

Short Research Communication

A Single Nucleotide Polymorphism in *HPGD* Gene Is Associated with Prostate Cancer Risk

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Abstract

Introduction: The *HPGD* gene was associated with some cancers, such as colorectal, breast, prostate, and bladder. However, detailed role of 15-hydroxyprostaglandin dehydrogenase (*HPGD*) gene remain unclear in prostate cancer. The study was to investigate the correlation between rs8752 that located in the 3'untranslated region (UTR) of the 15-hydroxyprostaglandin dehydrogenase (*HPGD*) gene and prostate cancer (PCa) risk.

Materials and Methods: 109 patients from the First Affiliate Hospital of Soochow University were recruited. According to the results of pathologic diagnosis, all patients were divided into two groups (prostate cancer and benign prostatic hyperplasia). The single-nucleotide polymorphism (SNP) rs8752 was genotyped in all samples by direct sequencing.

Results: 54 prostate cancer and 55 BPH patients were included with a median age of 70.41 and 67.62 years, respectively. No statistically significant difference between two groups in patient criteria. The frequency of the GG homozygote and AG+GG genotype were 37.74% and 62.26% in 54 prostate cancer samples, while in 55BPH patients, values were 62.50% and 37.50%. Compared with the GG genotype, the combined GA+AA genotypes had a significantly higher risk of prostate cancer (OR = 2.750; 95% CI: 1.266-5.971, $p = 0.011$). Furthermore, the risk effect was obtained in subgroups of PCa patient group, the AA+AG genotypes significantly associated with the higher Gleason score samples (AA+AG vs GG: OR = 3.50, 95%CI = 1.106-11.072, $p = 0.033$) and the risk of pathological stage (AA+AG vs GG: OR = 4.00, 95%CI = 1.253-12.767, $p = 0.019$).

Conclusions: rs8752 in the 3'untranslated region (UTR) of the 15-hydroxyprostaglandin dehydrogenase (*HPGD*) gene was found to be responsible for the susceptibility to prostate cancer in Chinese individuals.

Key words: prostate cancer; *HPGD* gene; single-nucleotide polymorphism; genetic susceptibility; testosterone.

Introduction

Prostate cancer (PCa) was the second most frequently diagnosed malignant tumor. It was a major cause of morbidity and mortality among men worldwide [1] and had become the leading cause of tumor-associated health hazards in men in the United States [2]. As a complex polygenetic disease, prostate cancer was thought as the comprehensive result of age, environmental influences and genetics [3, 4]. Methods for the accurate prediction of PCa are as critical as those for its treatment. The prognoses of

patients with PCa are predicted using tumor stage, Gleason grade, and PSA level [5]. However, the clinical applications of these predictive indicators are limited. As in other cancers, an increasing number of single nucleotide polymorphisms (SNPs) have been found to be associated with PCa [3]. Thus, the use of molecular markers as predictive indicators of the pre- and post-treatment prognoses of patients with PCa has received a considerable amount of interest.

In this study, we examined the correlation

between rs8752, a SNP in the *HPGD* gene, and PCa risk. The *HPGD* gene was located at chromosome 4q34-35 and encoded 15-hydroxyprostaglandin dehydrogenase (HPGD), a short-chain non-metalloenzyme that belongs to the family of alcohol dehydrogenases. The HPGD enzyme (15-hydroxyprostaglandin dehydrogenase) was responsible for the metabolism of prostaglandins, which participate in a variety of physiologic and pathologic metabolic processes, such as inflammation, angiogenesis and pathologic responses [6-8]. It was widely distributed in various human normal tissues such as breast, prostate, gut and bladder. Recent studies had identified that the *HPGD* gene was associated with colorectal cancers, breast cancers, prostate cancers, and bladder cancers [9-13]. rs8752 was located in the 3'untranslated region (UTR) of *HPGD* at 4q34-35 and was a miR-485-5p binding site [10]. We aimed to validate the existence of the rs8752 polymorphism in prostate tissue collected from patients with PCa. In addition, we investigated the function and clinical utility of the identified rs8752 as an indicator for the prediction and assessment of PCa risk.

Material and Methods

Study subjects

A total of 109 consecutive patients were included in this study. All patients had previously undergone prostate biopsy for detection of PCa at the First Affiliate Hospital of Soochow University over the period of 2014–2016. All patients were of the Chinese Han descent. The typical indications for prostate biopsy at our hospital were: (i) total PSA level >10.0 ng/ml; (ii) total PSA level of 4-10 ng/ml, and %fPSA ratio <0.16; (iii) abnormal digital rectal examination (DRE) for prostate nodule or significant prostate asymmetry; or (iv) presence of prostate nodules detected by magnetic resonance imaging (MRI) or ultrasound. Transrectal ultrasound (TRUS)-guided biopsy was performed using a 12-core scheme. All patients were divided into two groups on the basis of the results of pathologic diagnosis. Samples of peripheral venous blood were obtained from the patients after receiving consent from the patients or their family members. This study was approved by the ethic committees at local hospitals.

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturers' protocol. Primers for rs8752 was synthesized by primer-design software (Primer Premier 5.0) using sequences obtained from the

Ensembl online database and had the following sequences: forward primer: 5'-GGCAGTCAAGGAATAACTACAG-3'; reverse primer: 5'-TAAAGTGAGCAGAGGAAAGAAAT-3'. The PCR conditions used were as follows: 95°C for 5 min for initial denaturation, 35 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by 72°C for 5 min for extension. The amplified rs8752 gene of were subjected to direct DNA sequencing (GENEWIZ, Suzhou, China).

Statistical analysis

Statistical analyses were conducted using the Statistical Package of the Social Sciences software version 19.0 (SPSS, Inc.). A two-sided $p < 0.05$ was considered to be statistically significant. Hardy-Weinberg equilibrium was analyzed using a goodness-of-fit χ^2 test. The baseline characteristics, SNP genotypes, and allele frequency distributions of the PCa group and BPH group were compared. Continuous variables were analyzed using the *t*-test, and categorical data were analyzed using the χ^2 test. Furthermore, odds ratios (ORs) and 95% confidence intervals (95% CI) from a non-conditional logistic regression model, which had been adjusted for Gleason score and pathological stage, were also used to estimate the association between rs8752 genotypes and susceptibility to PCa.

Results

HPGD gene rs8752 genotypes and clinic characteristics

The characteristics of the 109 Chinese (53 patients and 56 controls) who underwent prostate needle biopsy were included in the final analysis. Their basic characteristics were shown in Table 1. Age was matched between cases and controls ($p = 0.51$). The preoperative PSA level ($p < 0.001$) and testosterone level ($p = 0.002$) of the cases were significantly different from those of the controls. Body mass index (BMI, $p = 0.598$), however, was not significantly different between groups.

Table 1. Basic characteristics of study objects. (N = 109).

Variable	Prostate biopsy patients (N=109)		p value
	PCa group (N=54)	BPH group (N=55)	
Mean age (years) (mean \pm SD)	70.41 \pm 6.30	67.62 \pm 8.32	*0.051
Preoperative PSA (ng/mL) (mean \pm SD)	31.41 \pm 40.50	9.95 \pm 9.20	*<0.001
Testosterone level (mean \pm SD)	5.79 \pm 3.39	7.60 \pm 2.56	*0.002
Body mass index (kg/m ²)			**0.598
≤ 23	18	34	
> 23	36	21	

**t*-test; **two-sided χ^2 test

Genotyping and association analysis

All study subjects were successfully genotyped by direct sequencing. The genotypic distributions in patients fit the Hardy-Weinberg equilibrium (goodness-of-fit $\chi^2=0.0075$, $p=0.931$), indicating that the study population could be further analyzed. As shown in Table 2, the frequency of the GG homozygote and AG+GG genotype in the patient group were 37.74% and 62.26%, respectively, and were 62.50% and 37.50% in the control group. After adjusting for age through logistic regression analysis, we found significant differences in susceptibility to PCa by genotype (AG+AA vs GG: $p=0.011$, OR=2.750, 95%CI =1.266-5.971).

Subjecting the data of the PCa group to stratification analysis on the basis of Gleason score, pathological stage and testosterone levels revealed that the AA+AG genotype was associated with a significantly increased Gleason score (AA+AG vs GG: OR=3.50, 95%CI= 1.106-11.072, $p=0.033$) and the risk of advanced pathological stage (AA+AG vs GG: OR = 4.00, 95%CI = 1.253-12.767, $p = 0.019$). Thus, the A allele may be a risk factor for PCa (Table 3). No significant difference was observed in the testosterone level of PCa group.

Table 2. Genotype distribution in PCa patient and BPH patient.

Genotypes	Prostate biopsy patients (N=109)		Odds ratio (95%CI)	p value
	PCa group (N=54)	BPH group (N=55)		
HPGD gene (rs8752)				
AA+AG (N = 53)	33 (62.26%)	20 (37.74%)	1 (reference)	0.011
GG (N = 56)	21 (37.50%)	35 (62.50%)	2.750 (1.266-5.971)	

Table 3. Genotype distribution in prostate cancer patient.

Genotypes	HPGD gene (rs8752)		Odds ratio (95%CI)	p value
	AA+AG (N = 33)	GG (N = 21)		
Prostate cancer patient				
Gleason score			3.50 (1.106-11.072)	0.033
high-grade (4+3 and ≥ 8)	21	7		
low-grade (3+4 and ≤ 6)	12	14		
Pathological stage			4.00 (1.253-12.767)	0.019
pT1-2	11	14		
\geq pT3	22	7		
Testosterone level (mean \pm SD)	6.19 \pm 3.37	5.16 \pm 3.41		0.283

Discussion

PCa was a complex, heterogeneous polygenic disease that is affected by numerous external environmental and genetic factors [4]. As a family of small noncoding RNAs, MicroRNAs were often reported to be up- or down-regulated in prostate cancer and played an important role in tumorigenesis, although their specific effects on cancer remain unclear [13-15]. However, microRNA-related SNPs affected base pairing between miRNAs and their targets, hence altering miRNA-target interactions; this effect may have functional consequences for cancer risk [13-15]. By utilizing the high-resolution melting method, Liu et al. [14] found that the presence of microRNA-related SNPs (rs11902171) in *ITGA ν* were associated with a decreased risk of prostate cancer (OR 0.57, 95% CI 0.35-0.93) in 347 Chinese Han patients with PCa and 367 age-matched healthy controls. Furthermore, the association between 2,169 microRNA-related SNPs and PCa risk had been investigated in a large-scale analysis of 22,301 cases and 22,320 controls of European ancestry. Twenty-two microRNA-related SNPs within the 3'UTR of the 16 genes had been found to be associated with PCa risk, and two microRNA-related SNPs, rs1010 in *VAMP8* and rs311497 in *GMEB2*, had been found to be associated with aggressive PCa [16].

HPGD was a potential tumor suppressor in breast cancer and modulates estrogen receptor signaling; the decreased expression of HPGD had been reported in lung, breast, colorectal, and prostate cancers [17]. Moreover, the expression of *HPGD* was associated with various malignant tumor. For example, Vainio *et al.* reported that *HPGD* gene was highly expressed in a subset of androgen receptor-over expressing advanced and metastatic prostate tumors that over-express androgen receptors. This result indicated that *HPGD* and this subset of typically incurable PCa were potentially related. In addition, the expression of HPGD protein in LNCaP cells increased in response to androgen stimulation [18].

The HPGD enzyme was involved in the metabolism of prostaglandin E₂, regulated prostaglandin level by converting them to their corresponding 15-ketos, and was responsible for the biological inactivation of PGs [19]. Moreover, HPGD was involved in the stages of carcinogenesis, such as proliferation, apoptosis, migration, invasion, and angiogenesis [18, 19]. In the present study, we found that the presence of the A allele in rs8752 polymorphism of the *HPGD* gene was indicative of a higher risk of PCa ($p = 0.011$, OR = 2.750, 95%CI = 1.266-5.971). Furthermore, the AA+AG genotype was significantly associated with increased Gleason score

(AA+AG vs GG: OR=3.50, 95%CI= 1.106-11.072, $p=0.033$) and advanced pathological stage (AA+AG vs GG: OR = 4.00, 95%CI = 1.253-12.767, $p = 0.019$) in patients with PCa. The SNP rs8752 (G/A) was located at the miR-485-5p binding site and likely disrupt the interaction between miR-485-5p and HPGD. This effect could upregulate the expression of HPGD protein and may be the possible mechanism that underlies the association of rs8752 with PCa risk.

This study is the first to investigate the association of polymorphisms in the *HPGD* gene with PCa. However, it is limited by time and geographical factors: First, all subjects were recruited from a single institution and the sample size was relatively small. Only preliminary conclusions could be drawn given that selection bias may exist and the representativeness of the study sample is relatively weak. Second, functional studies, which are critical to confirm the present findings, were not performed in the present work. Third, only subjects of Chinese Han descent were included in this study. Additional studies that include different populations should be conducted because allele sequences may vary in different ethnic groups.

In conclusion, the present findings suggested that the rs8752 in *HPGD* gene was significantly associated with an increased risk of PCa. Furthermore, the AA+AG genotype was associated with a high Gleason score and the risk of advanced pathological stage in patients with PCa. Thus the A allele may be association with the prognosis of patients with PCa. Further large-scale investigations are warranted to confirm the results of the present study and to define the potential mechanisms of rs8752 in PCa.

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Competing Interests

The authors have declared that no competing interest exists.

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