Prostate cancer is known as the second most common malignancy and the second most common cause of cancer related deaths among men in the European Union. As the importance of a strong family history, besides environmental factors, on prostate and breast cancer is observed and as it is known that men with one relative with prostate cancer had a double risk of developing prostate cancer, scientists are making many genetic studies to show the association between the genetic polymorphisms and outcome parameters.

Prostate tumors generally prognose poorly but the alterations in the tumor development and clinical outcome is still not well understood.

It is known that renin-angiotensin system (RAS) activation plays an important role in the progression of certain diseases like cardiac, renal, and hypertension. There are also many evidences that angiotensin-converting enzyme (ACE) also participates locally in the pathology of carcinomas. ACE degrades vasodilator kinins and generates angiotensin II (Ang II), one of the

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**Genetic polymorphism of angiotensin I–converting enzyme (ACE-I) and prostate cancer risk**

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We designed this study to investigate the effects of angiotensin I–converting enzyme (ACE I) genotype on prostate cancer risk in patients with prostate cancer in Turkish population.

Our study was carried out in 48 patients with prostate cancer and 51 healthy volunteers as controls. ACE I/D genotypes were determined by polymerase chain reaction (PCR), and restriction fragment length polymorphism techniques (RFLP).

When we examined the genotype distribution for ACE gene, the frequencies of II, DD, and ID genotypes among the patients with prostate cancer were 8.3%, 47.9%, and 43.8%, respectively; and among the control subjects, they were 23.5%, 35.3%, 41.2%, respectively. Although we found that patients have a higher level of DD genotype, the distribution of the ACE genotypes of the patient group didn’t differ significantly from the control group ($X^2 = 1.29, p = 0.25$) but when we examined the risk ratio we found that having a DD genotype increases the risk factor for prostate cancer 1.35 times (Odds ratio: 1.35 95% CI 0.78-2.33).

The result of our study supports the hypothesis that genetic factors related to ACE may increase the risk factors for human prostate cancer and studies on this gene polymorphism could be an ideal target for future interventions intended to early diagnosis for prostate cancer.

**Key words:** ACE gene, ACE I/D polymorphism, angiotensin, prostate cancer


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**Introduction**

Prostate cancer is known as the second most common malignancy and the second most common cause of cancer related deaths among men in the European Union. As the importance of a strong family history, besides environmental factors, on prostate and breast cancer is observed and as it is known that men with one relative with prostate cancer had a double risk of developing prostate cancer, scientists are making many genetic studies to show the association between the genetic polymorphisms and outcome parameters.

Prostate tumors generally prognose poorly but the alterations in the tumor development and clinical outcome is still not well understood.

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effector peptides of the RAS system. Although ACE synthesis by the prostate was reported by Krassnigg et al. and AT-1 receptor is known as the predominant Ang II prostatic receptor the pathophysiological role of ACE is not well understood. Either a regulatory role in the prostate stroma or paracrine function mediation in the human prostate is suggested for Ang II. It is also reported that inhibition of ACE activity suppresses tumor growth and angiogenesis in vitro and in vivo in animal models.

The ACE gene is located on chromosome 17 q 23 and a polymorphism in the ACE gene, consisting of the insertion I or deletion D of a 287 bp DNA fragment in intron 16, accounting 20% to 50% of the variance in ACE expression or activity in blood and tissue among individuals. Homozygote for the I allele (II) can display half of the level for the plasma ACE level compared with homozygote D allele (DD genotype).

In this study, we studied ACE I/D polymorphism and determined whether variants of this polymorphism associates with prostate cancer.

**Materials and Methods**

**Patients selection and clinical investigation**

48 prostate cancer and 51 healthy men were included in the study. The patients and the controls had similar distribution of age. All the subjects were selected from Istanbul Üsküdar Hospital and Haydarpasa Numune Education and Research Hospital, Department of Urology between 2005 and 2006 and the diagnosis of prostate carcinoma was confirmed by the clinical and laboratory examinations and confirmed pathologic examination. Control group were selected among the healthy volunteers. 10 ml blood samples were collected from the patients and the control group in the tubes with EDTA.

**DNA isolation**

Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with salting out procedure.

**ACE I/D polymorphism.**

Template DNA (0.5-1.0 µg) was used in a PCR under stringent conditions to avoid the possibility of false positives for ACE genotyping. Reactions were performed with 10 pmol of each primer: forward primer, 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'; and reverse primer, 5'-GAT GTG GCC ATC TTC GTC AGA T-3' in a final volume of 25 µL containing 1.5 mM MgCl₂, 25 mM KCI, 5 mM Tris-HCl (Ph 8.4), 0.25 mM each of DNTP (MBI Fermantes, Lithuania) and 1 unit of Taq polymerase (MBI Fermantes, Lithuania). Amplification was carried out in a DNA thermal cycler (MJ Research Techn, UK) for 30 cycles with denaturation extension at 72°C for 2 min. PCR products were separated on a 2% agarose gel, and DNA was visualized by ethidium bromide staining. The PCR product is a 190-bp fragment in the presence of the insertion (I) allele. Thus, each DNA sample revealed one of three possible patterns after electrophoresis: a 490-bp band (genotype II), a 190-bp band (genotype DD), or both 490-bp and 190-bp bands (genotype ID).

Restriction fragments were visualized after ethidium bromide staining of the agarose gel with the use of an ultraviolet transilluminator (Figure 1).

**Statistical analysis**

Statistical analyses were performed using the SPSS software package, version 10.0. Clinical laboratory data are expressed as means±SD. Mean values were compared between patients with prostat cancer and control subjects by the unpaired Student’s t-test. Differences in the distribution of ACE genotypes or alleles between cases and controls were tested using the chi-square statistic, respectively. ACE I/D allele frequencies were estimated by gene counting methods. P<0.05 was considered statistically significant.
Results

The patients and the controls had similar distribution of age, smoking and body mass index. The distributions of genotypes and alleles of ACE I/D are shown in Table 1. The frequencies of II, DD, and ID genotypes among the patients with prostate cancer were 8.3%, 47.9%, and 43.8%, respectively; among the control subjects, they were 23.5%, 35.3% and 41.2%, respectively. Although the patients have a higher level of DD genotype, the distribution of the ACE genotypes of the patient group didn’t differ significantly from the control group ($X^2 = 1.29$, $p=0.25$) but when the risk ratio was examined we found that having a DD genotype increases the risk factor for prostate cancer 1.35 times (Odds ratio: 1.35 %95 CI: 0.78-2.33).

Discussion

Prostate carcinoma (PCA) is one of the most common malignancy in men, and is one of the important leading cause of cancer mortality in Turkey. Various molecular mechanisms have been associated with prostate carcinoma and the aggressive behaviour of prostate tumor and the mechanism of the progression of prostate cancer is not clearly known. It is only known that prostate cancer is a complex disease and it is evident that the components of prostatic cancers form a cellular and molecular network. Prostate adenocarcinoma and its neoplastic progression are shown to be influenced by interactions between cells in the stromal and epithelial compartments that have been shown to accelerate local tumour growth and increase genetic instability of the tumour epithelium.

To our knowledge there are not many studies on the relation between ACE I/D genotype and prostate cancer risk, but Koh et al showed in one of their study that lower plasma ACE concentration leads a significantly reduced risk of breast cancer and a study by Lyall et al showed that Angiotensin II mediates proto-oncogene expression and acts through the AT1 receptor and may be important in angiotensin II-induced smooth muscle hypertrophy.

When a change in the human ACE gene occurs by an insertion (I) or a deletion (D) of a 287-bp Alu-repetitive sequence in intron 16, plasma ACE level changes, and the highest levels of circulating and tissue ACE activity are known to be found in carriers of the DD genotype. It is reported by Mederios et al that in DD carriers, chronic exposure to higher levels of angiotensin II during an individual’s lifetime may alter the onset of advanced disease and experimental studies demonstrate that angiotensin II can promote angiogenesis, an important determinant in the growth and spread of many human cancers, angiotensin II induces vascular endothelial growth factor (VEGF), which plays a pivotal role in tumour angiogenesis and correlates with aggressive behaviour and a poor prognosis.

In this study, we observed ACE I/D II, DD, and ID genotypes among the patients with prostate cancer were 8.3%, 47.9%, and 43.8%, respectively; among the control subjects, they were 23.5%, 35.3% and 41.2%, respectively. Although the patients have a higher level of DD genotype, the distribution of the ACE genotypes of the patient group didn’t differ significantly from the control group ($X^2 = 1.29$, $p=0.25$) but when the risk ratio was examined we found that having a DD genotype increases the risk factor for prostate cancer 1.35 times (Odds ratio: 1.35 %95 CI: 0.78-2.33).

Our results is consistent with the study by Mederios et al as we found that DD genotype increases the risk factor for prostate cancer 1.35 times.

Consequently, we hypothesized that ACE I/D polymorphism is associated with an overall risk of prostate cancer. In this study, we have provided further evidence that the ACE D allele is associated with the cancer development in a patient group with prostate cancer.

References


