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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 (*GAS5*) 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 *GAS5* 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, GAS5 = growth arrest-specific transcript 5, GWAF = genome-wide association analyses with family, GWAS = Whole Genome Association Analysis, lncRNA = 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-

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ciation studies, more than 50 SNP sites related to prostate cancer risk have been identified, and this dataset has grown with the dissemination and development of gene sequencing technology.[12] A genome-wide association study of prostate cancer patients in the Chinese population found that only 2 SNPs, 9q31.2 (rs817826) and 19q13.4 (rs103294), were associated with prostate cancer in the Chinese population, which also confirms that there is a difference in the genetic susceptibility to prostate cancer between the Chinese, European, and American populations.[12] We therefore have reason to believe that in the future as more genome-wide association studies are performed in patients with prostate cancer, SNPs will be more widely used to predict prostate cancer risk in the Chinese population.

The *GAS5* gene is located on human chromosome 1q25 and contains 12 exons. This gene does not encode a protein.[13] Studies have shown that long non-coding RNA (lncRNA) *GAS5* expression is different in a variety of human tumor tissues and in normal tissues. Although we cannot rule out the possibility that this differential expression level is a secondary change caused by tumors, studies have also confirmed that *GAS5* can play an important inhibitory role in the malignant transformation of cells by regulating a variety of cell signaling pathways.[14–16] Pickard et al[17,18] found that the expression level of *GAS5* in metastatic prostate cancer cell lines LNCaP and PC3 was lower than that in normal prostate cell lines and non-metastatic prostate cancer cell lines, and overexpression of *GAS5* not only increases the basal apoptosis rate of prostate cancer cell lines 22Rv1 and PC3, but also enhances the role of apoptosis stimulating factors. It is therefore speculated that *GAS5* is a potentially important target for prostate cancer treatment.

The association between *GAS5* polymorphism and tumor risk has been widely studied. For example, Lin et al[19] found that *GAS5* rs145204276 polymorphism is associated with a risk of lymph node metastasis in patients with prostate cancer. Wang et al[20] found that polymorphism at rs55829688 in the promoter region of *GAS5* is related to the risk of colorectal cancer and the down-regulation of *GAS5* expression levels.

In this study, we selected *GAS5* rs17359906 and rs1951625 from the lncRNASNP2 database (http://bioinfo.life.hust.edu.cn/lncRNASNP#!/). The selection of these 2 SNP sites was based on Genome-wide association analyses with family (GWAF) >0.05, and SNP-affected lncRNA and microRNA binding and lncRNA structure, and minor allele frequency (MAF) >0.05. The purpose of this study was to investigate the correlation between *GAS5* polymorphisms and the occurrence and prognosis of prostate cancer, and to provide evidence for the prevention and treatment of prostate cancer.

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 ± 12.04) 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. We 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 Zhuji 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, Valencia, 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 ATT GG-3' (Rv). rs1951625: 5'-ACT GCA CCC GCA GTT AAG AA-3′ (Fw); 5′-CTT AAG TGC CTG 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 Mg_2 + 0.8 μL, 10 mM forward and reverse primers 0.4 μL each, 1 U/μL Taq enzyme 0.15 μL, ddH_2O 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 Jinweizhi 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 χ^2-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 χ^2-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.

Table 1

Comparison of demographic and clinical data of prostate cancer patients and control groups.

Characteristic	Case (n = 218)	Control (n = 220)	*P* value
Age (yr, mean ± SD)	70.54 ± 12.04	70.64 ± 11.19*	.93
>70	128 (58.72%)	143 (65.00%)†	.18
≤70	90 (41.28%)	77 (35.00%)	
BMI (kg/m^2)			<.001
>24	115 (52.75%)	72 (32.73%)†	
≤24	103 (47.25%)	148 (67.72%)	
Plasma PSA (ng/mL, mean ± SD)	32.43 ± 14.49	10.18 ± 5.01*	<.01
>20	194 (88.99%)	9 (4.09%)†	<.01
≤20	24 (11.01%)	211 (95.91%)	
Smoking status			.18
Never	113 (51.83%)	128 (58.18%)†	
Ever	105 (48.17%)	92 (41.82%)	
Drinking status			.14
Never	137 (62.84%)	153 (69.55%)†	
Ever	81 (37.16%)	67 (30.45%)	
Clinical T stage			
>T2	124 (56.88%)		
≤T2	94 (43.12%)		
Gleason score			
≤7	129 (59.17%)		
>7	89 (40.83%)		

BMI = body mass index, PSA = prostate-specific antigen, SD = standard deviation.
* *t* test.
† χ^2 test.

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 control group of prostate cancer patients ($P > .05$). The BMI and plasma PSA levels of prostate cancer patients were significantly higher than those of the control group ($P < .05$). 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).

Table 2

Association of SNPs at rs17359906 and rs1951625 loci of *GAS5* with prostate cancer risk.

	Case (n = 218)	Control (n = 220)	HWE p[a]	HWE p[b]	Adjusted OR (95% CI)	*P* value
rs17359906			0.41	0.11		
Genotype						
GG	112 (51.38%)	178 (80.91%)			1.00 (reference)	
GA	85 (38.99%)	37 (16.82%)			3.65 (2.32–5.74)	<.001
AA	21 (9.63%)	5 (2.27%)			6.68 (2.45–18.21)	<.001
Allele						
G	309 (70.87%)	393 (89.32%)			1.00 (reference)	
A	127 (29.13%)	47 (10.68%)			3.44 (2.38–4.96)	<.001
rs1951625			0.10	0.09		
Genotype						
GG	98 (44.95%)	120 (54.55%)			1.00 (reference)	
GA	88 (40.37%)	78 (35.45%)			1.38 (0.92–2.07)	.144
AA	32 (14.68%)	22 (10.00%)			1.78 (0.97–3.26)	.083
Allele						
G	284 (65.14%)	318 (72.27%)			1.00 (reference)	
A	152 (34.86%)	122 (27.73%)			1.40 (1.05–1.86)	.027

a = case group, b = control group, CI = confidence interval, *GAS5* = growth arrest-specific transcript 5, HWE = Hardy-Weinberg equilibrium, OR = odds ratio.

Table 3

Correlation between *GAS5* SNP loci and the risk of prostate cancer in different genetic models.

SNP model	Case (n = 218)	Control (n = 220)	*P* value	Adjusted OR (95%CI)
rs17359906				
Dominance model			<.001	4.01 (2.61–6.16)
GG	112 (51.38%)	178 (80.91%)		
GA + AA	106 (48.62%)	42 (19.19%)		
Recessive model			.002	4.58 (1.70–12.39)
GG + GA	197 (30.73%)	215 (97.73%)		
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 (0.99–1.48)
GG	98 (44.95%)	120 (54.55%)		
GA + AA	120 (55.05%)	100 (45.45%)		
Recessive model			.179	1.55 (0.87–2.76)
GG + GA	186 (85.32%)	198 (90.00%)		
AA	32 (14.68%)	22 (10.00%)		
Additive model (GG vs GA vs AA)			.280	1.21 (0.88–1.68)

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

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

Table 4

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

	Case (n = 218)	Control (n = 220)	Adjusted OR (95% CI)	*P* alue
Age				
<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 (20.13%)	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.

Table 5

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

	Case (n = 218)	Control (n = 220)	Adjusted OR (95% CI)	*P* value
Age				
<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/m^2)				
>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.

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).

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.

				95% CI for Exp (*B*)	
Factors	***B***	***P***	**Exp (*B*)**	**Lower**	**Upper**
Plasma PSA	–3.08	.004	0.049	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.

Table 7

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 (9.57%)	2.17 (0.91–5.16)	.117
GA + AA	71 (57.26%)	49 (52.13%)	1.23 (0.72–2.11)	.537

CI = confidence interval, OR = odds ratio.

3.7. Correlation between SNPs at rs17359906 and rs1951625 of GAS5 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%). ($P = .025$, 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,

Table 8

Correlation between SNPs at the rs17359906 and rs1951625 loci of *GAS5* and Gleason score.

	Gleason score >7 (n = 89)	Gleason score ≤7 (n = 129)	Adjusted OR (95% CI)	*P* value
rs17359906				
GG	42 (44.68%)	70 (56.45%)	1.00 (reference)	
GA	39 (41.49%)	46 (37.10%)	1.41 (0.80–2.51)	.299
AA	8 (8.51%)	13 (10.48%)	1.03 (0.39–2.68)	.959
GA + AA	47 (50.00%)	59 (47.58%)	1.33 (0.77–2.28)	.374
rs1951625				
GG	32 (34.04%)	66 (53.23%)	1.00 (reference)	
GA	35 (37.23%)	53 (42.74%)	1.36 (0.75–2.48)	.392
AA	22 (23.40%)	10 (8.06%)	4.54 (1.92–10.71)	<.001
GA + AA	57 (60.64%)	63 (50.81%)	1.87 (1.07–3.25)	.038

CI = confidence interval, OR = odds ratio.

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	100 (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	29 (14.95%)	2 (22.22%)	1.19 (0.24–6.08)	0.831
≤20 ng/mL				
GG	13 (54.17%)	113 (53.55%)	1.00 (reference)	
GA	8 (33.33%)	78 (36.97%)	0.89 (0.35–2.25)	0.993
AA	3 (12.50%)	20 (9.48%)	1.30 (0.34–4.99)	0.982

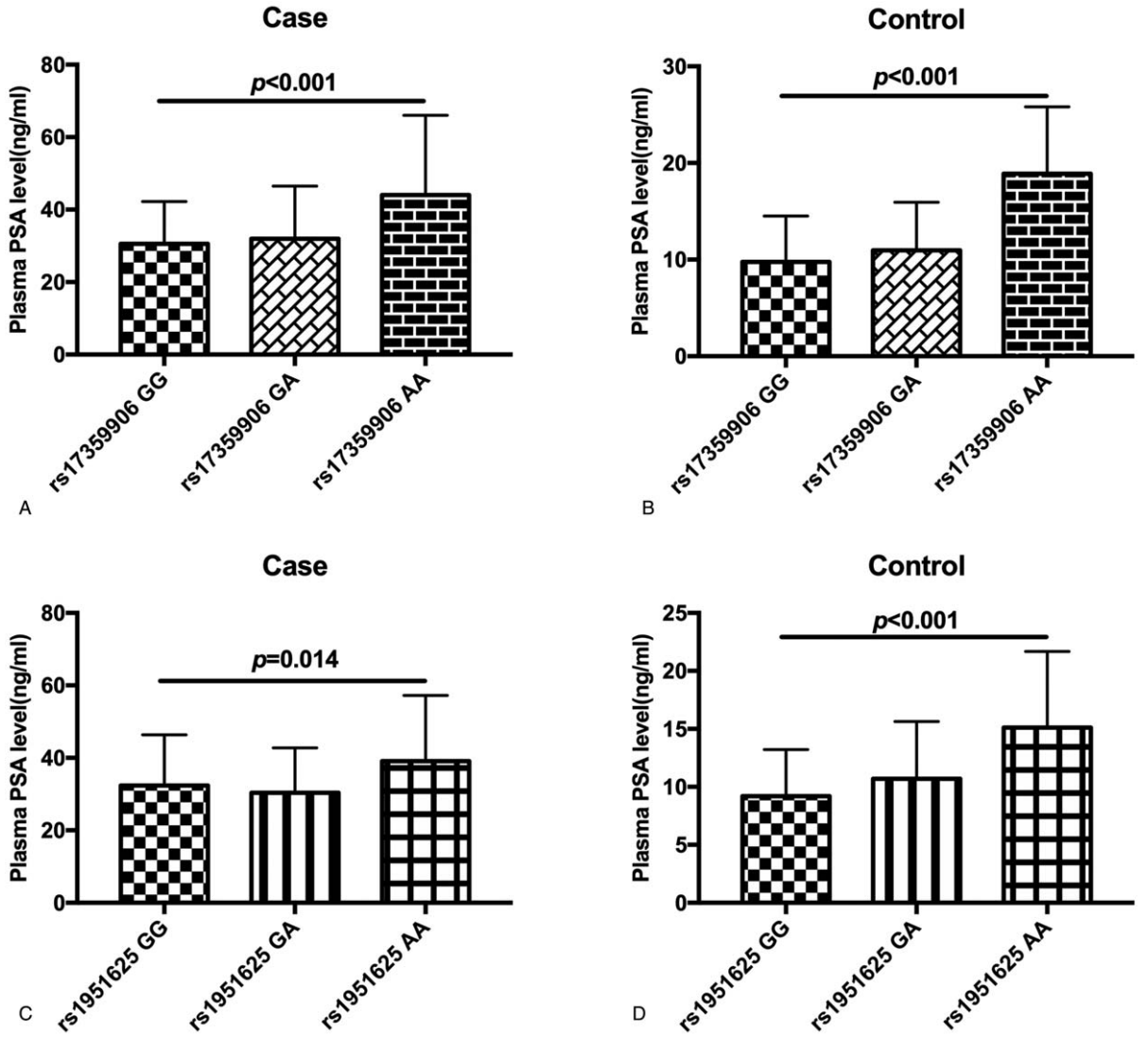

Figure 1. Comparison of plasma PSA levels in patients with different genotypes at the rs17359906 and rs1951625 loci of *GAS5*. (A) Comparison of plasma PSA levels in patients with prostate cancer with different genotypes at the *GAS5* rs17359906 locus. (B) Comparison of plasma PSA levels in the control 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.

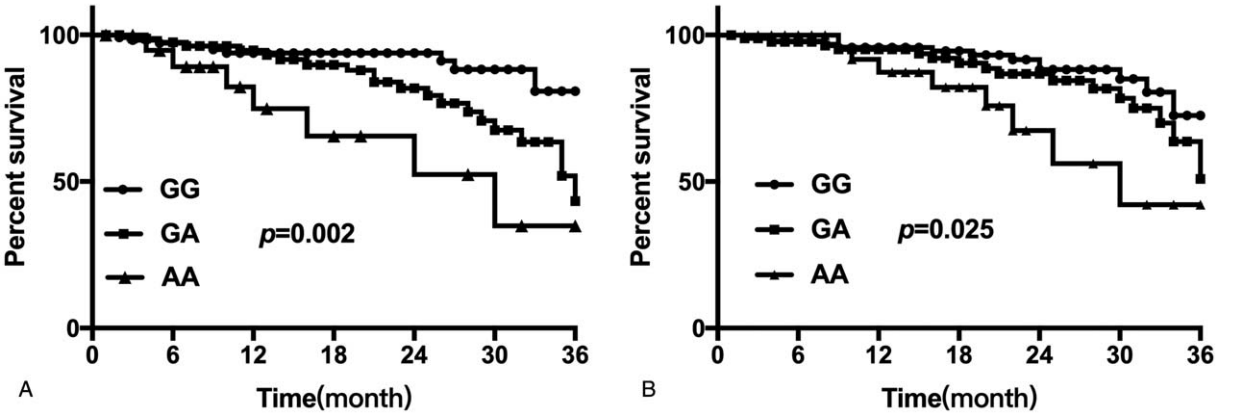

Figure 2. Kaplan-Meier curve analysis of 36-month recurrence-free survival rate of patients with prostate cancer. (A) Comparison of 36-month recurrence-free survival rate of patients with prostate cancer with different genotypes at the rs17359906 locus of *GAS5*. (B) Comparison of the 36-month relapse-free survival rate of patients with prostate cancer with different genotypes at the rs1951625 locus of *GAS5*.

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.[25,26] 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.[27–29] Likewise, it was found that lncRNA plays an important role in various biological processes such as cell differentiation, proliferation, apoptosis, migration, and infiltration.[30–32] The *GAS5* plays an important role in the occurrence and development of tumors and is abnormally expressed in various tumors, such as lung cancer,[33] gastric cancer,[34] and triple negative breast cancer (TNBC).[35] Studies have shown that lncRNA *GAS5* plays an important role in the occurrence of prostate cancer. For example, Xue et al[36] showed that lncRNA *GAS5* mediates the AKT/mTOR signaling pathway by targeting miR-103 in prostate cancer. In addition, Pickard et al[17] 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* rs17359906 locus dominant model, recessive model, and additive model were also significantly associated with increased prostate cancer risk, but no correlation was found between the *GAS5* rs1951625 locus dominant model, recessive model, additive model, and prostate cancer risk. 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.[37–39] Several studies have shown that PSA levels are positively related to an increase in postoperative Gleason score. The higher the PSA level after surgery, the higher the Gleason score. In this study, we found that *GAS5* rs17359906 G>A and rs1951625 G>A mutations were associated with elevated plasma PSA levels, which further confirmed that *GAS5* rs17359906 G>A and rs1951625 G>A were associated with poor prognosis of prostate cancer. Interestingly, from hierarchical analysis of plasma PSA levels, we found that 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. ($P > 0.05$). In subjects with plasma PSA levels below 20 ng/mL, the GA genotype at rs17359906 was associated with an increased risk of prostate cancer ($P < .05$). 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 ($P < .05$). In subjects with plasma

PSA levels below 20 ng/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 loci SNP and lncRNA *GAS5* expression levels and RNA structure. Therefore, there is a lack of mechanism research to support the findings of this study. Secondly, the correlation of research results in this study needs further demonstration in a study with a larger sample size. In addition, there is a lack of evidence from in vitro and in vivo studies to support the findings of this study. These are topics for future research.

5. Conclusion

The results of this study indicate that *GAS5* rs17359906 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 independent risk factors for prostate cancer.

Author contributions

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

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