

Effects of ACE I/D Polymorphism on Prostate Cancer Risk, Tumor Grade and Metastasis

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Abstract. The aim was to substantiate the putative significance of angiotensin-converting enzyme (ACE) (insertion/deletion) I/D polymorphism on prostate cancer risk, BTPSA-ATPSA (before treatment-after treatment prostate-specific antigen) levels and tumor development. Materials and Methods: 48 prostate cancer patients and 51 healthy volunteers were included. The ACE I/D genotypes were determined by PCR (polymerase chain reaction) and RFLP (restriction fragment length polymorphism) techniques. Results: The DD genotype may have detrimental and the II genotype may have protective effect on prostate cancer ($p=0.03$). The highest before treatment PSA (BTPSA) values were found in the patient group having the DD genotype ($p=0.017$). PSA-AT levels were higher in homozygous mutant DD than homozygous II and the decrease in PSA-AT level was found to be statistically significant in each genotype ($p=0.000$). Patients with the D allele showed a higher prevalence of late stage prostate carcinoma when compared to the patients with II genotype ($p=0.022$) and the detrimental effects of the D allele, both in lymph node metastases and distant metastasis were observed. Conclusion: The risk of prostate cancer development, the PSA level and tumor metastasis may be associated with genetic variation in the ACE I/D genotypes which may be used as an important biomarker for further studies.

Prostate cancer is an increasingly prevalent problem. It is responsible for 12% of all new cases of cancer diagnosed each year in Europe (1). Family history appears to play an important role, along with environmental factors (2). As

compared to men who have no close relative with the disease, the risk of developing prostate cancer doubles among men who have one affected close relative; men with two or three affected relatives have a five- to eleven-fold increased risk of developing the disease (3).

Genetic studies are beginning to delineate the association between genetic polymorphisms and likely outcomes. Prostate tumors generally have a poor prognosis but the connections between tumor development and clinical outcomes are still not well understood (4). These connections may be determined by genetic variation. There is a sizeable body of evidence that the renin-angiotensin system (RAS) and angiotensin-converting enzyme (ACE) participate locally in the pathology of carcinomas as well as in the progression of certain diseases such as cardiac and renal disease, and hypertension (5-8). It is known that ACE, by degrading the vasodilator kinins, generates one of the effector peptides of the RAS system, angiotensin II (Ang II) (4). Krassnigg *et al.* (9) have reported that ACE is synthesised by the prostate and the AT-1 (angiotensin II type 1) receptor is known as the predominant Ang II prostatic receptor, but the pathophysiological role of ACE is still not well understood (10). It is thought that it either has a regulatory role in the prostate stroma or paracrine function mediation in the human prostate (11). It has also been reported that inhibition of ACE activity suppresses tumor growth and angiogenesis *in vitro* and *in vivo* in animal models (12, 13).

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, leading to a change in the plasma ACE level. The highest levels of circulating and tissue ACE activity are known to be found in carriers of the DD genotype (14). Mederios *et al.* (4) have reported that in DD carriers, chronic exposure to high levels of angiotensin II during an individual's lifetime may affect the occurrence of advanced disease. In a previous study (15) we have shown that the ACE I/D polymorphism genotypes of patients with prostate cancer did not differ significantly from those of a control

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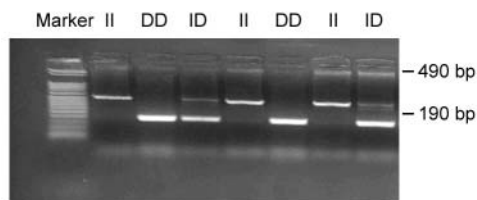


Figure 1. Direct visualization of ACE I/D PCR products, electrophoresed on 2% agarose gel, by ethidium bromide staining. A 490 base pair ACE I allele and 190 base pair ACE D allele is seen. Results from seven patients are shown. Lane 1 is the marker, lanes 2,5,7 II homozygote, lanes 3 and 6 DD homozygote and lanes 4 and 8 ID heterozygote.

group but the DD genotype increased the risk factor for prostate cancer 1.35-fold. Experimental studies have also demonstrated that angiotensin II can promote angiogenesis, an important determinant of the growth and spread of many human cancers (4).

Given this background, the present study focused on the effects of ACE I/D polymorphism on prostate cancer risk, and the effect of the genotype on prostate-specific antigen (PSA) level, tumor metastasis and late stage prostate carcinoma.

Materials and Methods

Patient selection and clinical investigation. Samples from 51 healthy men and 48 male patients with prostate cancer were included in this study. All the patients were selected between 2005 and 2006 from the Istanbul Uskudar Hospital and the Haydarpasa Numune Education and Research Hospital. The diagnosis of prostate carcinoma was confirmed by clinical and laboratory examinations and pathological examination. Control males were selected among healthy volunteers.

DNA isolation. Blood samples were collected in tubes containing EDTA (ethylene diamine tetra acetic acid) and DNA samples were extracted from whole blood with the salting-out procedure (16).

ACE I/D polymorphism. The template DNA (0.5-1.0 µg) was used in a PCR (polymerase chain reaction) under stringent conditions to avoid the possibility of false positives for ACE genotyping (17). The reactions were carried out 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 KCl, 5 mM Tris-HCl (pH 8.4), 0.25 mM each of (deoxyribonucleotide triphosphate) dNTP (MBI Fermantes) and 1 unit of Taq polymerase (MBI Fermantes). 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. The PCR products were separated on a 2% agarose gel and DNA was visualized by ethidium bromide staining. The PCR product is a 490-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).

Table I. Characteristics of the study population.

	Control (n=51)	Prostate cancer (n=47)	p-value
Age (years) (±SD)	69.02±7.89	70.55±9.68	NS
Smoking (%) (Yes/No)	11,76/88,24 (n=6/45)	34.04/65.96 (n=16/31)	0.028
Alcohol (%) (Yes/No)	10.8/89.2	28.0/72.0	0.082
BMI (kg/m ²) (±SD)	24.61±4.47	26.04±2.81	NS
BTPSA (ng/ml) (±SD)	1.45±1.10	32.2±34.13	0.002
ATPSA (ng/ml) (±SD)	-	6.55±17.58	
BTFPSA (ng/ml) (±SD)	0.40±0.44	9.95±30.75	0.112
ATFPSA (ng/ml) (±SD)	-	6.16±21.99	
Alkaline phosphates	90.75±18.30	191.85±73.50	<0.001
BUN	19.65±5.65	24.73±11.60	NS
Creatinine	0.87±0.19	1.06±0.31	0.029

BMI: body mass index, PSA: prostate-specific antigen, FPSA: free prostate specific antigen, BTPSA: before treatment PSA, ATPSA: after treatment PSA, BUN: blood urea nitrogen, NS: not significant, n: number of individuals.

Statistical analysis. Statistical analyses were performed using the SPSS software package, version 11.0. Clinical laboratory data are expressed as means±SD. Mean values were compared between patients with prostate 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 chi-square and paired *t*-test. ACE I/D allele frequencies were estimated by gene counting methods; *p*<0.05 was considered the threshold for statistical significance.

Results

The baseline characteristics of patients and controls are shown in Table I. The patients and the controls had similar distributions of age, before treatment free prostate specific antigen (BTFPSA), BUN (blood urea nitrogen), alcohol consumption and body mass index. The patient group had a significantly higher level of before treatment prostate specific antigen (BTPSA) (*p*=0.002), alkaline phosphates (*p*<0.001), creatinine (*p*<0.05) and smoking (*p*=0.028) compared with the controls (Table I).

The distributions of the genotypes and alleles of ACE I/D are shown in Table II. The DD genotype and D allele may have statistically significant detrimental effects and II genotype and I allele may have statistically significant protective effects on prostate cancer (*p*=0.03).

The association between the PSA levels before (BTPSA) and after (ATPSA) antiandrogen treatment and the ACE I/D genotype is shown Table III. Antiandrogenic treatment decreased the PSA levels, however, in prostate cancer patients, the PSA-BT levels were significantly higher in homozygous ACE DD mutants (25.54±33.52) than in homozygous wild type ACE II (9.82±10.95) (*p*=0.017). A statistically significant decrease of the PSA values after antiandrogenic treatment was shown (*p*<0.0001 for each genotype), as expected.

Table II. Genotype and allele frequencies of ACE I/D genes in study groups.

ACE I/D polymorphism genotype	Groups	
	Control (n=51)	Prostate cancer (n=48)
II	12 (23.5%)	4 (8.3%)
DD	15 (29.4%)	25 (52.1%)*
ID	24 (47.1%)	19 (39.6%)
Alleles		
I	48 (47.05%)	27 (28.12%)
D	54 (52.95%)	69 (71.88%)

ACE: angiotensin-converting enzyme, I/D: Insertion/ deletion; **p*=0.03.

Table III. ACE I/D polymorphism and PSA levels in the patient group.

ACE I/D polymorphism genotype	BTPSA (ng/ml)	ATPSA (ng/ml)
II	9.82±10.95	6.86±5.96**
DD	25.54±33.52*	9.82±10.95**
ID	22.47±32.08	2.48±5.15**
Alleles		
I	19.79±29.22	3.30±5.39**
D	25.54±33.52	6.52±18.37**

BTPSA: before treatment PSA, ATPSA: after treatment PSA, n: number of individuals, PSA: prostate specific antigen.**p*=0.017, ***p*<0.0001.

Table IV. ACE I/D polymorphism and metastasis type and tumor stage in prostate cancer patients.

ACE I/D polymorphism genotype	Early stage	Late stage	Lymph node metastasis			Distant metastasis	
	(T1 and T2)	(T3 and T4)	N0	N1	N2	M0	M1
II	19.0% (4)*	0%	11.1%	0%	–	5.6%	0%
DD	52.4% (11)	52.0% (13)	55.6%	75%	–	66.7%	54.5%
ID	28.6% (6)	48.0% (12)	33.3%	25.0%	100%	27.8%	45.5%

Lymph node metastasis: N0, negative; N1, 2, positive. Distant metastasis: M0, negative; M1, positive. **p*=0.022.

N1 and N2 lymph node metastases were observed in five patients: three of them had the DD and two had the ID genotype, whereas no patient with lymph node metastases had the homozygous II genotype. Distant metastases were recorded in 11 patients: six of them with the DD and five of them with the ID genotype, whereas no distant metastases were recorded in patients with the II genotype. Patients with the D allele (DD + ID genotypes) showed a higher prevalence of late stage prostate carcinoma when compared to the patients with II genotype ($\chi^2=5, 21; p=0.022$) (Table IV).

Discussion

Prostate carcinoma is one of the most common malignancies in men and is one of the leading causes of cancer mortality in Turkey. Neither the aggressive behavior of prostate tumors nor the mechanism of the progression of prostate cancer are well understood as yet (18).

Prostate adenocarcinoma and its neoplastic progression have been shown to be influenced by interactions between cells in the stromal and epithelial compartments (18-21) that have been shown to accelerate local tumor growth and increase genetic instability of the tumor epithelium (4, 19-21).

To our knowledge there are few studies of the relationship between the ACE I/D genotype and cancer risk, but in one study Koh *et al.* (22) showed that lower plasma ACE

concentration led to a significantly reduced risk of breast cancer. Moreover, a study by Lyall *et al.* (23) has shown that angiotensin II mediates proto-oncogene expression and acts through the AT1 receptor, and may be important in angiotensin II-induced smooth muscle hypertrophy.

It is known that angiogenesis is an important factor in the growth and spread of many types of human cancer, and experimental studies have demonstrated that angiotensin II can promote angiogenesis by inducing vascular endothelial growth factor (VEGF), which plays a pivotal role in tumor angiogenesis and correlates with aggressive behavior and a poor prognosis (24-29).

This study has shown that the DD genotype is statistically over-represented in prostate cancer and shows the detrimental effect of the D allele (*p*=0.03). This is consistent with the findings of Medeiros R *et al.* (4) who showed that the DD genotype was significantly associated with advanced disease, and by our previous study (15).

ACE I/D polymorphism is also associated with high BTPSA levels, lymph node metastasis and tumor development. The DD genotype has been shown to be associated with high ACE levels, leading to angiogenesis, in many studies (4, 12, 14, 21), and this angiogenesis could cause detrimental effects leading to lymph node metastasis. Based on all the studies on the ACE I/D genotype and circulating ACE levels, the use of ACE inhibitors may reduce the proliferation of tumor cells and tumor growth in prostate cancer.

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