Association of G/A Polymorphism, rs266882, in AREI Region of the Prostate-Specific Antigen Gene with Prostate Cancer Risk and Clinicopathological Features

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Purpose: To determine the association of prostate-specific antigen (PSA) 158A/G polymorphism with clinicopathologic characteristics of the disease and prostate cancer (PCa) risk.

Materials and Methods: Two hundred and six subjects, including 95 patients with PCa and 111 subjects with benign prostatic hyperplasia (BPH), were recruited in this study. Genotyping was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism method.

Results: Presence of GG genotype significantly increased the risk of PCa more than 2-fold compared to AG genotype (adjusted odds ratio = 2.4; $P = .03$). The percentages of G alleles of polymorphisms in patients with PCa were more than that in ones with BPH (odds ratio = 1.2; $P = .7$).

Conclusion: The GG genotype of PSA 158A/G polymorphism is a predisposing factor for PCa. But no association was observed between alleles and grade, stage, or age of diagnosis. Similarly, the rs266882 polymorphism was not associated with PSA plasma levels.

Keywords: prostate cancer, benign prostatic hyperplasia, prostate-specific antigen, genetics, polymorphism

INTRODUCTION

Prostate malignancy, the second most common cancer in men, accounts for 32% of all types of cancer and is the 4th most frequent cancer in the world. Although the main etiological cause of the disease is still unclear, it is known to be a multifactorial disease with a genetic component.[1]

Prostate-specific antigen (PSA), kallikrein-related peptidase 3, is a serine protease that is part of the kallikrein superfamily, mainly produced by prostate cells.[2] It has widely been used as a diagnostic marker of the prostate cancer (PCa) since the early 1990s. Serum level of PSA increases with benign prostatic conditions, but a higher surge of it is seen with PCa and that is why it is the most commonly used clinical biomarker for PCa diagnosis and follow-up.

Early detection through serum testing for PSA and improved procedures for surgical intervention and radiation therapy have significantly reduced the number of fatalities; however, there is still no effective cure for men with advanced disease. Therefore, much research has been dedicated to identifying prognostic markers that distinguish indolent versus aggressive forms of PCa.[3]

Recent technological advances have allowed investigators to interrogate thousands of single nucleotide polymorphisms (SNPs) across the genome to search for specific genetic markers associated with risk of developing this complex disease. The genes that may effect the cellular proliferation of the prostate gland are not well-known, but the gene for PSA has been the focus of several studies.[3]

The PSA gene is mapped on chromosome 19 (19q13.4).[4] The main regulators of PSA expression at the gene level are androgens. Production of PSA is mediated through binding of the androgen receptor (AR) to androgen response elements (ARE) in the promoter region of the PSA gene.[4] After binding of androgen to the AR, a cascade of cellular events happens, which causes the movement of AR to the nucleus, where it binds to AREs in the promoters of target genes to initiate PSA gene transcription. At least three AREs have been identified on the PSA gene promoter region.[5] The one nearest to the transcription start site is referred to as AREI.

Several polymorphisms have been reported which can influence the serum levels of PSA.[6] A recent study mentioned using this SNP as a predictive marker.[7] The PSA gene contains a polymorphism, substitution of a Guanine (G) by an Adenine (A), locating 158 bases upstream of the transcription site, which have transcriptional control role for AR-regulated expression. Therefore, this SNP has been related to serum levels of PSA.[3,6,8-10]

With regard to the role of PSA G158A SNP, a number of surveys have been focused on the association between this SNP, serum level of PSA, and PCa susceptibility in different populations. Since the PSA test in screening patients who had to undergo prostate biopsy encounters many challenges, using the PSA 158 A/G polymorphism genotypes combined with the result of serum PSA is a method for decreasing unnecessary biopsies.

This study investigated any relation between PSA 158 G/A polymorphism and risk of PCa, tumor stage and grade, and serum level of PSA for the first time in a group of Iranian patients with PCa.

MATERIALS AND METHODS

This case-control study consisted of 206 subjects, including 95 patients with PCa and 111 controls with benign prostatic hyperplasia (BPH), who were recruited from department of urology of Shahid Labbafinejad Medical Center, Tehran, Iran, between February 2010 and April 2011.

The present study was approved by the Urology and Nephrology Research Center Review Board (www.unrc.ir) affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran. After a written informed consent was obtained from each participant, a structured questionnaire was completed to gather information on potential risk factors, including age, body mass index, history of PCa in 1st-degree relatives, blood group, and total and free PSA level.

Blood samples were taken using sample tubes containing ethylenediaminetetraacetic acid (EDTA), and DNA extraction was performed according to the standard protocol.[11] All purified DNA samples were protected at +4 °C.

Open laparoscopic or radical prostatectomy was used to determine the tumor grade and stage and perineural and vascular invasion. Tumor stage and grade are determined according to 1997 TNM guideline[12] and Gleason grading

system, from 1 (most differentiated) to 5 (least differentiated), respectively.

Control groups with BPH had the following criteria in order to rule out the false diagnosis of PCa: 1) Either serum PSA < 4.0 ng/mL or negative pathological report of the prostate biopsy with serum PSA > 4.0 ng/mL; 2) Normal digital rectal examination; and 3) Negative pathological report of malignancy in resected prostatic tissues from open surgical prostatectomy. Exclusion criteria were family history of PCa in control group, consuming any PSA decreasing medication, hormone therapy, orchiectomy, and non-adenocarcinoma of the prostate.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis

Polymerase chain reaction (PCR) was performed using 20 pmol of forward primer (5'-TTG TAT GAA GAA TCG GGG ATC GT-3') and reverse primer (5'- TCC CCC AGG AGC CCT ATA AAA-3') in a 50-µL reaction volume containing 2 mM $MgCl_2$. The PCR program was 94 °C for 10 min followed by 35 cycles at 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 40 sec with a final extension at 72 °C for 10 min. After PCR, a restriction fragment length polymorphism (RFLP) method was used with NheI restriction enzyme (Roche), then, separated on a 2% agarose gel. The three genotypes were determined according to their size: AA (300 bp), AG (150, 300 bp), and GG (150 bp) (Figure 1).

The validity of these PCR-RFLP analyses was confirmed by direct sequencing of several PCR samples with each genotype (Figure 2).

Statistical Analyses

Data analysis was performed by STATA (V.11) software. Chi-Square test and Student's *t* test were used for evaluating the association between categorical variables and mean values of variables, respectively. Serum levels of PSA were log transformed, and linear regression model was fitted to estimate the effect of polymorphisms on serum total PSA. Multivariable logistic regression models were used to determine odds ratio (OR) for categorical dependent variables adjusting for potential confounders, such as age, family history of cancer, and grade and stage of tumor. Results were considered significant if *P* value was less than .05.

Using the PS Power and Sample Size Calculations software, Version 3.0, January 2009 (http://biostat.mc.vanderbilt.edu/PowerSampleSize) (William D. Dupont and Walton D. Plummer, 2009), our sample size in different subgroups provided maximum power of 0.79 (at significance level of .05) to detect an OR of 2.4.

RESULTS

Subjects' Characteristics

The average ± standard deviation age of the patients was 67 ± 8.7 years (range, 47 to 89 years). Total PSA levels were significantly higher in patients than in controls. The mean serum total PSA was 467 ng/dL more than that in patients with BPH ($P < .001$). In this group, the percentage of positive family history of cancer was significantly greater than that in patients with BPH (17.4% versus 1%; $P < .001$). In overall, the education level of patients with PCa was significantly higher than that of patients with BPH ($P < .001$; Table 1)

Among patients, 31% had poorly differentiated tumors (Gleason score ≥ 7) and 21% had advanced tumor stage at diagnosis (TNM stage III and IV) (Table 2).

PSA and Risk of PCa

Having considered the AG genotype as the reference group between PSA polymorphisms, patients with GG genotype had 1.2-fold greater risk of developing high-grade (Gleason Score ≥ 7) PCa (OR = 1.2), but it was not statistically significant ($P = .7$).

The OR between GG and AA genotypes and stages of PCa was 0.75 and 0.78, respectively, indicating that these polymorphisms had a protective effect on the high level of disease stages compared to AG genotype as the reference group. But the adjusted OR was slightly more than null value (Table 3). Results showed that there was not a statistical association between polymorphisms and vascular or perineural invasion.

The mean age in those who had different polymorphisms indicated that patients with GG and AA genotypes had higher mean ages compared to the reference group (AG genotype). It means that patients with these polymorphisms could get the PCa at higher ages compared to those without this polymorphism. In other words, it can be said that poly-

morphisms did not have statistically significant effects on reducing the onset and also the time of diagnosis of PCa. The mean total PSA in those who had GG and AA genotypes was around 1.2 and 0.2 ng/dL more than that in those with AG genotype (P = .9 and P = .2, respectively); adjusting for other factors did not change this difference considerably (Table 3).

PSA Genotypes and PCa

There were no significant differences between the patient and control groups for any of the PSA ARE-I genotypes. However, some kinds of polymorphisms, like GG genotype, had more frequencies in patients with PCa (32%) than in BPH group (22.6%). The OR between GG polymorphism and AG polymorphism (reference) was 1.76. Having adjusted for potential confounders, such as age and stage and grade of tumor, this gap became more prominent and statistically significant (OR = 2.4; P = .03); it means 2.4-fold greater risk of developing PCa in patients with GG geno-

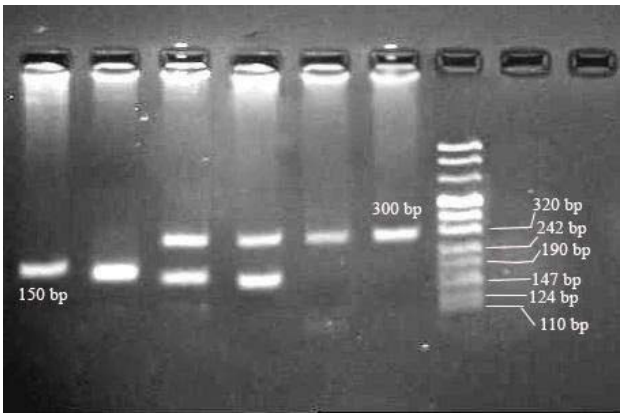

Figure 1. Representative screening for the prostate-specific antigen genotypes.
Left to right: line 1: GG, line 2: GG, line 3: AG, line 4: AG, line 5: AA, and line 6: AA

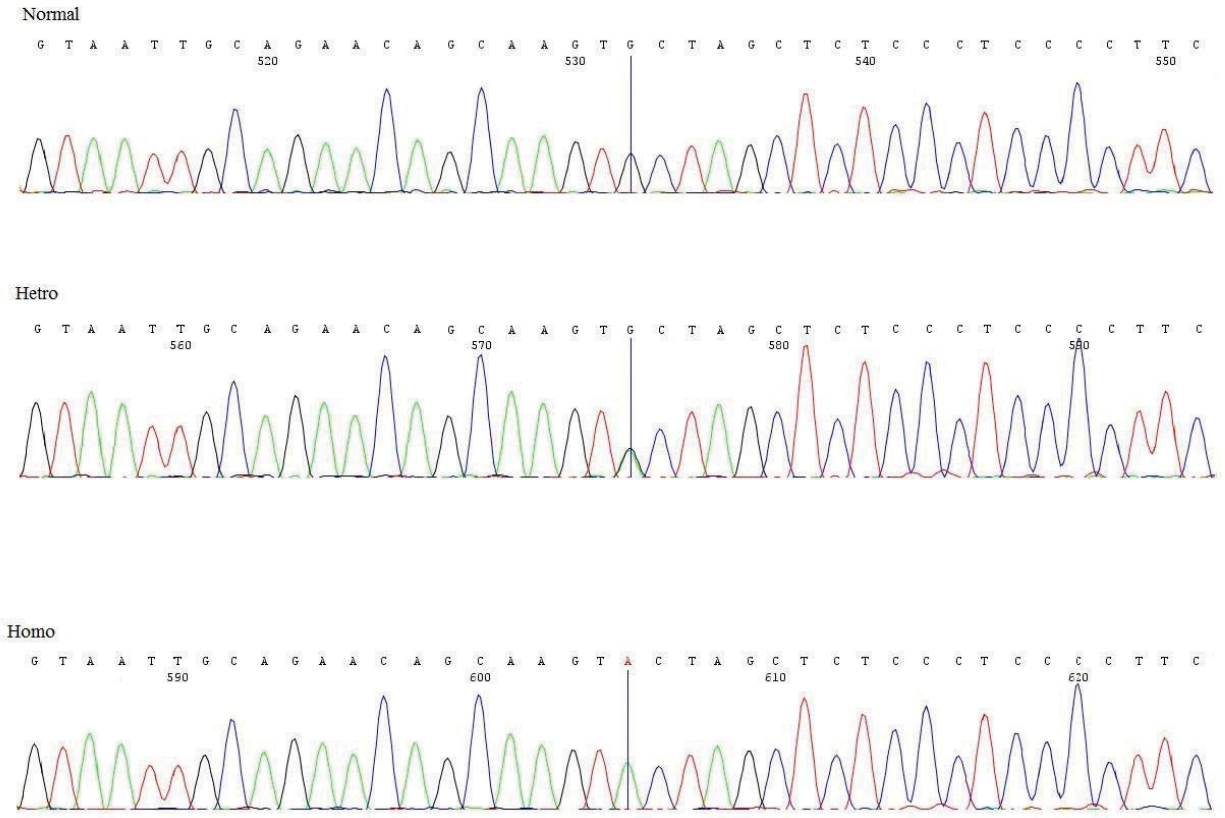

Figure 2. A sample of sequencing result for confirming prostate-specific antigen genotyping.
Arrow is the position of rs266882.

Table 1. Comparison of main variables in patients with prostate cancer and benign prostatic hyperplasia.*

Variables	Cancer		Benign prostatic hyperplasia		*P*
	number	percent	number	percent	
Smoking					
Never	63	67.7	88	83	
Stop	11	11.8	9	8.5	.029
Currently	19	20.4	9	8.5	
Drug history (finasteride)	14	14.7	55	49.5	< .001
Marital status	91	97.8	104	98	.89
Family history of prostate cancer	16	17.4	1	1	< .001
> 40 year's son (> 2 sons)	0	0	6	15.4	< .001
Education					
Illiterate	15	16.3	32	31.7	
Primary	36	39	4	53.5	< .001
Diploma	23	25	11	10.9	
Academic	18	19.6	4	4	
Blood group					
O	32	36.8	31	36	
A	39	45	34	39.5	.78
B	15	17.2	20	23.3	
AB	1	1.1	1	1.2	
Other disease					
None	75	78.9	102	92	
CVD	14	14.7	7	6.3	
HLP	2	2.1	0	0	.76
Breast cancer	1	1.1	0	0	
DM	3	3.2	2	1.8	
Abnormal PSA Ratio	45	50.6	14	17.9	< .001
	Mean (SD)		Mean (SD)		
Total PSA	474.4 (4299)		7.3 (7.11)		< .001
Age	63.2 (7.34)		70.3 (8.53)		< .001
Body mass index*	25 (3.11)		25.2 (3.50)		.67

*CVD indicates cardiovascular disease; HLP, hyperlipidemia; DM, diabetes mellitus; PSA, prostate-specific antigen; and SD, standard deviation.

type compared to AG genotype. No significant association was observed between AA genotype and PCa (Table 4).

The Allele Frequency of PSA Gene

Patients with PCa had a greater percentage of G alleles than the control BPH ones; however, it was not statistically significant (Table 4). The OR between G allele and development of PCa was 1.22, which means 1.2-fold greater risk of developing PCa compared to A allele, but that was not statistically significant.

Table 2. Frequency of different stages and grades of prostate cancer in the study population.

Grade and Stage	Number	Percent
Grade		
Gleason < 7	58	70.65
Gleason > 7	26	29.35
TNM Stage		
I and II	63	78.16
III and IV	17	21.84

Table 3. Relationship between AREI (PSA-158 G/A) genotype and prostate cancer stage and grade, total PSA level, perineural and vascular invasion of cancer, and age at diagnosis.*

Prostate Cancer	Overall *P*	AA Genotype	AG Genotype	GG Genotype
Stage	0.9 (Based on Chi-Square)			
Stages 1and 2, n (%)		20 (32)	22 (35)	21 (33)
Stages 3 and 4, n (%)		5 (29)	7 (41)	5 (29)
OR (*P*)				
Crude		0.78 (.7)	1	0.75 (.7)
Adjusted		1.04 (.9)	1	1.04 (.9)
Grade	0.9 (Based on Fisher's Exact test)			
Gleason < 7, n (%)		18 (31)	22 (37.9)	18 (31)
Gleason ≥ 7, n (%)		8 (30.7)	9 (34.6)	9 (34.6)
OR (*P*)				
Crude		1.08 (.9)	1	1.2 (.7)
Adjusted		1.51 (.5)	1	1.80 (.3)
Vascular invasion	0.4 (Based on Fisher's Exact test)			
Negative, n (%)		20 (31)	24 (37)	20 (31)
Positive, n (%)		1 (25)	3 (75)	0 (0)
OR (*P*)				
Crude		0.4 (.4)	1	-
Adjusted		0.6 (.7)	1	-
Perineural invasion	0.5 (Based on Fisher's Exact test)			
Negative, n (%)		1(12)	4 (50)	3 (37)
Positive, n (%)		24 (34.8)	26 (37.7)	19 (27)
OR (*P*)				
Crude		3.7 (.2)	1	0.97 (.9)
Adjusted		5.8 (.15)	1	1.2 (.8)
Age of diagnosis	0.7 (Based on ANOVA test)			
Number		58	77	49
Mean age (SD)		67.9 (8.9)	66.8 (9)	66.9 (7.9)
Mean difference (*P*)				
Crude		0.9 (.6)	0	3.3 (.09)
Adjusted		1.9 (.3)	0	3.9 (.05)
Total PSA level	0.6			
Number		46	63	45
Mean PSA (SD)		12 (15.6)	13 (17)	15.7 (24)
Mean difference				
Crude		0.2 (.2)	0	1.2 (.9)
Adjusted		0.2 (.2)	0	1.06 (.9)

*OR indicates odds ratio; SD, standard deviation; and PSA, prostate-specific antigen.

DISCUSSION

In previous studies, it was demonstrated that PSA gene homozygous for the G allele is associated with higher serum PSA concentration and some other tumors[3,6,13-15] whereas the homozygous for the A allele is associated with a higher level of total PSA in men with or without PCa.[9,10] In a case-control analysis of 500 Caucasian cases and 676 controls, there was no significant association between rs266882 and total and free PSA plasma levels.[16] In the present study, the total PSA levels in those who had GG genotypes

Table 4. Genotype and allele frequency and odds ratio between different polymorphisms in patients with prostate cancer and benign prostatic hyperplasia.[£]

Polymorphism	Cancer, no (%)	Benign prostatic hyperplasia, no (%)	Overall *P** 95%CI	Odds Ratio (*P*)	
				Crude	Adjusted
PSA Polymorphism					
AG	33 (38)	50 (47)	.27	1	1
GG	28 (32.2)	24 (22.6)		1.76 (.1)	2.4 (.03)
AA	26 (29.9)	32 (30.2)		1.23 (.5)	1.54 (.3)
Polymorphism Allele					
G	89 (51)	98 (46.2)	.3		
A	85 (49)	114 (53.8)			

*Based on Chi-Square test.
[£]CI indicates confidence interval; and PSA, prostate-specific antigen.

was 1.2 ng/dL more than that in those who had A/G, but like Turkish and Japanese population, it was not statistically significant.[17]

There are two different reports on the association of this SNP with serum PSA level in Japanese population. One showed association between higher PSA level and GG genotypes and the other showed no association between -158 G/A polymorphism and the serum PSA level.[18,19]

A functional study has indicated that the PSA -158 G/A polymorphism has no functional effect on the activity of the PSA promoter in vitro and in vivo,[20] which supports our findings.

It has been proposed that rs266882 polymorphism is associated with disease stage or grade.[6,9,21-26] The GG genotype in Taiwanese is associated with larger tumor volume and higher pathological stage.[14] A large case-control study of Caucasian-Australians (821 patients and 734 controls) found a significant association between the G allele and stage III to IV tumors,[15] whereas a larger case-control study on a white American population found that the GG genotype is associated with the lower stage of the disease.[22] In this study, there was no significant association between rs266882 and high grade or advanced stage of the disease. The GG genotype was associated with a 1.2-fold greater risk of developing high-grade (Gleason score ≥ 7) PCa (OR = 1.2; P = .7). Another study which investigated the association of rs266882 SNP with PCa risk found that this SNP was not associated with cancer grade.[27]

Several investigators have studied the relation of the rs266882 polymorphism with PCa susceptibility. An initial case-control study of non-Hispanic white men (57 patients and 156 controls) reported a positive association of homozygous variant GG genotype of rs266882 with a three-fold risk of advanced cancer suggesting that the PSA promoter activity under the control of allelic variation is an androgen-dependent event.[28] Subsequent studies on this polymorphism also reported increased risk of PCa with the G allele in Taiwan (122 patients and 84 controls),[14] Scotland (97 patients and 144 controls),[24] and Turkey (49 patients and 47 controls). In a larger sibling-based case-control study on a predominantly white American population, the association between PCa susceptibility and the G allele was confirmed.[22]

A case-control study on patients with Turkish origin reported that ARE-I PSA polymorphism has a significant influence on PCa and BPH risk.[29] Another study in 2008 found that the GG genotype carriers have a higher risk of developing PCa than those with the AG and AA genotypes.[30] Other studies refuted these findings and found an association between the A allele and increased risk of Pca.[10,23] Similarly, a larger study of Australian Caucasian men (209 patients and 223 controls) found a three-fold risk of PCa with the AA allele.[25]

Our study showed that the presence of GG genotype signifi-

cantly increased the risk of cancer more than 2-fold compared to AG genotype (Adjusted OR = 2.4; P = .03), which is in line with several studies.[18,31,32] The percentages of G alleles of polymorphisms in patients with PCa were more than that in those with BPH. However, we found a 70% increased risk of PCa with GG polymorphism.

In patients with PCa, allele frequency of the PSA polymorphism at position -158 (A 0.49, G 0.51) was similar to African American men (A 0.52, G 0.48), non-Hispanic white men (A 0.48, G 0.52), and Hispanic white men (A 0.37, G 0.63),[9] and different from Turkish (A 0.63, G 0.36) and Japanese men (A 0.22 G 0.78).

The exact reason why previous studies and ours on the PSA polymorphisms provided such inconsistent results cannot be fully understood, but some factors, such as ethnicity, life-style, and/or gene–gene, and gene–environment interactions may explain this issue. Because of the small sample size of this study, our results clearly need to be confirmed by studies with larger number of advanced cases.

CONCLUSION

We found a significant positive association between the GG genotype of PSA polymorphism and PCa risk compared to AG genotype. But no association was observed between alleles and grade, stage, or age of diagnosis. Similarly, the rs266882 polymorphism was not associated with PSA plasma levels and cancer risk. The difference in results for PSA AREI polymorphisms between studies may be minimized using larger study groups.

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CONFLICT OF INTEREST

None declared.

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