RESEARCH ARTICLE

Association Between Single Nucleotide Polymorphisms in the *XRCC1* Gene and Susceptibility to Prostate Cancer in Chinese Men

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Abstract

Background: Prostate cancer (Pca) is one of the most common complex and polygenic diseases in men. The X-ray repair complementing group 1 gene (*XRCC1*) is an important candidate in the pathogenesis of Pca. The purpose of this study was to evaluate the association between single nucleotide polymorphisms in the *XRCC1* gene and susceptibility to Pca. Materials and Methods: *XRCC1* gene polymorphisms and associations with susceptibility to Pca were investigated in 193 prostate patients and 188 cancer-free Chinese men. Results: The c.910A>G variant in the exon9 of *XRCC1* gene could be detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing methods. Significantly increased susceptibility to prostate cancer was noted in the homozygote comparison (GG versus AA: OR=2.95, 95% CI 1.46-5.42, χ^2=12.36, P=0.001), heterozygote comparison (AG versus AA: OR=1.76, 95% CI 1.12-2.51, χ2=4.04, P=0.045), dominant model (GG/AG versus AA: OR=1.93, 95% CI 1.19-2.97, χ2=9.12, P=0.003), recessive model (GG versus AG+AA: OR=2.17, 95% CI 1.33-4.06, χ2=8.86, P=0.003) and with allele contrast (G versus A: OR=1.89, 95% CI 1.56-2.42, χ2=14.67, P<0.000). Conclusions: These findings suggest that the c.910A>G polymorphism of the *XRCC1* gene is associated with susceptibility to Pca in Chinese men, the G-allele conferring higher risk.

Keywords: Prostate cancer - *XRCC1* gene - single nucleotide polymorphism - susceptibility - Chinese men

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Introduction

Prostate cancer (Pca) is the most commonly diagnosed cancer among men, accounting for 10% of male cancer-related mortality (Jemal et al., 2008). The aetiology of PCa remains poorly understood and the genetic and environmental factors have been proved with the susceptibility to PCa (Pienta et al., 1993; Lichtenstein et al., 2000; Schaid, 2004). A number of studies suggested that X-ray repair complementing group 1 (*XRCC1*) is an important candidate gene for mediating the genetic influence on Pca (Hirata et al., 2007; Geng et al., 2009; Agalliu et al., 2010; Mandal et al., 2010; Dhillon et al., 2011; Kuasne et al., 2011; Langsenlehner et al., 2011; Wei et al., 2011; Wei et al., 2011; Berhane et al., 2012; Mittal et al., 2012). It has been reported that several SNPs, such as C26304 (Arg194Trp), G27466A (Arg280His), and G28152A (Arg399Gln) et al., were associated with Pca (Hirata et al., 2007; Geng et al., 2009; Mandal et al., 2010; Dhillon et al., 2011; Langsenlehner et al., 2011; Wei et al., 2011; Wei et al., 2011; Berhane et al., 2012; Mittal et al., 2012). However, up to date, the association analysis between the c.910A>G variant of *XRCC1* gene and susceptibility to Pca have not been analyzed. Thus, the objective of the current study was to examine the c.910A>G variant and evaluate its effects on the susceptibility to Pca in Chinese men.

Materials and Methods

Subjects

This present case-control study was performed on a total of 381 subjects (Han Chinese), including 193 prostate patients and 188 cancer-free volunteers. The diagnosis of prostate cancer was confirmed by the doctor according to the clinical, pathological and laboratory examinations. The general characteristics for these subjects, including age, smoking status, drinking status, body mass index (BMI), family history of prostate cancer, Gleason glade and PSA level (ng/ml), were summarized (Table 1). This study was approved by the local ethics committee and all subjects signed the study informed consent forms.

Genotyping

Genomic DNA was extracted from peripheral blood samples by standard phenol/chloroform/isoamyl alcohol

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extraction protocol. One pair of primers was designed using Primer Premier 5.0 software. Primers sequences, annealing temperature, region and fragment sizes were shown in Table 2. Polymerase chain reaction (PCR) amplifications were performed on 20 μl reaction mixture containing 50 ng mixed DNA template, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was 95°C for 5 min followed by 32 cycles of 94°C for 30 s, 60.9°C annealing for 30 s, and72°C for 30 s, and a final extension at 72°C for 10 min. The PCR-restriction fragment length polymorphism (PCR-RFLP) method was used to analyze the c.910A>G genotype. Aliquots of 5 μl PCR products were digested with 2U restriction enzyme at 37°C for 10 h. The digested products were separated by electrophoresis in 2.5% agarose gel. The PCR amplified products were sequenced by ABI 3730 sequencer (Bioasia Biotechnology Co., Ltd. Shanghai, China).

Statistical analysis

The Statistical Package for Social Sciences software (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA) was used to analyze the data. Allele and genotype frequencies were calculated by counting. The Chi-squared (χ^2) test was utilized to evaluate the significant departure from the Hardy-Weinberg equilibrium. Odds ratios (OR) and 95% confidence intervals (95% CI) adjusted for age, smoking status, drinking status, body mass index (BMI), family history of prostate cancer were then estimated by multiple logistic regression analysis to investigate the association between the *XRCC1* polymorphism and susceptibility of prostate cancer. P value<0.05 was considered statistically significant.

Results

General characteristics

A total of 381 subjects were recruited in this study, including 193 prostate cancer patients and 188 healthy controls. The general characteristics for these subjects were performed in Table 1. No significant difference between prostate cancer patients and healthy controls were detected in terms of age (P=0.5356). Additionally, no significant differences were detected in BMI, smoking and drinking status between the prostate cancer patients and healthy controls (P=0.7732, P=0.2626 and P=0.2952, respectively).

Genotyping of XRCC1 polymorphism

In this work, a novel allelic variant corresponding to the A→G mutations and Threonine (Thr) to Alanine (Ala) amino acid replacement in the exon9 of *XRCC1* gene could be detected by PCR-RFLP. The PCR product of c.910A>G was digested with HhaI enzyme. Three possible genotypes were defined by three distinct banding patterns: AA (225 bp), AG (225, 138 and 87 bp), and GG (138 and 87 bp). The allelic and genotypic frequencies of the c.910A>G polymorphism were presented in Table 3. The polymorphism site was fitted with Hardy-Weinberg equilibrium (P>0.05). Allele frequencies in Pca patients and healthy controls were 0.5570 and 0.6915 for A allele, and 0.4430 and 0.3085 for G allele, respectively. Frequencies of the AA, AG, and GG genotypes were 0.3471, 0.4197, and 0.2332 in prostate cancer patients, while the frequencies of those genotypes in healthy subjects were performed to be 0.5000, 0.3830, and 0.1170. The allele and genotype frequencies of prostate cancer

Table 1. Characteristics of the Prostate Cancer Cases and Controls

Characteristics	Cases (n)		Controls (n)		χ^2-value	P-Value
Number	193	%	188	%		
Age(years)					0.3837	0.5356
mean±SD	72.78±8.82		71.59±8.66			
≥70	126	0.6528	117	0.6223		
<70	67	0.3472	71	0.3777		
Smoking status					1.2551	0.2626
Non smoker	89	0.4611	76	0.4043		
Smoker	104	0.5389	112	0.5957		
Drinking status					1.0959	0.2952
Non drinker	119	0.6166	106	0.5638		
Drinker	74	0.3834	82	0.4362		
Body mass index (BMI) (kg/m^2)					0.083	0.7732
≥23	131	0.6788	125	0.6649		
<23	62	0.3212	63	0.3351		
Family history of prostate cancer						
Yes	46	0.2383	-			
Never	147	0.7617	-			
Gleason glade						
≥7	129	0.6684	-			
<7	64	0.3316	-			
PSA level (ng/ml)						
≥10	137	0.7098	-			
<10	56	0.2902	-			

Table 2. PCR and PCR-RFLP Analysis for the c.910A>G Variant in XRCC1 Gene

Primer sequences	Annealing temperature (°C)	Amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
5'-TGCCAGCTCCAACTCGTACC-3' 5'-GATACTTGGCCCCAAGCTCTAG-3'	60.9	225	Exon9	HhaI	AA:225 AG:225,138,87 GG: 138,87

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism

Table 3. Genotype and Allele Frequencies of XRCC1 Gene c.910A>G Polymorphism in the Subjects

	Genotype frequencies						Allele frequencies				χ^2	P
	AA		AG		GG		A		G			
	n	%	n	%	n	%	n	%	n	%		
Case Group (n=193)	67	0.3471	81	0.4197	45	0.2332	215	0.557	171	0.443	4.3177	0.1155
Control Group (n=188)	94	0.5	72	0.383	22	0.117	260	0.6915	116	0.3085	1.9701	0.3733
Total (n=381)	161	0.4226	153	0.4016	67	0.1758	475	0.6234	287	0.3766	7.9877	0.0184
	χ^2=12.8895, P=0.0016						χ^2=14.6745, P=0.0001					

Table 4. Association Between the c.910A>G Variant of XRCC1 Gene and Susceptibility of Prostate Cancer

Comparisons	OR (95% CI)	χ^2-value	P-value
Homozygote comparison (GG vs. AA)	2.870(1.577-5.221)	12.36	0.001
Heterozygote comparison (AG vs. AA)	1.578(1.010-2.466)	4.04	0.045
Dominant model (GG/AG vs. AA)	1.881(1.246-2.839)	9.12	0.003
Recessive model (GG vs. AG/AA)	2.294(1.316-4.000)	8.86	0.003
Allele contrast (G vs. A)	1.783(1.325-2.399)	14.67	0

vs., versus; OR, odds ratio; CI, confidence interval

patients were significantly different from those of the healthy controls (χ^2=14.6745, p=0.0001 and χ^2=12.8895, p=0.0016, respectively, Table 3).

XRCC1 polymorphism and susceptibility to prostate cancer

The association analysis between the c.910A>G polymorphism and the susceptibility to Pca was performed in Table 4. Significantly increased susceptibility to Pca were found in the homozygote comparison (GG versus AA: OR=2.95, 95% CI (1.46-5.42), χ^2=12.36, P=0.001), heterozygote comparison (AG versus AA: OR=1.76, 95% CI 1.12-2.51, χ^2=4.04, P=0.045), dominant model (GG/AG versus AA: OR=1.93, 95% CI 1.19-2.97, χ^2=9.12, P=0.003), recessive model (GG versus AG+AA: OR=2.17, 95% CI 1.33-4.06, χ^2=8.86, P=0.003) and allele contrast (G versus A: OR=1.89, 95% CI 1.56-2.42, χ^2=14.67, P<0.000).

Discussion

Pca is a complex disease and the genetic factors play a key role in contributing to the susceptibility. Common genetic SNPs in the cancer-related gene play a major role in tumorigenesis. The present study firstly indicated that the *XRCC1* c.910A>G polymorphism was associated with susceptibility to PCa. The G allele might be a risk allele for PCa in Chinese men (G versus A: OR=1.89, 95% CI 1.56-2.42, χ^2=14.67, P<0.000, Table 4). As shown in Table 3, the allelic and genotypic frequencies were statistically associated with the risk of PCa between patients and controls (P=0.0001 and P=0.0016, respectively). Our findings suggested that the GG genotype was strongly associated with increased susceptibility to Pca compared to AA genotype and AG/AA carriers (OR=2.95, 95% CI 1.46-5.42, P=0.001 and OR=2.17, 95% CI 1.33-4.06, P=0.003, Table 4). Previous studies have been evaluated the relationship between the susceptibility of Pca and SNPs in *XRCC1* gene, such as the (Hirata et al., 2007; Geng et al., 2009; Mandal et al., 2010; Dhillon et al., 2011; Langsenlehner et al., 2011; Wei et al., 2011; Wei et al., 2011; Berhane et al., 2012; Mittal et al., 2012), but not including the c.910A>G variant. To our knowledge, this is the first report demonstration the susceptibility of the *XRCC1* c.910A>G polymorphism with Pca. Results from this study provided more information to help understand and determine the role of *XRCC1* gene in Pca. However, the results from these studies still remain inconsistent. Therefore, further investigation will be necessary to clarify the role of the c.910A>G and other polymorphisms of *XRCC1* gene in susceptibility to Pca on larger different ethnic populations.

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The author(s) declare that they have no competing interests.

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