuals agreed to participate (participation rate: 90.2%). Controls were age-matched (± 5 years) to cases. All subjects were asked to provide 5 mL blood for genotyping and signed written informed consent.

SNP selection and genotyping

Five milliliters of venous blood was drawn from each case and control. The blood was kept at -20°C, and 1.5-2.2 mg/mL ethylenediaminetetraacetic acid (EDTA) was used as anticoagulant. The DNA was extracted using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Genotyping of the *XPG* rs2296147, *XPG* rs2094258, *CSB* rs2228526, *XPC* rs2228001, *CCNH* rs2266690, and *MMS19L* rs29001322 polymorphisms were performed on a 384-well plate on the MassARRAY platform (Sequenom, San Diego, CA, USA), which combined polymerase chain reaction (PCR) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry technologies. PCR and single-base extension primers were designed using the Sequenom Assay Design 3.1 software (Sequenom) according to manufacturer instructions. The cycling program involved preliminary denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, and annealing at 64°C for 30 s, with a final extension at 72°C for 10 min. PCR products were verified by 1.0% agarose gel electrophoresis, and the PCR products were visualized using ethidium bromide staining. For quality control, genotyping was repeated for a random sample of 5% of the cases and controls, and the results were 100% concordant.

Statistical analysis

All statistical analyses were performed using the SAS software (version 9.1; SAS Institute, Cary, NC, USA). Continuous variables are reported as means ± SD and were analyzed using the independent sample Student *t*-test. Categorical variables are reported as the proportion of subjects (%) and were analyzed using the χ^2 test. The Hardy-Weinberg equilibrium and between-group comparisons of genotype distributions were analyzed using a goodness-of-fit χ^2 test. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to assess the effect of each SNP on prostate cancer risk. Unconditional multivariate logistic regression models were adopted to calculate the OR (95%CI) after adjusting for age, family history of prostate cancer, smoking status, drinking status, and body mass index (BMI). All comparisons were two-sided, and P < 0.05 was regarded as statistically significant.

RESULTS

The mean ages of the cases and controls were 66.7 ± 8.2 years and 67.3 ± 7.5 years, respectively (Table 1). We did not find a significant effect of smoking status, drinking status, or BMI on the risk of prostate cancer. However, we found that prostate cancer cases were more likely to have a family history of cancer compared with the controls (7.5 *vs* 0.8%, P < 0.05).

The genotype distributions of the *XPG* rs2296147 and *CSB* rs2228526 SNPs were significantly different between cases and controls (Table 2). Associations between the polymorphisms of the six genes and risk of prostate cancer were analyzed using conditional logistical regression, with frequency matched by age. For *XPG* rs2296147, the variant genotype TT was moderately significantly associated with a higher risk of prostate

cancer when compared to the wild-type genotype CC, with an adjusted OR (95%CI) of 1.79 (1.01-3.25). For *CSB* rs2228526, individuals carrying the GG genotype were associated with an increased risk of prostate cancer (OR = 1.95, 95%CI = 1.02-3.74).

Table 1. Characteristics of the prostate cancer cases and controls.

Characteristics	Cases	%	Controls	%	χ^2 value	P value
Age (years, means ± SD)	66.7 ± 8.2		67.3 ± 7.5			
<65	81	35.4	90	37.8		
≥65	148	64.6	148	62.2	0.3	0.584
Smoking status						
Non-smoker	143	62.4	166	69.7		
Smoker	86	37.6	72	30.3	2.78	0.09
Drinking status						
Non-drinker	153	66.8	172	72.3		
Drinker	76	33.2	66	27.7	1.64	0.2
Body mass index (kg/m²)						
<23	140	61.3	143	60.1		
≥23	89	38.7	95	39.9	0.05	0.82
Family history of prostate cancer						
No	17	7.5	2	0.8		
Yes	212	92.5	236	99.2	12.9	<0.001

Table 2. Genotype distributions and association with prostate cancer.

Gene	Cases	%	Controls	%	OR (95%CI)[1]	P value
XPG rs2296147						
CC	143	62.3	167	70.2	-	-
CT	49	21.2	46	19.3	1.28 (0.79-2.07)	0.29
TT	38	16.5	25	10.5	1.79 (1.01-3.25)	0.04
XPG rs2094258						
AA	93	40.7	105	44.1	-	-
AG	75	32.6	75	31.4	1.13 (0.72-1.77)	0.57
GG	61	26.7	58	24.5	1.19 (0.74-1.92)	0.46
CSB rs2228526						
AA	90	39.5	113	47.4	-	-
AG	105	45.7	103	43.2	1.52 (0.81-2.92)	0.17
GG	34	14.8	22	9.4	1.95 (1.02-3.74)	0.03
XPC rs2228001						
AA	158	69.2	170	71.4	-	-
AC	38	16.6	37	15.5	1.12 (0.65-1.89)	0.69
CC	33	14.2	31	13.1	1.15 (0.65-2.03)	0.62
CCNH rs2266690						
CC	159	69.3	158	66.4	-	-
CT	40	17.4	41	17.1	0.96 (0.58-1.63)	0.9
TT	30	13.3	39	16.5	0.76 (0.44-1.33)	0.31
MMS19L rs29001322						
CC	99	43.4	116	48.7	-	-
CT	93	40.7	92	38.7	1.19 (0.79-1.80)	0.4
TT	36	15.9	30	12.6	1.41 (0.78-2.55)	0.23

[1]Adjusted for age and family history of prostate cancer.

A further association analysis was conducted to identify interactions of the susceptibility-associated genes, *XPG* rs2296147 and *CSB* rs2228526, and their impact on prostate cancer risk (Table 3). The combination genotype of the *XPG* T allele and the *CSB* G allele was associated with a moderately higher risk of prostate cancer, with an OR (95%CI) of 1.84 (1.06-3.20).

Table 3. Interactions of *XPG* rs2296147 and *CSB* rs2228526 in cases and controls on prostate cancer risk.

Single nucleotide polymorphism	Cases (N = 229)	%	Controls (N = 238)	%	OR (95%CI)[1]	P value
XPG rs2296147/*CSB* rs2228526						
CC/AA	59	31.6	85	33.6	-	-
T allele/AA	31	17.4	28	19.2	1.60 (0.83-3.07)	0.13
CC/G allele	84	36.3	82	40.2	1.48 (0.92-2.38)	0.09
T allele/G allele	55	14.7	43	7.0	1.84 (1.06-3.20)	0.02

[1]Adjusted for age and family history of prostate cancer.

DISCUSSION

In this case-control study in a Chinese population, we identified the individual and combined effects of polymorphisms in *XPG* rs2296147, *XPG* rs2094258, *CSB* rs2228526, *XPC* rs2228001, *CCNH* rs2266690, and *MMS19L* rs29001322 on the risk of prostate cancer. We found that *XPG* rs2296147 and *CSB* rs2228526 were strongly associated with prostate cancer risk, both individually and in combination.

To the best of our knowledge, our study is the first to describe the associations of these DNA repair gene polymorphisms with prostate cancer risk in a Chinese population. Three previous studies conducted in Indian, Caucasian-American, and African-American populations, respectively, reported associations between the *XPG* Asp1104His polymorphism and risk of prostate cancer (Hyytinen et al., 1999; Hooker et al., 2008; Berhane et al., 2012). Our findings strongly indicate that polymorphisms in *XPG* rs2296147 contribute to prostate cancer susceptibility, which is in line with results of previous studies showing that the *XPG* rs2296147 TT genotype is associated with increased prostate cancer risk. Berhane et al. (2012) reported that the His/His genotype of *XPG* was associated with a strongly increased risk of prostate cancer in an Indian population, with an OR of 2.53. Another study conducted in the United States reported that *XPG* Asp1104His plays an important role in the development of prostate cancer (Hyytinen et al., 1999). However, other studies have yielded conflicting results. One population-based study including 254 African-American prostate cancer cases and 301 healthy controls from the United States reported a non-significant association between *XPG* polymorphisms and the risk of developing prostate tumors (Hooker et al., 2008). The inconsistency of these studies may be explained by differences in genetic background, population background, source of controls, and sample sizes, or due to chance.

Our study found a moderately increased risk of prostate cancer for patients with the *CSB* rs2228526 GG genotype. Similar associations have been identified for other types of cancer, including skin cancer, bladder cancer, lung cancer, and oral cancer (Chiu et al., 2008; Chang et al., 2009; Ma et al., 2009; Wheless et al., 2012). Polymorphisms in DNA repair genes may be associated with differences in the repair of DNA damage, and thus influence the risk of developing tumors. A recent study in an African-American population found that variants of *CSB* did not influence the development of prostate cancer (Hooker et al., 2008). Further studies are greatly warranted to confirm these associations. The combination of the *XPG* rs2296147 T allele and the *CSB* rs2228526 G allele was also strongly associated with prostate cancer in our study. This combination effect could be explained by the additive effect of the two genotypes.

There are two limitations in our study. First, since our study was only conducted in one place, selection bias is inevitable and the results may not be extractable to other popula-

tions. Second, the sample size in our study was relatively small, which might have reduced the statistical power to detect differences in *XPC*, *CCNH*, and *MMS19L* between groups. Therefore, further large sample multicenter studies are greatly needed.

In conclusion, we found that polymorphisms in *XPG* rs2296147 and *CSB* rs2228526 were significantly associated with prostate cancer susceptibility in the Chinese population, and that the combination of the *XPG* rs2296147 T allele and the *CSB* rs2228526 G allele were strongly associated with an increased risk of prostate cancer. Our results support the hypothesis that naturally occurring genetic variation of DNA repair genes increases susceptibility to prostate cancer.

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