Association of long-chain non-coding RNA GAS5 gene polymorphisms with prostate cancer risk and prognosis in Chinese Han population

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Abstract

Background: To investigate the correlation between growth arrest-specific transcript 5 (GASS) gene polymorphism and the risk and prognosis of prostate cancer in Chinese Han population.

Methods: Sanger sequencing was used to analyze genotypes at the rs17359906 and rs1951625 loci of the GASS gene in 218 prostate cancer patients and 220 healthy controls. The follow-up period was from August 2016 to August 2019, and the relationships between GAS5 gene polymorphisms at the rs17359906 and rs1951625 loci and the recurrence-free survival rate of prostate cancer patients were analyzed.

Results: GAS5 A-allele carriers at the rs17359906 locus were 3.44 times more likely to develop prostate cancer than G-allele carriers (95% confidence interval [CI]: 2.38–4.96, P < .001). Carriers of the GAS5 A allele at the rs1951625 locus had a 1.40-fold higher risk of prostate cancer than carriers of the G allele (95% CI: 1.05–1.86, P = .027). Plasma prostate-specific antigen (PSA), body mass index (BMI), and rs17359906 and rs1951625 loci were independent risk factors for prostate cancer. GAS5 AA genotype and A-allele carriers (GA + AA) at the rs1951625 locus were significantly correlated with Gleason scores > 7 (P < .05). GAS5 genes rs17359906 G > A and rs1951625 G > A were associated with high plasma PSA levels. The recurrence-free survival rate of patients with prostate cancer with AA genotype at the rs17359906 locus of GAS5 (66.67%) was significantly lower than that of the GA genotype (76.47%), whereas the GG genotype was the highest (91.96%), and the difference was statistically significant (P = .002). The recurrence-free survival rate of patients with prostate cancer with the AA genotype at the rs1951625 locus of GAS5 (75.00%) was significantly lower than that of the GA genotype (81.82%), whereas the GG genotype was the highest (87.76%) with a statistically significant difference (P = .025).

Conclusion: GAS5 rs17359906 G > A and rs1951625 G > A are significantly associated with an increased risk of prostate cancer and a reduction in three-year relapse-free survival.

Abbreviations: BMI = body mass index, CI = confidence interval, GASS = growth arrest-specific transcript 5, GWAF = genome-wide association analyses with family, GWAS = Whole Genome Association Analysis, IncRNA = long non-coding RNA, MAF = minor-allele frequency, OR = odds ratio, SNPs = single nucleotide polymorphisms, TNBC = triple negative breast cancer.

Keywords: gene polymorphisms, growth arrest-specific transcript 5, prostate cancer, relapse-free survival

1. Introduction

Prostate cancer is a common solid malignant tumor in men. In recent years, the incidence of prostate cancer worldwide has increased.1–3 With the increasing aging trend of the Chinese population and changes in diet, the incidence of prostate cancer in China has also increased.4,5 Radical prostatectomy is one of the most effective treatments for early and middle stage prostate cancer, however about 20% of patients will experience biochemical recurrence within 5 years after surgery.6 Most patients experiencing postoperative biochemical recurrence have reduced life expectancies. A large proportion of patients experience local tumor recurrence or distant metastasis after surgery.7 Biochemical recurrence occurs in 20% to 50% of patients after radical radiotherapy.8 Therefore, further research on the pathogenesis of prostate cancer is of great significance in the prevention and treatment of the disease.

The incidence of prostate cancer is strongly associated with family history,9,10 suggesting that there may be genetic susceptibility to the pathogenesis of prostate cancer. With the development of the Human Genome Project and Whole Genome Association Analysis (GWAS),11 there is increasing evidence that the susceptibility of prostate cancer is related to single nucleotide polymorphisms (SNPs). Through genome-wide asso-
et al\[17,18\] found that the expression level of lymph node metastasis in patients with prostate cancer. Wang rs145204276 polymorphism is associated with a risk of has been widely studied. For example, Lin et al\[19\] found that in patients with prostate cancer in the Chinese population, which also limited the population to members of the Chinese Han population to reduce the possibility of population stratification. Demographic data, collected for all patients, included: age, body mass index (BMI), smoking status, drinking status, and plasma PSA levels. We also collected clinical pathological data such as Clinical T stage and Gleason score in patients with prostate cancer. This study was performed with the approval of the Medical Ethics Committee of Zhujin People’s Hospital of Zhejiang Province, and all subjects signed written informed consent forms prior to the commencement of the study.

2.2. GAS5 gene polymorphism analysis

In this study, we analyzed the polymorphisms at the rs17359906 and rs1951625 loci of GAS5. We used the QiAamp DNA Blood Mini Kit (Qiagen, Vista, CA, USA) to extract genomic DNA from peripheral venous blood of the patients, and used the extracted genomic DNA as a template to perform a PCR reaction to synthesize DNA fragments containing SNP sites. The primer information was: rs17359906: 5’-ATC TGC ACC CAG CAC CAT AC-3’ (Fw); 5’-TGG TAT GTT ACC TGC ATC ATC ATT GG-3’ (Rv); rs1951625: 5’-ACT GCA CCC GCA GTT AAG AA-3’ (Fw); 5’-CTT AAG TGG TGT CAT TCC GC-3’ (Rv). The PCR reaction system contained about 50 ng of genomic DNA, 10× buffer 2.0 μL, 2 mM dNTP 0.4 μL, 25 mM MgCl2 + 0.8 μL, 10 mM forward and reverse primers 0.4 μL each, 1 U/μL Taq enzyme 0.15 μL, ddH2O 14.85 μL, and a total volume of 20 μL. Conditions for PCR amplification: 95°C for 5 minutes, then 95°C for 30 seconds; 58°C for 30 seconds; 72°C for 30 seconds, for a total of 35 cycles, and then 72°C for 5 minutes. The PCR products were sequenced by Sanger, and the sequencing work was completed by Suzhou Jinweihi Biotechnology Co., Ltd. Five percent of the samples were randomly selected for repeated verification, and the results of the 2 verifications were found to be consistent.

2.3. Follow-up

The follow-up of 218 patients with prostate cancer in this study was performed by outpatient and telephone follow-up. The deadline for follow-up was August 2019, and no patients were lost to follow-up. The follow-up time ranged from 6 to 36 months.

2.4. Statistical analysis

Statistical analysis of the data in this study was performed using SPSS 20.0 software (SPSS Inc., Chicago). The goodness-of-fit χ2-test was used to assess whether the genotype of the SNP loci conformed to Hardy-Weinberg equilibrium. Continuous variables were expressed as (mean±SD), and statistical analysis was performed by t-test. The categorical variables were expressed as [n (%)], and the statistical analysis was performed using the χ2-test. Logistic regression was used to analyze the correlation between GAS5 rs17359906 and rs1951625 loci and the risk of prostate cancer, calculated odds ratio (OR) and 95% confidence interval (CI), and adjusted age, BMI, smoking status, and drinking status.

2. Materials and methods

2.1. Patients

In this study, we enrolled 218 male prostate cancer patients (case groups), who were treated in our hospital from January 2015 to August 2016, aged 45 to 89 years, with an average age of (70.54 ± 11.19) years. Prostate cancer was confirmed in all patients by pathological examination. We recruited 220 healthy men, aged 45 to 87 years, with an average age of (70.64 ± 11.19) years, to form the control group. Patients were included in the control group provided that they had not been diagnosed with cancer before entering the study and had PSA test scores in the normal range. Patients with a history of tumors such as bladder cancer, lung cancer, liver cancer, prostate transitional cell carcinoma, squamous cell carcinoma, fibroadenomas, basal cell carcinoma, undifferentiated tumors, or sarcomatoid carcinoma, and patients with vascular diseases, severe endocrine diseases, liver diseases, or severe nutritional metabolic diseases were excluded.
Kaplan-Meier Curve in Graphpad prism 7 (GraphPad Software, Inc., San Diego, CA, USA) was used to analyze the three-year relapse-free survival of patients with different genotypes of GAS5 at the rs17359906 and rs1951625 loci.

3. Results

3.1. Demographic and clinical data
Table 1 compares the demographic and clinical data of prostate cancer patients and the control group in this study. There were no differences in the age, smoking status, drinking status, and clinical T stages of prostate cancer patients were as follows: 48 were T1, 46 were T2, 55 were T3, and 69 were T4. Among patients with prostate cancer, 129 had a Gleason score ≤7, and 89 had a Gleason score >7.

3.2. GAS5 gene polymorphism locus genotype and allele frequency
The rs17359906 and rs1951625 loci of GAS5 in the case and control groups were all in Hardy-Weinberg equilibrium (P > .05) (Table 2). Taking the GAS5 rs17359906 locus GG genotype as a reference, after adjusting for age, BMI, smoking status, and drinking status, GA and AA genotype patients had a significantly greater risk of prostate cancer. The risk in the GA genotype was 3.65 times (95% CI: 2.32–5.74, P < .001) and in the AA genotype the risk was 6.68 times (95% CI: 2.45–18.21, P < .001) greater than in the GG genotype. Carriers of the A allele were 3.44 times more likely to have prostate cancer than those of the G allele (95% CI: 2.38–4.96, P < .001). Taking the GAS5 rs1951625 locus GG genotype as a reference, after adjusting for age, BMI, smoking status, and drinking status, there was no significant correlation between GA and AA genotypes and the risk of prostate cancer (P > .05). However, carriers of the A allele had a 1.40-fold higher risk of prostate cancer than carriers of the G allele (95% CI: 1.05–1.86, P = .027) (Table 2).

3.3. Different genetic patterns of GAS5 SNP loci and risk of prostate cancer
GAS5 rs17359906 locus dominant model (adjusted OR = 4.01, 95% CI: 2.61–6.16, P < .001), recessive model (adjusted OR = 4.58, 95% CI: 1.70–12.39, P = .002), and additive model (adjusted OR = 1.58, 95% CI: 1.17–2.13, P = .004) were all significantly associated with an increased risk of prostate cancer (Table 3). The rs1951625 locus dominant model, recessive model, and additive model all showed no significant correlation with prostate cancer risk (P > .05, Table 3).
3.4. Hierarchical analysis

Stratified analysis of general patient data showed that the stratification of age, BMI, smoking status, and drinking status did not affect the association between the SNP at the GAS5 rs17359906 site and the risk of prostate cancer. The populations of GA and AA genotypes at the rs17359906 locus had a greater risk of developing prostate cancer than those of GG genotypes ($P < .05$, Table 4).

BMI and drinking status affected the association between SNPs at the GAS5 rs1951625 locus and prostate cancer risk. Among patients with BMI $\leq 24$ kg/m$^2$ and those who never drink, the risk of prostate cancer is high for GA and AA genotypes when they carry the GAS5 at the rs1951625 locus. However, there was no significant increase in the risk of prostate cancer in men with a BMI $> 24$ kg/m$^2$ and those who had never had a drink when they carried GA and AA genotypes at the rs1951625 locus.

### Table 3

| SNP model                        | Case (n = 218) | Control (n = 220) | P value | Adjusted OR (95% CI) |
|----------------------------------|---------------|-------------------|---------|----------------------|
| rs17359906                       |               |                   |         |                      |
| Dominance model                  |               |                   |         |                      |
| GG                               | 112 (51.38%)  | 178 (80.91%)      | .001    | 4.01 (2.61–6.16)     |
| GA + AA                          | 106 (48.62%)  | 42 (19.19%)       |         |                      |
| Recessive model                  |               |                   |         |                      |
| GG + GA                          | 197 (30.73%)  | 215 (97.73%)      | .002    | 4.58 (1.70–12.39)    |
| AA                               | 21 (9.63%)    | 5 (2.27%)         |         |                      |
| Additive model (GG vs GA vs AA)  |               |                   | .004    | 1.58 (1.17–2.13)     |
| rs1951625                        |               |                   |         |                      |
| Dominance model                  |               |                   | .056    | 1.21 (0.90–1.48)     |
| GG                               | 98 (44.95%)   | 120 (54.55%)      |         |                      |
| GA + AA                          | 120 (55.05%)  | 100 (45.45%)      | .179    | 1.55 (0.87–2.76)     |
| Recessive model                  |               |                   |         |                      |
| GG + GA                          | 186 (85.32%)  | 198 (90.00%)      | .280    | 1.21 (0.88–1.68)     |
| AA                               | 32 (14.68%)   | 22 (10.00%)       |         |                      |

CI = confidence interval, OR = odds ratio, SNP = single nucleotide polymorphism.

### Table 4

Hierarchical analysis of the association between SNPs at the rs17359906 locus of GAS5 and prostate cancer risk.

| Age                  | Case (n = 218) | Control (n = 220) | Adjusted OR (95% CI) | P value |
|----------------------|---------------|-------------------|----------------------|---------|
| <70                  |               |                   |                      |         |
| GG                   | 52 (57.78%)   | 59 (76.62%)       | 1.00 (reference)     |         |
| GA + AA              | 38 (42.22%)   | 18 (23.38%)       | 2.40 (1.22–4.70)    | .016    |
| [0.1-5] ≥70          |               |                   |                      |         |
| GG                   | 60 (46.88%)   | 119 (83.22%)      | 1.00 (reference)     |         |
| GA + AA              | 68 (53.13%)   | 24 (16.78%)       | 5.62 (3.21–9.81)    | <.001   |
| BMI (kg/m$^2$)       |               |                   |                      |         |
| >24                  |               |                   |                      |         |
| GG                   | 58 (40.43%)   | 58 (80.56%)       | 1.00 (reference)     |         |
| GA + AA              | 57 (49.57%)   | 14 (19.44%)       | 4.07 (2.05–8.11)    | <.001   |
| [0.1-5] ≤24          |               |                   |                      |         |
| GG                   | 54 (52.43%)   | 120 (81.08%)      | 1.00 (reference)     |         |
| GA + AA              | 49 (47.57%)   | 28 (18.92%)       | 3.89 (2.21–6.84)    | <.001   |
| Smoking status       |               |                   |                      |         |
| Never                |               |                   |                      |         |
| GG                   | 51 (45.13%)   | 102 (79.69%)      | 1.00 (reference)     |         |
| GA + AA              | 62 (54.87%)   | 26 (20.31%)       | 4.78 (2.70–8.42)    | <.001   |
| Ever                 |               |                   |                      |         |
| GG                   | 61 (58.10%)   | 76 (82.61%)       | 1.00 (reference)     |         |
| GA + AA              | 44 (41.90%)   | 16 (17.39%)       | 3.43 (1.76–6.66)    | <.001   |
| Drinking status      |               |                   |                      |         |
| Never                |               |                   |                      |         |
| GG                   | 69 (50.36%)   | 123 (80.39%)      | 1.00 (reference)     |         |
| GA + AA              | 68 (49.64%)   | 30 (19.61%)       | 4.04 (2.40–6.80)    | <.001   |
| Ever                 |               |                   |                      |         |
| GG                   | 43 (53.09%)   | 55 (82.09%)       | 1.00 (reference)     |         |
| GA + AA              | 38 (46.91%)   | 12 (17.91%)       | 4.05 (1.89–8.68)    | <.001   |

BMI = body mass index, CI = confidence interval, GAS5 = growth arrest-specific transcript 5, OR = odds ratio, SNP = single nucleotide polymorphism.
The genotypes of GAS5 at the rs1951625 locus (P > .05, Table 5). The risk of prostate cancer in patients with GA and AA genotypes of the GAS5 gene at the rs1951625 locus and who were <70 or ≥70 years of age, never smoked, or ever smoked, and never drank or ever drank, compared favorably with the risk of patients with GG genotypes. There was no significant difference in the risk of developing prostate cancer in these men (P > .05, Table 5).

### 3.5. Multivariate analysis of the risk of prostate cancer

Plasma PSA, BMI, rs17359906, rs1951625, and other variables were included in the logistic regression model for multivariate correlation regression analysis. The results showed that plasma PSA, BMI, rs17359906, and rs1951625 were independent risk factors for prostate cancer (Table 6).

#### Table 5

Hierarchical analysis of the association between the SNP at the rs1951625 locus of GAS5 and the risk of prostate cancer.

| Age      | Case (n = 218) | Control (n = 220) | Adjusted OR (95% CI) | P value |
|----------|----------------|-------------------|----------------------|---------|
| <70      |                |                   |                      |         |
| GG       | 42 (46.67%)    | 46 (59.74%)       | 1.00 (reference)     |         |
| GA + AA  | 48 (53.33%)    | 31 (40.26%)       | 1.70 (0.92–3.14)     | .126    |
| ≥70      |                |                   |                      |         |
| GG       | 56 (43.75%)    | 74 (51.75%)       | 1.00 (reference)     |         |
| GA + AA  | 72 (56.25%)    | 69 (48.25%)       | 1.38 (0.85–2.23)     | .232    |
| BMI (kg/m²) |             |                   |                      |         |
| >24      |                |                   |                      |         |
| GG       | 51 (44.35%)    | 32 (44.44%)       | 1.00 (reference)     |         |
| GA + AA  | 64 (55.65%)    | 40 (55.56%)       | 1.01 (0.56–1.82)     | .990    |
| <24      |                |                   |                      |         |
| GG       | 47 (45.63%)    | 88 (59.46%)       | 1.00 (reference)     |         |
| GA + AA  | 56 (54.37%)    | 60 (40.54%)       | 1.75 (1.05–2.90)     | .042    |
| Smoking status |         |                   |                      |         |
| Never    |                |                   |                      |         |
| GG       | 50 (44.25%)    | 73 (57.03%)       | 1.00 (reference)     |         |
| GA + AA  | 63 (55.75%)    | 55 (42.97%)       | 1.67 (1.00–2.79)     | .064    |
| Ever     |                |                   |                      |         |
| GG       | 48 (45.71%)    | 47 (51.09%)       | 1.00 (reference)     |         |
| GA + AA  | 57 (54.29%)    | 45 (48.91%)       | 1.24 (0.71–2.17)     | .542    |
| Drinking status |       |                   |                      |         |
| Never    |                |                   |                      |         |
| GG       | 58 (42.34%)    | 86 (56.21%)       | 1.00 (reference)     |         |
| GA + AA  | 79 (57.66%)    | 67 (43.79%)       | 1.75 (1.10–2.79)     | .025    |
| Ever     |                |                   |                      |         |
| GG       | 40 (49.38%)    | 34 (50.75%)       | 1.00 (reference)     |         |
| GA + AA  | 41 (50.62%)    | 33 (49.25%)       | 1.06 (0.55–2.02)     | .869    |

BMI = body mass index, CI = confidence interval, GAS5 = growth arrest-specific transcript 5, OR = odds ratio, SNP = single nucleotide polymorphism.

### 3.6. Correlation between the SNPs of the rs17359906 and rs1951625 loci of GAS5 and clinical parameters

The distribution of GAS5 genotypes at the rs17359906 and rs1951625 loci between Clinical T stage > T2 and Clinical T stage ≤ T2 is shown in Table 7. The results indicate that there was no significant correlation between SNPs at the GAS5 rs17359906 and rs1951625 loci and Clinical T stage (P > .05, Table 7). The distribution of GAS5 genotypes at the rs17359906 and rs1951625 loci for patients with Gleason score ≤ 7 and Gleason score > 7 are shown in Table 8. The results indicate that there was no significant correlation between SNPs at the rs17359906 locus of GAS5 and Gleason score (P > .05), and that the AA genotype at the rs1951625 locus of GAS5 and the carrier of the A allele (GA + AA) were significantly associated with a Gleason score > 7 (P < .05, Table 8).

#### Table 6

Multivariate analysis of the risk of prostate cancer.

| Factors | B   | P      | Exp (B) | 95% CI for Exp (B) |
|---------|-----|--------|---------|-------------------|
| Plasma PSA | -3.08 | .004 | 0.040 | 0.021 – 0.125 |
| BMI     | 2.86 | .035 | 2.52 | 1.99 – 4.03 |
| rs17359906 | 4.12 | .002 | 3.12 | 2.45 – 5.51 |
| rs1951625 | 3.87 | .003 | 3.04 | 2.27 – 5.75 |

BMI = body mass index, CI = confidence interval.
Correlation between SNPs at the GAS5 rs17359906 and rs1951625 loci and clinical T stage.

|                    | Clinical T stage ≤T2 (n = 124) | Clinical T stage >T2 (n = 94) | Adjusted OR (95% CI) | P value |
|--------------------|---------------------------------|--------------------------------|----------------------|---------|
| rs17359906         |                                 |                                |                      |         |
| GG                 | 66 (53.23%)                     | 46 (48.94%)                    | 1.00 (reference)     |         |
| GA                 | 44 (35.48%)                     | 41 (43.62%)                    | 0.75 (0.42–1.32)     | .391    |
| AA                 | 14 (11.29%)                     | 7 (7.45%)                      | 1.39 (0.52–3.72)     | .673    |
| GA + AA            | 58 (46.77%)                     | 48 (51.06%)                    | 0.84 (0.49–1.44)     | .624    |
| rs1951625          |                                 |                                |                      |         |
| GG                 | 53 (42.74%)                     | 45 (47.87%)                    | 1.00 (reference)     |         |
| GA                 | 48 (38.71%)                     | 40 (42.55%)                    | 1.02 (0.57–1.82)     | .949    |
| AA                 | 23 (18.55%)                     | 9 (8.57%)                      | 2.17 (0.91–5.16)     | .117    |
| GA + AA            | 71 (57.26%)                     | 49 (62.13%)                    | 1.23 (0.72–2.11)     | .537    |

CI = confidence interval, OR = odds ratio.

3.7. Correlation between SNPs at rs17359906 and rs1951625 loci and plasma PSA levels

Correlation between SNPs at GAS5 rs17359906 and rs1951625 loci and plasma PSA levels in the case and control groups was analyzed. The results of univariate analysis of variance showed that there was a statistically significant difference in plasma PSA levels between the case and control groups with different genotypes at the rs17359906 and rs1951625 loci of GAS5 (P < .05). However, in subjects with plasma PSA levels exceeding 20 ng/mL, there was no correlation between the frequency of different genotypes at rs17359906 and the risk of prostate cancer (P > .05) (Table 9). In subjects with plasma PSA levels below 20 ng/mL, the GA genotype at rs17359906 was associated with an increased risk of prostate cancer (P < .05) (Table 9). In subjects with plasma PSA levels exceeding 20 ng/mL, the GA genotype frequency at rs1951625 was associated with an increased risk of prostate cancer (P < .05) (Table 9). In subjects with plasma PSA levels below 20 ng/mL, the genotype frequency at rs1951625 was not associated with prostate cancer risk (P > .05) (Table 9). The mean plasma PSA level of the allele carriers of point A was significantly higher than that of the G-allele carriers (Fig. 1), suggesting that the GAS5 gene rs17359906 G > A and rs1951625 G > A mutations are associated with elevated plasma PSA levels.

3.8. GAS5 SNPs at the rs17359906 and rs1951625 loci and recurrence-free survival in patients with prostate cancer

In this study, we followed the progress of disease in 218 patients with prostate cancer using outpatient and telephone follow-up methods. As of August 2019, no patients were lost to follow-up. The follow-up time ranged from 6 months to 36 months. Of the 218 patients, 36 patients relapsed during follow-up, and the 36-month recurrence-free survival rate was 83.49%. The recurrence-free survival rate of patients with prostate cancer of the AA genotype at the GAS5 rs17359906 locus (66.67%) was significantly lower than that of the GA genotype (76.47%) at the same locus, and the recurrence-free survival rate of patients with the GG genotype at this locus was the highest (91.96%). (P = .002, Fig. 2A). The recurrence-free survival rate of patients with prostate cancer of the AA genotype at the GAS5 rs1951625 locus (75.00%) was significantly lower than that of the GA genotype (81.82%) at the same locus. The recurrence-free survival rate of patients with the GG genotype at this locus was the highest (87.76%). (P = .023, Fig. 2B).

4. Discussion

In this study, we examined 2 SNP loci on the GAS gene associated with the risk of prostate cancer, namely rs17359906 and rs1951625. Our results indicate that GAS5 rs17359906 G > A and rs1951625 G > A mutations are significantly associated with an increased risk of prostate cancer and a reduction in three-year relapse-free survival rates in patients with prostate cancer.

Prostate cancer has surpassed lung cancer in the United States and is now first among malignant tumors that endanger men’s health.[21,22] Although the incidence of prostate cancer in Asia is lower than in Europe and the United States, it has increased significantly in recent years. Prostate cancer usually progresses slowly, and tumor-bearing patients often have good long-term survival rates,[23,24] however some patients’ tumors grow rapidly,
Figure 1. Comparison of plasma PSA levels in patients with different genotypes at the rs17359906 and rs1951625 loci of GAS5. (A) Comparison of plasma PSA levels in patients with prostate cancer with different genotypes at the GAS5 rs17359906 locus. (B) Comparison of plasma PSA levels in the control group with different genotypes at the GAS5 rs17359906 locus. (C) Comparison of plasma PSA levels in patients with prostate cancer with different genotypes at the GAS5 rs1951625 locus. (D) Comparison of plasma PSA levels in the control group with different genotypes at the GAS5 rs1951625 locus.

Table 9
Correlation between GAS5 rs17359906 and rs1951625 SNP and prostate cancer risk in subjects with different plasma PSA levels.

|                   | Case (n = 218) | Control (n = 220) | Adjusted OR (95%CI) | p-value |
|-------------------|----------------|-------------------|---------------------|---------|
| rs17359906        |                |                   |                     |         |
| >20 ng/mL         |                |                   |                     |         |
| GG                | 108 (51.55%)   | 7 (77.78%)        | 1.00 (reference)    |         |
| GA                | 75 (38.66%)    | 2 (22.22%)        | 2.63 (0.53-13.00)   | 0.380   |
| AA                | 19 (9.79%)     | 0 (0%)            | 1.07 (0.88-1.07)    | 0.546   |
| ≤20 ng/mL         |                |                   |                     |         |
| GG                | 12 (50.00%)    | 171 (81.04%)      | 1.00 (reference)    |         |
| GA                | 10 (41.67%)    | 35 (16.59%)       | 4.07 (1.63-10.16)   | 0.004   |
| AA                | 2 (8.33%)      | 5 (2.37%)         | 4.36 (0.70-13.37)   | 0.147   |
| rs1951625         |                |                   |                     |         |
| >20 ng/mL         |                |                   |                     |         |
| GG                | 85 (43.81%)    | 7 (77.78%)        | 1.00 (reference)    |         |
| GA                | 80 (41.24%)    | 0 (0%)            | 1.08 (1.01-1.08)    | 0.033   |
| AA                | 28 (14.95%)    | 2 (22.22%)        | 1.19 (0.24-6.88)    | 0.831   |
| ≤20 ng/mL         |                |                   |                     |         |
| GG                | 13 (54.17%)    | 113 (53.55%)      | 1.00 (reference)    |         |
| GA                | 8 (33.33%)     | 76 (36.97%)       | 0.89 (0.35-2.25)    | 0.983   |
| AA                | 3 (12.50%)     | 20 (9.48%)        | 1.30 (0.34-4.99)    | 0.982   |
and may metastasize and transform into castration-resistant prostate cancer in a short time. In such cases traditional endocrine therapy is no longer effective and the patient's condition deteriorates rapidly. The cause of this heterogeneity in prostate cancer is currently unknown. Therefore, it is of great significance to investigate the pathogenesis of prostate cancer, to find new pathogenic factors and prognostic evaluation factors, which will facilitate individualized treatment to patients according to the unique nature of their disease.

Initially, non-coding RNA was thought to be “transcription noise” and have no biological function. However, as research into epigenetics and genomics intensified, researchers discovered that these RNAs can regulate gene expression at epigenetic, transcription, and post-transcription levels. Likewise, it was found that lncRNA plays an important role in various biological processes such as cell differentiation, proliferation, apoptosis, migration, and infiltration. The GAS5 plays an important role in the occurrence and development of tumors and is abnormally expressed in various tumors, such as lung cancer, gastric cancer, and triple negative breast cancer (TNBC). Studies have shown that lncRNA GAS5 plays an important role in the occurrence of prostate cancer. For example, Xue et al. showed that lncRNA GAS5 mediates the AKT/mTOR signaling pathway by targeting miR-103 in prostate cancer. In addition, Pickard et al. found that lncRNA GAS5 promotes the apoptosis of prostate cancer cells, and abnormally low expression of lncRNA GAS5 may significantly reduce the effectiveness of chemotherapy drugs.

In this study, we used bioinformatics techniques to find 2 structures that may affect GAS5 binding to microRNA and lncRNA GAS5. The MAFs of these 2 SNP sites is >0.05. The MAF of the rs17359906 locus in the 1000 genomes database is 0.081 and the MAF of the rs1951625 locus in the 1000 genomes database is 0.2714, which is not significantly different from the findings in this research paper, indicating that the selected population is representative.

Case-control studies found that the GAS5 rs17359906 locus A allele and the GAS5 rs1951625 locus A allele were significantly associated with an increased risk of prostate cancer. The GAS5 rs1951625 locus dominant model, recessive model, additive model were also significantly associated with increased prostate cancer risk, but no correlation was found between the GAS5 rs1951625 locus and the risk of prostate cancer. This indicates that the rs17359906 locus and the rs1951625 locus are susceptible factors for the development of prostate cancer. Further research shows that rs17359906 and rs1951625 are similar independent risk factors for prostate cancer to plasma PSA and BMI. To our knowledge, there have as yet been no research studies conducted on the correlation between rs17359906 and rs1951625 loci and prostate cancer risk, hence the association between these loci and the risk of prostate cancer has not yet been fully elucidated. This study is the first to focus on these 2 SNP loci.

We analyzed the correlation between the GAS5 rs17359906 and rs1951625 loci and Gleason scores and Clinical T stages to determine the relationship between GAS5 polymorphisms and disease progression. The results showed that the GAS5 rs1951625 locus A allele was associated with a Gleason score >7, indicating that carriers of the GAS5 rs1951625 locus A allele were likely to have highly malignant disease with a poor prognosis. The results of follow-up analysis also confirmed that the carriers of GAS5 rs17359906 locus A allele and rs1951625 locus A allele had poor prognoses. Although we did not find a significant correlation between the rs17359906 locus SNP and the Gleason score, we speculate that this may be related to our relatively small sample size.

At present, PSA is the most widely used indicator for prostate cancer screening. It is used clinically to guide biopsy, evaluate the degree of malignancy of prostate cancer, and detect recurrence. Several studies have shown that PSA levels are positively related to the degree of malignancy of prostate cancer, and detect recurrence. Several studies have shown that PSA levels are positively related to the degree of malignancy of prostate cancer, and detect recurrence. Several studies have shown that PSA levels are positively related to the degree of malignancy of prostate cancer, and detect recurrence. Several studies have shown that PSA levels are positively related to the degree of malignancy of prostate cancer, and detect recurrence. Several studies have shown that PSA levels are positively related to the degree of malignancy of prostate cancer, and detect recurrence. Several studies have shown that PSA levels are positively related to the degree of malignancy of prostate cancer, and detect recurrence.

Figure 2. Kaplan-Meier curve analysis of 36-month recurrence-free survival rate of patients with prostate cancer. (A) Comparison of 36-month recurrence-free survival rate of patients with prostate cancer with different genotypes at the rs17359906 locus of GAS5. (B) Comparison of the 36-month relapse-free survival rate of patients with prostate cancer with different genotypes at the rs1951625 locus of GAS5.
PSA levels below 20ng/mL, the genotype frequency of rs1951625 was not associated with prostate cancer risk ($P > .05$). The reason for the analysis may be related to the small sample size. In the stratified study of plasma PSA levels, the sample size of some genotype subjects is small, which affects the objectivity of statistical analysis. It is necessary to further expand the sample size for the study.

There are some limitations to this study. First, we did not analyze the correlation between GAS5 rs17359906 and rs1951625 G > A and rs1951625 G > A were significantly associated with increased risk of prostate cancer and reduced three-year relapse-free survival. Furthermore, the GAS5 rs17359906 and rs1951625 loci are important risk factors for prostate cancer.

### Author contributions

**Study design:** Lisha Zhao, Weihong Zheng, Chen Li.

**Data collection:** Lisha Zhao, Weihong Zheng.

**Data analysis:** Lisha Zhao, Weihong Zheng, Chen Li.

**Interpretation of data:** Lisha Zhao, Weihong Zheng, Chen Li.

**Draft manuscript:** Lisha Zhao.

**Review manuscript:** Chen Li.

### References

[1] Zhai Z, Zheng Y, Li N, et al. Incidence and disease burden of prostate cancer from 1990 to 2017: results from the Global Burden of Disease Study 2017. Cancer 2020;126:1969–78. doi: 10.1002/cncr.32733.

[2] Sharma R. The burden of prostate cancer is associated with human development index: evidence from 87 countries, 1990–2016. EPMA J 2019;10:137–32.

[3] Teoh JYC, et al. Global incidence of prostate cancer in developing and developed countries with changing age structures. PLoS One 2019;14: e0221775.

[4] Du D, Zha Y. Epidemiology of prostate cancer in China: a overview and clinical implication. Zhonghua Wai Ke Za Zhi 2015;53:249–52.

[5] Zhao F, et al. Trends in treatment for prostate cancer in China: preliminary patterns of care study in a single institution. J Cancer 2018;9:797–803.

[6] Freedland SJ, Moul JW. Prostate specific antigen recurrence after definitive therapy. J Urol 2007;177:1985–91.

[7] Gimpburg X, et al. Prostate cancer biochemical recurrence rates after robotic-assisted laparoscopic radical prostatectomy. JSLS 2012;16:443–50.

[8] Pollack A, et al. Prostate cancer radiotherapy dose response: an update of the fox chase experience. J Urol 2004;171:1132–6.

[9] Baez AJ, Vermeulen D. The complexity of epigenetic diseases. J Pathol 2016;238:333–44.

[10] Ishak MB, Giri VN. A systematic review of replication studies of prostate cancer susceptibility genetic variants in high-risk men originally identified from genome-wide association studies. Cancer Epidemiol Biomarkers Prev 2011;20:1599–610.

[11] Stegeman S, et al. A large-scale analysis of genetic variants within putative miRNA binding sites in prostate cancer. Cancer Discov 2015;5:368–79.

[12] Xu J, et al. Genome-wide association study in Chinese men identifies two new prostate cancer risk loci at 9q31.2 and 19q13.4. Nat Genet 2012;44:1231–5.

[13] Chen X, et al. Long non-coding RNA GAS5 and ZFAS1 are prognostic markers involved in translation targeted by miR-940 in prostate cancer. Oncotarget 2018;9:1048–62.

[14] Huang W, et al. LncRNA GAS5-AS1 inhibits glioma proliferation, migration, and invasion via miR-106b-5p/TUSC2 axis. Hum Cell 2020;33:416–26.

[15] Li J, et al. LncRNA GAS5 inhibits Th17 differentiation and alleviates immune thrombocytopenia via promoting the ubiquitination of STAT3. Int Immunopharmacol 2020;80:106127.

[16] Li G, et al. LncRNA GAS5 regulates the proliferation, migration, and invasion and apoptosis of brain glioma cells through targeting GSTM1 expression. The effect of LncRNA GAS5 on glioma cells. J Neurooncol 2019;14:352–36.

[17] Pickard MR, Mourtada-Maarabouni M, Williams GT. Long non-coding RNA GAS5 regulates apoptosis in prostate cancer cell lines. Biochim Biophys Acta 2013;1832:1613–23.

[18] Mourtada-Maarabouni M, et al. GAS5, a non-protein-coding RNA, controls apoptosis and is downregulated in breast cancer. Oncogene 2009;28:195–208.

[19] Lin CY, et al. Impact of GAS5 genetic polymorphism on prostate cancer susceptibility and clinicopathologic characteristics. Int J Med Sci 2019;16:1424–9.

[20] Wang Y, Wu S, Yang X, et al. Association between polymorphism in the promoter region of IncRNA GAS5 and the risk of colorectal cancer [published correction appears in Biosci Rep. 2019 May 21;39(5)]. Biosci Rep 2019;394: (BSR20190091). Published 2019 Apr 16. doi:10.1042/ BSR20190091.

[21] Boring CC, Squires TS, Health CW Jr. Cancer statistics for African Americans: Prostate cancer. CA Cancer J Clin 1992;42:47–7.

[22] DeSantis CE, et al. Cancer statistics for African Americans, 2016: progress and opportunities in reducing racial disparities. CA Cancer J Clin 2016;66:290–308.

[23] Boorjian SA, et al. A critical analysis of the long-term impact of radical prostatectomy on cancer control and function outcomes. Eur Urol 2012;61:664–75.

[24] Thompson I, et al. Guideline for the management of clinically localized prostate cancer: 2007 update. J Urol 2007;177:2106–31.

[25] D’Amico AV, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. JAMA 1998;280:969–74.

[26] Bader DA, McGuire SE. Long noncoding RNA in genome regulation: prospects and mechanisms. RNA Biol 2010;7:582–5.

[27] Ringani MT, et al. Long non-coding RNA SNHG6 as a potential biomarker for hepatocellular carcinoma. Pathol Oncol Res 2018;24:329–37.

[28] Chen B, Huang S. Circular RNA: an emerging non-coding RNA as a regulator and biomarker in cancer. Cancer Lett 2018;418:1–7.

[29] Wang Y, Wu S, Yang X, et al. Association between polymorphism in the FSHR gene and clinicopathologic characteristics. Int J Med Sci 2019;16:1424–9.

[30] Wang Y, Wu S, Yang X, et al. Association between polymorphism in the FSHR gene and clinicopathologic characteristics. Int J Med Sci 2019;16:1424–9.

[31] Lian Y, et al. Knockdown of pseudogene derived from lncRNA DUXAP10 inhibits cell proliferation, migration, invasion, and promotes apoptosis in pancreatic cancer. J Cell Biochem 2018;119:3671–82.

[32] Chen S, et al. Macrophage infiltration promotes invasiveness of breast cancer cells via activating long non-coding RNA UCA1. Int J Exp Pathol 2015;8:9052.

[33] Dong L, et al. Upregulation of long non-coding RNA GAS5 inhibits lung cancer cell proliferation and metastasis via miR-203/PTEN axis. Med Sci Monit 2019;25:2311–9.

[34] Li Y, Xu J, Lu H. The GAS5/miR-222 axis regulates proliferation of gastric cancer cells through the PTEN/Akt/mTOR pathway. Dig Dis Sci 2017;62;3426–37.

[35] Li S, et al. Long non-coding RNA GAS5 suppresses triple negative breast cancer progression through inhibition of proliferation and invasion by competitively binding miR-196a-5p. BioMed Pharmacother 2018;104:451–7.

[36] Xue D, Zhou C, Lu H, et al. LncRNA GAS5 inhibits proliferation and progression of prostate cancer by targeting miR-103 through AKT/ mTOR signaling pathway [published online ahead of print, 2016 Oct 14]. Tumour Biol 2016;doi: 10.1007/s13277-016-5429-8.

[37] Mao Z, et al. Diagnostic performance of PCA3 and kH2 in combination with serum PSA for prostate cancer. Medicine (Baltimore) 2018;97:e12806.

[38] Chen B, Macapinlac HA, Lu Y. Superscan 18F-fluorodeoxyglucose PET/CT of PSA-negative prostate cancer bone metastases. Clin Nucl Med 2019;44:337–8.

[39] Armstrong AJ, et al. A phase 2 multimodality trial of docetaxel/prednisone with sunitinib followed by salvage radiation therapy in men with PSA recurrent prostate cancer after radical prostatectomy. Prostate Cancer Prostatic Dis 2016;19:100–6.