

ARE-I Polymorphism on PSA Gene in Prostate Cancer Patients of a Turkish Population

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Abstract. *Background:* The gene for kallikrein-like serin protease, prostate-specific antigen (PSA), has been a candidate gene in several studies. It is known that androgens are important in the proliferation and development of prostate gland and they are the main regulators of PSA expression. The polymorphism of the ARE-I locus on the PSA gene was studied in prostate cancer patients to determine a possible relationship of that locus to prostate cancer risk. *Patients and Methods:* Forty-nine prostate cancer patients and forty-seven healthy control subjects were compared. Total and free PSA levels were measured by an enzymatic immunassay method. PSA ARE-I polymorphism analyses were performed using a previously described PCR-restriction fragment length polymorphism (RFLP) method. *Results:* There were no significant differences between the control and patient groups for any of the PSA-AREI genotypes, but the G allele carriers had a 2-fold higher risk of developing advanced prostate cancer. *Conclusion:* G allele carriers have a higher risk of developing advanced prostate cancer. With further research, such PSA-AREI polymorphism analyses may help in follow-up and in deciding the prognosis of prostate cancer patients.

Prostate malignancy accounts for 32% of all types of cancer and is the fourth most frequent cancer in the world (1). It is known to develop from the acinar epithelium and 70% of cases from the peripheral zone of the gland (1). Although the main etiological cause of the disease is still unknown, it is known to be a multifactorial disease with a genetic component (2).

Androgens are important in the proliferation and development of the prostate gland from early fetal life.

Huggins and Hodges have shown that after orchiectomy and estrogen treatment, metastases of prostate cancer regressed and their study proved the enhancing effects of androgens in prostate cancer (3).

Testosterone is converted to its more active form, dihydrotestosterone, by the catalysis of the enzyme 5α-reductase in the prostate gland and the relationship of 5α-reductase activity to prostate cancer has been investigated (4-6). It is thought that differences in prostate cancer incidence in different populations might be related to the levels of dihydrotestosterone; for example, Japanese men generally have low 5α-reductase activity and also lower prostate cancer incidence (3).

The degree of differentiation and the tumor volume are the most important factors that determine the aggressivity of the cancer; the usual system for classifying the prostate differentiation level is the Gleason scoring system, which is shown in Table I (7).

Prostate cancer cases have decreased in the last decade because of practical screening methods using prostate-specific antigen (PSA) as the main parameter (1, 2). PSA is a 33 kDa glycoprotein containing a polypeptide of 240 amino acids that is mainly synthesized by the epithelial cells of the prostate gland (8-10). Serum PSA levels can be increased in benign prostatic cases because increased synthesis of PSA and/or structural disorders of the prostate may cause leakage into the general circulation, but it is more increased in malignancy and is useful for diagnosis or to determine the follow-up or prognosis of prostate cancer patients (11, 12). Although PSA has been used as a tumor marker for a long time, its role in prostate pathologies has not yet been precisely determined. The genes that may effect the cellular proliferation of the prostate gland are not well known, but the gene for kallikrein-like serin protease, PSA, has been the focus of several studies (11, 13, 14). The PSA gene is situated on chromosome 19 (19q13.4) (9, 15). The main regulators of PSA expression at the gene level are androgens (12), and androgen receptors (AR) change their

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Table I. Gleason scoring system.

| Gleason scores (in total) | Level of differentiation |
|---------------------------|-------------------------------|
| 2-4 | Well-differentiated |
| 4-6 | Intermediately differentiated |
| 7 | Intermediate-undifferentiated |
| 8-10 | Undifferentiated |

conformation after binding to these ligands, travel into the nucleus and bind to specific nucleotide strands on the promotor regions of their specific genes. The dihydroandrostenedion-androgen receptor complex (DHT-AR) is one of the main regulators of gene transcription of specific promotor regions of the PSA gene, enhancing PSA gene transcription (16, 17). The binding sites on the nucleotide are known as androgen-response elements (ARE) (12, 16, 18, 19). Cleutjens *et al.* have determined at least three ARE (ARE-I, ARE-II, ARE-III) on the PSA gene promoter region (20). Xue *et al.* defined a single nucleotide polymorphism on the ARE-I sequence of the PSA gene that was related to the risk of developing prostate cancer (16).

The polymorphism of the ARE-I locus of the PSA gene and PSA levels in prostate cancer patients were investigated to determine a possible relationship of that locus to prostate cancer risk.

Patients and Methods

All patients (n:49) were followed-up by the Uskudar State Hospital. The control group subjects (n:47) were chosen from healthy men that were more than 50 years old. All of the subjects were diagnosed clinically and also confirmed by investigation of their prostatic specimens. For DNA isolation, blood samples were taken using sample tubes containing EDTA and isolation was performed using precipitation method and all purified DNA samples were protected at +4°C.

Fifty μ L PCR reaction mixture containing 1 U Taq polymerase (Promega), 0.3 μ L of each primer (AR1-5 TTG TAT GAA GAA TCG GGG ATC GT-3', AR2- 5'- TCC CCC AGG AGC CCT ATA AAA-3') (MBI Fermentas), 50 mM KCl, 10 mM Tris-HCl, 4 mM MgCl₂, 0.1 mM deoxynucleoside triphosphate (dNTP) (MBI Fermentas) was used with 500 ng of DNA (14). The PCR reaction was carried out as follows: first the denaturation step at 94°C for 10 min; then 35 cycles of denaturation (1 min at 94°C); annealing (1 min at 59°C and extension (40 sec at 72°C) and finally extension at 72°C for 10 min. The final product was expected to be 300 bp.

After PCR, a restriction fragment length polymorphism (RFLP) method was used with NheI restriction enzyme (MBI Fermentas) and all the final samples were evaluated by agarose gel electrophoresis (2%). After this restriction step, there were three possible band forms on the gel electrophoresis: 300 bp for AA, 150 bp for GG and both for the heterozygote AG genotypes.

The PSA levels were measured by an enzymatic immunoassay method (Elecsys 2010 Hitachi Boehringer, Mannheim, Germany).

Table II. Characteristics of the patient and control groups.

| | Control group (n:47) | Patient group (n:49) |
|---|-------------------------|-------------------------|
| Age (x±SD) | 63.00±8.835 | 66.00±8.14 |
| Body mass index (kg/m ²) (x±SD) | 26.25±3.03 | 26.07±2.70 |
| Total-PSA (ng/dL) (x±SD) | 1.26±0.92 | 89.43±253.52* |
| Free-PSA (ng/dL) (x±SD) | 0.30±0.29 | 4.25±9.02* |
| Gleason score | - | 6.80±1.13 |

The intergroup significance levels were evaluated by student *t*-test. PSA: Prostate Specific Antigen, **p*<0.05.

Table III. The distribution of PSA ARE-I genotypes and allele frequencies in patient and control groups.

| Group | Control (n:47) | Patient (n:49) |
|-----------------|-------------------|-------------------|
| Genotype | | |
| AA | 28 (59.6%) | 22 (44.9%) |
| GG | 4 (8.5%) | 9 (18.4%) |
| AG | 15 (31.9%) | 18 (36.7%) |
| Allele | | |
| A | 71 (75.53%) | 62 (63.20%) |
| G | 23 (24.46%) | 36 (36.70%) |

The statistical analyses were performed with the SPSS 10.0 program using Chi-square, Fisher's and Student's *t*-tests.

Results

The characteristics of control and patient groups are given in Table II. There was no significant difference between ages or body mass indices (BMI) of the two groups.

The total PSA and free PSA levels were significantly higher in the patient group than in control cases as expected, and there were also significant differences in total and free PSA levels in all the genotype groups between the control and patient groups. The distributions of the PSA ARE-I genotypes and the allele frequencies in the patient and control groups are given in Table III. There were no significant differences between the control and patient groups for any of the PSA-AREI genotypes (*p*>0.05).

The ARE-I PSA genotype distribution of the patient group according to the Gleason Score System using the cut-off level of 7 are given in Table IV. G allele carriers had a 2-fold greater risk of developing advanced (Gleason \geq 7) prostate cancer.

The total PSA levels were higher in the prostate cancer patients carrying the G allele than in those without the G allele (Figure 1), but the difference did not reach significance (*p*=0.143).

Table IV. *ARE-I PSA genotype distribution [n (%)] of the patient group according to the Gleason score (cut-off of Gleason score 7).*

| Genotype | AG | GG | AA | GG+AG |
|------------------------|-----------|----------|-----------|-----------|
| Patient group n (%) | 18 (36.7) | 9 (18.4) | 22 (44.9) | 27 (55.1) |
| Gleason<7 | 6 (33.3) | 4 (44.4) | 12 (54.5) | 10 (45.5) |
| Gleason≥7 | 12 (66.7) | 5 (55.6) | 10 (45.5) | 17 (63.0) |

OR (95% CI): 2.04 (0.648-6.42).

Discussion

The few previous studies on the ARE-I gene polymorphism have produced differing results. Xue *et al.* investigated the relationship between the G and A alleles at the 158 position on the PSA ARE-I gene promoter in prostate cancer and found that the G allele was more frequent in prostate cancer patients; they also found that the GG genotype significantly (60%) increased prostate cancer risk (16). In contrast, Medeiros *et al.* found that the AA genotype was more frequent in prostate cancer patients than control subjects (21). In some studies, no statistically significant difference was found between any clinical and endocrinological parameters and the PSA-AREI genotypes (22). In the present results, although the G allele seemed to be more frequent in the patient group, there were no statistically significant differences between the patient and control groups for any alleles or genotypes (Table III).

In most of the previous studies, it was demonstrated that there was no difference in serum PSA levels between any allele and/or genotype groups (8,11). However in some studies it was shown that people with an AA genotype had increased PSA levels compared to other genotypes (16, 21). It was thus concluded that the A allele increased the expression level of the PSA protein (21, 23). In the present, the G allele was related to higher levels of PSA, but this was not proven statistically ($p>0.05$) (Figure 1). The significant increment in free PSA levels in the AA ($p=0.011$) and GG ($p=0.041$) patients than in the corresponding control cases was thought to be related to the general increment of PSA levels in prostate cancer patients compared to the control group.

Dos Santos *et al.* found that the GG genotype carriers had a higher risk of developing prostate cancer than those with the AG and AA genotypes (24). Xue *et al.* found that the GG genotype patients had a 3-fold higher risk of developing advanced prostate cancer (25). The present results were compatible with theirs, since the G allele carriers had a 2-fold higher risk of advanced prostate cancer according to the

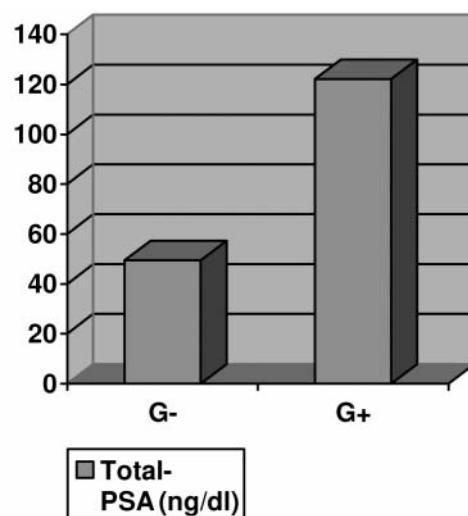


Figure 1. Total PSA levels in G+ (49.36 ± 135.96) and G- (122.08 ± 318.21) patients ($p=0.143$).

Gleason score system (Table IV). Gsur *et al.* investigated the relationship between PSA polymorphisms and Gleason scores and found that the patient group with high Gleason scores (≥ 7) had AG and GG genotypes more frequently than did the low-score (<7) patients (14). In contrast, Lai *et al.* demonstrated that the AA genotype increased the prostate cancer risk three-fold (26).

The present study was the first in a Turkish population. The difference in results for PSA ARE-I polymorphisms between studies may be minimized by using larger study groups. Further evaluations may illuminate the effects of polymorphisms on both the PSA levels and the stages of prostate cancer and thus help the follow-up and prognostic determinations for prostate cancer patients.

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