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Original Article

Single Nucleotide Polymorphism *WRN* Leu1074Phe Is Associated with Prostate Cancer Susceptibility in Chinese Subjects

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Deficiencies in the human DNA repair gene *WRN* are the cause of Werner syndrome, a rare autosomal recessive disorder characterized by premature aging and a predisposition to cancer. This study evaluated the association of *WRN* Leu1074Phe (rs1801195), a common missense single nucleotide polymorphism in *WRN*, with prostate cancer susceptibility in Chinese subjects. One hundred and forty-seven prostate cancer patients and 111 male cancer-free control subjects from 3 university hospitals in China were included. Blood samples were obtained from each subject, and the single nucleotide polymorphism *WRN* Leu1074Phe was genotyped by using a Snapshot assay. The results showed that *WRN* Leu1074Phe was associated with the risk of prostate cancer in Chinese men and that the TG/GG genotype displayed a decreased prevalence of prostate cancer compared with the TT genotype (OR = 0.58, 95% CI: 0.35–0.97, $p = 0.039$). Through stratified analysis, more significant associations were revealed for the TG/GG genotype in the subgroup with diagnosis age ≤ 72 yr (OR = 0.27, 95% CI: 0.12–0.61, $p = 0.002$) and in patients with localized diseases (OR = 0.36, 95% CI: 0.19–0.70, $p = 0.003$). However, no statistically significant difference was found in the subgroup with age > 72 yr or in patients with advanced diseases. We concluded that the genetic variant Leu1074Phe in the DNA repair gene *WRN* might play a role in the risk of prostate cancer in Chinese subjects.

Key words: polymorphism, prostatic neoplasms, single nucleotide, susceptibility, WRN

Being the most common malignancy among men, prostate cancer (PCa) constitutes a major health problem in developed countries. The incidence of prostate cancer in China is low [1]. However, along with lifestyle changes and the process of westernization, the morbidity of prostate cancer has increased dramatically in recent years, and it is becoming a great health burden in China [2, 3].

Accumulating evidence suggests that genetics play an important role in the etiology of prostate cancer

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[4, 5]. Through many large-scale and genome-wide genetic studies, many single nucleotide polymorphisms (SNPs) have been found to be associated with an increased risk of prostate cancer, yet these SNPs only had moderate effects individually, and the responsible genes remain largely unidentified [6, 7].

The DNA repair gene *WRN* is located at chromosome 8p11–12. WRN protein is an important member of the RecQ helicase family, which is involved in multiple DNA repair pathways, protecting the genome from incorrect recombination during mitosis and maintaining its stability [8]. Deficiencies in the human RecQ helicase gene *WRN* are the cause of Werner syndrome, a rare autosomal recessive disorder characterized by premature aging and a predisposition to cancer. Since mutations in the gene *WRN* lead to accelerated aging and cancer susceptibility, it has been reasoned that polymorphisms in the gene *WRN* may also be associated with age-related pathologies, including a high incidence of cancer.

WRN Leu1074Phe (rs1801195), one of the common missense SNPs in the gene *WRN* [9], usually manifests a G to T variation and leads to a protein variation of 1074Leu to Phe. The *WRN* Leu1074Phe polymorphism located in the C-terminus of *WRN*. The variant protein 1074Phe/Phe was reported to exhibit a small decrease in helicase and exonuclease activities relative to the wild-type protein [10]. In previous studies, *WRN* Leu1074Phe had been found to be associated with many diseases, including breast cancer [9, 11].

Prostate cancer manifests an obvious increase in morbidity along with aging, and its relationship with the Werner syndrome gene *WRN* has never been investigated. In the present study, we discuss the association of the polymorphism *WRN* Leu1074Phe with the risk of PCa in Chinese men for the first time.

Materials and Methods

Study subjects. The subjects studied came from 2 cities in China: Beijing, in the north of China and Hangzhou, in the south of China. Participants were recruited from 2 university hospitals in Beijing and one university hospital in Hangzhou between January 2009 and June 2010. The inclusion criterion for cases was pathologically verified adenocarcinoma of the prostate, whether newly diagnosed or not. An informed consent form was signed by both the physician and the recruited patient before a 2-ml blood sample was obtained from each patient. A questionnaire, which included questions regarding prostate cancer family history, diet (frequencies of eating meats, fish, seafoods, milk, vegetables, fruits, beans and frumentum in daily life), smoking status (never/ever, years of smoking history and cigarettes/day), drinking status (never/ever, years of drinking history, grams of alcohol/day) and clinical information, such as age, Gleason score and prostate-specific antigen (PSA) level at the time of diagnosis, tumor-node-metastasis (TNM) staging, pathology, treatment (radical prostatectomy, hormone therapy, radiation therapy, combined therapy, *etc.*) and current situation (survival, death, relapse, metastasis, *etc.*) was also sent to each eligible case patient. TNM staging was determined after an operation or based on imaging modalities and biopsy result.

In total, 166 prostate cancer patients were invited to participate; of those, 147 (88.6%) agreed to participate by donating a blood sample and/or answering the questionnaire. The patients were classified according to localized PCa — T1–2 and N0/NX, M0/MX, Gleason sum 2–7, and PSA level less than 50 ng/ml or advanced PCa — T3/4 or N+ or M+ or Gleason sum 8–10 or PSA level greater than 50 ng/ml.

The control subjects were male cancer-free patients or healthy men. They were randomly selected from the above-mentioned 3 hospitals during the recruitment stage and frequency matched according to the expected age distribution (within 5 years) and geographic origin of the case patients. The PSA value was also checked for each control subject as a screening marker, and only those with a PSA value < 4 ng/ml were included. After informed consent was obtained from the control subjects, a 2-ml blood sample was obtained and a questionnaire was administered to each subject just as for the case patients. Of the 137 randomly selected control subjects invited, 111 (81.0%) agreed to participate by contributing a blood donation and/or completing the questionnaire.

All procedures were carried out with the adequate understanding and written consent of each subject. The study was also approved by ethics committee of each hospital.

Blood samples and DNA extraction. Strict

procedures were followed for blood handling and transport to ensure consistency. A 2-ml sample of donated blood was obtained from each participant and it remained at room temperature for no more than 6 hours. The blood samples were then stored at −20℃ and transported to our laboratory in Peking University People's Hospital, Beijing, using a dry ice box within 1 month. After the blood samples arrived at the laboratory, genomic DNA was extracted using the phenol-chloroform method as soon as possible. Overall, 100% of the samples had sufficient DNA for the study.

Genotyping. Genotyping was conducted by Yingjun Biotechnology Co., Ltd in Shanghai, China. A method adapted to the ABI SNaPshot® multiplex system was used to genotype the polymorphism *WRN* Leu1074Phe as well as 6 other SNPs (not mentioned in this paper).

Multiplex PCR (first PCR). Multiplex PCR amplification was carried out on an ABI 9700 Thermocycler using 1 μl genomic DNA (10–50 ng) as a template, 1 μl 10 × PCR buffer, 0.8 μl dNTPs (2.5 mM each), 0.05 μl rTaq (5U/μl), 1.4 μl first primer mix and water to a final reaction volume of 10 μl. The program used was an initial denaturation step of 94℃ for 3 min followed by 32 cycles of 94℃ for 30 sec, 60℃ for 30 sec and 72℃ for 30 sec. The final extension step was carried out for 7 min at 72℃. The first PCR primers for WRN Leu1074Phe were: upper primer 5'-AAACGGTGTAGGAGTCTGC CT-3', and lower primer 5'-TTCATTTTACTTGTGAGA GGCCT-3'.

The PCR products were checked on a 2% agarose gel. To avoid participation in a subsequent primer extension reaction, the primers and unincorporated dNTPs of the preliminary PCR reaction were removed before genotyping: 5 μl of PCR product was incubated with 2 units of ExoSAP for 60 min at 37℃, followed by 15 min at 75℃ for enzyme inactivation.

SNaPshotTM reaction. The typing PCR (second PCR) primer used for *WRN* Leu1074Phe was 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTT CTAATGAAGAATTGTG TCCAAAGAAGTT-3'. Noting that all the primers were used simultaneously for seven SNP targets, many T residues were attached to the typing primer of *WRN* Leu1074Phe to discriminate it from the other targets. The single typing primer was used for typing a single SNP genotype with ddNTP end-labeling.

SNaPshot reactions were carried out using 1.5 μl purified first PCR products as a template, typing primer mix, 2.5 μl of SNaPshot multiplex ready reaction mix (ABI), and water to a final reaction volume of 5 μl. Reactions were carried out in the ABI 9700 Thermocycler at 96℃ for 10 sec, 50℃ for 5 sec and 60℃ for 30 sec, and repeated for a total of 30 cycles. Following cycling, the samples were treated with 1 U of shrimp alkaline phosphatase (SAP) at 37℃ for 1 h and heat-inactivated at 72℃ for 15 min.

In an ABI optical plate, 9 μl of HI-DI formamide, 0.5 μl of Genescan 120 LIZ size standard (ABI) and 0.5 μl of the reaction mixture were combined, denatured at 95℃ for 5 min, and immediately placed on ice for 2 min. Samples were then loaded on the ABI PRISM 3100 Genetic Analyzer and analyzed using the Genescan (version 3.1) software.

Statistical analysis. Data were given as means ± standard deviation (SD) for variables with normal distribution, and otherwise as medians. A test for Hardy-Weinberg equilibrium was performed among the control subjects with the use of Chi-squared tests or the exact test if any of the cell counts were < 5. The differences of allele and genotype frequencies between case patients and control individuals were analyzed using Pearson's χ^2 test. Genotype data were analyzed with the homozygotes of the common allele as the reference group. Odds ratios (ORs), 95% confidential intervals (CIs) and corresponding P values for the association between prostate cancer risk and genotypes (or alleles) were calculated using logistic regression analysis by comparing the genotypes between case patients and control subjects. In addition, we stratified our analyses by age at diagnosis (> 72 and ≤ 72 yrs), and by aggressiveness of the disease (localized and advanced cases, as described above). For all analyses, genetic effects were adjusted for age (at time of diagnosis for case subjects and at time of ascertainment for controls), smoking status and alcohol use. All statistical tests were performed using SPSS program version 11.5 for Windows. A p value of < 0.05 was considered statistically significant (two-tailed).

Results

The clinical characteristics of the study partici-

pants are presented in Table 1. The mean ages (age at diagnosis for case patients and age at inclusion for control subjects) for case patients and control subjects were 71.8 and 70.2 years, respectively. PSA values of control subjects were all below 4 ng/ml, with a median value of 1.21 ng/ml.

Subjects were recruited from 2 separate areas of China. Considering the huge total area of the nation

Table 1 Clinical and demographic characteristics of the subjects

Characteristic	Advanced PCa (n = 91)	Localized PCa (n = 56)	All Case Subjects (n = 147)	Control Subjects (n = 111)	*P* value*
Age (yr)					
Mean age	72.2 ± 9.1	71.0 ± 9.8	71.8 ± 9.3	70.2 ± 9.8	0.19
Age at diagnosis [n (%)]					
≤ 72	37 (40.7)	31 (55.4)	68 (46.3)	51 (45.9)	0.96
> 72	54 (59.3)	25 (44.6)	79 (53.7)	60 (54.1)	
Smoking status					
Never	57 (62.6)	34 (60.7)	91 (61.9)	65 (58.6)	0.59
Ever	34 (37.4)	22 (39.3)	56 (38.1)	46 (41.4)	
Drinking status					
Never	67 (73.6)	42 (75.0)	109 (74.1)	81 (73.0)	0.83
Ever	24 (26.4)	14 (25.0)	38 (25.9)	30 (27.0)	
PSA level [n (%)]					
No. of subjects	90	52	142	109	
≤ 4.0 ng/ml	2 (2.2)	3 (5.8)	5 (3.5)	109 (100.0)	
4.1–9.9 ng/ml	5 (5.6)	17 (32.7)	22 (15.5)	0	
10.0–19.9 ng/ml	10 (11.1)	22 (42.3)	32 (22.5)	0	
20.0–49.9 ng/ml	21 (23.3)	10 (19.2)	31 (21.8)	0	
50.0–99.9 ng/ml	13 (14.4)	0	13 (9.2)	0	
≥ 100.0 ng/ml	39 (43.3)	0	39 (27.5)	0	
Missing data	1	4	5	2	
Tumor stage [n (%)]					
No. of subjects	90	55	145		
T1	7 (7.8)	19 (34.5)	26 (17.9)		
T2	25 (27.8)	36 (65.5)	61 (42.1)		
T3	44 (48.9)	0	44 (30.3)		
T4	14 (15.6)	0	14 (9.7)		
Missing data	1	1	2		
Nodal stage [n (%)]					
No. of subjects	79	56	135		
N0	53 (67.1)	56 (100.0)	109 (80.7)		
N1	26 (32.9)	0	26 (19.3)		
Could not be assessed	12	0	12		
Metastasis stage [n (%)] (%)					
No. of subjects	90	55	145		
M0	46 (51.1)	55 (100.0)	101 (69.7)		
M1	44 (48.9)	0	44 (30.3)		
Could not be assessed	1	1	2		
Gleason score [n (%)]					
No. of subjects	73	52	125		
2–6	8 (11.0)	19 (36.5)	27 (21.6)		
7	18 (24.7)	33 (63.5)	51 (40.8)		
8–10	47 (64.4)	0	47 (37.6)		
Missing data	18	4	22		

*Calculated based on data from control and all case subjects

of China, the genetic backgrounds of these 2 areas are of great interest. We compared the genotype frequencies of *WRN* Leu1074Phe between the 2 populations but found no significant differences (Table 2). We therefore combined data from the 2 populations in the following statistical calculations.

The allele and genotype frequencies of *WRN* Leu1074Phe among case and control subjects are listed in Table 3. The results of allelic and genotypic tests are also shown. The genotype frequency did not deviate statistically significantly from Hardy-Weinberg equilibrium ($p \geq 0.05$).

Prostate cancer subjects had a lower proportion of the G allele (32.3% vs. 38.3%) and TG/GG genotype (53.1% vs. 65.8%) than control subjects. The TG/GG genotype was supposed to be a protective genotype with decreased risk for prostate cancer in the present study. The estimated odds ratio (OR) for carriers with the TG/GG genotype was 0.58 (95%CI: 0.35–0.97, $p = 0.039$) compared with the TT genotype, after being adjusted for age, smoking status and alcohol use. However, the difference between the G and T alleles was not significant (OR = 0.76, 95% CI: 0.52–1.09, $p = 0.138$).

In the subsequent stratified analyses, we chose a diagnosis age of 72 as the dividing line so there would be equal numbers of older and younger cases. As shown in Table 4, more significant associations of the TG/GG genotype (OR = 0.27, 95%CI: 0.12–0.61, $p = 0.002$) were found in the early onset subgroup. The G allele also manifested a significant association with PCa in this subgroup (OR = 0.44, 95%CI: 0.25–0.76, $p = 0.004$). However, no statistically significant association of both risk genotype and allele was found in the subgroup with diagnosis age >72. Interestingly, through stratification by aggressiveness, the decreased risk for carriers of the G allele (OR = 0.49, 95%CI: 0.29–0.82, $p = 0.007$) and TG/GG genotype (OR = 0.36, 95%CI: 0.19–0.70, $p = 0.003$) was accentuated in patients with localized disease, but vanished in those with advanced disease (Table 5).

Table 6 shows that the genotype distribution of *WRN* Leu1074Phe was not significantly associated with a list of clinical characteristics of prostate cancer such as Gleason score, serum PSA level at diagnosis, or age at diagnosis. Interaction analysis between the polymorphism and other potential risk factors, such as smoking and drinking status, also had negative results. However, subjects with localized disease seem to have a higher distribution of risk genotype TT compared with patients with advanced disease ($p = 0.023$).

Table 2 Comparison of genotype frequencies of *WRN* Leu1074Phe between subjects from Hangzhou and Beijing

	Control			Cases		
Area	Hangzhou	Beijing	*P* value	Hangzhou	Beijing	*P* value
Genotype	n = 40	n = 71	0.86	n = 57	n = 90	0.27
TT	15 (37.5)	23 (32.4)		31 (54.4)	38 (42.2)	
GT	21 (52.5)	40 (56.3)		19 (33.3)	42 (46.7)	
GG	4 (10.0)	8 (11.3)		7 (12.3)	10 (11.1)	

Table 3 Association of *WRN* Leu1074Phe with prostate cancer susceptibility

SNP	Gene	Genotype or Allele	Controls (%) n = 111	Cases (%) n = 147	Crude OR (95%CI)	*P* value	Adjusted OR† (95%CI)	*P* value
Leu1074Phe (rs1801195)	WRN	TT	38 (34.2)	69 (46.9)	1.00 (Ref.)		1.00 (Ref.)	
		TG	61 (55.0)	61 (41.5)	0.55 (0.32–0.94)	0.028	0.55 (0.32–0.94)	0.029
		GG	12 (10.8)	17 (11.6)	0.78 (0.34–1.80)	0.56	0.74 (0.32–1.72)	0.49
		TG or GG	73 (65.8)	78 (53.1)	0.59 (0.35–0.98)	0.041	0.58 (0.35–0.97)	0.039
		T	137 (61.7)	199 (67.7)	1.00 (Ref.)		1.00 (Ref.)	
		G	85 (38.3)	95 (32.3)	0.77 (0.53–1.11)	0.16	0.76 (0.52–1.09)	0.14

† Adjusted for age, smoking status and alcohol use in a logistic regression model

Table 4 *WRN* Leu1074Phe genotype and allele frequencies [n (%)] and adjusted OR stratified by age at diagnosis

Genotype or Allele	Age at diagnosis ≤ 72				Age at diagnosis > 72			
	Controls (n = 51)	Cases (n = 68)	Adjusted OR† (95%CI)	*P* value	Controls (n = 60)	Cases (n = 79)	Adjusted OR† (95%CI)	*P* value
TT	13 (25.5)	37 (54.4)	1.00		25 (41.7)	32 (40.5)	1.00	
TG	32 (62.7)	28 (41.2)	0.29 (0.13–0.66)	0.003	29 (48.3)	33 (41.8)	0.95 (0.46–2.00)	0.90
GG	6 (11.8)	3 (4.4)	0.18 (0.039–0.88)	0.034	6 (10.0)	14 (17.7)	0.53 (0.62–5.88)	0.26
TG + GG	38 (74.5)	31 (45.6)	0.27 (0.12–0.61)	0.002	35 (58.3)	47 (59.5)	1.11 (0.56–2.22)	0.76
T	58 (56.9)	102 (75.0)	1.00		79 (65.8)	97 (61.4)	1.00	
G	44 (43.1)	34 (25.0)	0.44 (0.25–0.76)	0.004	41 (34.2)	61 (38.6)	1.25 (0.75–2.08)	0.39

† Adjusted for age, smoking status and alcohol use in a logistic regression model

Table 5 *WRN* Leu1074Phe genotype and allele frequencies [n (%)] and adjusted OR stratified by aggressiveness

Genotype or Allele	Controls (n = 111)	Localized cases (n = 56)	Adjusted OR† (95%CI)	*P* value	Controls (n = 111)	Advanced cases (n = 91)	Adjusted OR† (95%CI)	*P* value
TT	38 (34.2)	33 (58.9)	1.00		38 (34.2)	36 (39.6)	1.00	
TG	61 (55.0)	20 (35.7)	0.38 (0.19–0.76)	0.006	61 (55.0)	41 (45.1)	0.73 (0.40–1.33)	0.31
GG	12 (10.8)	3 (5.4)	0.29 (0.075–1.11)	0.071	12 (10.8)	14 (15.4)	1.19 (0.48–2.94)	0.71
TG + GG	73 (65.8)	23 (41.1)	0.36 (0.19–0.70)	0.003	73 (65.8)	55 (60.4)	0.81 (0.45–1.45)	0.47
T	137 (61.7)	86 (76.8)	1.00		137 (61.7)	113 (62.1)	1.00	
G	85 (38.3)	26 (23.2)	0.49 (0.29–0.82)	0.007	85 (38.3)	69 (37.9)	0.98 (0.65–1.47)	0.92

† Adjusted for age, smoking status and alcohol use in a logistic regression model

Table 6 Association of *WRN* Leu1074Phe with clinical characteristics

WRN Leu1074Phe	Aggressiveness of disease- no. (%)		Gleason score [n (%)]			PSA level at diagnosis	Age at diagnosis	Smoking status		Drinking status	
	Localized	advanced	2–6	7	8–10	Median (ng/ml)	Mean (yr)	Never	Ever	Never	Ever
No. of subjects	56	91	27	51	47	n = 142‡	n = 147	156	102	190	68
Reference: TT	33 (58.9)	36 (39.6)	18 (66.7)	22 (43.1)	19 (40.4)	25.0 (n = 66)	70.8 (n = 69)	60 (38.5)	47 (46.1)	79 (41.6)	28 (41.2)
Association: TG + GG	23 (41.1)	55 (60.4)	9 (33.3)	29 (56.9)	28 (59.6)	34.2 (n = 76)	72.7 (n = 78)	96 (61.5)	55 (53.9)	111 (58.4)	40 (58.8)
P value	0.023		0.070†			0.20	0.23	0.23		0.95	

† Calculated by linear association; ‡ Data of 5 cases missed

Discussion

In the present case–control study, we studied the effect of *WRN* Leu1074Phe, a missense SNP in DNA repair gene *WRN*, on the risk of prostate cancer among 147 prostate cancer patients and 111 cancer-free controls, and found it to be significantly associated with prostate cancer susceptibility in Chinese subjects. To our knowledge, this is the first study to reveal the association between DNA repair gene *WRN* and the risk of prostate cancer. Moreover, in subsequent stratified analysis, we observed statistically more significant associations in the subset of cases with localized disease and in case subjects with a diagnosis age ≤ 72 yr. These findings suggested that the genetic variant *WRN* Leu1074Phe might play a role in prostate cancer development in Chinese men.

Previous studies on the gene *WRN* have identified

Table 7 Reported *WRN* Leu1074Phe genotype frequencies [n (%)] in normal control subjects from different ethnic groups

Study group	n	Genotype (%)			Allele (%)		Ref.
		GG	GT	TT	G	T	
HapMap-CEU	120	36.7	36.7	26.7	55.0	45.0	
HapMap-HCB	90	17.8	55.6	26.7	45.6	54.4	
HapMap-JPT	90	11.1	40.0	48.9	31.1	68.9	
German	1,780	35.0	46.6	18.4	58.3	41.7	(16)
Chinese female	1,008	38.9	44.7	16.4	61.3	38.7	(11)
Taiwanese female	1,538	11.5	46.8	41.7	34.9	65.1	(15)
Chinese	139	12.9	48.9	38.1	37.4	62.6	(17)
Chinese male, present study	111	10.8	55.0	34.2	38.3	61.7	

many specific polymorphisms, which were thought to be associated with aging and with the risk of myocardial infarction, osteoporosis and breast cancer [9, 11–14]. For Leu1074Phe, one of the most common missense SNPs in *WRN*, many previous studies also revealed its association with various diseases. A study involving Finnish and Mexican populations reported an age-dependent decline of the 1074Phe/Phe genotype, suggesting a beneficial effect of the Leu allele in aging populations [9]. Two studies have addressed the relationship of *WRN* Leu1074Phe with breast cancer, but with different results. Wang Z *et al.* [11] reported a significant association of the *WRN* 1074 Phe/Phe genotype with breast cancer risk in Chinese women (OR = 1.36, 95%CI: 1.06–1.74) and a 3.58-fold increased risk of breast cancer (OR = 3.58, 95% CI: 2.54–5.05) for subjects carrying the TT genotype and with earlier menarche at the same time. Another case-control study of 935 primary breast cancer patients and 1,545 healthy controls revealed that *WRN* Leu1074Phe was not associated with the risk of breast cancer in Taiwanese females [15]. Studies of the association with other cancers are rare. A recent large population-based case-control study failed in finding a significant association with the risk of colorectal cancer in a German population [16].

In the present study, we discussed the polymorphism *WRN* Leu1074Phe and PCa susceptibility in a relatively small sample of Chinese men. Similar to previous results obtained for East Asian people from HapMap and Taiwanese females, we observed a significantly lower proportion of G alleles compared with T alleles in Chinese men. This was in contrast to studies performed on Caucasian populations, which showed a relative predominance of G alleles (Table 7, Ref. 11, 15–17). However, our genotyping results seem to be different than those of Wang Z *et al.* [11], who detected a predominance of G alleles in Chinese women. This confusing phenomenon might be explained by gender and geographic differences.

We examined the putative impact of the *WRN* Leu1074Phe genotype and found a significant association with the risk of prostate cancer in a Chinese population, suggesting that the effect of *WRN* Leu1074Phe on prostate cancer risk might be strongly evident in Chinese men. In a study by Kamath-Loeb and colleagues [10], a small decrease in helicase and exonuclease (about 1/3 in quantification) was found for variant protein 1074Phe/Phe compared to the wild-type protein 1074Leu, indicating that the latter had a higher repair capacity and thus was more beneficial for sustaining the stability of the genome. The results of the present study showed that the Leu+ (G+) genotypes had significant protective effects against the risk of prostate cancer; these results were consistent with those of Kamath-Loeb [10].

In contrast with previous studies [18, 19], we chose 72 years of age as the dividing line for an analysis stratified by different diagnosis age. The purpose for choosing age 72 was to make the number of cases as equal as possible between the older and younger subgroups. Finally, a significant association was noted in subjects with an early diagnosis age (≤ 72), but not in subjects with a later diagnosis age (> 72). This indicated that the polymorphism *WRN* Leu1074Phe might play an important role in promoting the early onset of the disease.

Moreover, when stratifying the case group by the aggressiveness of the disease, the present study found that *WRN* Leu1074Phe was significantly associated

with localized prostate cancer, but not with advanced disease. We also observed that localized subjects had a lower distribution frequency of the protective genotype TG/GG compared with advanced subjects (Table 6). This result was interesting when compared with those of other studies [18, 20], in which the correlations between SNPs and cancers were usually more significant in advanced diseases than localized ones. One possible explanation was that the genetic variant *WRN* Leu1074Phe may not play an important role in the progression and metastasis of prostate cancer; another possible explanation was that the number of cases in our subgroup analysis was relatively small, and its power to explain the differences was limited. The third possible explanation was that there was no routine screening of the PSA level for people older than 50 or 60 years in China, and in some of our case subjects the diseases were already advanced when they were initially diagnosed. This might have caused localized and advanced diseases to be mixed to a certain extent. Except for the aggressiveness, no other clinical characteristics such as Gleason score, PSA level at diagnosis or age at diagnosis were found to be associated with *WRN* Leu1074Phe. Interactions between the polymorphism and other potential risk factors, such as smoking and drinking status, were also assessed in this study. However, no positive result was found, as shown in Table 6.

Several limitations existed in our case-control study. Firstly, the sample size may limit the statistical power of our study, especially for subgroups with different diagnosis ages and levels of aggressiveness. Secondly, our prostate cancer patients were from hospitals and the controls were randomly selected from among cancer-free patients in these same hospitals, so whether they formed a representative control population needs to be assessed.

In conclusion, our study indicated that the single nucleotide polymorphism *WRN* Leu1074Phe was significantly associated with prostate cancer risk in Chinese men, suggesting that it may be a useful biomarker of the predisposition for prostate cancer. Larger well-designed studies with sufficient explanatory power are needed to confirm our findings.

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References

1. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin (2005) 55: 74–108.
2. Ye DW and Li CL: Epidemiological trends of prostate cancer: retrospect and prospect. China Oncology (2007) 17: 177–180 (in Chinese).
3. Sim HG and Cheng CW: Changing demography of prostate cancer in Asia. Eur J Cancer (2005) 41: 834–845.
4. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A and Hemminki K: Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med (2000) 343: 78–85.
5. Edwards SM and Eeles RA: Unravelling the genetics of prostate cancer. Am J Med Genet C Semin Med Genet (2004) 129C: 65–73.
6. Hsing AW and Chokkalingam AP: Prostate cancer epidemiology. Front Biosci (2006) 11: 1388–1413.
7. Schaid DJ: The complex genetic epidemiology of prostate cancer. Hum Mol Genet (2004) 13: R103–121.
8. Brosh RM Jr and Bohr VA: Human premature aging, DNA repair and RecQ helicases. Nucleic Acids Res (2007) 35: 7527–7544.
9. Castro E, Edland SD, Lee L, Ogburn CE, Deeb SS, Brown G, Panduro A, Riestra R, Tilvis R, Louhija J, Penttinen R, Erkkola R, Wang L, Martin GM and Oshima J: Polymorphisms at the Werner locus: II. 1074Leu/Phe, 1367Cys/Arg, longevity, and atherosclerosis. Am J Med Genet (2000) 95: 374–380.
10. Kamath-Loeb AS, Welcsh P, Waite M, Adman ET and Loeb LA: The enzymatic activities of the Werner syndrome protein are disabled by the amino acid polymorphism R834C. J Biol Chem (2004) 279: 55499–55505.
11. Wang Z, Xu Y, Tang J, Ma H, Qin J, Lu C, Wang X, Hu Z, Wang X and Shen H: A polymorphism in Werner syndrome gene is associated with breast cancer susceptibility in Chinese women. Breast Cancer Res Treat (2009) 118: 169–175.
12. Ogata N, Shiraki M, Hosoi T, Koshizuka Y, Nakamura K and Kawaguchi H: A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. J Bone Miner Metab (2001) 19: 296–301.
13. Ye L, Miki T, Nakura J, Oshima J, Kamino K, Rakugi H, Ikegami H, Higaki J, Edland SD, Martin GM and Ogihara T: Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population. Am J Med Genet (1997) 68: 494–498.
14. Wirtenberger M, Frank B, Hemminki K, Klaes R, Schmutzler RK, Wappenschmidt B, Meindl A, Kiechle M, Arnold N and Weber BH: Interaction of Werner and Bloom syndrome genes with p53 in familial breast cancer. Carcinogenesis (2006) 27: 1655–1660.
15. Ding SL, Yu JC, Chen ST, Hsu GC and Shen CY: Genetic variation in the premature aging gene WRN: a case–control study on breast cancer susceptibility. Cancer Epidemiol Biomarkers Prev (2007) 16: 263–269.
16. Frank B, Hoffmeister M, Klopp N, Illig T, Chang-Claude J and Brenner H: Colorectal cancer and polymorphisms in DNA repair genes WRN, RMI1 and BLM. Carcinogenesis (2010) 31: 442–445.
17. Shen M, Lan Q, Zhang L, Chanock S, Li G, Vermeulen R,

Rappaport SM, Guo W, Hayes RB, Linet M, Yin S, Yeager M, Welch R, Forrest MS, Rothman N and Smith MT: Polymorphisms in genes involved in DNA double-strand break repair pathway and susceptibility to benzene-induced hematotoxicity. Carcinogenesis (2006) 27: 2083–2089.

18. Lindmark F, Zheng SL, Wiklund F, Bensen J, Bälter KA, Chang B, Hedelin M, Clark J, Stattin P, Meyers DA, Adami HO, Isaacs W, Grönberg H and Xu J: H6D polymorphism in macrophage-inhibitory cytokine-1 gene associated with prostate cancer. J Natl Cancer Inst (2004) 96: 1248–1254.
19. Zheng SL, Sun J, Wiklund F, Smith S, Stattin P, Li G, Adami HO, Hsu FC, Zhu Y, Bälter K, Kader AK, Turner AR, Liu W, Bleecker ER, Meyers DA, Duggan D, Carpten JD, Chang BL, Isaacs WB, Xu J and Grönberg H: Cumulative association of five genetic variants with prostate cancer. N Engl J Med (2008) 358: 910–919.
20. Park K, Kim JH, Jeon HG, Byun SS and Lee E: Influence of IGFBP3 gene polymorphisms on IGFBP3 serum levels and the risk of prostate cancer in low-risk Korean men. Urology (2010) 75: 1516. e1–7.