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Research article

Angiotensin-converting-enzyme gene insertion-deletion polymorphism and renin angiotensin aldosterone system activity in different phenotypes of polycystic ovary syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common condition. Its pathophysiology involves an interaction between genetic and environmental factors, resulting in different reproductive and metabolic subtypes. Genetic variation in the angiotensin converting enzyme (ACE) gene has been implicated in the pathophysiology of the syndrome. Our project aims to investigate the relationship between the ACE I/D (insertion/deletion) polymorphism and circulating levels of ACE activity, renin and aldosterone in PCOS.

Patients and methods: PCOS and healthy donors were enrolled over 2 years. The Rotterdam criteria were used to segregate 4 phenotypes. Enrolled controls were matched to PCOS patients for body mass index. Clinical data were recorded and blood samples were taken for analysis of ACE I/D polymorphism and biochemical parameters. Hardy-Weinberg equilibrium evaluation, mean/median comparison and Spearman correlation were performed.

Results: A total of 102 women with PCOS and 107 controls were involved in the study. The most common polymorphism was ID, both in the control and PCOS groups. Renin levels in PCOS patients were positively correlated with luteinizing hormone (LH) and anti-mullerian hormone (AMH) in the ID genotype and with testosterone, free androgen index and insulin in the DD genotype.

Conclusion: The ACE I/D variation may influence the pathophysiology of PCOS subtypes, together with an indirect relationship with renin. Our findings may provide valuable therapeutic insights into the management of PCOS, particularly regarding the potential need for ACE inhibitors or renin inhibitors tailored to ACE gene I/D genotypes or specific subtypes.

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1. Introduction

Polycystic ovary syndrome (PCOS) is a condition that affects women of reproductive age and is characterized by reproductive, endocrine and/or skin problems. Four phenotypes are commonly described based on the combination of hyperandrogenism (HA), oligoanovulation (OA) and polycystic ovaries on ultrasound (PCOU) [1]. Insulin resistance and an inverted LH/FSH ratio are also associated with PCOS [2]. The pathophysiology of PCOS involves interactions between environmental factors and genetic backgrounds, resulting in various subtypes and phenotypes. In a cohort primarily composed of Caucasians, the main genes under study exhibited variability in the genetic structure of PCOS. This variance enabled the identification of PCOS subtypes, diverging from the phenotypes proposed by Rotterdam [3]. The three subtypes were reproductive, metabolic, and indeterminate. In fact, valuable clinical and biochemical traits were assessed to distinguish these subtypes, such as body mass index (BMI), insulin, luteinizing hormone (LH), testosterone (Testo)/sex hormone-binding globulin (SHBG), and anti-müllerian hormone (AMH) [3,4].

At the end of a normal follicular phase, follicle atresia is observed subsequent to a thecal androgen secretion triggered by the LH surge and the release of ovarian angiotensin peptides [5,6]. In PCOS, the ovarian secretion of androgens displays a hyperresponsiveness to LH enhanced by insulin resistance. The insulin and LH signaling pathways are suggested to be modulated by key factors of the renin-angiotensin-aldosterone system (RAAS), which is abnormally expressed in PCOS [7–9]. The RAAS is actually a cascade of enzymatic cleavage starting with angiotensinogen and ending with angiotensin II or with other angiotensin peptides through the classical or the novel pathway, respectively. The ACE is required in both classical and novel RAAS pathways [9]. The ACE gene is located on chromosome 17q23. Although, thousands of polymorphisms were recorded by the National Center for Biotechnology Information (NCBI), the most studied polymorphism was the ACE gene polymorphism (rs4340) insertion/deletion (I/D) a modulator of ACE mRNA stability and as a consequence the enzymatic activity. It is a 287 bp Alu repeat sequence in intron 16 (Table 1), It is a 287 bp Alu repeat sequence in intron 16 (Table 1) and is linked to several cardiovascular risk factors, pregnancy complications, and various health conditions [10–12]. In the context of polycystic ovary syndrome (PCOS), it stands out as the only polymorphism of ACE gene examined as a candidate gene for PCOS pathogenesis [13]. Robust meta-analyses indicate that this polymorphism may promote the development of PCOS and is potentially associated to insulin resistance [14,15].

The three possible genotypes described are homozygote insertion/insertion (I/I), heterozygote insertion/deletion (I/D), and homozygote deletion/deletion (D/D). The D allele is associated with high ACE activity, enhanced steroidogenesis, and elevated prevalence of insulin resistance, which may interfere with PCOS phenotypes and/or proposed subtypes [16,17].

This mysterious syndrome, involving metabolic and reproductive hormones as well as the RAAS, drives us to investigate its associated circulating RAAS factors and ACE I/D polymorphism, in order to find clues to complete the puzzle and infer perspectives in therapeutic insights.

The main objective of our work was to investigate the relationship between ACE I/D polymorphism and circulating levels of ACE activity, renin, and aldosterone in PCOS.

2. Patients and methods

We conducted a cross-sectional study from 2019 to 2020, approved by the personal protection committee of southern Tunisia (ethics approval number CPP SUD N°0197/2019). Women with PCOS aged between 18 and 45 years were included. The diagnosis of PCOS was made according to the Rotterdam criteria [18]. Four phenotypes were defined according to the following criteria.

1 OligoAnovulation OA + HyperAndrogenism HA + Polycystic Ovaries On Ultrasound PCOU
2 OligoAnovulation OA + HyperAndrogenism HA
3 HyperAndrogenism HA + Polycystic Ovaries On Ultrasound PCOU
4 OligoAnovulation OA + Polycystic Ovaries On Ultrasound PCOU.

We included a control group to compare ACE genotypes, consisting of BMI-matched healthy volunteers with regular menstrual cycles, normal ovaries on ultrasound and no hyperandrogenism [19]. Age and anthropometric parameters were also recorded. Cohort exclusion criteria included use of oral contraceptive pills (OCPs) and any other medication, especially those affecting the RAAS. All participants gave written consent before being included.

Blood was collected on the 3rd to the 5th day of menses, between 8 and 10 a.m. Participants were asked to keep fasting for 12 h.

Table 1
Features of ACE I/D polymorphism study.

Gene sequence accession number	rs4340
ACE I/D polymorphism location	11 698 bp
Deletion allele primer	
Forward [F1]	5′-CTGGAGACCACTCCCATCCTTTCT-3′
Reverse [R1]	5′-GATGTGGCCATCACATTCGTCAGAT-3′
Insertion allele primer	
Forward [F2]	5′-TGGGACCACAGCGCCCGCCACTAC-3′
Reverse [R2]	5′-TCGCCAGCCCTCCCATGCCCATAA-3′

Table 1 displays characteristics of ACE I/D gene polymorphism and information on allele primers.

Several biochemical parameters were assessed: The measurement of glucose, insulin, gonadotrophins (FSH, LH), antimüllerian hormone (AMH), estradiol, testosterone (Testo), and sex hormone-binding globulin (SHBG) were made on COBAS-6000 (Roche Diagnostics, Penzberg, Germany). ACE activity was assessed by spectrophotometry on COBAS-6000 (Roche Diagnostics, Penzberg, Germany) using an ACE kinetic kit (Buhulmann Laboratories, Baselstrasse, Switzerland) [20]. Renin, aldosterone (Aldo), androstenedione (A4), dehydroepiandrosterone sulfate (DHEAS), and 17-hydroxyprogesterone (17-OH-prog) were analyzed using an ELISA kit (DRG, Germany). It should be noted that ACE activity and SHBG were only assessed in PCOS patients.

Homeostasis HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was assessed by multiplying glucose (mmol/L) by insulin (mU/L) and dividing the result by 22.5. Free androgen index (FAI) was calculated by dividing the product of Testo (nmol/L) and 100 by SHBG (nmol/L) [21].

To evaluate the ACE polymorphism, DNA was isolated from whole blood using phenol/chloroform extraction on EDTA (ethylenediaminetetraacetic acid) followed by two PCRs and amplification products were separated on 2 % agarose gel to study ACE genotypes [22]. The primers for the first PCR were specific for the D allele, whereas the second PCR targeted the I allele. The primers are listed in Table 1.

Statistical Analysis: The SPSS 25 software was used to process data. Variables were presented as mean ± standard deviation or median (interquartile range). Normality was evaluated using the Shapiro-Wilk test. Means/medians were compared by student test or Mann-Whitney *U* Test in terms of variable distribution. The differences in the various tested variables among the three genotypes were evaluated on a two-by-two basis. The X2 test was employed to examine the Hardy-Weinberg equilibrium by comparing observed frequencies to expected frequencies, with cases weighted by the frequency of the tested variable. Both X2 test and Fisher's exact test were used to compare the distribution of the following three genetic models: (II as reference vs ID + DD), (ID as reference vs II + DD), and (DD as reference vs II + ID). Spearman correlation was applied to test the relationship between ACE activity, aldosterone, renin, and aldosterone to renin ratio (A/R) and PCOS subtype traits (BMI, insulin, LH, FAI, and AMH). We opted to dismiss the null hypothesis with a significance level set at $p < 0.05$.

3. Results

To investigate the ACE gene insertion/deletion polymorphism and renin levels in polycystic ovary syndrome, we enrolled 209 participants: 102 with PCOS and 107 controls. We started with a complete clinical examination. Most of the biochemical parameters analyzed were not significantly different between the groups, except that PCOS patients were significantly younger and more insulin resistant than controls with an undoubtedly high LH/FSH ratio, 17OHprog, testosterone, and A4 (Table 2). The comparison between the four phenotypes showed that phenotype 4 exhibited a higher median age compared to phenotype 1 and lower median androgen levels compared to hyperandrogenic phenotypes (1, 2, and 3), in contrast to the uniform fat distribution and similar glucose

Table 2
Basal characteristics of PCOS patients and controls.

	Unit	Control N = 107	PCOS N = 102	p
Age	years	27.0 [24–34]	25.0 [22–28]	0.001
BMI	kg/m^2	24.0 [21.5–27.5]	24.3 [21.8–29.3]	0.602
WC	cm	88.0 [79.0–96.0]	99.0 [89.0–108.0]	0.306
W/H	–	0.81 [0.82–0.94]	0.90 [0.85–0.93]	0.233
FSH	mUI/mL	6.5 [5.7–8.2]	5.8 [5.3–7.0]	0.002
LH	mUI/mL	6.3 [4.5–7.3]	8.4 [6.3–11.7]	<0.0001
LH/FSH	–	0.95 [0.63–1.21]	1.46 [1.06–1.95]	<0.0001
Estradiol	pmol/L	151.2 [92.9–201.5]	160.0 [103.6–191.3]	0.240
Testosterone	nmol/L	0.79 [0.55–1.07]	1.13 [0.79–1.63]	<0.0001
SHBG	nmol/L	–	43.6 [26.7–74.8]	–
FAI	–	–	2.31 [15.8–4.95]	–
17OHprog	nmol/L	2.4 [1.5–3.3]	3.4 [2.6–4.0]	<0.0001
A4	nmol/L	6.1 [3.5–7.4]	7.2 [5.6–8.8]	<0.0001
SDHEA	µmol/L	5.2 [3.4–6.6]	5.9 [4.3–7.7]	0.127
AMH	pmol/L	–	33.5 [21.5–45.5]	–
Insulin	mUI/L	9.2 [6.9–13.9]	10.7 [8.3–17.1]	0.011
Glucose	mmol/L	5.1 [4.6–5.5]	5.1 [4.7–5.5]	0.847
HOMA-IR	–	2.00 [1.48–3.10]	2.29 [1.84–3.96]	0.036
Aldosterone	ng/L	108.9 [65.5–137.7]	123.9 [78.6–187.1]	0.479
Renin	ng/L	11.9 [7.1–19.3]	12.2 [6.1–17.9]	0.152
Aldo/R	ng/ng	9.4 [5.1–15.7]	9.3 [5.1–18.0]	0.363
ACE	UI/L	–	39.8 ± 14.0	–

17OHprog: 17-hydroxyprogesterone, A4: androstenedione, ACE: converting enzyme, Aldo/R: aldosterone to renin ratio, AMH: anti-mullerian hormone, BMI: body mass index, FSH: follicle stimulating hormone, FAI: free androgen index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, LH: luteinizing hormone, PCOS: polycystic ovary syndrome, SDHEA: dehydroepiandrosterone-sulfate, SHBG: sex hormone-binding globulin, Testo: testosterone, WC: waist circumference, W/H:waist to hip ratio.

Table 2 shows that age, LH, LH/FSH, testosterone, 17 hydroxyprogesterone, androstenedione, insulin, HOMA-IR were significantly elevated in PCOS patients compared to controls.

metabolism profile (Table 3). We then looked at the ACE gene polymorphism in the PCOS patients. There were three ACE possible genotypes, DD (homozygote deletion/deletion), ID (heterozygote insertion/deletion) and II (homozygote insertion/insertion).

The polymorphism was determined by two consecutive PCRs. The first PCR was performed to amplify 190bp (the D allele), 490bp (the I allele) or both (the ID genotype). The second PCR was performed on samples with only 190bp products. If a fragment of 335bp is present, it confirms the presence of the insertion and in this case the genotype is ID. If the 335bp fragment is absent, the sample is DD (Fig. 1A and B). Then we quantified the ACE I/D gene polymorphism distribution in the control group versus PCOS group (Fig. 1C) and, in order to correlate the different PCOS phenotypes and ACE polymorphism, in the four PCOS subgroup (Fig. 1 D and Table 4).

No Hardy-Weinberg imbalance was observed in either the control or PCOS groups ($p > 0.05$).

The most present polymorphism was the ID (heterozygote insertion/deletion), both in control or PCOS group. Moreover, when comparing the different PCOS subgroup phenotypes, the phenotype 3 (HA + PCOU) was the subgroup where ID polymorphism was the most present, compared to the others phenotypes (Table 4).

We decided then to investigate the correlation that could exist between the clinical feature of the patients with the ACE polymorphism and the different PCOS phenotypes. In PCOS patients, ACE activity was significantly elevated in D allele carriers, unlike the matched aldosterone, renin, and Aldo/renin ratio levels. In addition, we observed an elevation of androgens, estrogen, AMH, and LH levels associated with the I allele, and of anthropometric measures, insulin, and HOMA-IR levels associated with the D allele, although without statistically significant differences (Table 5).

In PCOS, renin, aside from Aldo, was positively correlated to LH and AMH in the ID genotype, and to testosterone, FAI, and insulin in the DD genotype (Table 6). We should however highlight that ACE activity was neither correlated to PCOS subtype traits, nor renin, aldosterone, or aldosterone/renin ratio (Aldo/R).

4. Discussion

This study identifies a clear relationship between circulating renin and the pathophysiology of PCOS, dependent on ACE I/D genotype distribution. The novelty was the interdependence of these two important factors, which have previously been examined separately and individually associated with PCOS.

Literature suggests a potential link between ACE I/D genotypes and the characteristics of PCOS. Some studies have indicated that

Table 3
Clinical and biological characteristics of PCOS phenotypes.

	Unit	PCOS phenotypes [N = 102]			
		1	2	3	4
		33	14	31	24
Age	years	25 [21–27]‡	25.5 [22.5–28.7]	25 [22–29]	28 [23–33]‡
BMI	kg/m^2	24.2 [21.0–27.5]	24.3 [21.4–31.1]	23.9 [22.0–27.9]	26.4 [22.6–29.4]
WC	cm	90.0 [79.0–99.2]	91.0 [80.0–108.0]	87.0 [77.5–99.5]	89.0 [86.5–95.0]
W/H		0.84 [0.78–0.90]	0.88 [0.76–0.90]	0.84 [0.78–0.90]	0.86 [0.79–0.90]
FSH	mUI/mL	5.6 [4.9–7.2]	6.0 [5.0–6.5]	6.0 [5.4–7.0]	6.1 [5.5–7.0]
LH	mUI/mL	8.0 [6.1–15.9]	8.3 [6.4–10.7]	8.5 [6.7–11.7]	7.9 [6.1–11.3]
Estradiol	pmol/L	155.6 [96.9–195.6]	147.9 [88.9–166.6]	161.1 [129.9–197.1]	145.7 [10.0–179.1]
Testosterone	nmol/L	1.49 [1.01–1.67]‡	1.09–0.86-1.66]††	1.18 [0.94–1.51]††	0.76 [0.48–0.99]‡,††,††
SHBG	nmol/L	43.7 [24.9–74.2]	43.7 [21.5–64.6]	39.0 [25.8–25.0]	55.2 [34.5–93.6]
FAI		2.6 [1.7–5.3]	2.6 [1.8–5.3]	2.8 [1.6–5.1]	1.6 [0.6–3.6]
17OHprog	nmol/L	3.6 [2.4–4.0]	3.0 [2.1–3.4]**	3.6 [3.1–4.3]**,††	2.8 [1.9–4.0]††
A4	nmol/L	7.4 [5.1–8.8]	8.8 [6.0–9.4]††	7.7 [6.2–8.8]††	5.8 [4.8–7.1]††,††
SDHEA	µmol/L	6.1 [5.1–8.8]‡	6.2 [3.3–7.9]	6.6 [4.3–8.5]††	4.5 [2.5–5.8]‡,††
AMH	pmol/L	33.6 [23.9–41.6]	34.1 [20.6–45.5]	35.0 [18.9–50.9]	34.4 [23.1–42.8]
Insulin	mUI/L	10.2 [7.9–17.6]	10.7 [8.9–25.3]	11.3 [9.2–17.1]	10.8 [7.6–15.3]
Glucose	mmol/L	5.1 [4.7–5.6]	5.3 [4.6–5.5]	4.8 [4.7–5.4]	5.2 [4.8–5.5]
HOMA-IR		2.16 [1.08–4.06]	2.32 [1.90–6.91]	2.55 [1.92–3.88]	2.26 [1.84–3.38]
ACE activity	UI/L	40.8 ± 17.4	36.4 ± 15.6	39.3 ± 14.1	40.6 ± 12.4
Aldosterone	ng/L	124.4 [71.4–182.3]	90.6 [42.0–132.2]**	142.0 [88.7–173.5]**	129.9 [83.2–223.0]
Renin	ng/L	15.4 [8.3–22.2]*	7.4 [4.3–13.5]*,**	13.1 [7.6–18.8]**	10.2 [4.8–16.0]
Aldo/R	ng/ng	9.0 [4.5–12.4]	12.0 [3.4–19.4]	9.3 [5.4–20.0]	10.5 [6.0–26.9]

*: significant difference between 1 vs. 2 phenotype.
┼: significant difference between 1 vs. 3 phenotype.
╪: significant difference between 1 vs. 4 phenotype.
**: significant difference between 2 vs. 3 phenotype.
┼┼: significant difference between 2 vs. 4 phenotype.
╪╪: significant difference between 3 vs. 4 phenotype.
17OHprog: 17-hydroxyprogesterone, A4: androstenedione, ACE: converting enzyme, Aldo/R: aldosterone to renin ratio, AMH: anti-mullerian hormone, BMI: body mass index, FSH: follicle stimulating hormone, FAI: free androgen index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, LH: luteinizing hormone, PCOS: polycystic ovary syndrome, SDHEA: dehydroepiandrosterone-sulfate, SHBG: sex hormone-binding globulin, Testo: testosterone, WC: waist circumference, W/H:waist to hip ratio.
Table 3 shows clinical and biological characteristics of PCOS phenotypes.

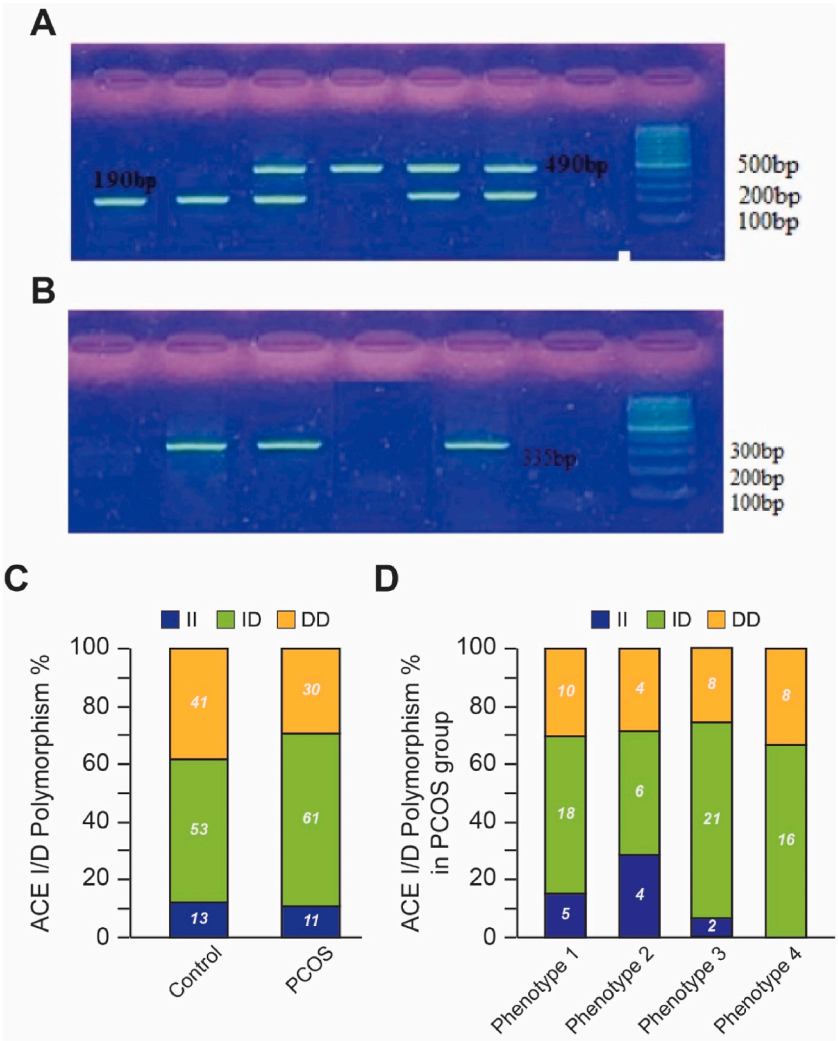

Fig. 1. Investigation of the ACE gene insertion/deletion polymorphism. A-B. PCR's results of ACE polymorphism.Gel [A] shows three types of profiles after the first PCR: samples with two bands [190bp and 490bp] corresponding to the ID genotype, samples with only one band of 490bp corresponding to the II genotype, and samples with one 190bp band.Gel [B] displays the result of the second PCR on samples showed a 190 pb with the first PCR, to discriminate between the ID or DD genotype. The absence of amplification reveals the DD genotype while appearance of a band of 335bp means an ID genotype.C-D. Quantification of ACE polymorphism Graph [C] represents ACE gene insertion/deletion polymorphism quantitative data in both control group [107 healthy women] and PCOS group [102 patients] while graph [D] display the ACE polymorphism in the four phenotypes dispatched from PCOS. Numbers in every graph represents the number of patient/control in every condition while graph display them in percentage.

Table 4
Distribution of genetic models in controls and PCOS patients.

	Controls	PCOS total group	Phenotype 1	Phenotype 2	Phenotype 3	Phenotype 4
[II vs ID + DD] p value	[13 vs 94]	[11 vs 91]	[5 vs 28]	[4 vs 10]	[2 vs 29]	[0 vs 24]
		0.757	0.652	0.096	0.369	0.072
[DD vs II + ID] p value	[41 vs 66]	[30 vs 72]	[10 vs 23]	[4 vs 10]	[8 vs 23]	[8 vs 16]
		0.174	0.403	0.478	0.200	0.648
[ID vs II + DD] p value	[53 vs 54]	[61 vs 41]	[18 vs 15]	[6 vs 8]	[21 vs 10]	[16 vs 8]
		0.136	0.615	0.638	0.073	0.129

Table 4 displays the distribution of genetic models in the PCOS total group and its four phenotypes compared to controls.

Table 5
Clinical and biological characteristics of PCOS according to ACE I/D genotype.

	Unit	ACE polymorphism in PCOS [N = 102]		
		II	ID	DD
		11	61	30
Age	years	25 [23–28]	25 [22–28]	25 [21.5–30.5]
BMI	kg/m^2	22.8 [20.9–25.4]	24.2 [24.2–33.2]	26.5 [21.0–30.2]
WC	cm	82.0 [77.5–105.0]	89.5 [79.0–100.0]	90.0 [82.0–97.0]
W/H		0.81 [0.77–0.93]	0.85 [0.77–0.90]	0.84 [0.78–0.91]
FSH	mUI/mL	5.8 [5.4–6.5]	5.9 [5.3–6.8]	6.3 [4.4–7.3]
LH	mUI/mL	10.0 [4.6–16.5]	8.5 [6.4–11.9]	8.0 [6.0–10.4]
Estradiol	pmol/L	160.0 [133.9–177.6]	157.1 [103.6–199.3]	148.3 [81.3–183.7]
Testosterone	nmol/L	1.42 [0.97–1.73]	1.14 [0.78–1.61]	1.07 [0.71–1.61]
SHBG	nmol/L	48.5 [21.8–80.8]	43.8 [24.9–76.6]	40.0 [27.4–72.3]
FAI		3.91 [1.97–5.11]	2.26 [1.56–4.40]	2.38 [1.17–4.67]
17OHprog	nmol/L	3.9 [2.8–4.3]	3.4 [2.8–3.9]	3.2 [2.4–4.0]
A4	nmol/L	8.5 [5.8–11.6][d]	7.0 [5.8–11.6][d]	7.2 [5.4–8.8]
SDHEA	µmol/L	6.2 [4.7–9.2]	6.1 [4.3–7.7]	5.4 [3.4–7.5]
AMH	pmol/L	36.7 [22.1–55.0]	34.4 [19.2–45.6]	32.1 [20.9–41.1]
Insulin	mUI/L	8.5 [7.5–12.3]	11.2 [8.4–18.1]	10.4 [9.0–16.3]
Glucose	mmol/L	5.3 [4.6–5.5]	5.1 [4.7–5.5]	5.1 [4.8–5.7]
HOMA-IR		1.84 [1.56–2.71]	2.48 [1.85–4.07]	2.03 [1.98–3.95]
ACE activity	UI/L	25.5 [21.2–28.7]*†	36.0 [30.2–48.2]*‡	45.0 [33.2–56.2]†‡
Aldosterone	ng/L	92.4 [68.1–126.8]	141.5 [88.7–199.8]	108.6 [74.0–211.5]
Renin	ng/L	9.9 [7.3–19.1]	12.1 [7.2–19.1]	12.2 [5.8–21.1]
Aldo/R	ng/ng	7.5 [4.4–14.0]	10.3 [5.6–18.6]	7.5 [5.0–20.2]

*: significant difference between II vs. ID genotypes.
†: significant difference between II vs. DD genotypes.
‡: significant difference between ID vs. DD genotypes.
17OHprog: 17-hydroxyprogesterone, A4: androstenedione, ACE: converting enzyme, Aldo/R: aldosterone to renin ratio, AMH: anti-mullerian hormone, BMI: body mass index, FSH: follicle stimulating hormone, FAI: free androgen index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, LH: luteinizing hormone, PCOS: polycystic ovary syndrome, SDHEA: dehydroepiandrosterone-sulfate, SHBG: sex hormone-binding globulin, Testo: testosterone, WC: waist circumference, W/H:waist to hip ratio.
Table 5 shows clinical and biological characteristics of PCOS according to ACE I/D genotype.

Table 6
Significant correlations observed between subtypes traits and circulating RAAS studied parameters in PCOS according to ACE I/D genotypes.

	ID			DD		
	Aldo	Renin	A/R	Aldo	Renin	A/R
ACE activity	–	–	–	–	–	–
BMI	0.263 0.042	–	0.252 0.050	0.372 0.043	–	–
WC	0.313 0.022	–	0.324 0.019	–	–	–
LH	–	0.263 0.043	–	–	–	–
Testo	–	–	–	–	0.483 0.007	–
A4	0.357 0.009	–	–	–	–	–
FAI	–	–	–	–	0.482 0.023	–
Estradiol	–	0.300 0.022	–	–	–	–
AMH	–	0.291 0.028	−0.367 0.005	–	–	–
Insulin	–	–		–	0.406 0.026	–

A4: androstenedione, ACE: converting enzyme, Aldo: aldosterone, Aldo/R: aldosterone to renin ratio, AMH: anti-mullerian hormone, BMI: body mass index, FAI: free androgen index, LH: luteinizing hormone, PCOS: polycystic ovary syndrome, Testo: testosterone, WC: waist circumference.
Table 6 shows significant correlations observed between subtypes traits and circulating RAAS studied parameters in PCOS.

the frequencies of ACE I/D genotypes in the total PCOS group deviate from the control group, while other reports have found that the ACE I/D polymorphism distributions in PCOS and controls were similar [17,23–28]. Other reports stressed out that the degree of involvement in PCOS onset might vary according to the population's ethnicity [16,23,29–31].

In line with the literature, ACE activity was increased in the D allele [32–34]. Interestingly, this heightened activity could potentially augment the activation of the RAAS and insulin resistance in PCOS [15,35–37]. Insulin resistance is widely reported in PCOS and linked to more severe conditions. It interferes with hormonal control inducing LH excess [38]. Its negative effect on SHBG synthesis has been found to enhance hyperandrogenemia [2]. Since 2012, a clinical classification of PCOS has been proposed, including four phenotypes (1, 2, 3, and 4) based on the combination of clinical features (1: HA + OA + PCOU, 2: OA + HA, 3: HA + PCOU, 4: OA + PCOU) [39]. Considering that the clinical phenotypes 1, 2, and 3 would be more concerned with hyperandrogenemia, it is reasonable to anticipate a higher prevalence of the D allele in these phenotypes. However, ACE I/D genotype distribution did not reflect this expectation according to this clinical classification. This could emphasize the weakness of phenotypic stratification based on Rotterdam criteria in the comprehension of the underlying pathophysiology of this syndrome, which is consistent with the inadequacies in the literature comparing clinical phenotypes of PCOS and ACE I/D polymorphism distribution [3,24]. Phenotypic stratification demonstrates the diversity of PCOS expression but lacks any biological specificity, contrasting with the clear biological expression of ACE I/D polymorphism genotypes [39]. The DD genotype exhibited significantly increased insulin levels, while ID genotype showed elevated LH/FSH ratio [27,30,40]. In 2020, the genetic approach of PCOS have identified three distinct biological subtypes based on genetic architecture: a metabolic subtype characterized by obesity and insulin resistance, a reproductive subtype marked by elevated LH and AMH levels, and an indeterminate subtype [3,41]. Upon rereading the results of previous reports, we were able to connect the metabolic subtype to the DD genotype and the reproductive subtype to the ID genotype [16,27,40]. Our results supported this tendency, as indicated by the correlations of renin with the primary traits of each subtype.

Indeed, Alphan et al. [36] highlighted an independent association between renin and insulin, regardless of aldosterone and ACE activity in PCOS. Androgens could activate renal RAAS and elevate renin levels [42]. Interestingly, in PCOS with the DD genotype, renin showed a positive correlation with insulin, and patients exhibited higher BMI and waist circumference compared to II and ID genotypes. This association aligns with the metabolic subtype of PCOS characterized by obesity and metabolic disturbance [3,41]. In the metabolic subtype, insulin resistance is the primary phenomenon enhancing thecal androgen production.

Conversely, in the ID genotype, renin was positively correlated with LH and AMH, and pleads in favor of the association between the ID genotype and the reproductive subtype; characterized by leanness, lower insulin resistance, and elevated LH and AMH levels [3,41]. We may raise concerns about the efficacy of RAAS inhibitors, particularly ACE inhibitors, which are currently used off-label in PCOS, and about the role of renin inhibitors, a topic that has not yet been explored [42,43]. However, the benefit of ACE inhibitors is well established in PCOS patients who experience hypertension or insulin resistance [5,44–47].

Previous research has underscored the impact of ACE I/D polymorphism on RAAS in plasma and relevant tissues [48]. However, it is worth noting that plasma ACE activity may not accurately reflect intra-ovarian changes [49]. The specific androgen variations within each subtype remain a topic of debate, and an inverse relationship between LH and androgen levels has been proposed, stemming from the negative feedback of elevated testosterone on LH [3]. The association between the II genotype and specific subtypes remains inconclusive, likely due to its lower prevalence, as reported in previous Tunisian studies [34,50–52]. However, it could potentially reflect the indeterminate subtype. Indeed, the subtyping method employed by Dapas et al. holds promise for enhanced reliability [3]. But, the definition of these subtypes lacks standardized codification, and there remains a lack of unified cutoffs for quantitative parameters.

Our study represents the first instance of highlighting ACE gene polymorphism within PCOS patients in North Africa. Future large-scale studies employing random selection and ensuring comparable representation of phenotypes are planned to further validate our findings. We should however stress out that the Rotterdam classification criteria might complicate the detection of genetic associations in PCOS [32]. In this study, our focus was on evaluating ACE activity, renin, and aldosterone as a reflection of peripheral RAAS activity in association with ACE I/D polymorphism. This choice arises from the broad availability of these assays in laboratories, which could facilitate easier access for patients. The lack of ACE activity, SHBG, and AMH levels in the control group represents a limitation of our study. These data would have been particularly valuable for genotype comparison and correlation analysis.

5. Conclusion

Our study highlights the association between ACE I/D polymorphism and PCOS, which could potentially influence the selection and occurrence of different subtypes in relation to both environmental factors and genetic background. Furthermore, the close association between renin and the pathophysiology of PCOS subtypes suggests that alteration of RAAS pathways may be a fundamental aspect of PCOS. Our findings highlight the need to investigate ACE and renin inhibitors to improve the reproductive and metabolic characteristics of the subtypes and de facto underline the potential for targeted therapeutic interventions in the management of PCOS.

CRediT authorship contribution statement

Khansa Chaabouni: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Amana Saadallah-Kallel:** Validation, Resources, Investigation. **Samia Ben Brahim:** Methodology, Formal analysis. **Kais Chaabane:** Visualization, Supervision, Investigation. **Madiha Frikha:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Mouna Mnif:** Supervision, Investigation. **Leila Keskes:** Writing – original draft, Resources. **Fatma Abdelhedi:** Writing – review & editing, Software. **Fatma Ayadi:** Writing – review & editing, Project administration, Funding acquisition.

Ethics statement

This study was carried out in accordance with the Declaration of Helsinki, and approval from the Personal Protection Committee of Southern Tunisia (ethics approval number CPP SUD N°0197/2019) was obtained. All participants provided written informed consent prior to inclusion.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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