Relationship between multidrug resistance 1 polymorphisms and the risk of prostate cancer in Chinese populations

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ABSTRACT. Prostate cancer is one of the most common malignancies in men. The multidrug resistance 1 gene (*MDR1*) is an important candidate gene for prostate cancer. The aim of this study was to evaluate the association between *MDR1* gene polymorphisms and the risk of prostate cancer. *MDR1* gene polymorphism and its association with the risk of prostate cancer were investigated in 357 Chinese men. A novel c.1465C>T polymorphism was detected with created restriction site-polymerase chain reaction and DNA sequencing. We found a significantly increased risk of prostate cancer in the homozygote comparison [TT *vs* CC: odds ratio (OR) = 2.300, 95% confidence interval (95%CI) = 1.261-4.196, chi-square = 7.53, P = 0.007], heterozygote comparison (TC *vs* CC: OR = 1.667, 95%CI = 1.049-2.648, chi-square = 4.71, P = 0.030), dominant model (TT/

TC *vs* CC: OR = 1.835, 95%CI = 1.197-2.815, chi-square = 7.81, P = 0.005), recessive model (TT *vs* TC/CC: OR = 1.776, 95%CI = 1.023-3.085, chi-square = 4.23, P = 0.041), and allele contrast (T *vs* C: OR = 1.625, 95%CI = 1.199-2.202, chi-square = 9.87, P = 0.002). These findings suggested that the c.1465C>T polymorphism of *MDR1* may be risk factors for prostate cancer in Chinese men.

Key words: Association analysis; Prostate cancer; Risk factors; Multidrug resistance 1 gene; Single-nucleotide polymorphism;

INTRODUCTION

Prostate cancer (Pca) is among the most common malignancies in men and the second leading cause of cancer-related death (Jemal et al., 2008). The pathogenesis of Pca is still largely unknown, with genetic and environmental factors likely contributing to increased risk of the disease (Pienta and Esper 1993; Lichtenstein et al., 2000; Schaid, 2004). Several candidate genes have been suggested to be associated with Pca, including multidrug resistance 1 (*MDR1*) (van Brussel and Mickisch, 2003; Sanchez et al., 2009; Sanchez et al., 2011), X-ray repair complementing group 1 (Hirata et al., 2007; Agalliu et al., 2010; Dhillon et al., 2011; Kuasne et al., 2011; Langsenlehner et al., 2011), xeroderma pigmentosum group D gene (Mandal et al., 2010), APEX nuclease 1 gene (Agalliu et al., 2010; Kuasne et al., 2011; Mittal et al., 2012), Toll-like receptor 4 (Jing et al., 2012), axis inhibition protein 2 (Pinarbasi et al., 2011), 2-5A-dependent RNase (Wei et al., 2012), complementation group G gene (Berhane et al., 2012), and N-acetyltransferase types 1 (Gong et al., 2011) and 2 (Gong et al., 2011; de Lima Junior et al., 2012). *MDR1* is an important candidate gene for Pca. Evidence from previous studies has suggested that common polymorphisms in *MDR1* are associated with the risk of Pca (van Brussel and Mickisch, 2003; Sanchez et al., 2009; Sanchez et al., 2011). Several *MDR1* single-nucleotide polymorphisms (SNPs) such as C2677T and C3435T have been detected to affect the risk of Pca (Narter et al., 2006). However, no similar association analysis has been carried out for the c.1465C>T variant in *MDR1* and risk of Pca. The current study aimed to investigate the *MDR1* c.1465C>T variant distribution and evaluate its effect on the risk of Pca in Chinese men.

MATERIAL AND METHODS

Subjects

A total of 176 Pca patients and 181 healthy controls were recruited for this study. The subject characteristics are summarized in Table 1 and included age, drinking status, smoking status, body mass index, family history of Pca, serum prostate-specific antigen level (ng/mL), and Gleason grade. All subjects were genetically unrelated ethnic Han Chinese men. The diagnosis of Pca was confirmed with pathological, clinical, and laboratory examinations. This study was approved by the local ethics committee, and written informed consent forms were obtained from all subjects.

Table 1. General characteristics of the prostate cancer patients and healthy controls.

Characteristics	Cases (N)		Controls (N)		χ^2-value	P
	176	%	181	%		
Age years, means ± SD	72.83 ± 9.79		71.76 ± 9.58		2.2391	0.1346
≥ 75	110	62.50	99	54.70		
< 75	66	37.50	82	45.30		
Drinking					0.7425	0.3888
Yes	61	34.66	55	30.39		
No	115	65.34	126	69.61		
Smoking					1.6020	0.2056
Yes	106	60.23	97	53.59		
No	70	39.77	84	46.41		
Body mass index (BMI) (kg/m^2)					0.5043	0.4776
≥ 25	98	55.68	94	51.93		
< 25	78	44.32	87	48.07		
Family history of prostate cancer						
Yes	42	23.86	-			
No	134	76.14	-			
Serum PSA level (ng/mL)						
≥ 10	128	72.73	-			
< 10	48	27.27	-			
Gleason score						
≥ 7	116	65.91	-			
< 7	60	34.09	-			

Genotype analysis

Blood samples were collected from all subjects, and genomic DNA was extracted according to a standard protocol. Specific polymerase chain reaction (PCR) primers were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). Primers, annealing temperature, region, fragment size, and selected restriction enzymes (MBI Fermentas, St. Leon-Rot, Germany) are presented in Table 2. PCR amplifications were performed with 20-µL reaction mixtures containing 50 ng mixed DNA template, 10 pM each primer, 0.20 mM deoxyribonucleotide triphosphate, 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, 59.2 °C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The c.1465C>T variant was detected with a created restriction site-PCR (CRS-PCR) method with 1 of the primers containing a nucleotide mismatch, which enabled the use of restriction enzymes for discriminating sequence variations (Haliassos et al., 1989; Zhao et al., 2003; Yuan et al., 2012; Yuan et al., 2013; Yuan et al., 2013). Following the supplier manual, we digested 5-µL aliquots PCR amplified products with 2 U restriction enzyme at 37°C for 10 h. The digested products were separated via electrophoresis in 2.5% agarose gel, and the genotype of the c.1465C>T polymorphism was based on the various electrophoretic patterns. The PCR-amplified products were sent to the Bioasia Biotechnology Co., Ltd. (Shanghai, China) for sequencing on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA 94404, USA) to verify the findings of the CRS-PCR analysis.

Statistical analysis

The chi-square test was used to evaluate Hardy-Weinberg equilibrium and compare

the genotype frequencies between patients and controls. The odds ratio (OR) and 95% confidence intervals (95%CI) were obtained through multiple and logistic regression analyses to investigate the association between the *MDR1* polymorphism and susceptibility to Pca. All statistical analyses were performed using SPSS (Windows version release 15.0; SPSS Inc.; Chicago, IL, USA). A P value of less than 0.05 was regarded as statistically significant.

Table 2. Primer and CRS-PCR analysis for detecting the c.1465C>T polymorphism in *MDR1* gene.

Primer sequences	Annealing temperature (°C)	Amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
5'-CACCACGATAGCTGAAAACAT<u>C</u>-3' 5'-TTAGGATTTCCCTTCTTCCGAT-3'	59.2	220	Exon14	*Aci*I	CC: 198, 22 CT: 220, 198, 22 TT: 220

CRS-PCR = polymerase chain reaction-created restriction site-polymerase chain reaction; underlined nucleotides mark nucleotide mismatches enabling the use of the selected restriction enzymes for detecting sequence polymorphism.

RESULTS

General characteristics of the subjects

In total, 357 subjects were evaluated in this study and their characteristics are presented in Table 1. No significant differences were found between Pca patients and healthy controls in terms of age, drinking, smoking, and body mass index (P = 0.1346, P = 0.3888, P = 0.2056, and P = 0.4776, respectively).

Genotyping of *MDR1* polymorphism

We founded the c.1465C>T variant within exon14 of the human MDR1 gene using CRS-PCR and DNA sequencing methods. Sequence analysis showed that the c.1465C>T polymorphism was caused by a C to T mutation, resulting in arginine (Arg) to cysteine (Cys) amino acid replacement (p.Arg489Cys; reference sequences GenBank IDs NG_011513.1, NM_000927.4, and NP_000918.2). The PCR product of the c.1465C>T variant was digested with *Aci*I enzyme and divided into 3 genotypes: CC (198 and 22 bp), CT (220, 198, and 22 bp), and TT (220 bp; see Table 2). The allelic and genotypic frequencies of the c.1465C>T polymorphisms appear in Table 3. The chi-square test suggested that the c.1465C>T polymorphism was in Hardy-Weinberg equilibrium in the cases and controls (P > 0.05). Allele frequencies were 0.5568 and 0.6713 for the C allele, and 0.4432 and 0.3287 for the T allele in Pca patients and healthy controls, respectively. Frequencies of the CC, CT, and TT genotypes were 0.3352, 0.4432, and 0.2216 in cases, whereas the frequencies of those genotypes in controls were 0.4807, 0.3812, and 0.1381. The allelic and genotypic frequencies of patients were significantly different from those of controls (chi-square = 9.8720, P = 0.0017, and chi-square = 8.9151, P = 0.0116, respectively; see Table 3).

Association analysis of *MDR1* polymorphism with risk of Pca

Table 4 shows the association between the c.1465C>T polymorphism and the risk of

Pca. Significantly increased risk of Pca occurred in the homozygote comparison (TT *vs* CC: OR = 2.300, 95%CI = 1.261-4.196, chi-square = 7.53, P = 0.007), heterozygote comparison (TC *vs* CC: OR = 1.667, 95%CI = 1.049-2.648, chi-square = 4.71, P = 0.030), dominant model (TT/TC *vs* CC: OR = 1.835, 95%CI = 1.197-2.815, chi-square = 7.81, P = 0.005), recessive model (TT *vs* TC/CC: OR = 1.776, 95%CI = 1.023-3.085, chi-square = 4.23, P = 0.041), and allele contrast (T *vs* C: OR = 1.625, 95%CI = 1.199-2.202, chi-square = 9.87, P = 0.002).

Table 3. Genotype and allele frequencies of the *MDR1* gene c.1465C>T polymorphism in the prostate cancer patients and controls.

	Genotypes			Alleles		χ^2	P
	CC	CT	TT	C	T		
Case Group (N = 176)	59 (0.3352)	78 (0.4432)	39 (0.2216)	196 (0.5568)	156 (0.4432)	1.8326	0.4000
Control Group (N = 181)	87 (0.4807)	69 (0.3812)	25 (0.1381)	243 (0.6713)	119 (0.3287)	3.3585	0.1865
Total (N = 357)	146 (0.4090)	147 (0.4117)	64 (0.1793)	439 (0.6148)	275 (0.3852)	6.0894	0.0476
		χ^2 = 8.9151, P = 0.0116		χ^2 = 9.8720, P = 0.0017			

Table 4. Association between the susceptibility of prostate cancer and the c.1465C>T variant in *MDR1* gene.

Comparisons	Test of association		
	OR (95%CI)	χ^2	P
TT *vs* CC (homozygote comparison)	2.300 (1.261-4.196)	7.53	0.007
TC *vs* CC (heterozygote comparison)	1.667 (1.049-2.648)	4.71	0.030
TT/TC *vs* CC (dominant model)	1.835 (1.197-2.815)	7.81	0.005
TT *vs* TC/CC (recessive model)	1.776 (1.023-3.085)	4.23	0.041
T *vs* C (allele contrast)	1.625 (1.199-2.202)	9.87	0.002

OR = odds ratio; 95%CI = 95% confidence interval.

DISCUSSION

Pca is a multi-factorial disease resulting from complex factors, and genetic factors play an important role in the risk of Pca. Previous studies have demonstrated that *MDR1* is an important candidate gene in the pathogenesis of osteoporosis (van Brussel and Mickisch, 2003; Sanchez et al., 2009; Sanchez et al., 2011). To the best of our knowledge, the current study is the first to investigate the prevalence of the c.1465C>T variant, which is located in exon14 of *MDR1*, and evaluate its relationship with Pca risk. Our data showed that the *MDR1* c.1465C>T variant is associated with the risk of Pca in Chinese men. The genotypic and allelic frequencies between patients and healthy controls were statistically associated with this risk (P = 0.0116 and P = 0.0017, respectively; see Table 3). The TT genotype was strongly associated with increased risk of Pca compared to that accompanying the CC genotype and CT/CC carrier status (OR = 2.300, 95%CI = 1.261-4.196, P = 0.007, and OR = 1.776, 95%CI = 1.023-3.085, P = 0.041; see Table 4). In addition, the T allele increased the risk of Pca (T *vs* C: OR = 1.625, 95%CI = 1.199-2.202, P = 0.002; see Table 4). Similar research has reported that several *MDR1* SNPs, such as C2677T and C3435T (Narter et al., 2006), are associated with the risk of Pca, but the c.1465C>T variant was not included in that study. Results from this study provide more evidence for the role of *MDR1* in Pca. However, the mechanism underly-

ing the association between the *MDR1* SNPs and the risk of Pca is still poorly understood. Thus, further studies are necessary to obtain more reliable results in larger populations and to augment the etiology in the pathogenesis of Pca.

Conflicts of interest

The authors declare no conflicts of interests.

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