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Association of a common genetic variant (insertion/deletion) in *ACE* gene with prostate cancer susceptibility in a Tunisian population

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Abstract

Background: Angiotensin-converting enzyme (*ACE*) plays a pivotal role in several pathologies including cancers. The association of insertion/deletion (I/D) polymorphism of the *ACE* gene with prostate cancer (PC) risk remains controversial. We aimed to investigate for the first time, to our Knowledge, in North Africa the potential relationship between *ACE* I/D polymorphism with PC susceptibility and clinical outcomes of PC patients.

Methods: This case-control study included 143 healthy individuals and 124 patients diagnosed with PC. Using genomic DNA, the samples were genotyped for *ACE* I/D polymorphism by polymerase chain reaction (PCR).

Results: We found that The D allele is significantly associated with an increased risk of PC and D/D + D/I genotypes were at 3 times increased risk of PC ([$p = 0.005$], OR = 2.95, IC 95% = 1.26–7.09) compared with I/I genotype ($p = 0.003$, OR = 0.3, IC 95% = 0.12–0.74). We observed an association between D/D and D/I genotypes with advanced age (≥70 years) ($p = 0.014$; $r^2 = 0.22$). Furthermore, there is a significant prediction of advanced Gleason score ≥8 based on epidemiological parameters and *ACE* genotype ($p = 0.000$; $R^2 = 0.349$), although no significant association was observed with stage and metastasis.

Conclusion: The *ACE* I/D polymorphism is likely to predispose to PC and could play a role in PC progression and aggressiveness.

KEYWORDS
Alu repeat sequence, angiotensin conversion enzymes, North Africa, polymorphism, prostate cancer, Tunisia

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1 | INTRODUCTION

Prostate cancer (PC) is the most frequent cancer diagnosed among men aged more than 40 years.[1] In 2018, this malignancy affects 1.6 million new cases per year in the world.[2] Despite the PC mortality rates have been steadily declining in most western countries including North America, as well as in Western and North Europe causing 358,989 deaths (3.8% of all deaths caused by cancer in men) in 2018, the PC death still important.[2–4] In Tunisia, PC is considered the third diagnosed cancer in men aged between 40 and 85 years with an incidence of 810 new cases per year.[3] Epidemiological and environmental factors appear to play an important role in the development of this Neoplasm.[3,5–8] Moreover, several studies highlight the importance of single nucleotide polymorphisms (SNPs) in the genetic susceptibility, development, and/ or progression of PC.[1,5,6,9,10] Among these polymorphisms, the genetic variants of the Renin-Angiotensin System (RAS) were well studied in association with cancers.[11–16] RAS includes Angiotensin-Converting Enzyme (*ACE*), the key effector peptide of RAS, converts angiotensin-I to angiotensin-II and splits bradykinin into inactive fragments.[11] The alteration of *ACE* enzyme activity was associated with the development of many diseases such as COVID,[17,18] hypertension, and cardiovascular diseases (CVDs),[19,20] renal pathology,[21] Alzheimer disease,[22,23] polycystic ovarian syndrome,[24] and cancers.[25]

An Insertion/Deletion polymorphism in the *ACE* gene is well described; it consists of either presence or absence of 287 bp *Alu* repetitive sequences (**rs4646994**) inserted in the intron 16 of *ACE* gene, which was reported to account for nearly half of the phenotypic variance of enzyme serum concentration.[26–28] This genetic variation results in three genotypes I/I, ID, and D/D.[26,28]

ACE I/D polymorphism has widely been studied in association with genetic susceptibility of various cancers including lung, gastric, breast, malignant glioma, laryngeal, hepatocellular carcinoma, renal, and prostate.[29–35] Previous studies demonstrated the association between this polymorphism and increasing PC risk development in Asiatic and Latino populations.[29,36] However, its impact on the Caucasian population is still debated.[29]

There is a sizeable body of evidence that delineates the implication of *ACE* I/D polymorphism in the pathogenesis as well as its potential prognosis value in several cancers.[37–39] It has also been reported that *ACE* genetic polymorphism in PC is associated with disease progression.[40]

To our knowledge, the relationship between *ACE* I/D polymorphism and PC in North Africa has yet to be explored. Thus, in the present study, we aimed to examine the relationship between *ACE* I/D gene polymorphism and PC susceptibility, clinicopathological features, and clinical outcomes of a Tunisian population.

2 | MATERIALS AND METHODS

2.1 | Cohort definition

All participants included in this research provided written informed consent, and the study was approved by the local committee of Charles Nicolle Hospital (17–03–2016).

Rigorous inclusion and exclusion criteria were adopted to ensure the credibility of the results (Table S1). A total of 277 unrelated subjects were enrolled in our study, including 124 pathologically diagnosed PC patients and 143 age and geographic origin-matched cancer-free men controls. The volunteers' control included benign prostatic hyperplasia (BPH) and healthy individuals. BPH samples were deprived of any signs of malignancy according to the pathology report and represent a value of antigen-specific of prostate (PSA) inferior to 4 ng/ml after treatment. Healthy controls presented a normal digital rectal examination (DRE) and serum PSA inferior to 0.2 ng/ml. PC cases were recruited from the Department of Urology at the Charles Nicole Hospital of Tunis, Tunisia. The clinical, anatomopathological, and epidemiological parameters of PC patients were obtained from the medical record and are summarized in Tables 1 and 2.

2.2 | Molecular analysis

Peripheral blood samples were obtained in Ethylenediaminetetraacetic acid (EDTA) at pH = 8 tubes. Genomic DNA was extracted using the standard phenol-chloroform protocol.[41] *ACE* (I/D) genotype was determined using a polymerase chain reaction, as described previously,[42] followed by electrophoresis. The PCR primers; that target the region of the *Alu* insertion or deletion of the gene ACE were designed as described[42] and listed in Table S2. PCR reaction was performed in a volume of 25 µl containing 100 ng of genomic DNA, 1X of DNA polymerase Taq buffer, 2.5 mM of $Mgcl_2$, 0.2 mM of dNTP, 0.32 µM of each primer, and 1 U of Eurobio Taq ADN polymerase (Eurobio-Ingen). PCR conditions are an initial denaturation of 94°C for 10 min followed by 30 cycles of 94°C for 30s, 58°C for 30s and 72°C for 45 s, and final elongation at 72°C for 10 min. The PCR products were revealed by electrophoresis in a 2% agarose gel. PCR products could give rise to a band of 490 bp for I allele (I: Insertion), or a band of 190 bp for D allele (D: Deletion), giving 3 genotypes: the homozygous D/D or I/I genotypes or the heterozygous I/D genotype (Figure 1).

2.3 | Statistical analysis

The Hardy Weinberg (HW) was determined using the software package Arlequin (version 3.01). The difference between genotypes and alleles frequencies between PC patients and controls was determined by the $\chi 2$ test. Furthermore, odds ratios (ORs) and their 95% Confidence Intervals (IC 95%) were calculated as a measure of the association of the polymorphic sites with PC risk. A *p*-value was considered significant at <0.05. All statistical analyses except H.W were carried out with version 21 of the SPSS software.

3 | RESULTS

Genotype frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$) (Table 3). The frequencies of the *ACE**D allele demonstrate

TABLE 1 Clinical parameters of prostate cancer patients and controls

Clinical parameters	Prostate cancer (PC)	Controls
Samples sizes	124	143
Age at diagnosis (years)	70.78 ± 9.258	69.67 ± 9.03
PSA mean (ng/ml)	**161.90 ± 150.217**	
Gleason score		
Low and Intermediate score (Gleason score < 8)	**62 (55.2%)**	
High score (Gleason score ≥ 8)	52 (44.8%)	
ISUP (International Society of Urology Pathology) grading		
G1	32 (27.8%)	
G2	13 (11.3%)	
G3	25 (20.3%)	
G4	21 (17.1%)	
G5	30 (24.4%)	
Tumor stage		
Localized	44 (35.5%)	
Locally advanced	17 (13.7%)	
Metastasis	42 (33.9%)	
Not available information	21 (16.9%)	
Asymptomatic		
Asymptomatic	7 (5.7%)	
Moderate	51 (41.5%)	
Severe	43 (28.7%)	
Not available information	22 (17.9%)	
Rectal touch (TR)		
T1	42 (33.9%)	
T2	35 (28.2%)	
T3	17 (13.7%)	
T4	22 (17.7%)	
Not available information	8 (6.5%)	
Metastasis		
Yes	42 (52.5%)	
No	28 (35%)	
Not available information	10 (12.5%)	
Radical prostatectomy		
Yes	17 (13.7%)	
No	93 (75%)	
Not available information	14 (11.3%)	
Treatment		
Curative	48(38.7%)	
Palliative	63(50.8%)	
Not available information	13(10.5%)	
First and second follow-up		
Yes	80 (65%)	
No	44 (35%)	

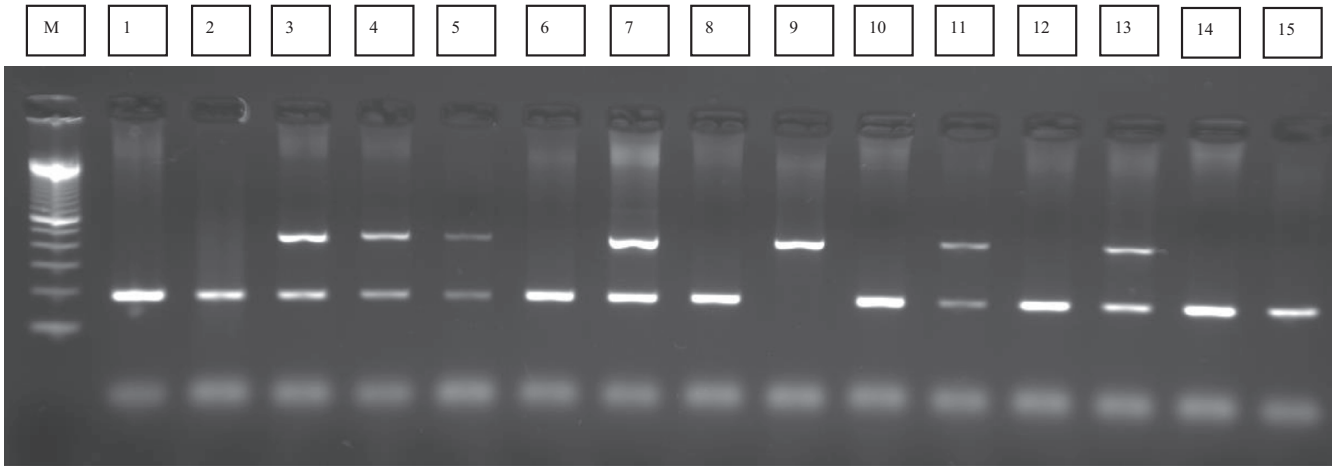
Abbreviations: PSA, Prostate Specific Antigen; TNM, Tumor Nodes Metastasis.

TABLE 2 Epidemiological parameters of prostate cancer patients and controls

Epidemiological parameters	Prostate cancer (*N* = 124)	Controls (*N* = 143)
Smoking status		
Smokers	88 (58.3%)	86 (60.1%)
Non-smokers	24 (15.9%)	41 (28.7%)
Not available information	12 (7.9%)	16 (11.2%)
Number of pack/years		
<20 PY	25 (28.4%)	27 (31.4%)
≥20 PY	63 (71.6%)	59 (68.6%)
Alcohol consumption		
Drinkers	59 (47.6%)	45 (31.1%)
No drinkers	53 (42.70%)	76 (53.5%)
Not available information	12 (9.7%)	22 (15.4%)
Diabetes		
Yes	30 (24.2%)	
No	76 (61.3%)	
Not available information	18 (14.5%)	
HTA		
Yes	26 (17.3%)	
No	86 (57.3%)	
Not available information	12 (8%)	

Abbreviations: HTA, arterial hypertension; PY, Packet year.

a different distribution of this allele between controls and cases (0.608 and 0.723, respectively) (Table 3). The analysis of the genotypic distribution showed that, in the recessive model, carries of (D/D + D/I) genotypes were found to be at increased risk of PC compared to control subjects harboring the *ACE* I/I genotype ($p = 0.005$, OR = 2.95, IC 95% = 1.26–7.09) (Table 3). Moreover, *ACE**D allele was significantly associated with increased risk of PC ($p = 0.005$ and OR = 1.69). Conversely, in the co-dominant model, the insertion genotype I/I was associated with a statistically significant reduced risk of PC ($p = 0.003$, OR = 0.3, IC 95% = 0.12–0.74). However, in the dominant model, no significant association between D/D and (I/I + D/I) genotypes and PC risk was observed (Table 3). Furthermore, the relationship between *ACE* genotypes and epidemiological parameters was explored. We found a positive association between D/D or D/I genotypes and advanced age (≥70 years) ($p = 0.014$; $r^2 = 0.22$). However, D/D or D/I genotypes in PC patients were not associated



M: DNA ladder marker 1000bp
Lanes 1, 2, 6, 8, 10, 12, 14&15: D/D homozygote genotype
Lanes 3, 4, 5, 7, 11&13: D/I heterozygote genotype
Lane 9: I/I homozygote genotype

FIGURE 1 Determination of *ACE* (I/D) polymorphism by PCR

TABLE 3 Comparison of genotypes distribution of ACE I/D (rs4646994) in controls and prostate cancer patients (PC) for Tunisian population

	Controls	PC patients	Controls group VS PC patients		
Genotypes	***N* = 143**	***N* = 124**	***p*-value**	**OR**	**95% CI**
Co-dominant model					
D/D	**58**	**64**	—	**1***	—
D/I	**58**	**50**	**0.35**	**0.78**	**0.45–1.36**
I/I	**27**	**10**	**0.003***	**0.30**	**0.12–0.74**
Dominant model					
D/D	58	64	—	1*	—
I/I+D/I	85	60	0.06	0.63	0.38–1.05
Recessive model					
I/I	27	10	—	1*	—
DD +D/I	116	114	0.005**	2.95	1.26–7.09
Alleles					
D (Wild)	174	178	—	1*	—
I (Mutant)	112	70	0.005***	0.59	0.40–0.87
p-value for HWE test	0.3350	0.5812	—	—	—

1* Reference group; *, **, *** *p*-value.

Abbreviations: 95% CI, Confidence Interval; HWE, Hardy-Weinberg EquilibriumOR, Odds ratio; PC, Prostate Cancer.

with the increased risk of PC in smokers compared to non-smokers (Table 4). Furthermore, we investigate the potential prognostic role of *ACE* genotypes and epidemiological parameters. We achieved a multivariate logistic regression analysis including stage, Grade (Gleason Score ≥ 8) or metastasis status, *ACE* I/D genotypes, and epidemiological parameters (consumption of alcohol, Tobacco status, and the presence or absence of arterial hypertension (HTA) and diabetes). We did found a significant correlation between epidemiological parameters, genotypes (D/D and D/I), and advanced Gleason score (≥8) ($p = 0.000$, $R^2 = 0.349$) (Table 5). However, the association between epidemiological parameters, genotypes, and advanced stage or metastasis did not yield significant results ($p > 0.05$).

4 | DISCUSSION

Activation of RAS plays a crucial role in many tissues including, normal and cancer prostate tissues (Human and cell lines). These changes lead to the expression of receptors and ACE enzyme and an increase in the local concentration of Ang II.[43–47] These investigations

TABLE 4 Comparison of genotypes distribution of ACE D/I (rs4646994) in PC patients according to tobacco status

Tobacco status	Genotypes	Controls	Cases	*p*-value	OR (95% CI)
Non-smokers	D/D	19	13	—	1*
	D/I	15	11	0.87	1.09 (0.34–3.44)
	I/I	7	0	0.08	0.00 (0.00–1.29)
Smokers	D/D	36	44	0.18	1.73 (0.71–4.25)
	D/I	31	35	0.28	1.58 (0.63–3.98)
	I/I	19	9	0.30	0.57 (0.17–1.90)
Highly smokers (≥ 20 PY)	D/D	26	31	0.16	1.83 (0.71–4.71)
	D/I	21	27	0.22	1.74 (0.65–4.72)

1* Reference group.

Abbreviations: 95% CI, Confidence Interval; OR, Odds ratio; PC, Prostate Cancer; PY, Packet year.

suggest that the RAS could be a good regulator of prostatic function. Moreover, it has been revealed that the level of RAS particular elements, and sensitivity of tissues on angiotensin could be modulated by the steroid hormones such as androgens. These androgens are responsible for the maintenance and growth of prostate gland. In addition, an inverse association has been shown between the risk of a PC incident and the use of any antihypertensive medication.[48] Moreover, a significant association has been established between *ACE* I/D gene polymorphism and serum enzyme concentration.[49] Thus, questioning the biological rationale underlying the selection of this candidate polymorphism gene.

Our study aimed to investigate whether *ACE* I/D gene polymorphism is associated with the occurrence of PC and with clinical and epidemiological parameters in a Tunisian population. This is to our knowledge, the first study performed in North Africa to this concern. In this Tunisian cohort, the frequencies of the D allele were 0.608 and 0.723 in controls and patients, respectively, which were different from Latino and Asian ethnicities.[29] We found that the D allele frequency of Tunisian men controls is close to The Iranian men controls and that the D allele frequency of PC Tunisian patients is near to Turkish PC patients.[29] Indeed, carrying of the D allele was associated with increased PC risk. Patients with D/D + D/I genotypes were at 3 times increased risk of PC development. Conversely, carriers of the I/I genotype were associated with a significantly lower risk of PC, which may demonstrate a protective effect of I/I genotype on PC development. These findings indicate that the *ACE* I/D gene polymorphism could be associated with PC susceptibility in the Tunisian population. Several Genetic association studies on *ACE* I/D gene polymorphism and its potential relationship with PC risk have been reported that *ACE* I/D polymorphism greatly varies between populations. Our findings were in agreement with some studies, which outlined an association between *ACE* I/D polymorphism and PC risk[29] in Asian,[50] Latino,[51] Turkish and Portuguese populations.[36] The *ACE**I allele and I/I genotype were significantly associated with a reduced risk of PC development compared to subjects with the D allele and D/D genotype in the Asian population.[29] Moreover, the *ACE**D allele and D/D genotype were associated with five times increased risk of PC development in comparison with patients harboring *ACE** I allele and I/I and D/I genotypes in the Latino population[51] and it's also associated with increased risk of PC development in Turkish and Portuguese populations.[36] However, our data contrast multiple studies in which no significant association has been reported in other ethnic groups: the Netherlands and Lebanon populations.[52,53] Thus, the effect of *ACE* I/D polymorphism in PC susceptibility in the Caucasian population[29] remain under debate. These discrepancies may be explained by genetic or/and environmental factors that interact with *ACE* gene polymorphism to determine PC susceptibility.

The prostate gland has an important role in the synthesis of the *ACE* enzyme.[54] Previous studies reported that I/D polymorphism is responsible in part of *ACE* enzyme serum level,[49,55] and that D/D carries have a higher enzymatic *ACE* level compared with I/I carries. The deletion genotype D/D is described as a growth factor and participates in several pathologies such as prostate cancer by the activation of various signaling pathways involved in cell proliferation and inhibition of apoptosis.[56–58] Furthermore, the prostate gland needs androgens to function correctly. Androgen secretions influence the synthesis and the function of AngII, a substrate of *ACE*, in a genomic and a rapid non-genomic way. Androgen has procancerogenic feature and can contribute to PC progression.[43,59,60] Thus, in addition to the D/D genotype, the hormonal context may represent one of the mechanism(s), which modulate the angiotensin expression contributing to PC progression. To summarize, the implication of *ACE* polymorphisms including *ACE* I/D can increase *ACE* gene expression, Angiotensin II (Ang II) affecting angiogenesis and the progress of PC oncogenesis. Furthermore, it has been demonstrated that homozygote genotype II can display as little as half of the plasma ACE level compared with DD homozygote genotype, however, the ID heterozygote display an intermediate level.[49] Thus, we think that D allele could play a major role on angiogenesis, proliferation and progress of PC disease.

Moreover, we explore the potential prognostic value of *ACE* polymorphism in PC. We found a positive association between D/D or D/I genotypes and advanced age (≥70 years). We were also able to detect a significant correlation between advanced Gleason grade (≥8) and epidemiological parameters with D/D or D/I genotypes. However, we failed to demonstrate any association between *ACE* I/D gene polymorphism and tumor status, metastasis, and other epidemiological parameters in our cohort. Some researchers have characterized *ACE* I/D polymorphism as a prognostic factor that could predict PC progression and clinical outcome[29,36]; In a chine

TABLE 5 Logistic regression: effect of prostate cancer risk factors on Gleason score

Gleason score[a]		B	Std. Error	Wald	dl	Sig	Exp(B)	95% Confidence Interval for Exp(B)	
								Lower Bound	Upper Bound
≥8	Constant	−2.125	1.059	4.023	1	0.045			
	[Age < 70]	−0.439	0.410	1.148	1	0.284	0.644	0.288	1.440
	[Age class = 2]	0[b]			0				
	[Tobacco status = S]	1.405	0.592	5.639	1	0.018	4.075	1.278	12.995
	[Tobacco status = NI]	1.703	1.049	2.634	1	0.105	5.493	0.702	42.965
	[Tobacco status = NS]	0[b]			0				
	[Alcohol Consumption = NI]	0[b]			0				
	[Alcohol Consumption = No]	0.488	0.449	1.179	1	0.277	1.628	0.675	3.925
	[Alcohol Consumption = Yes]	0[b]			0				
	[Diabetes = NI]	−17.712	6197.851	0.000	1	0.998	2.032E-008	0.000	0.0000
	[Diabetes = No]	0.553	0.475	1.354	1	0.245	1.738	0.685	4.410
	[Diabetes = Yes]	0[b]			0				
	[HTAcardio = No]	−0.206	0.516	0.159	1	690	0.814	0.296	2.238
	[HTAcardio = Yes]	0[b]			0				
	[Genotype = D/D]	0.686	0.739	0.861	1	0.353	1.985	0.466	8.451
	[Genotype = D/I]	0.563	0.738	0.582	1	0.446	1.755	0.414	7.452
	[Genotype = I/I]	0[b]			0				
ND	Constant	−65.967	2875.473	0.001	1	0.982			
	[Age < 70]	−31.238	1449.595	0.000	1	0.983	1.271E-013	0.000	0.0000
	[Age ≥ 70]	0[b]			0				
	[Tobacco status = S]	16.903	2039.693	0.000	1	0.993	21930272.314	0.000	0.0000
	[Tobacco status = NI]	50.486	2498.178	0.000	1	0.984	843106649742839 2000000.000	0.000	0.0000
	[Tobacco status = NS]	0[b]			0				
	[Alcohol Consumption = NI]	0[b]			0				
	[Alcohol Consumption = No]	16.161	1185.214	0.000	1	0.989	10440772.664	0.000	0.0000
	[Alcohol Consumption = Yes]	0[b]			0				
	[Diabetes = NI]	−16.959	0.000		1		4.311E-008	4.311E-008	4.311E-008
	[Diabetes = No]	1.709	1.961	0.760	1	0.383	5.525	0.118	257.702
	[Diabetes = Yes]	0[b]			0				
	[HTA = No]	14.428	822.077	0.000	1	0.986	1845599.638	0.000	0.0000
	[HTA = Yes]	0[b]			0				
	[Genotype = D/D]	16.098	822.078	0.000	1	0.984	9800575.860	0.000	0.0000
	[Genotype = D/I]	17.218	822.081	0.000	1	0.983	30042073.687	0.000	0.0000
	[Genotype = I/I]	0[b]			0				

Note: Logistic regression: Number of observation = 124, Chi-square: 53.235, $p = 0.000$, R^2: 0.349;-2 Log likelihood = 96.953.

Abbreviations: HTA, arterial hypertension; NI, Not available information; NS, Non-Smoker; S, Smoker.

[a]The reference category is PC with Gleason score < 8.

[b]This parameter is set to zero because it is redundant.

population, D/D or D/I genotype was found to be associated with advanced age ≥71 years and aggressive stage[29]; and with high-grade tumor and high PSA serum in Portugal.[36] Nevertheless, the I/I genotype was associated with the protective role against early stage of PC and there is no significant association between D/D or D/I genotype and advanced tumor stage, in the Turkish population.[29] Indeed, many functional studies highlight that *ACE* is a vasoconstrictor that could affect cancer progression.[55,61] Our results highlight that *ACE* I/D gene polymorphism could be implicated in PC progression and may improve the aggressive of disease in the Tunisian population.

We could explain this heterogeneity of results by the difference of genetic pool between ethnicities and populations and the impact

of environmental and lifestyle, which could interact with *ACE* genetic variants.

A limitation of our study is the relatively small sample size in part due to the low incidence of the disease in Tunisia. Further analysis on a larger cohort could help better to understand the significance of *ACE* I/D polymorphism in the pathobiology of PC and its contribution to the aggressiveness of the disease.

5 | CONCLUSION

In summary, our study provides the first evidence of a correlation between *ACE* I/D gene polymorphism and PC susceptibility in a Tunisian population and its potential prediction of clinical behavior. *ACE* I/D polymorphism may improve PC prognostication. Further studies are needed to explore other *ACE* gene polymorphisms and their expression level, in the Tunisian population, and the mechanisms by which they contribute to PC development and prognosis.

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CONFLICT OF INTEREST

The authors all report that they have no conflicts of interest in this study.

AUTHOR CONTRIBUTIONS

RS, SB, and SO conceived and designed the study. RS, MC, AD, and MC provided clinical samples. SO, SB, and LC provided technical support. RS, SZ collected clinical parameters. RS, FA conducted the experiment. RS, RJ, SO analyzed data and prepared all the tables. RS, RJ, and SO wrote the manuscript. All authors approved the final manuscript submitted for publication.

DATA AVAILABILITY STATEMENT

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

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REFERENCES

1. Tabayoyong W, Abouassaly R. Prostate cancer screening and the associated controversy. *Surg Clin North Am*. 2015;95(5):1023-1039.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7-30.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
4. Rawla P. Epidemiology of prostate cancer. *World J Oncol*. 2019;10(2):63-89.
5. Cooney KA. Inherited predisposition to prostate cancer: from gene discovery to clinical impact. *Trans Am Clin Climatol Assoc*. 2017;128:14-23.
6. Forrest MS, Edwards SM, Houlston R, et al. Association between hormonal genetic polymorphisms and early-onset prostate cancer. *Prostate Cancer Prostatic Dis*. 2005;8(1):95-102.
7. Gong Z, Kristal AR, Schenk JM, Tangen CM, Goodman PJ, Thompson IM. Alcohol consumption, finasteride, and prostate cancer risk. *Cancer*. 2009;115(16):3661-3669.
8. Steinberg GD, Carter BS, Beaty TH, Childs B, Walsh PC. Family history and the risk of prostate cancer. *Prostate*. 1990;17(4):337-347.
9. Agalliu I, Kwon EM, Salinas CA, Koopmeiners JS, Ostrander EA, Stanford JL. Genetic variation in DNA repair genes and prostate cancer risk: results from a population-based study. *Cancer Causes Control*. 2010;21(2):289-300.
10. Benafif S, Kote-Jarai Z, Eeles RA. A review of prostate cancer Genome-Wide Association Studies (GWAS). *Cancer Epidemiol Biomark Prev*. 2018;27(8):845-857.
11. Escobar E, Rodriguez-Reyna TS, Arrieta O, Sotelo J. Angiotensin II, cell proliferation and angiogenesis regulator: biologic and therapeutic implications in cancer. *Curr Vasc Pharmacol*. 2004;2(4):385-399.
12. Xiao Y, Dong Z, Zhu J, You J, Fan J. Association between ACE A240T polymorphism and cancer risk: a meta-analysis. *J Int Med Res*. 2019;47(12):5917-5925.
13. Abdeahad H, Avan A, Khazaei M, et al. Angiotensin-converting enzyme gene polymorphism and digestive system cancer risk: a meta-analysis based on 9656 subjects. *J Cell Biochem*. 2019;120(12):19388-19395.
14. Cheng Z, Liu Z. Renin-angiotensin system gene polymorphisms and colorectal cancer risk: a meta-analysis. *J Renin Angiotensin Aldosterone Syst*. 2019;20(4):147032031988193.
15. Pringle KG, Delforce SJ, Wang YU, et al. Renin-angiotensin system gene polymorphisms and endometrial cancer. *Endocr Connect*. 2016;5(3):128-135.
16. Raba G, Zawlik I, Braun M, et al. Evaluation of the association between angiotensin converting enzyme insertion/deletion polymorphism and the risk of endometrial cancer in and characteristics of Polish women. *Adv Clin Exp Med*. 2020;29(5):581-585.
17. Li M, Schifanella L, Larsen PA. Alu retrotransposons and COVID-19 susceptibility and morbidity. *Hum Genomics*. 2021;15(1):2.
18. Yamamoto N, Ariumi Y, Nishida N, et al. SARS-CoV-2 infections and COVID-19 mortalities strongly correlate with ACE1 I/D genotype. *Gene*. 2020;758:144944.
19. Das S, Zhang E, Senapati P, et al. A novel angiotensin II–induced long noncoding RNA giver regulates oxidative stress, inflammation, and proliferation in vascular smooth muscle cells. *Circ Res*. 2018;123(12):1298-1312.
20. Messerli FH, Bangalore S, Bavishi C, Rimoldi SF. Angiotensin-converting enzyme inhibitors in hypertension: to use or not to use? *J Am Coll Cardiol*. 2018;71(13):1474-1482.
21. Ruggenenti P, Perna A, Remuzzi G. ACE inhibitors to prevent end-stage renal disease: when to start and why possibly never to stop: a post hoc analysis of the REIN trial results. *J Am Soc Nephrol*. 2001;12(12):2832-2837.
22. Achouri-Rassas A, Ali NB, Cherif A, et al. Association between ACE polymorphism, cognitive phenotype and APOE E4 allele in a Tunisian population with Alzheimer disease. *J Neural Transm (Vienna)*. 2015;123(3):317-321.
23. Shoaib RMS, Hammad A, Yahia S, Elsaid A, Abdel-Malak CA. Angiotensin II type 1 receptor gene polymorphism and serum angiotensin-converting enzyme level in Egyptian children with systemic lupus erythematosus. *Clin Rheumatol*. 2018;37(12):3309-3317.

24. Cintra MTR, Balarin MAS, Tanaka SCSV, et al. Polycystic ovarian syndrome: rs1799752 polymorphism of ACE gene. *Revista Da Associação Médica Brasileira*. 2018;64:1017-1022.
25. Zhang K, Cheng D, Yi L, Shi H, Zhen G. Association between angiotensin I-converting enzyme gene polymorphism and susceptibility to cancer: a meta analysis. *Int J Clin Exp Pathol*. 2014;7(9):6291-6300.
26. Haiman CA, Henderson SO, Bretsky P, Kolonel LN, Henderson BE. Genetic variation in angiotensin I-converting enzyme (*ACE*) and breast cancer risk. *The Multiethnic Cohort*. 2003;63(20):6984-6987.
27. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest*. 1990;86(4):1343-1346.
28. Haiman CA, Henderson SO, Bretsky P, Kolonel LN, Henderson BE. Genetic variation in angiotensin I-converting enzyme (ACE) and breast cancer risk: the multiethnic cohort. *Can Res*. 2003;63(20):6984-6987.
29. Wang ZY, Li HY, Jiang ZP, Zhou TB. Relationship between angiotensin-converting enzyme insertion/deletion gene polymorphism and prostate cancer susceptibility. *J Cancer Res Ther*. 2018;14(Supplement):S375-S380.
30. Cheng Z, Ma R, Tan W, Zhang L, Tan Q. Lack of association between ACE insertion/deletion polymorphism and lung cancer: a meta-analysis. *J Renin Angiotensin Aldosterone Syst*. 2015;16(2):453-458.
31. Deckers IA, van den Brandt PA, van Engeland M, et al. Polymorphisms in genes of the renin-angiotensin-aldosterone system and renal cell cancer risk: interplay with hypertension and intakes of sodium, potassium and fluid. *Int J Cancer*. 2015;136(5):1104-1116.
32. Han CD, Ge WS. Up-regulation of Angiotensin-Converting Enzyme (ACE) enhances cell proliferation and predicts poor prognosis in laryngeal cancer. *Med Sci Monit*. 2016;22:4132-4138.
33. Moghimi M, Kargar S, Jafari MA, et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with breast cancer risk: a meta-analysis. *Asian Pac J Cancer Prev*. 2018;19(11):3225-3231.
34. Pandith AA, Qasim I, Zahoor W, Shah P, Bhat AR. ACE I/D sequence variants but not MTHFR C677T, is strongly linked to malignant glioma risk and its variant DD genotype may act as a promising predictive biomarker for overall survival of glioma patients. *Gene*. 2018;639:62-68.
35. Zha Y, Gan P, Liu Q, Tan J. Relationship between polymorphism of angiotensin-converting enzyme gene insertion/deletion and risk of hepatocellular carcinoma in a Chinese Dai population. *J Renin Angiotensin Aldosterone Syst*. 2015;16(3):695-699.
36. Yigit B, Bozkurt N, Narter F, Yilmaz H, Yucebas E, Isbir T. Effects of ACE I/D polymorphism on prostate cancer risk, tumor grade and metastatis. *Anticancer Res*. 2007;27(2):933-936.
37. Yuan F, Zhang L-S, Li H-Y, Liao M, Lv M, Zhang C. Influence of angiotensin I-converting enzyme gene polymorphism on hepatocellular carcinoma risk in China. *DNA Cell Biol*. 2013;32(5):268-273.
38. Lukic S, Nikolic A, Alempijevic T, et al. Angiotensin-converting enzyme gene insertion/deletion polymorphism in patients with chronic pancreatitis and pancreatic cancer. *Dig Surg*. 2011;28(4):258-262.
39. Namazi S, Monabati A, Ardeshir-Rouhani-Fard S, Azarpira N. Association of angiotensin I converting enzyme (insertion/deletion) and angiotensin II type 1 receptor (A1166C) polymorphisms with breast cancer prognostic factors in Iranian population. *Mol Carcinog*. 2010;49(12):1022-1030.
40. Medeiros R, Vasconcelos A, Costa S, et al. Linkage of angiotensin I-converting enzyme gene insertion/deletion polymorphism to the progression of human prostate cancer. *J Pathol*. 2004;202(3):330-335.
41. Evans GA. Volumes 1, 2, and 3. Current protocols in molecular biology. Volumes 1 and 2: By J. Sambrook, E. F. Fritsch, and T. Maniatis. *Molecular cloning: A laboratory manual*. 2nd ed. Cold Spring Harbor Laboratory Press; 1989: 1626. $115.00. Edited by Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Greene Publishing Associates and John Wiley & Sons; 1989: 1120. $255.00. Cell. 1990;61(1):17-8.
42. Zhu X, Bouzekri N, Southam L, et al. Linkage and association analysis of angiotensin I-converting enzyme (ACE)-gene polymorphisms with ACE concentration and blood pressure. *Am J Hum Genet*. 2001;68(5):1139-1148.
43. Domińska K, Kowalski A, Ochędalski T, Rębas E. Effects of testosterone and 17β-estradiol on angiotensin-induced changes in tyrosine kinase activity in the androgen-independent human prostate cancer cell line, DU145. *Int J Mol Med*. 2017;40(5):1573-1581.
44. Domińska K, Piastowska-Ciesielska AW, Płuciennik E, Lachowicz-Ochędalska A, Ochędalski T. A comparison of the effects of Angiotensin IV on androgen-dependent and androgen-independent prostate cancer cell lines. *J Renin Angiotensin Aldosterone Syst*. 2012;14(1):74-81.
45. Domińska K, Piastowska-Ciesielska AW, Lachowicz-Ochędalska A, Ochędalski T. Similarities and differences between effects of angiotensin III and angiotensin II on human prostate cancer cell migration and proliferation. *Peptides*. 2012;37:200-206.
46. Uemura H, Hoshino K, Kubota Y. Engagement of renin-angiotensin system in prostate cancer. *Curr Cancer Drug Targets*. 2011;11(4):442-450.
47. Uemura H, Kubota Y. Role of renin-angiotensin system in prostate cancer. *Gan to Kagaku Ryoho*. 2009;36(8):1228-1233.
48. Fitzpatrick AL, Daling JR, Furberg CD, Kronmal RA, Weissfeld JL. Hypertension, heart rate, use of antihypertensives, and incident prostate cancer. *Ann Epidemiol*. 2001;11(8):534-542.
49. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Investig*. 1990;86(4):1343-1346.
50. Wang X, Wang S, Lin Y-W, et al. Angiotensin-converting enzyme insertion/deletion polymorphism and the risk of prostate cancer in the Han population of China. *Med Oncol*. 2012;29(3):1964-1971.
51. Sierra Díaz E, Sánchez Corona J, Rosales Gómez RC, et al. 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*. 2009;10(4):241-246.
52. van der Knaap R, Siemes C, Coebergh JW, van Duijn CM, Hofman A, Stricker BH. Renin-angiotensin system inhibitors, angiotensin I-converting enzyme gene insertion/deletion polymorphism, and cancer: the Rotterdam Study. *Cancer*. 2008;112(4):748-757.
53. El Ezzi AA, Clawson JM, El-Saidi MA, et al. Association of angiotensin I converting enzyme insertion/287 bp deletion polymorphisms and proliferative prostatic diseases among lebanese men. *Prostate Cancer*. 2020;2020:5959134.
54. Danilov SM, Kadrev AV, Kurilova OV, et al. Tissue ACE phenotyping in prostate cancer. *Oncotarget*. 2019;10(59):6349-6361.
55. Suehiro T, Morita T, Inoue M, Kumon Y, Ikeda Y, Hashimoto K. Increased amount of the angiotensin-converting enzyme (ACE) mRNA originating from the ACE allele with deletion. *Hum Genet*. 2004;115(2):91-96.
56. Singh A, Srivastava N, Amit S, Prasad SN, Misra MP, Ateeq B. Association of AGTR1 (A1166C) and ACE (I/D) polymorphisms with breast cancer risk in North Indian population. *Transl Oncol*. 2018;11(2):233-242.
57. Chen J, Sun M, Zhou M, Lu R. Associations between I/D polymorphism in the ACE gene and lung cancer: an updated systematic review and a meta-analysis. *BMC Cancer*. 2021;21(1):158.
58. Amankwah EK, Sellers TA, Park JY. Gene variants in the angiogenesis pathway and prostate cancer. *Carcinogenesis*. 2012;33(7):1259-1269.

59. Bachmann J, Feldmer M, Ganten U, Stock G, Ganten D. Sexual dimorphism of blood pressure: possible role of the renin-angiotensin system. *J Steroid Biochem Mol Biol*. 1991;40(4-6):511-IN2.
60. Derkx FH, Steunkel C, Schalekamp MP, Visser W, Huisveld IH, Schalekamp MA. Immunoreactive renin, prorenin, and enzymatically active renin in plasma during pregnancy and in women taking oral contraceptives. *J Clin Endocrinol Metab*. 1986;63(4):1008-1015.
61. Danilov SM, Metzger R, Klieser E, Sotlar K, Trakht IN, Garcia JGN. Tissue ACE phenotyping in lung cancer. *PLoS One*. 2019;14(12):e0226553.

SUPPORTING INFORMATION

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