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ORIGINAL ARTICLE

POLYMORPHISMS OF HPC2/ELAC2 AND SRD5A2 (5α-Reductase Type II) GENES IN PROSTATE CANCER

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ABSTRACT

Prostate cancer is the proliferation of malignant cells in the prostate gland. The HPC2/ELAC2 gene on chromosome 17p11.2 and SRD5A2 gene on chromosome 2p22-23 are predisposing genetic factors. We examined the relationship between Ser217Leu and Ala541Thr polymorphisms of the former gene, and Ala49Thr and Val89Leu polymorphisms of the latter gene to prostate cancer in Turkish men, using the polymerase chain reaction (PCR) method and appropriate restriction enzymes. The HPC2/ ELAC2 gene Ser217-Leu and SRD5A2 gene Ala49Thr polymorphisms were associated with an increased risk of prostate cancer in Turkish men [for the HPC2/ELAC2 gene Ser217Leu polymorphism: odds ratio (OR) 2.7; confidence interval 95% (CI 95%) 1.6-4.8; p 0.000<0.05, and for the SRD5A2 gene Ala49Thr polymorphism: OR 2.4; CI 95% 1.2-4.9; p 0.004<0.05].

Key words: Prostate cancer, HPC2/ELAC2 gene, SRD5A2 gene, Polymorphism

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INTRODUCTION

Prostate cancer results from alteration of the balance between cell proliferation and cell death in the prostate gland. This cancer accounts for 32% of total cancers in men [1]. Prostate cancer is caused by hormonal, dietary, environmental and genetic factors. The genes HPC2/ ELAC2, SRD5A2 (5α-reductase type II), HPC1 (hereditary prostate cancer 1), AR (androgen receptor), PSA (prostate specific antigen) are among the genes responsible for it [1].

The HPC2/ELAC2 gene belongs to a family of prostate cancer susceptibility genes which encode an evolutionarily conserved metal-dependent hydrolase [1] that is 3' processing endoribonuclease and to interact a component of the mitotic apparatus is a γ tubulin. Thus, it is suggested that the ELAC2 gene is associated with cell cycle control [2,3]. The HPC1/ELAC2 protein contains 826 amino acids. Its gene is located on chromosome 17p11.2 and contains 24 exons. The are includes two recurrent missense mutations (Ser217Leu and Ala541Thr) [4].

Steroid 5α-reductase type II irreversibly converts testosterone to dihydrotestosterone in prostatic cells [5,6]. Steroid 5α-reductase type II consists of 254 amino acids [7]. Its gene SRD5A2 contains five exons [8] and 56.4 kb and is located on chromosome 2p22-23. The single nucleotide polymorphisms in this gene that have been studied in relation to prostate cancer are Ala49Thr and Val89Leu [9]. We have investigated the

relationship between Ser217Leu and Ala541Thr polymorphisms in the HPC2/ELAC2 gene and Ala49Thr and Val89Leu polymorphisms in the SRD5A2 gene in Turkish men with prostate cancer.

MATERIALS AND METHODS

We studied 64 prostate cancer patients (mean age 65.24 ± 8.63) who had adenocarcinoma and 34 healthy controls (mean age 49.83 ± 18.29) without a family history of cancer. All came from the Çukurova region of southern Turkey. Their clinical data were on record at Çukurova University, Faculty of Medicine Hospitals, Adana, Turkey. The research protocol was approved by the Ethical Committee of Çukurova University, Faculty of Medicine. The obtained venous blood samples were collected into CBC tubes and stored at 4°C. DNA was extracted from whole blood samples using the salting-out procedure [8].

For the Ser217Leu polymorphism in the HPC2/ELAC2 gene, the polymerase chain reaction (PCR) amplification used primers and conditions as described in [4]. The 276 bp PCR product was digested overnight with *Taq*Iα at 65°C. Subsequently, genotypes were visualized on a 10% polyacrylamide gel.

For the Ala541Thr polymorphism of the HPC2/ELAC2 gene, the PCR amplification used primers and conditions as previously described in [4]. The 419 bp PCR product was digested for 3 hours with *Fnu*4HI at 37°C. Genotypes were visualized on a 10% polyacrylamide gel.

For the Ala49Thr and Val89Leu polymorphisms in the SRD5A2 gene, the PC reactions were carried out using a master mix that consisted of 2.5 μL of 10X PCR buffer, 0.2 μL of 25 mM dNTPs, 0.2 μL of each 20mM primer, and 1 μL Taq polymerase (5U/μL) and ddH_2O, for a total reaction volume of 25 μL. Nested PC reactions were used to amplify the regions containing the polymorphisms of interest. The forward (5'-TGG CCT TGT ACG TCG CGA AG-3') and reverse (5'-AGC AGG GCA GTG CGC TGC ACT-3') primers were used to amplify the region containing the Ala49Thr and Val89Leu polymorphisms. These were amplified with a 35-cycle protocol at 95°C for 3 min. for one cycle; 96°C for 30 seconds, 62°C for 1 min., and 72°C for 1 min. for 35 cycles; followed by an elongation cycle at 72°C for 10 min. Both primers amplified with a 261 bp region. Finally, 5 μL of the final 276 bp PCR product was digested with *Mwo*I and *Rsa*I overnight at 65°C. Genotypes were visualized on a 10% polyacrylamide gel and stained in ethidium bromide for 5 min., visualized using the UviTech Gel documentation system and then evaluated [9].

Statistical analyses of the data were performed by a SPSS (11.5 version) program. The Pearson Chi-Square test was used to compare the ratios, and values of $p < 0.05$ were considered statistically significant.

RESULTS

The allele frequencies of Ser217Leu and Ala541Thr polymorphisms at the HPC2/ELAC2 gene in the cancer and the control subjects were in Hardy-Weinberg equilibrium. The allele frequencies of Ser217 and Leu217 in the cancer patients were 52.3 and 47.7%, respectively, and for the control groups were 82.4 and 17.6%, respectively (Table 1). The difference between the patients and controls for the Ser217Leu polymorphism was significant. This shows that there was a noteworthy relation at risk of prostate cancer between cases and controls for the HPC2/ELAC2 gene Ser217Leu polymorphism [odds ratio (OR) 2.7; confidence interval 95% (CI 95%) 1.6-4.8; p 0.000<0.05].

Allele frequencies for Ala541 and Thr541 in the patients were 95.3 and 4.7%, respectively, and 97.1 and 2.9% respectively, for the controls (Table 2). There was no difference between the patient and control groups regarding the Ala541Thr polymorphism (OR 1.4; CI 95% 0.4-0.7; p 0.556, $p > 0.05$).

The genotype frequencies for the Ala49Thr and Val89 Leu polymorphisms in the SRD5A2 gene were in Hardy-Weinberg equilibrium. Allele frequencies for Ala49 and Thr49 in the patients were 71.9 and 28.1%, respectively, and those for both polymorphisms in the controls were 89.7 and 10.3%, respectively. We demonstrated a remarkable difference between the patients and controls for the Ala49Thr polymorphism (OR 2.4; CI 95% 1.2-4.9; p 0.004 <0.05). For the Thr49 allele, there was an obvious, significantly higher risk in the patients.

For the SRD5A2 gene Val89Leu polymorphism, there was not any statistical difference (OR 1.2; CI 95% 0.8-1.8; p 0.248, $p > 0.05$). The frequencies, according to our study, of the Val89 and Leu89 genotypes for patients and controls are shown in Table 4.

DISCUSSION

We found significant differences between the patient and control groups for Ser217Leu in the HPC2/

Table 1. Frequency distribution of variables between the HPC2/ELAC2 gene Ser217Leu polymorphism and prostate cancer

Genotype	Prostate Cancer *n* (%)	Control *n* (%)	*p* Value	OR (CI 95%)
Ser217	67 (52.3)	56 (82.4)	<0.001	2.7 (1.6-4.8)
Leu217	61 (47.7)	12 (17.6)		
Total	128 (100.0)	68 (100.0)		

Table 2. Frequency distribution of variables between the HPC2/ELAC2 gene Ala541Thr polymorphism and prostate cancer

Genotype	Prostate Cancer *n* (%)	Control *n* (%)	*p* Value	OR (CI 95%)
Ala541	122 (95.3)	66 (97.1)	0.566, >0.05	1.4 (0.4-0.7)
Thr541	6 (4.7)	2 (2.9)		
Total	128 (100.0)	68 (100.0)		

Table 3. Frequency distribution of variables between the SRD5A2 gene Ala49Thr polymorphism and prostate cancer

Genotype	Prostate Cancer *n* (%)	Control *n* (%)	*p* Value	OR (CI 95%)
Ala49	92 (71.9)	61 (89.7)	0.004, <0.05	2.4 (1.2-4.9)
Thr49	36 (28.1)	7 (10.3)		
Total	128 (100.0)	68 (100.0)		

Table 4. Frequency distribution of variables between the SRD5A2 gene Val89Leu polymorphism and prostate cancer

Genotype	Prostate Cancer *n* (%)	Control *n* (%)	*p* Value	OR (CI 95%)
Val89	35 (27.4)	24 (35.3)	0.248, >0.05	1.2 (0.8-1.8)
Leu89	93 (72.6)	44 (64.7)		
Total	128 (100.0)	68 (100.0)		

ELAC2 gene and for Ala49Thr in the SRD5A2 gene. However, we did not see any difference between the patient and control groups for Ala541Thr in the HPC2/ELAC2 gene and for Val89Leu in the SRD5A2 gene.

There is controversy on the role of HPC2/ELAC2 and SRD5A2 genes in prostate cancer. They focus on different populations or populations from different regions in the same country.

There are several studies which reveal that there is correlation between prostate cancer patients and their ethnic backgrounds. Some of the investigated populations were Caucasian, African-American, Asian,

Canadian, American-Caucasians and British for the Ser217Leu polymorphism in the HPC2/ELAC2 gene [4,12,15]. Our findings in the Turkish population for the correlation between the Ser217Leu polymorphism in the HPC2/ELAC2 gene and prostate cancer risk agree with those of the above-mentioned studies. In contrast with those findings, no association was found between Ser217Leu polymorphism in the HPC2/ELAC2 gene in Finnish, Caucasian, African-American and American-Caucasian prostate cancer patients [16-18].

The other polymorphism is Ala541Thr for the HPC2/ ELAC2 gene which has been studied by a number of investigators for different populations. Some of these results for Canadian, Japanese, African-American and American-Caucasian and Asian populations were statistically significant [4,13,15,19]. However, some results of Caucasian, Finnish and British men with prostate cancer were not statistically significant which are in agreement with our findings [14,16,17].

There are a number of studies based on the investigation of different populations with the Ala49Thr polymorphism in the SRD5A2 gene. Some studies for African-American, Hispanic, Caucasians, French and American men [9,11,20,21] had similar results which indicates a significant difference between the Ala49Thr polymorphism in the SRD5A2 gene and prostate cancer patients. However, negative findings were reported by several studies about Chinese and Turkish populations [20,21]. In contrast, we showed significant differences between patients and controls regarding the Thr49 allele.

In a previously published study, the Val89Leu polymorphism was investigated for Japanese, Indian, French, Caucasian, Chinese and Turkish populations. It has recently been shown that there was no correlation between the Val89Leu genotype and prostate cancer [9,21-23,26, 27]. Our results also support the findings of these studies based on the SRD5A2 gene in Turkish men with prostate cancer. According to statistical data, some of the screened populations were significantly different. They were Canadian, American and Caucasian populations [11,24,25]. However, Soderstrom *et al.* [28] published that the Leu89 allele decreased the risk of prostate cancer about 6.67-fold.

In conclusion, genetic polymorphisms are affected by ethnic backgrounds. In order to reveal the correlation between some polymorphisms and prostate cancer, we investigated the Ser21Leu and Ala541Thr polymorphisms in the HPC2/ ELAC2 gene and Ala49Thr and Val89Leu polymorphisms in the SRD5A2 gene in a Turkish population with prostate cancer. We found correlations between the Ser217Leu and Ala49Thr polymorphisms and the disease according to our sample size. In order to get a certain result about the relation, larger samples are needed; depending on this, further studies may achieve the exact correlation.

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