Association between single nucleotide polymorphisms on chromosome 17q and the risk of prostate cancer in a Chinese population

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Abstract

In European populations, 7 single nucleotide polymorphisms (SNPs) on chromosome 17q, 3 SNPs on 17q12, and 4 SNPs on 17q24.3 were recently identified to be closely related to the risk of prostate cancer by a genome-wide association study. In Japanese populations, the correlation between 2 SNPs on 17q and the risk of prostate cancer and tumor aggressiveness was also confirmed by a large-scale experiment. However, whether 17q is associated with prostate cancer and its clinical manifestations in Chinese populations is still unknown. Therefore, we conducted a case-control study in a northern Chinese population and tested 2 SNPs, rs4430796 and rs1859962, on 17q in 124 prostate cancer patients and 111 controls using polymerase chain reaction-high resolution melting curve (PCR-HRM) combined with sequencing. We analyzed the association of the 2 SNPs with the risk of prostate cancer as well as patients’ lifestyles, onset ages, Gleason scores, PSA levels, and pathologic stages. We found a significant difference in the G allele of SNP rs1859962 (P = 0.035, OR = 1.51, 95% CI = 1.03–2.21) but not in the rs4430796 genotype frequency or allele frequency distribution between prostate cancer patients and the controls (P > 0.05). Neither of the SNPs was significantly associated with the onset age, Gleason score, PSA level, pathologic stage, or other clinical indicators of patients with prostate cancer (P > 0.05). Our results show that polymorphism of the G allele of SNP rs1859962 is associated with the risk of prostate cancer in a Chinese population.

Key words Prostate cancer, 17q, single nucleotide polymorphisms (SNPs), association, risk gene

Prostate cancer is the most common cancer of the male reproductive system in American men, and its incidence varies among different ethnic groups [1]. More specifically, the incidence of this disease has been reported to be relatively high in European and American populations, second only to lung cancer, which is the second leading cause of cancer-related deaths in men [2]. In contrast, prostate cancer incidence is relatively low in China, occurring at a rate of only 2.41 cases per 100 000 males. However, in recent years, this rate has increased rapidly (3.7 cases/100 000 males in Shanghai and 4.0 cases/100 000 males in Beijing in 1995) with the aging population and the improvement of living conditions [3]. Prostate cancer is ranked third highest among malignancies of the male urinary and reproductive system in China. Thus, as this disease gradually affects the quality of life and life expectancy of men over 50 years of age in Chinese populations [4], it is important to investigate its risk factors.

More than 20 years ago, researchers proposed that genetic factors play a key role in the pathogenesis of prostate cancer [5]. Subsequently, many studies have shown that familial genetic factors, such as close consanguinity with patients, the number of patients in family members, and age of onset (less than 50 years),

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are the main risk factors of prostate cancer[5-9]. Research on susceptibility genes is one of the hot issues in risk factors of prostate cancer. Nevertheless, the confirmation of prostate cancer susceptibility genes has been challenging. In deed, several significant gene regions identified by association analysis could not be confirmed in subsequent studies[10,11]. Similarly, the methods of identifying candidate genes have been shown not to work effectively[12]. However, advances in genomic technologies and detection methods have improved the ability to study the underlying genetics of prostate cancer risk. A large number of gene mutations on many chromosomes were shown to be associated with prostate cancer risk in European and American populations in recent genome-wide association studies (GWAS)[13-15]. Based on 3 known risk loci of prostate cancer, SNPs rs4242382 and rs6983267 on 8q24 and SNP rs4430796 on 17q in the hepatocyte nuclear factor 1B (HNF1B) gene, 4 SNPs associated with prostate cancer, rs10993994 on chromosome 10 in the microseminoprotein-beta (MSMB) gene, rs4962416 on chromosome 10 in the C-terminal–binding protein 2 (CTBP2) gene, rs10896449 on 11q13, and rs10486567 on chromosome 7 in the juxtaposed with another zinc finger protein 1 (JAZF1) gene, were also identified in a GWAS in the United States in 2007[14]. In subsequent studies, 31 SNPs were found to be associated with prostate cancer in European and American populations[13-18]. The same GWAS was conducted in Japan and revealed that 15 of these 31 risk SNPs also exist in Japanese populations[19]. Among the risk loci identified in European, American, and Japanese populations were SNPs on chromosome 17q. Both SNP rs4430796 on 17q12 and SNP rs1859962 on 17q24 were associated with prostate cancer in European and American populations, whereas only SNP rs4430796 was associated with prostate cancer in Japanese populations[19]. The genetics of Chinese and Japanese populations are not generally believed to be significantly different. Consistent with this, the frequency of risk allele A of SNP rs4430796 in Chinese populations is very similar to its frequency in Japanese populations (0.722 and 0.667, respectively). However, the frequency of risk allele G of SNP rs1859962 in Chinese populations is significantly different from that in Japanese populations (0.489 and 0.200, respectively). This significant difference in risk allele frequency between Japanese and Chinese populations may indicate that the association between SNP rs1859962 and the risk of prostate cancer is different between these two populations. To test this hypothesis and investigate the association of SNPs rs4430796 and rs1859962 with the risk of prostate cancer and clinical indicators of patients, we conducted the following case-control study.

Materials and Methods

Subjects and materials

Study subjects A total of 124 patients with pathologically confirmed prostate cancer were selected from outpatients and inpatients at the Department of Urology, Beijing Hospital of the Ministry of Health. Patients’ ages ranged from 51 to 86 years, with a median age of 73.5 years. Their mean serum level of prostate-specific antigen (PSA) was (25.1 ± 3.7) ng/mL. A total of 111 people undergoing routine physical examination in the Medical Center, Beijing Hospital of the Ministry of Health were selected as controls. All controls were men who had no family history of prostate cancer, whose ages ranged from 60 to 88 years (with a median age of 68 years), and whose mean serum PSA value was (1.4 ± 1.3) ng/mL. Written informed consent and peripheral blood for testing were obtained from each subject.

Reagents Whole genomic DNA extraction kits were purchased from Bio Chain Company. Taq DNA polymerase and dNTP were purchased from Beijing Dingguo Biological Technology Co., Ltd. LCGreen Plus saturated fluorescent dyes were obtained from Idaho Company, USA. Primers were synthesized by Shanghai Sangon Biotech Development Co., Ltd. Gene sequencing was completed at the Department of Sequencing, Beijing Genomics Institute.

Main instruments The main instruments used in this study include the following: polymerase chain reaction (PCR) PTC-225 Thermo Cycler system manufactured by the MJ Company, USA; high resolution melting (HRM) gene mutation/genotyping system LightScanner TMHR-I 96 manufactured by the Idaho Company, USA; protein/nucleic acid colorimeter BioPhotometer manufactured by Eppendorf Company, Germany; gel imaging system Gel DOC-2000 manufactured by Bio-Rad Company, USA; and centrifuge Allegra TM 21R manufactured by Beckman Company, USA.

Experimental methods

Genomic DNA extraction and quantification DNA was extracted from peripheral blood samples (0.5 mL) anti-coagulated by EDTA using the whole genome DNA extraction kit. The concentration and purity of the extracted DNA was assayed using a protein/nucleic acid colorimeter. According to the measured results, DNA samples were diluted to working solutions of 20 ng/μL and then stored in a refrigerator at 4°C.

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**PCR reaction** The vicinal gene sequences of SNPs rs4430796 and rs1859962 were obtained by searching the GenBank. The primers were designed by Oligo 6.0 and Primer 5.0 software. The terminal bases of the internal reference oligonucleotides were closed by carboxyl terminal 3 (C3) to prevent the extension reaction. PCR reactions were performed in a total volume of 10 μL containing 1 μL of DNA template, 1 μL of 10x PCR Buffer, 0.2 μL of 10 mmol/L dNTP, 0.2 μL of Taq DNA polymerase (5 U/μL), 0.1 μL of upstream and downstream primers (10 pmol/μL) respectively, 1 μL of 1x LC Green Plus saturated fluorescent dye, 0.2 μL of internal reference oligonucleotide (including 0.05 μL of each of the four fragments of high and low temperature internal reference oligonucleotides), and deionized water. PCR reaction was conducted as follows: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at various temperatures (Table 1) for 30 s, followed by extension at 72°C for 6 s. After 35 PCR cycles, the product was maintained at 72°C for 7 min for final extension. Denaturation and renaturation were conducted as follows before HRM analysis: 95°C for 30 s, 25°C for 2 min, 94°C for 30 s, and 24°C for 4 min.

**HRM analysis** The PCR product was transferred into HRM-specific 96-well plates, and then HRM analysis was conducted using the LightScanner TMHR-I 96. A melting curve plotted from readings collected between 45°C and 98°C had a slope of 0.3°C/s[10]. LightScanner Call IT software was used for analyzing the collected melting data and determining genotype.

**Sequencing test** Genotypes were determined according to the HRM curves. Five samples randomly selected from individuals of different genotypes were sequenced for verification. The samples for sequencing were re-amplified by PCR with the following primers: rs4430796, upstream sequence 5’-TTGCAATGAGGCAGAT-3’ and downstream sequence 5’-GCCCCATTTAGAGATTAAG-3’, with an annealing temperature of 56°C; rs1859962, upstream sequence 5’-CGCGAG GCAAAATACAAATTTAAG-3’ and downstream sequence 5’-GCCCGATTATAATTAGGCAAATTTAAG-3’, with an annealing temperature of 53.5°C. PCR was performed in a total volume of 50 μL containing 5 μL of DNA template, 5 μL of 10x PCR Buffer, 1 μL 10 mmol/L dNTP, 1 μL of Taq DNA polymerase (5 U/μL), and 0.5 μL of 10 pmol/μL upstream and downstream primers, respectively, and deionized water. PCR conditions were as follows: pre-denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C (rs4430796) or 53.5°C (rs1859962) for 45 s, and extension at 72°C for 60 s, and a final extension at 72°C for 7 min. PCR products were subjected to electrophoresis on 8% polyacrylamide gels, after which PCR products were visualized using the gel imaging system and then sequenced by the Department of Sequencing, Beijing Genomics Institute.

**Statistical analyses**

A representative group of samples was tested by the Hardy-Weinberg equilibrium (HWE) test. The relative risks were evaluated by odds ratio (OR) and 95% confidence interval (95% CI). The measurement data are presented as mean ± standard deviation (SD). Genotype and allele frequencies between groups were compared using the Fisher’s or Pearson’s chi-square (χ²) test, and values of $P < 0.05$ were considered significantly different.

| SNP      | Primer sequence (5’–3’)                      | Annealing temperature (°C) | Product length (bp) |
|----------|-----------------------------------------------|----------------------------|---------------------|
| rs4430796| Upstream: AGAGAGGAGACAAGACT                  | 63.7                       | 52                  |
|          | Downstream: GCCCTGCCCAATTTAAG                 |                            |                     |
| rs1859962| Upstream: AGACTTTTCCAAATCCCTG                 | 53.5                       | 67                  |
|          | Downstream: GCCCGATTATAATTAGAATCTTG          |                            |                     |
| LTC      | Upstream: TTAATAATATAAATTATATATATTAATTTAATT  | 60                         | 50                  |
|          | ATATATATATATATAATATAATATAATATAATTAATTAATTTAT|                            |                     |
|          | Downstream: TATATAATATATATATATATATATATATAATT|                            |                     |
|          | ATATAATATATAATATAATTAATTAATTAATTAATTAATTAAT |
| HTC      | Upstream: GGGCGCGGGCGACCTGACCCCGAGCTGAGCT   | 92                         | 63                  |
|          | GGGAGCTAGGAGGAGGAACCGAGGGAGGGCGGGGC-G3       |                            |                     |
|          | Downstream: CCCGGGCGCGCCCCGCCCCGCCCAGCGCT   |                            |                     |
|          | CAGAGTCTCGGGTAATGGCGGCGGC-G3                 |                            |                     |

SNP, single nucleotide polymorphism; LTC, low temperature calibrator; HTC, high temperature calibrator.

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Results

Association of candidate genes with the risk of prostate cancer in a Chinese population

Results of HRM analysis  Part of the negative derivative curves and melting curves of SNPs rs4430796 and rs1859962 obtained from HRM analysis are shown in Figure 1. Among the 105 samples of SNP rs4430796 that were successfully genotyped, genotypes GG, AG, and AA were identified in 12, 34, and 59 cases, respectively, whereas those genotypes were respectively identified in 6, 34, and 38 of the 78 successfully genotyped control samples. Among the 119 samples of SNP rs1859962 that were successfully genotyped, genotypes TT, GT, and GG were identified in 36, 60, and 23 cases, respectively, whereas those genotypes were identified respectively in 44, 49, and 12 of the 105 control samples that were successfully genotyped (Table 2).

Sequencing results  Five DNA samples collected from individuals with different genotype of SNPs rs4430796 and rs1859962 were tested by sequencing. The results showed that HRM analysis was consistent with the sequencing data, with an accuracy of 100% (Figure 1).

Correlation statistics  The genotype distribution of the two SNPs in this study was consistent with HWE ($\chi^2$ < 1). The frequency of the G allele of SNP rs1859962 was 106 (47.3%) and 73 (34.8%) in the patient group and control group, respectively, with a significant difference between the two groups ($P = 0.035$, OR = 1.51, 95% CI = 1.03–2.21), suggesting that the mutation of allele T to G in SNP rs1859962 may be associated with increased risk of prostate cancer in Chinese populations. Notably, the risk of prostate cancer in allele G carriers was 1.51 times that of non-G carriers. In contrast, no significant difference in the genotype and allele frequency of rs4430796 was observed between the patient and control groups, indicating that this SNP may not be associated with the risk of prostate cancer in Chinese populations.

Association of prostate cancer quality traits with the genotypes in a Chinese population

The quality traits (phenotype) of prostate cancer patients, including the patient’s diet, education, family history of cancer, addiction to tea, tobacco and alcohol, and ten other categorical indicators, were investigated. Our results showed that these indicators were not significantly associated with the genotypes of these two loci (Table 3).

Association of the clinical characteristics of prostate cancer with the genotypes in a Chinese population

We also analyzed the association of the three

| Table 2, Association of alleles and genotypes of SNPs rs4430796 and rs1859962 with the risk of prostate cancer |
|--------------------------------------------------|----------|---------|-----|---|--------|
| SNP                  | Number of cases (%) | Number of controls (%) | $P$ | OR | 95% CI |
|----------------------|---------------------|-----------------------|-----|----|--------|
| rs4430796            |                     |                       |     |    |        |
| Allelic frequency    | 0.73                | 1.10                  | 0.69–1.73 |
| A                    | 152 (72.4)          | 110 (70.5)            | 0.32 | 0.74 | 0.41–1.33 |
| G                    | 58 (27.6)           | 46 (29.5)             | 0.32 | 0.74 | 0.41–1.33 |
| Genotypic frequency  |                     |                       |     |    |        |
| G/G                  | 12 (11.4)           | 6 (7.6)               | 0.32 | 0.74 | 0.41–1.33 |
| A/G                  | 34 (32.3)           | 34 (43.6)             | 0.32 | 0.74 | 0.41–1.33 |
| A/A                  | 59 (56.3)           | 38 (48.8)             | 0.32 | 0.74 | 0.41–1.33 |
| G/G + A/G            | 46 (43.7)           | 40 (51.2)             | 0.32 | 0.74 | 0.41–1.33 |
| rs1859962            |                     |                       |     |    |        |
| Allelic frequency    | 0.035               | 1.51                  | 1.03–2.21 |
| G                    | 106 (47.3)          | 73 (34.8)             | 0.069 | 1.66 | 0.96–2.88 |
| T                    | 132 (52.7)          | 137 (65.2)            | 0.069 | 1.66 | 0.96–2.88 |
| Genotypic frequency  |                     |                       |     |    |        |
| G/G                  | 23 (19.3)           | 12 (11.4)             | 0.069 | 1.66 | 0.96–2.88 |
| G/T                  | 60 (50.4)           | 49 (46.7)             | 0.069 | 1.66 | 0.96–2.88 |
| T/T                  | 36 (30.3)           | 44 (41.9)             | 0.069 | 1.66 | 0.96–2.88 |
| G/G + G/T            | 83 (69.7)           | 61 (58.1)             | 0.069 | 1.66 | 0.96–2.88 |

OR, odds ratio; CI, confidence interval.
Figure 1. Sequencing and genotyping of single nucleotide polymorphisms (SNPs) rs4430796 and rs1859962. Panels A and C represent homozygotes, B represents the heterozygote, and D displays the normalized melting curves and genotyping results using LightScanner Call IT software. Blue curves in panel D represent GG genotype, gray curves represent AA or TT genotypes, and red curves represent heterozygous types (AG/TG).

Table 3. Association of the genotypes of SNPs rs4430796 and rs1859962 with histories of patients with prostate cancer

| Item          | rs4430796 |          | rs1859962 |          |
|---------------|-----------|----------|-----------|----------|
|               | AA        | AG + GG  | P         | TG + GG  | TT       | P         |
| History†      |           |          |           |          |          |           |
| Yes           | 11        | 5        | 0.12      | 13       | 5        | 0.67      |
| No            | 27        | 31       |           | 49       | 24       |           |
| Education     |           |          | 0.96      |          | 0.47     |           |
| Low           | 15        | 12       |           | 22       | 7        |           |
| High          | 39        | 30       |           | 53       | 24       |           |
| Smoking       |           |          | 0.45      |          | 0.35     |           |
| Often         | 23        | 20       |           | 37       | 12       |           |
| Seldom        | 30        | 19       |           | 35       | 18       |           |
| Milk          |           |          | 0.33      |          | 0.23     |           |
| Often         | 38        | 23       |           | 44       | 22       |           |
| Seldom        | 16        | 15       |           | 28       | 8        |           |
| Onion         |           |          | 0.33      |          | 0.05     |           |
| Often         | 23        | 12       |           | 21       | 15       |           |
| Seldom        | 31        | 25       |           | 50       | 15       |           |
| Meat          |           |          | 0.22      |          | 0.24     |           |
| Red meat      | 20        | 19       |           | 33       | 10       |           |
| Others        | 34        | 19       |           | 39       | 20       |           |
| Tomato        |           |          | 0.6       |          | 0.27     |           |
| Often         | 41        | 27       |           | 50       | 24       |           |
| Seldom        | 13        | 11       |           | 22       | 6        |           |
| Bean good     |           |          | 0.58      |          | 0.58     |           |
| Often         | 31        | 24       |           | 45       | 17       |           |
| Seldom        | 23        | 14       |           | 27       | 13       |           |
| Tea           |           |          | 0.18      |          | 0.14     |           |
| Often         | 27        | 14       |           | 29       | 17       |           |
| Seldom        | 26        | 24       |           | 42       | 13       |           |
| Drinking      |           |          | 0.89      |          | 0.09     |           |
| Often         | 5         | 4        |           | 6        | 6        |           |
| Seldom        | 47        | 34       |           | 64       | 23       |           |

*History refers to family history of cancer. †The values are presented as number of cases.
genotypes of both loci with prostate cancer Gleason score, PSA level, tumor stage, and onset age and found no significant correlation (Table 4).

**Discussion**

In this study, we investigated the association between SNPs rs4430796 on 17q12 and rs1859962 on 17q24 and the risk and clinical manifestations of prostate cancer in a Chinese population. By reviewing HapMap data, we found that SNP rs4430796 was located in a non-coding region of the HNF1B gene, which had no significant effect on the expression of the HNF1B protein. However, SNP rs1859962 was located in a gene scarce region of 17q24 and no biological functions have been found so far.

Gudmundsson et al. [21] first reported the association of SNPs rs4430796 and rs1859962 with sporadic prostate cancer in 2007. Subsequently, in a study including 1015 men (542 cases and 473 controls) from 403 non-Hispanic Caucasian families, Levin et al. [22] found that the allele A of rs4430796 was correlated with increased risk of prostate cancer ($P = 0.006$, OR = 1.40, 95% CI = 1.09–1.81). In particular, rs4430796 was closely correlated with prostate cancer in patients younger than 50 years ($P = 0.006$) but not in patients older than 50 years ($P = 0.118$). Furthermore, the prostate cancer risk of young SNP carriers was double that of young non–SNP carriers. In contrast, no significant difference in rs1859962 allele frequency was observed between the case and control groups ($P = 0.13$).

Waters et al. [23] detected 13 risk gene mutations in 2768 prostate cancer patients and 2359 controls including African-Americans, European-Americans, Latinos, Japanese-Americans, and Indigenous Hawaiians. No significant positive correlation between SNP rs1859962 and prostate cancer was observed in any population. However, the rs4430796 A allele frequency was significantly different among these races and exhibited remarkable positive correlation with prostate cancer among all populations except African-Americans and Japanese-Americans (the two largest groups), suggesting that this variation is not closely related to the risk of prostate cancer in African-Americans and Japanese-Americans. Helfand et al. [24] detected the genotypes of SNPs rs4430796 and rs1859962 in 759 Caucasian patients with prostate cancer and 790 corresponding healthy volunteers in the United States and analyzed these data using logistic regression analysis. They found that the frequency of the two SNPs was significantly higher in the case group than in the control group (OR = 1.32 and 1.15, respectively) and that the carriers of the risk gene SNP rs4430796 exhibited a higher Gleason score and pathologic stage [24].

In a replicated study in a Japanese population (311 cases and 1035 controls), SNP rs4430796 but not rs1859962 was found to be associated with the risk of prostate cancer ($P < 0.001$) [25]. Moreover, rs4430796 was associated with the increased probability of prostate cancer incidence in patients under the age of 66 years ($P = 0.034$). However, no significant association of the two loci with Gleason score and tumor progression was observed [25]. We compared the results of the previous studies and found the association between rs4430796 and prostate cancer was much stronger in the Japanese population than in the United States population ($P = 0.006$).

### Table 4. Association of the genotypes of rs4430796 and rs1859962 with the clinical indicators of patients with prostate cancer

| Clinical phenotype | rs4430796 | P | rs1859962 | P |
|--------------------|-----------|---|-----------|---|
| **Gleason score**  |           |   |           |   |
| ≥7                 | 4         | 23| 11        | 24|
| <7                 | 6         | 26| 11        | 24|
| **PSA level (ng/mL)** |          |   |           |   |
| ≥10.0              | 7         | 32| 12        | 34|
| 4.0–9.9            | 4         | 20| 9         | 18|
| <4.0               | 0         | 4 | 2         | 3 |
| **Cancer stage**   |           |   |           |   |
| >T2                | 6         | 33| 13        | 26|
| ≤T2                | 9         | 27| 10        | 33|
| **Onset age (years)** |          |   |           |   |
| ≥75                | 8         | 39| 19        | 40|
| <75                | 4         | 39| 11        | 29|

*The values are presented as number of cases.*
0.049). One possible explanation is that the risk of some alleles varies in different populations although these alleles have a risk in all populations. In another study in a Japanese population (518 sporadic cases, 109 potential prostate cancer patients, and 323 controls), Liu et al. found that, after age adjustment, the frequency of allele A of SNP rs4430796 was significantly higher in prostate cancer patients than in controls (OR = 1.55 and 1.35, respectively). The risk of prostate cancer increased with the number of risk alleles present. They also found that SNP rs4430796 was related to progressive prostate cancer, whereas SNP rs1859962 was associated with non-progressive prostate cancer.

In the present study, our results showed that the genotype and the frequency of allele of SNP rs4430796 was not significantly associated with the risk of prostate cancer in a Chinese population. This result was not consistent with results in European and American populations as discussed above. There are several possible reasons for this difference. First, the methods used in this study were not sensitive enough to detect small differences. Second, the degree to which this SNP influence prostate cancer may vary according to population-specific interactions between genes or genes and the environment. Third, some genetic variations appeared as the risk genes only in specific populations, but did not increase the risk in other populations. In this study, we also found that the allele G of SNP rs1859962 was associated with prostate cancer risk in a Chinese population, which was consistent with the GWAS result in a European population but inconsistent with that in a Japanese population. The HapMap database showed that the frequency of risk allele G of SNP rs1859962 was 0.489, 0.508, and 0.20 in Chinese, European, and Japanese populations, respectively, which was similar to the result in our study (0.47), the European GWAS (0.51), and the Japanese GWAS (0.27). The various risk allele frequencies among different populations may explain the differences in the risk of prostate cancer caused by allele G of SNP rs1859962. We conducted HapMap mapping in a 35 kb span including rs1859962 and found that the block covered region in which rs1859962 located was not identical among these three populations (Figure 2). Further studies are needed to determine whether this leads to a difference in linkage disequilibrium between rs1859962 and the real prostate cancer risk gene, thereby influencing the risk of prostate cancer.

Although both located on 17q, SNPs rs4430796 and rs1859962 are not in close proximity (rs4430796 is located in 17q12, whereas rs1859962 in 17q24) and are two independent loci. We analyzed the haplotypes of rs4430796 and rs1859962 but found no significant difference in their distribution between the groups (P > 0.05) (Table 5). We analyzed the cumulative effect of risk allele and found the frequencies of allele A of rs4430796 and allele G of rs1859962 were higher in the case group than in the control group. Furthermore, the risk of prostate cancer increased by 62% in patients carrying allele A of SNP rs4430796 or allele G of SNP rs1859962 (P = 0.039, OR = 1.62, 95% CI = 1.02–2.57), and it increased 1.56 times in carriers with both alleles compared to that in non-carriers (P = 0.002, OR = 2.56, 95% CI = 1.41–4.63) (Table 6).

Our results showed that in a Chinese population, there was no association between the SNP genotypes and the clinical indicators of prostate cancer, including Gleason score, PSA level, tumor stage, and onset age, whereas most studies indicated that rs4430796 was correlated with patients’ age at diagnosis. However, the various results among studies may be due to different study populations and races. For example, Levin et al. analyzed the data from prostate cancer patients selected based on family as the study population and found a strong association of SNP rs4430796 with patients’ ages of onset. The relationship between rs4430796 and rs1859962 and pathologic stage is controversial probably because of differing synergy between these and other SNPs in the incidence of prostate cancer among distinct populations.

In a genome linkage study on 17q21–22, Lange et al. identified two loci located within the BRCA1 gene, SNPs rs1799950 and rs3773559, that associated with early-onset prostate cancer and familial prostate cancer, respectively. The two loci were far from SNPs rs4430769 and rs1859962 (1–5 Mb) and had no linkage, suggesting that one or more independent prostate cancer susceptibility loci exist in the region. Mitra et al. found that prostate cancer patients with both BRCA1 and BRCA2 mutations had a higher Gleason score than did controls (patients without BRCA1 or BRCA2 mutation) (P = 0.016). Further studies are needed to determine whether the different synergistic effects of SNPs rs4430769 and rs1859962 plus BRCA-related SNPs affect their relationship to tumor stage.

In the investigation of cancer risk, the role of the gene-environment interaction cannot be ignored. Rodrigues et al. found that certain genes can increase the risk of prostate cancer in people with a long-term history of consuming red meat, such as beef and mutton that are red before cooking. In this study, we analyzed the association of SNPs rs4430769 and rs1859962 with patients’ diet, degree of education, family history of cancer, addiction to tea, tobacco, and alcohol, as well as other factors and did not find significant association.

Nevertheless, our experiment is limited due to a relatively small sample size. Some SNPs lack statistical significance probably because of different modes of
Figure 2. Sketch map of linkage disequilibrium of rs1859962 among three populations. A, a Chinese population; B, an European population; C, a Japanese population.

Table 5. Haplotype analysis of SNPs rs4430796 and rs1859962

| Haplotype | Theoretical number of cases (%) | Theoretical number of controls (%) | \( P \) | OR (95% CI) |
|-----------|---------------------------------|-----------------------------------|--------|-------------|
| A G       | 62.41 (30.3)                    | 35.82 (23.6)                      | 0.159  | 1.409 (0.874–2.273) |
| A T       | 86.59 (42.0)                    | 72.18 (47.5)                      | 0.305  | 0.802 (0.526–1.223)  |
| G G       | 29.59 (14.4)                    | 15.18 (10.0)                      | 0.215  | 1.512 (0.783–2.920)  |
| G T       | 27.41 (13.3)                    | 28.82 (19.0)                      | 0.146  | 0.656 (0.370–1.161)  |
linkage disequilibrium. That is, although the pathogenic SNP in different populations may have the same impact, the relationship between the SNPs we detected and the true pathogenic SNP varies in different populations. The correlation coefficient detected may increase or decrease in patients with different degrees of linkage disequilibrium. This may explain why, in our experiments, no significant correlation between SNP rs4430796 and clinical indicators in patients with prostate cancer was observed, whereas allele G of rs1859962 was found to be significantly associated with the risk of prostate cancer in a Chinese population.

### Conclusion

In summary, our results indicated that the polymorphism of allele G in SNP rs1859962 locus may be associated with the risk of prostate cancer in Chinese populations.

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### Table 6. Cumulative effect of two independent risk variants of prostate cancer

| Number of risk alleles | Prostate cancer cases (%) | Controls (%) | P       | OR (95% CI) |
|------------------------|---------------------------|--------------|---------|-------------|
| rs4430796(A) and rs1859962(G) |                           |              |         |             |
| 0                      | 43 (17.8)                 | 60 (28.0)    |         |             |
| 1                      | 144 (59.5)                | 124 (58.0)   | 0.039#  | 1.62 (1.02–2.57) |
| 2                      | 55 (22.7)                 | 30 (14.0)    | 0.002#  | 2.56 (1.41–4.63) | 0, no risk allele; 1, one risk allele; 2, two risk alleles. *Compared with subgroup 0.

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