ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(6): 1043-1049
doi: 10.25083/rbl/24.6/1043.1049

**Received for publication, May, 14, 2018**
**Accepted, July, 06, 2018**

*Original paper*

# *Influences of angiotensin I-converting enzyme gene insertion/deletion polymorphism on prostate cancer risk in Romania*

**ANCA GABRIELA PAVEL[1,2,*], DANAI STAMBOULI[1], CONSTANTIN GINGU[3,4], ADRIAN PREDA[3], ISMAIL GENER[3], CATALIN BASTON[3,4], GABRIELA ANTON[2]**

[1]Molecular Genetics & Cytogenetics Department, Cytogenomic Medical Laboratory, Bucharest, Romania
[2]Stefan S. Nicolau Institute of Virology, The Romanian Academy, Bucharest, Romania
[3]Center of Internal Medicine-Nephrology, Fundeni Clinical Institute, Bucharest, Romania
[4]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

**Abstract**

The contribution of genetic variants to prostate cancer risk has been documented. Mutations in ACE (angiotensin I-converting enzyme) gene are found to be related with prostate cancer. The purpose of the present study was to characterize ACE genotypes in prostate cancer cases and to investigate any association between I/D polymorphisms of ACE gene and clinical characteristics in a group of Romanian patients. A total of 46 healthy men and 46 male patients diagnosed with prostate cancer were included in the study. ACE I/D polymorphisms were determined by polymerase chain reaction methodology. The highest genotype frequencies found within the prostate cancer group were detected for DD genotype (55.6%) with a significant difference in the allele frequencies ($p = 0.006$), compared to the control group, where no significant differences were observed ($p > 0.05$). The DD genotype was statistically significant associated with the advanced tumor stage and Gleason score higher than 8. Our data suggest that the ACE gene polymorphism can be associated with the evolution of prostate cancer disease, more precisely that DD genotype represented a risk factor for prostate cancer.

**Keywords** Prostate cancer, *ACE* gene, genotype, polymorphism, risk factor.

**To cite this article:** PAVEL AG, STAMBOULI D, GINGU C, PREDA A, GENER I, BASTON C, ANTON G. Influences of angiotensin I-converting enzyme gene insertion/deletion polymorphism on prostate cancer risk in Romania. *Rom Biotechnol Lett*. 2019; 24(6): 1043-1049. DOI: 10.25083/rbl/24.6/1043.1049

*Corresponding author: ANCA GABRIELA PAVEL, Stefan S. Nicolau Institute of Virology, The Romanian Academy, Bucharest, Romania
E-mail: pavel.anca.gabriela@gmail.com

# Introduction

Prostate cancer (PC) is one of the most commonly diagnosed malignancies in the world and continues to be a major cause of morbidity and mortality in men over 50 years. It is acknowledged that PC is a multifactorial disease, and despite the high prevalence, the disease etiology remains largely unknown. In addition to increasing age and ethnicity, genetic variation or polymorphisms existing in the human genome can confer genetic susceptibility to cancer. A series of polymorphisms where described as being involved in the development of different pathologies in Romanian population (A.C. BULGAR & al [1], M. TOMA & al [2]). The involvement of different polymorphisms in the pathogenesis of PC (R. EELES & al [3], L. TANG & al [4], D. KACHAKOVA & al [5]) and the detection of these modifications may provide useful molecular indicators of prognosis.

Angiotensin I-converting enzyme (ACE), a key enzyme in the renin-angiotensin system (RAS), plays an important role in regulating blood pressure and circulatory homeostasis. This enzyme is also able to degrade bradykinin, a potent vasodilator (J.H. VAN BERLO & al. [6]). ACE has been also implicated in the pathogenesis of several malignancies such as lung cancer, breast cancer, prostate cancer, gastric cancer and oral cancer (S. DEVİĆ PAVLİĆ & al [7], E. VAİRAKTARİS & al [8], M. NACAK & al [9], M. SUGİMOTO & al [10], B. YİGİT & al [11], C.A. HAİMAN & al [12]). It has been suggested that Ang II is involved in cellular proliferation (A. MUSCELLA & al. [13]) and has a significant role in tumor growth, angiogenesis and metastasis (M. FUJİTA & al. [14], M. FUJİTA & al [15]).

The *ACE* gene maps to human chromosome 17q23 and contains many variations. The most widely studied polymorphism is the Insertion/Deletion (I/D) variant located on intron 16, described by Rigat et al. [16]. The presence or absence of a 287-bp nonsense repetitive DNA domain leads to three genotypes: II, ID and DD. The I/D polymorphism accounts for 20% to 50% of the variance in *ACE* gene expression or activity in blood and tissues among individuals (C. RÖCKEN & al [17]). It is well known that different genotypes associate diverse ACE levels, and that DD genotype induces the highest ACE levels in blood (B. RİGAT & al [16]).

The implications of *ACE* polymorphisms in the progression of human PC remain to be established. Thus, the purpose of the present study was to characterize *ACE* genotypes in PC cases and to investigate any relation between *ACE* I/D polymorphism, the risk of PC and clinical characteristics in a group of Romanian patients with PC.

# Materials and Methods

**Patient selection and clinical investigation.** Peripheral blood samples were collected in tubes containing EDTA (ethylene diamine tetra acetic acid) from 46 healthy men, age 55 – 75 years (mean age 65.2 ± 6.22) and 46 male patients with PC, age 55 – 76 years (mean age 65.5 ± 3.5). All PC patients were selected between 2014 and 2016 from the Fundeni Hospital, Bucharest. The diagnosis of prostate carcinoma was confirmed by clinical and laboratory examination. Clinical characteristics of PC patients are presented in **Error! Reference source not found.**. PSA (prostate specific antigen) cut-off values and Gleason grading were considered according to Lotan and Epstein (T.L. LOTAN & al. [18]). Control male were selected among healthy volunteers. All samples were obtained with the informed consent of the participants prior to their inclusion in the study.

**Table 1.** Clinical characteristics of patients with prostate carcinoma

| | Cases ($n$ = 46) |
|---|---|
| Age | Mean 65.5±3.5 |
| < 70 | 40 |
| ≥ 70 | 6 |
| PSA values (ng/ml) | Mean 15±5 |
| < 10 | 14 |
| ≥ 10 | 32 |
| Tumor stage | |
| Early | 4 |
| Advanced | 42 |
| Gleason score | |
| < 8 | 25 |
| ≥ 8 | 21 |

**DNA analysis.** Genomic DNA from PC patients and control groups was isolated from whole blood samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

The *ACE* genotype was determined by PCR (polymerase chain reaction) methodology as described previously (V. SHANMUGAM & al. [19]) with some modifications. Briefly, genomic DNA samples were subjected to PCR amplification using the following primer pair sequence that target the *ACE* I/D polymorphism: 5'-CTG GAG AGC CAC TCC CAT CCT TTC T-3' (sense) and 5'-GAC GTC GCC ATC ACA TTC GTC AGA T-3' (antisense) synthetized by ThermoFisher Scientific (Carlsbad, CA). Amplification was carried out in a 25 µl volume containing template DNA (0.5–1.0 µg), primers (1 µM of each), dNTPs (0.2 mM each), 1X PCR buffer and 0.04 U/µl of Taq polymerase Recombinant (Invitrogen by ThermoFisher Scientific, Carlsbad, CA). After initial denaturation at 95°C for 5 min, the DNA was amplified by 30 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 45 s and primer extension at 72 °C for 45 s. Final extension was performed at 72°C for 10 min. The PCR products were visualized under UV trans-illuminator following electrophoresis on 2% agarose gel and staining with ethidium bromide.

**Statistical analysis.** Statistical analysis was performed using the SPSS software package, version 22.0 for Windows

(SPSS Inc, Chicago, IL, USA). Clinical laboratory data were presented as mean ± standard deviation. Hardy–Weinberg equilibrium (HWE) test was conducted for alleles and genotypes distribution. Chi-square test ($\chi 2$) and chi-square goodness-of-fit test were used to compare the distribution of genotypes and alleles in cases and control groups. To analyze the association of I/D *ACE* genotypes and clinicopathological features (tumor stage, PSA serum levels and Gleason score) Fisher's exact test was used. A p value < 0.05 was considered the threshold for statistical significance.

# Results and Discussion

**Frequency of ACE polymorphisms.** The I/D *ACE* genotype was determined by the length of the PCR product: 190-bp in case of the deletion and 490-bp in the presence of the insertion. Each DNA sample revealed one of three 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) (Figure 1).

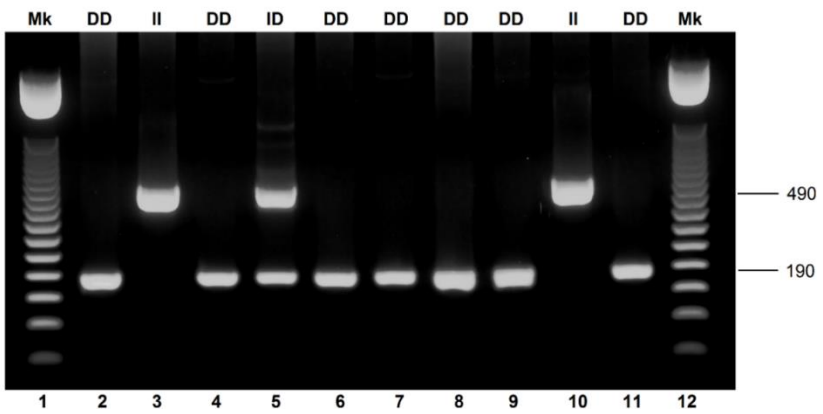


**Figure 1.** *ACE* genotypes by PCR amplification. PCR products are a 190 bp fragment (D allele) and a 490 bp fragment (I allele). Results from 11 patients are shown. Lanes 1 and 12 DNA ladder, lanes 2,4,6,7,8,9 and 11 genotype DD, lanes 3 and 10 genotype II, and lane 5 genotype ID.

A total of 46 pathological samples and 46 controls were genotyped. I/D *ACE* polymorphism could not be amplified in one of the PC samples due to poor DNA quality. Allele frequencies and I/D *ACE* genotype distribution for each study group are presented in **Error! Reference source not found.**. The observed genotype distributions are consistent with Hardy–Weinberg equilibrium among the control group (p = 0.926) and PC cases (p = 0.256).

**Table 1.** Genotype and allele frequencies of ACE I/D polymorphism

| *ACE* genotype | Study groups | |
|---|---|---|
| | Control (*n*=46) | Prostate cancer (*n*=45) |
| II | 8 (17.4%) | 5 (11.1%) |
| ID | 22 (47.8%) | 15 (33.3%) |
| DD | 16 (34.8%) | 25 (55.6%) |
| Allele | | |
| I | 38 (41.3%) | 25 (27.8%) |
| D | 54 (58.7%) | 65 (72.2%) |

The distribution of *ACE* genotypes and alleles for the PC group did not differ significantly from the control group (genotype: $\chi^2 = 3.981$, p = 0.136; allele: $\chi^2 = 3.677$, p = 0.055), although the patients group had a more frequent DD genotype (55.6%) compared to the control group (34.8%). The highest genotype frequencies found within the control group were detected for the ID heterozygous genotype for *ACE* (47.8%). For the PC group there were significant differences in the allele distribution (p = 0.006). No significant differences were observed between I and D alleles (p = 0.095) in the control group.

**Correlation between clinicopathological features of PC and *ACE* polymorphism.** The majority of PC patients included in this study were diagnosed with advanced tumor stages. Fisher's exact test was conducted and did not detect a statisticaly significant association between the tumor stage and I/D *ACE* genotype of the patients (p = 0.184). Because of the small number of individuals with early tumor stages (only 8.7%), there were used only the data

obtained from the patients diagnosed with advanced tumor stages (91.3%), and the chi-square goodness-of-fit test showed a significant difference between the three genotypes in the PC group with advanced tumor stage ($\chi^2 = 21.732$, $df = 2$, $p < 0.001$). The DD genotype is most frequent associated with the advanced tumor stage, followed by ID genotype and II genotype with the lowest frequency (Figure 2).

Subsequent analysis evaluated the association of the *ACE* I/D genotypes and the PSA serum levels (Figure 3) and the Fisher's exact test revealed a two-tailed p value of 0.1821 that is not statistically significant.

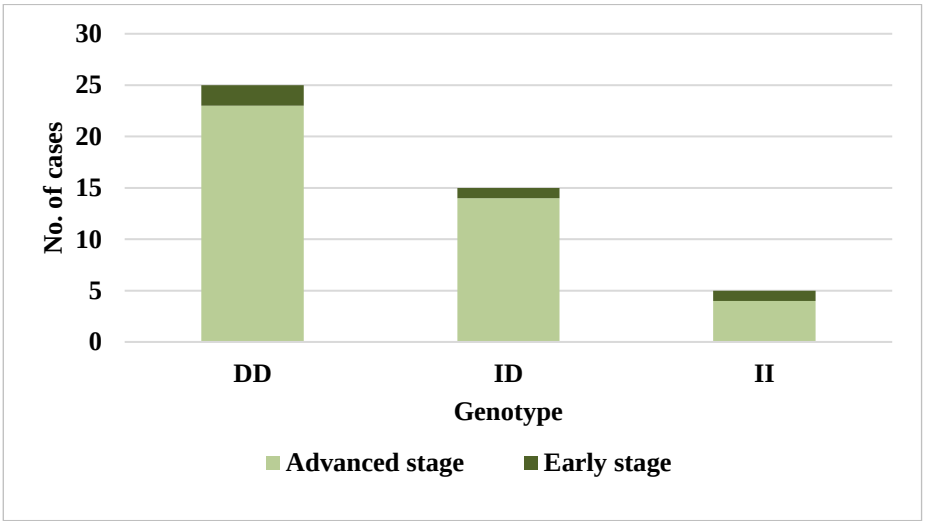


**Figure 2.** Frequency of *ACE* I/D polymorphism according to PC tumor stage

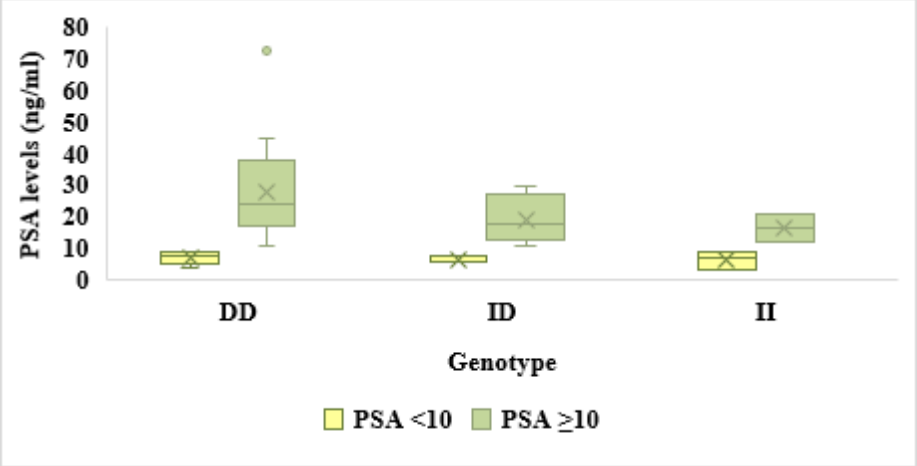


**Figure 3.** Distribution of PSA serum levels in PC group according to *ACE* genotypes

Data regarding the association of *ACE* I/D genotypes and the Gleason score (Figure 4), Gleason score < 8 (low-grade tumor) and Gleason score ≥ 8 (high-grade tumor), showed that there is a statistically significant association between the two subgroups ($p = 0.035$); otherwise, Gleason score ≥ 8 is more frequently associated with the DD genotype (64%) compared to ID and II genotypes (27% and 20% respectively).

## Discussions

Despite years of cancer genes research, the mechanisms of PC progression and the molecular base of tumor cells remain largely unknown. As a multifactorial disorder, cancer is influenced by genetic, as well as environmental factors, life style and age. Among genetic factors, beside oncogenes and tumor suppressor genes, polymorphisms in more than 100 genes, which confer moderate or high risk of cancer, have been identified (N. RAHMAN [20]). Such an example is the RAS, which regulates cell proliferation, angiogenesis, blood pressure and cardiovascular homeostasis and has also been involved in various malignancies (P.R. GARD [21], A.J. GEORGE & al [22], X. WANG & al [23]). The role of ACE in the development of human cancers and in the progression of PC mostly, is still under debate. Studies in various populations (Chinese, Turkish, Mexican and Netherlands) have produced conflicting results

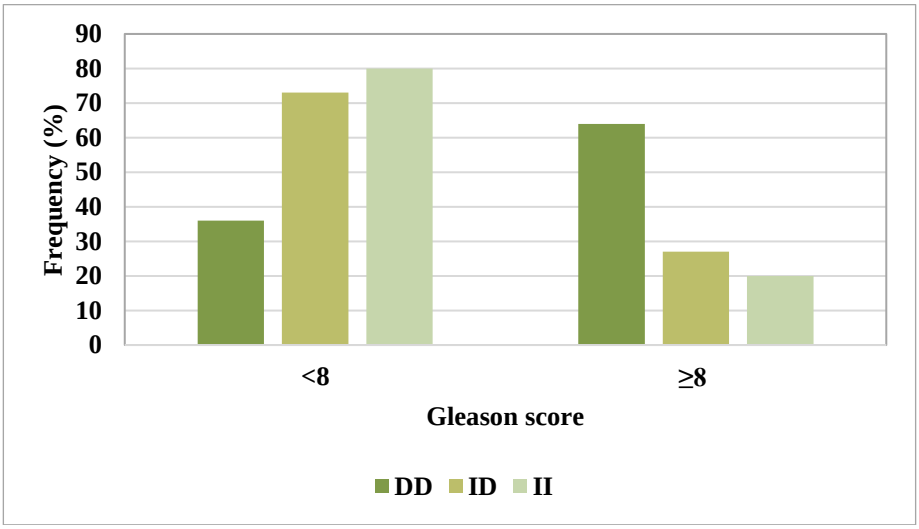


**Figure 4.** Graphical display of Gleason score associated with DD, ID and II genotypes in PC group

(Y. XİE & al [24]). Ruiter et al. [25], in a meta-analysis of 24 studies, found an increased risk of PC for the carriers of *ACE* DD genotype, while noting a large variation in the analyzed results. Another meta-analysis study conducted by Xie et al [24], suggested that *ACE* I/D polymorphism could be a potential genetic biomarker for risk of PC.

In this study, we analyzed the relation between *ACE* I/D polymorphism and clinical characteristics in a group of Romanian patients diagnosed with PC.

The highest levels of circulating and tissue ACE activity are known to be found in carriers of DD genotype (B. RIGAT & al [16]). It is reported by Medeiros et al [26] that the DD genotype was significantly associated with advanced disease and experimental studies demonstrated that Ang II can promote angiogenesis, an important determinant in the growth and progression of many human cancers. Another study showed that different genotypes of *ACE* I/D polymorphism found in patients with PC did not differ significantly from the control group, but when the risk ratio was examined, having a DD genotype increases the risk factor for PC 1.35 times (B. YİGİT & al [27]).

In the present study, we observed a high frequency of DD genotype compared to the other genotypes (55.6%) in patients with PC; these patients had a high probability to be diagnosed with an advanced tumor stage compared with ID and II genotype carriers. When comparing allele frequencies in PC and control groups, the D allele showed the highest frequency in the PC group. We observed a higher frequency of DD genotype among the PC patients with Gleason score ≥ 8. Consequently, compared with the DD genotype and D allele, the ID and II genotype can be associated with a decreased risk of PC. Similar results were found in the previous studies which indicated the I allele could have a protective effect against carcinogenesis (B. YİGİT & al [11], X. WANG & al [23], R. MEDEİROS & al [26], E. SİERRA DÍAZ & al [28], R. VAN DER KNAAP & al [29]).

Yigit et al [11] have shown that DD genotype is significantly present in PC and demonstrated that the DD genotype is statistically over-represented in PC. Sierra Díaz et al [28] suggested that D allele, particularly the presence of DD homozygous genotype, is a risk factor for PC. Contrariwise, the results of Hasanzad et al [30] suggested that the D allele has a protective effect on PC development, although not significantly ($p = 0.4$), and the presence of II genotype increased the risk of cancer more than 38% compared with DD genotype.

Previous studies suggested that the DD genotype can be associated with high ACE levels, leading to angiogenesis (B. YİGİT & al [11], B. RİGAT & al [16], R. MEDEİROS & al [26], E. SİERRA DÍAZ & al [28]), which could cause detrimental effects leading to lymph node metastasis. The association between *ACE* I/D polymorphism and clinicopathological parameters in PC patients revealed that a statistically significant difference was present between the advanced tumor stage and distribution of the DD genotype, which shows a potential relationship between the two variables.

This investigation is the first addressing the I/D polymorphism of *ACE* gene in Romanian population and complements previous studies targeting the association of D allele with the PC progress, in various ethnic backgrounds, as this polymorphism is viewed as a potential risk factor in carcinogenesis.

## Conclusion

I In this study, we have provided further evidence that polymorphism of *ACE* gene is associated with the progression of disease in PC patients, more precisely that DD genotype represented a risk factor for PC. The statistical analysis revealed that there is an association between the *ACE* polymorphism and clinicopathological parameters of PC patients.

Some limitations of our study should be considered: the relatively small number of recruited PC patients and the lack of data regarding family history, that could lead to a better understanding of the relation between genetic variants and prostate malignancy.

Therefore, future studies with larger groups of subjects are needed to establish if *ACE* I/D polymorphism can be used in developing a genetic profile that may be useful in the prediction of the outcome of PC.

Furthermore, additional studies concerning the association of different genetic factors involved in angiogenesis and inflammatory processes related to oncogenesis are of great importance. This type of findings could lead to the development of preventive measures concerning individuals that are at risk for PC.

## Acknowledgements

Paper supported by the Partnerships in Priority Domains program – PN II, implemented with the support of MEN – UEFISCDI, project code PN-II-PT-PCCA-2013-4-1851 and by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/187/1.5/S/156040.

## References

1. A.C. BULGAR, E.V. FUIOR, A.V. GAFENCU, A.C. BREHAR, F.M. BREHAR, D.L. PĂUN, C. DUMITRACHE, Association of TCF7L2 rs7903146 and rs290487 polymorphisms and type 2 diabetes in Romanian subpopulation. *Romanian Biotechnological Letters*, 22: 12520-12530 (2017).
2. M. TOMA, O.A. ALEXIU, P.M. SORIN, A.M. CRACIUN, A. STANISLAV, I. RADU, L.M. BERCA, G.D. CIMPONERIU, C. CRISTESCU, M. NICULESCU, L-selectin gene P213S polymorphism involvement in some human pathologies. *Romanian Biotechnological Letters*, 22: 12521-12537 (2017).
3. R. EELES, C. GOH, E. CASTRO, E. BANCROFT, M. GUY, A.A. AL OLAMA, D. EASTON, Z. KOTE-JARAİ, The genetic epidemiology of prostate cancer and its clinical implications. *Nat Rev Urol*, 11: 18 (2014).
4. L. TANG, M.E. PLATEK, S. YAO, C. TİLL, P.J. GOODMAN, C.M. TANGEN, Y. WU, E.A. PLATZ, M.L. NEUHOUSER, F.Z. STANCZYK, J.K. REİCHARDT, Associations between polymorphisms in genes related to estrogen metabolism and function and prostate cancer risk: results from the Prostate Cancer Prevention Trial. *Carcinogenesis*, 1: 9 (2017).
5. D. KACHAKOVA, A. MİTKOVA, E. POPOV, O. BELTCHEVA, A. VLAHOVA, T. DİKOV, S. CHRİSTOVA, V. MİTEV, C. SLAVOV, R. KANEVA, Polymorphisms in androgen metabolism genes AR, CYP1B1, CYP19, and SRD5A2 and prostate cancer risk and aggressiveness in Bulgarian patients. *Turk J Med Sci*, 46: 626-640 (2016).
6. J.H. VAN BERLO, Y.M. PİNTO, Polymorphisms in the RAS and cardiac function. *Int J Biochem Cell Biol.*, 35: 932-943 (2003).
7. S. DEVİĆ PAVLİĆ, S. RİSTİĆ, V. FLEGO, M. KAPOVİĆ, A. RADOJČİĆ BADOVİNAC, Angiotensin-converting enzyme insertion/deletion gene polymorphism in lung cancer patients. *Genet Test Mol Biomarkers*, 16: 722-725 (2012).
8. E. VAİRAKTARİS, C. YAPİJAKİS, C. TSİGRİS, S. VASSİLİOU, S. DERKA, E. NKENKE, S. SPYRİDONİDOU, A. VYLLİOTİS, E. VORRİS, V. RAGOS, F.W. NEUKAM, Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with increased risk for oral cancer. *Acta Oncol*, 46: 1097-1102 (2007).
9. M. NACAK, İ. NACAK, M. ŞANLI, M. ÖZKUR, M. PEKTAŞ, A.Ş. AYNACIOĞLU. Association of angiotensin converting enzyme gene insertion/deletion polymorphism with lung cancer in Turkey. *Cancer Genet*, 198: 22-26 (2010).
10. M. SUGİMOTO, T. FURUTA, N. SHİRAİ, M. IKUMA, H. SUGİMURA, A. HİSHİDA, Influences of chymase and angiotensin I-converting enzyme gene polymorphisms on gastric cancer risks in Japan. *Cancer Epidemiol Biomark Prev*, 15: 1929-1934 (2006).
11. B. YİGİT, N. BOZKURT, F. NARTER, H. YİLMAZ, E. YUCEBAS, T. ISBİR, Effects of ACE I/D polymorphism on prostate cancer risk, tumor grade and metastatis. Anticancer Res, 27: 933-936 (2007).
12. C.A. HAİMAN, S.O. HENDERSON, P. BRETSKY, L.N. KOLONEL, B.E. HENDERSON, Genetic variation in angiotensin I-converting enzyme (ACE)

and breast cancer risk: the multiethnic cohort. *Cancer Res*, 63: 6984-6987 (2003).
13. A. MUSCELLA, M.G. GRECO, M.G. ELİA, C. STORELLİ, S. MARSİGLİANTE, Angiotensin II stimulation of Na+/K+ ATPase activity and cell growth by calcium-independent pathway in MCF-7 breast cancer cells. *J Endocrinol*, 173: 315-323 (2002).
14. M. FUJİTA, I. HAYASHİ, S. YAMASHİNA, M. ITOMAN, M. MAJİMA, Blockade of angiotensin AT1a receptor signaling reduces tumor growth, angiogenesis, and metastasis. *Biochem Biophys Res Commun*, 294: 441-447 (2002).
15. M. FUJİTA, I. HAYASHİ, S. YAMASHİNA, A. FUKAMİZU, M. ITOMAN, M. MAJİMA, Angiotensin type 1a receptor signaling-dependent induction of vascular endothelial growth factor in stroma is relevant to tumor-associated angiogenesis and tumor growth. *Carcinogenesis*, 26: 271-279 (2005).
16. B. RİGAT, C. HUBERT, F. ALHENC-GELAS, F. CAMBİEN, P. CORVOL, F. SOUBRİER, An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Investig*, 86: 1343-1346 (1990).
17. C. RÖCKEN, U. LENDECKEL, J. DİERKES, S. WESTPHAL, S. CARL-MCGRATH, B. PETERS, S. KRÜGER, P. MALFERTHEİNER, A. ROESSNER, M.P. EBERT, The number of lymph node metastases in gastric cancer correlates with the angiotensin I–converting enzyme gene insertion/ deletion polymorphism. *Clin Cancer Res*, 11: 2526-2530 (2005).
18. T.L. LOTAN, J.I. EPSTEİN. Gleason grading of prostatic adenocarcinoma with glomeruloid features on needle biopsy. *Hum Pathol*, 40: 471-477 (2009).
19. V. SHANMUGAM, K.W. SELL, B.K. SAHA, Mistyping ACE heterozygotes. *Genome Res*, 3: 120-121 (1993).
20. N. RAHMAN, Realizing the promise of cancer predisposition genes. Nature, 505: 302 (2014).
21. P.R. GARD, Implications of the angiotensin converting enzyme gene insertion/deletion polymorphism in health and disease: a snapshot review. *Int J Mol Epidemiol Genet*, 1: 145 (2010).
22. A.J. GEORGE, W.G. THOMAS, R.D. HANNAN, The renin–angiotensin system and cancer: old dog, new tricks. *Nat Rev Cancer*, 10: 745 (2010).
23. X. WANG, S. WANG, Y.W. LİN, J. WU, H. CHEN, Y.Q. MAO, X.Y. ZHENG, C. ZHOU, L.P. XİE, Angiotensin-converting enzyme insertion/deletion polymorphism and the risk of prostate cancer in the Han population of China. *Med Oncol*, 29: 1964-1971 (2012).
24. Y. XİE, C. YOU, J. CHEN. An updated meta-analysis on association between angiotensin I-converting enzyme gene insertion/deletion polymorphism and cancer risk. *Tumor Biol*, 35: 6567-6579 (2014).
25. R. RUİTER, L. VİSSER, C. VAN DUİJN, The ACE insertion/deletion polymorphism and risk of cancer, a review and meta-analysis of the literature. *Curr Cancer Drug Targets*, 11: 421-30 (2011).
26. R. MEDEİROS, A. VASCONCELOS, S. COSTA, D. PİNTO, F. LOBO, A. MORAİS, J. OLİVEİRA, C. LOPES, Linkage of angiotensin I-converting enzyme gene insertion/deletion polymorphism to the progression of human prostate cancer. *J Pathol*, 202: 330-335 (2004).
27. B. YİGİT, N. BOZKURT, F. NARTER, H. YİLMAZ, E. YUCABAS, T. ISBİR, Genetic polymorphism of angiotensin I-converting enzyme (ACE) and prostate cancer risk. *Adv Mol Med*, 2: 65-68 (2006).
28. E. SİERRA DÍAZ, J. SÁNCHEZ CORONA, R.C. ROSALES GÓMEZ, S.A. GUTİERREZ RUBİO, J.G. VÁZQUEZ CAMACHO, H. SOLANO MORENO, M.C. MORÁN MOGUEL, Angiotensin-converting enzyme insertion/deletion and angiotensin type 1 receptor A1166C polymorphisms as genetic risk factors in benign prostatic hyperplasia and prostate cancer. *J Renin Angiotensin Aldosterone Syst*, 10: 241-246 (2009).
29. R. VAN DER KNAAP, C. SİEMES, J.W. COEBERGH, C.M. VAN DUİJN, A. HOFMAN, B.H. STRİCKER, Renin-angiotensin system inhibitors, angiotensin I-converting enzyme gene insertion/deletion polymorphism, and cancer. *Cancer*, 112: 748-757 (2008).
30. M. HASANZAD, M. SAMZADEH, S.H. JAMALDİNİ, A.A. HAGHDOOST, M. AFSHARİ, S.A. ZİAEİ, Association of angiotensin I converting enzyme polymorphism as genetic risk factor in benign prostatic hyperplasia and prostate cancer. *Genet Test Mol Biomarkers*, 16: 770-774 (2012).