Increased frequency of the DI genotype of the angiotensin-I converting enzyme and association of the II genotype with insulin resistance in polycystic ovary syndrome

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Abstract

Objective: The polycystic ovary syndrome (PCOS) is a common and complex disease with unclear pattern of inheritance, characterized by an androgen excess, while hyperinsulinemia and insulin resistance (IR) are common features of the syndrome. The angiotensin I converting enzyme (ACE) insertion (I)/deletion (D) gene polymorphism was proved to be involved in many pathophysiological conditions, including hypertension and IR.

Design: The purpose of this study was to evaluate the involvement of the ACE gene polymorphism in the pathogenesis of PCOS.

Methods: In a case–control association study involving 801 PCOS women and 266 healthy controls, hormonal determinations and ACE polymorphism genotyping were performed. The PCOS women were classified into three groups: Group A presented biochemical hyperandrogenism, combined with anovulation and polycystic ovarian morphology; Group B, clinical hyperandrogenism combined with anovulation and polycystic ovarian morphology; and Group C, chronic anovulation and polycystic ovarian morphology.

Results: A significant increase in the frequency of the DI genotype of the ACE polymorphism was detected in PCOS women as a whole (P<0.035), in PCOS Group A (P=0.039) and Group B (P=0.010), while there was no difference in Group C (P=0.939). Significant difference was also observed in hyperandrogenic PCOS women as a whole (Group A+B) (P=0.017). The II genotype was positively correlated with HOMA-IR and QUICKI and with fasting insulin and glucose/insulin ratio in these groups.

Conclusions: The association study of the ACE I/D polymorphism in PCOS women demonstrates an increase in the DI genotype incidence and an association of the II genotype with IR.

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Introduction

The polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women of reproductive age, affecting 6–10% of the population (1, 2, 3). PCOS is a heterogeneous disorder of androgen excess production with varying hormonal and metabolic abnormalities (4). It is characterized by hirsutism, anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasound (5). It is known that hyperinsulinemia, insulin resistance (IR), and obesity are common syndromes (6). PCOS appears to be a common and complex disease without a clear pattern of inheritance (7, 8, 9, 10). The efforts to elucidate the genetic component of PCOS pathogenesis are focused on identifying genetic changes in enzymes and proteins directly or indirectly involved in various aspects of the syndrome.

The renin–angiotensin system (RAS) is a fundamental regulator of cardiovascular function and blood pressure (11, 12). The angiotensin converting enzyme (ACE) metabolizes angiotensinogen (AGT) to form angiotensin II, which can act as angiotensin II type 1 receptor (AGTR1) to modify blood pressure by mechanisms including direct effects on vascular tone and indirect effects via alterations of renal function.

The RAS is also involved in the ovarian and adrenal steroidogenesis, maturation of oocytes and ovulation, as well as development of corpus luteum through complex interactions with other systems (13). ACE, one of the key players of the RAS, is also expressed in many tissues, including ovary (14). It was demonstrated that...
the RAS products are associated with angiogenesis in ovarian endothelium (15) and ovarian steroidogenesis (16), as well as with the maturation of ovarian follicles in mice (17).

It was shown that the variability of the plasma ACE concentration is associated with an insertion (I)/deletion (D) polymorphism in intron 16 of the ACE gene. This is called the ACE I/D polymorphism (106180.0001, rs4340) (18, 19, 20). Since then, this polymorphism was proved to be involved in many pathophysiological conditions such as hypertension, myocardial infarction, hypertrophic cardiomyopathy, and diabetic or nondiabetic nephropathy (21).

The aim of this study was to evaluate the involvement of the ACE I/D polymorphism in the pathogenesis of PCOS leading to increased genetic predisposition for long-term cardiovascular events.

Patients and methods

Patients

The study included 801 Caucasian women with PCOS with a mean age of 24.05 ± 5.78 years and a mean body mass index (BMI) of 26.59 ± 7.07 kg/m² and 266 regularly menstruating, ovulatory women. BMI did not differ statistically between PCOS and control women. Although a statistically significant difference was observed concerning the mean age between PCOS and control women, it had no effect on the study as the control group was only used to compare genotype frequencies of the I/D ACE polymorphism that are not influenced by age. All the participants share the same Greek origin. All women with PCOS were recruited from the outpatient Endocrine and Human Reproduction Clinic of the Second Department of Obstetrics and Gynecology of Aristotle University of Thessaloniki between the years 2000 and 2010. Normal controls were voluntarily selected from the cohort of patients visiting the outpatient clinic for medical consulting. Furthermore, no woman reported use of any medication that could interfere with the normal function of the hypothalamic–pituitary–gonadal axis during the past 6 months.

The diagnosis of PCOS was based on the presence of at least two out of three features of biochemical and/or clinical hyperandrogenemia and/or chronic anovulation and/or polycystic ovarian morphology on ultrasound, thus meeting the criteria of the Rotterdam European Society for Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) experts meeting in Rotterdam in 2003 (Rotterdam, 2003). Exclusion criteria were congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing syndrome. All subjects had normal thyroid, kidney, and liver function and none had excessive alcohol intake. All members of the control population had regular ovulation, progesterone serum > 10 ng/ml in the luteal phase of their menstrual cycle, and no evidence of clinical hyperandrogenemia.

All women with PCOS were classified into three groups: Group A (n = 592) presented biochemical hyperandrogenemia, combined with chronic anovulation and/or polycystic ovarian morphology; Group B (n = 129), clinical hyperandrogenism combined with chronic anovulation and/or polycystic ovarian morphology; and Group C (n = 92), chronic anovulation and polycystic ovarian morphology (Fig. 1). Group A + B (n = 721) represents the sum of PCOS women with biochemical or clinical hyperandrogenism, combined with chronic anovulation and/or polycystic ovarian morphology.

Biochemical hyperandrogenism was defined as increased serum testosterone and/or free androgen index (FAI), above two S.D. from average values of population control. Clinical hyperandrogenism was defined as the presence of hirsutism, acne, or androgenic alopecia. Hirsutism was defined by Ferriman–Gallwey scores of ≥ 8 (22).

Methods

Blood samples were taken between the third and fourth day of the cycle in healthy controls and between the 3rd and 4th day after spontaneous bleeding in women with PCOS, after overnight fasting. For women with menstrual interval > 90 days, micronized progesterone (100 mg) was given for 10 days to achieve an induced menstruation (<5% of the total population of PCOS women included in the study).

Figure 1 Schematic representation of PCOS women cohort. PCOS Group A presented biochemical hyperandrogenemia, anovulation, and/or polycystic ovarian morphology; PCOS Group B presented clinical hyperandrogenism, anovulation, and/or polycystic ovarian morphology; and PCOS Group C presented chronic anovulation and polycystic ovarian morphology.
Hormonal assays included serum LH, FSH, testosterone, free testosterone, androstenedione, 17-OH progesterone and insulin, and biochemistry: total cholesterol, triglycerides, HDL- and LDL-cholesterol, hematocrit, number of white blood cells, total protein, albumin, fibrinogen, and glucose levels.

Hormonal measurements were performed by chemiluminescence (Elecys 2010; Roche Diagnostics) with the exception of serum androstenedione (A4), DHEAS, sex hormone binding globulin (SHBG), and 17-OH progesterone that were measured by RIA (BioSource, B-1400 Nivelles, Belgium). Serum lipids were measured with an automatic biochemical analyzer (Olympus, Medicon System Reagent Hellas). The normal ranges for hormonal measurements are as follows: FSH 3.5–12.5 mIU/ml, LH 2.4–12.6 mIU/ml, DHEA-S 940–4070 mIU/ml, SHBG 24.6–122 nmol/l, 17-OH progesterone 0.1–1.1 ng/ml, androstenedione 0.47–940–4070 ng/ml, testosterone 4.79–23.3 ng/ml, and insulin 3–17 mU/l. The intra-assay coefficients of variation (CV) were 1.5% for FSH, 0.7% for LH, 2.7% for prolactin, 3.8% for insulin, 4.1% for 17α-OH progesterone, 1.3% for testosterone, 5.9% for androstenedione, 9.4% for DHEA-S, and 5.8% for SHBG. The average interassay CV were 3.2% for FSH, 1.7% for LH, 3.4% for prolactin, 4.4% for insulin, 6.3% for 17α-OH progesterone, 2.2% for testosterone, 9.2% for androstenedione, 12.1% for DHEA-S, and 7.8% for SHBG.

IR was defined by measuring the levels of fasting insulin, fasting glucose, the ratio of fasting glucose/insulin, and indices HOMA-IR and QUICKI. All women with PCOS and type 2 diabetes were excluded from the study.

The FAI was calculated using the following formula: testosterone (nmol/l) × 100 / SHBG (nmol/l).

IR was calculated from the indices HOMA-IR and QUICKI.

The calculation of indices HOMA-IR and QUICKI was based on the following equations:

\[
\text{HOMA-IR} = \text{fasting insulin} \times \text{fasting glucose} / 22.5 \\
\text{QUICKI} = 1 / \log(\text{fasting insulin}) + \log(\text{glucose})
\]

IR was considered in individuals with both HOMA-IR ≥ 2.5 and QUICKI ≤ 0.333.

Written consent was given by all participants according to the guidelines of the Institutional Review Council (Institutional Review Board) School of Medicine, University of Patras and the Aristotle University of Thessaloniki. The study complied with the principles of the Declaration of Helsinki.

**Genotyping**

Genomic DNA was extracted from whole peripheral blood by the standard method of phenol/chloroform. All DNA samples were genotyped by a first PCR with specific primers, and the amplification product was analyzed by gel electrophoresis. The primers, used in the first PCR, were ACEF1 (forward): 5’-CTG GAG ACC ACT CCC ATC CTT TCT-3’ and ACER1 (reverse): 5’-GAT GTG GCC ATC ACA TTC GTC AGA T-3’. Samples yielding exclusive amplification of the D allele (and therefore potentially typed as DD) were subjected to a second independent amplification to confirm the genotype. The primers, used in the second PCR, were ACEF2 (forward): 5’-TTT GAG ACG GAG TCT CGC TC-3’ and ACER1 (reverse): 5’-GAT GTG GCC ATC ACA TTC GTC AGA T-3’. Oligonucleotide primers were synthesized by VBC-Genomics Bioscience Research GmbH, Vienna, Austria. Reactions were carried out using the following parameters (33 cycles): DNA denaturation at 94 °C for 10 min in first cycle and for 30 s in all subsequent cycles, annealing at 54 °C for the first reaction and at 58 °C for the second reaction for 30 s and extension at 72 °C for 30 s with a final elongation of 10 min. Amplification was confirmed by gel agarose electrophoresis.

**Statistical analysis**

Values are expressed as mean ± s.d. The genotype frequency distributions between groups and controls were compared using the χ² test. All variables (anthropometric, clinical, and hormonal) were tested for normal distribution using the Kolmogorov–Smirnov test. Distribution comparison across groups was done using parametric methods (independent t-test and ANOVA) for variables following normal distribution and nonparametric methods (Mann–Whitney and Kruskal–Wallis) for variables not following normal distribution. Bonferroni adjustment for multiple comparisons was applied. Values were considered to be statistically significant at P <0.05. Statistical analysis was performed using PASW 18 for Windows (IBM SPSS Statistics, IBM software, Chicago, IL, USA).

**Results**

For the I/D ACE polymorphism tested in this study, genotype distributions were estimated using Hardy–Weinberg equilibrium in both patients and controls (all P values >0.05). Genotype frequencies of the I/D ACE polymorphism were compared between PCOS women and ovulatory women used as controls. An association of the DI genotype with PCOS syndrome was detected (Fig. 2 and Table 1): 39.1% (313) of all PCOS women were homozygous for the DD genotype, 49.3% (395) heterozygous for the DI genotype, and 11.6% (93) homozygous for the II genotype. In PCOS Group A, 40.9% (242) were homozygous DD, 48.1% (285) heterozygous DI, and 11% (65) homozygous II. In PCOS Group B, the frequency of DD genotype was 31% (40), DI 58.1% (75), and II 10.9% (14). In PCOS Group C, 38.8% were homozygous for the DD genotype (31).
The II genotype was also positively correlated with fasting insulin and glucose/insulin ratio in PCOS women as a whole (Group A+B+C) ($P=0.035$), for PCOS Group A ($P=0.039$), for PCOS Group B ($P=0.010$), and for PCOS Group A+B ($P=0.017$), while there was no difference for PCOS Group C ($P=0.939$).

Clinical and biochemical features of the study cohort concerning PCOS women and controls are presented in Table 1.

The heterozygosity of I/D ACE polymorphism was associated with decreased levels of 17-OH progesterone in PCOS Group A+B ($P=0.032$; Table 3). No association of I/D ACE polymorphism was detected in PCOS women as a whole, PCOS Group A, PCOS Group B, PCOS Group C, PCOS Group A+B, or controls with FAI ($P=0.887$, $P=0.127$, $P=0.812$, $P=0.103$, $P=0.652$, and $P=0.964$ respectively) and testosterone level ($P=0.290$, $P=0.151$, $P=0.670$, $P=0.086$, $P=0.112$, and $P=0.630$ respectively).

The I/D ACE polymorphism was associated with increased IR, as assessed by HOMA-IR and QUICKI of IR, for the PCOS Group A+B (PCOS women with biochemical and clinical hyperandrogenemia) ($P=0.022$ and $P=0.022$ respectively), as well as for the Group A ($P=0.018$ and $P=0.008$ respectively). The II genotype was also positively correlated with fasting insulin and glucose/insulin ratio in PCOS Group A+B ($P=0.032$ and $P=0.046$ respectively) as well as in Group A ($P=0.011$ and $P=0.018$ respectively) (Table 3).

Subsequently, we divided all PCOS women with biochemical and clinical hyperandrogenemia into two subgroups, namely, those with or without IR and then we compared the frequency of I/D ACE polymorphism between them. Women with IR were considered as presenting simultaneously QUICKI < 0.33 and HOMA-IR > 2.5, and without IR as having simultaneously QUICKI > 0.33 and HOMA-IR < 2.5. A statistically significant increase in the II genotype was observed in PCOS women with IR ($P=0.016$; Fig. 3 and Table 4).

### Discussion

The results of this study showed a statistically significant difference in the frequency of genotypes of ACE gene I/D polymorphism (I/D ACE) in women with PCOS compared with the control Greek population. The different distribution of the ACE I/D genotypes regarded PCOS women with hyperandrogenism, while it disappeared in PCOS women with anovulation and polycystic ovarian morphology. To our knowledge, it is the first time that the involvement of I/D polymorphism of ACE gene in the pathogenesis of PCOS has been highlighted.

The criteria for determining the diagnosis of PCOS are subject to ongoing debate and great concern among both clinicians and researchers trying to elucidate the pathogenesis of the syndrome. The introduction of polycystic ovarian morphology on ultrasound has expanded the most stringent criteria of NIH (26), adding two additional categories of patients (5). A number of studies have shown that these later categories are milder forms of the syndrome presenting a more similar profile to women with chronic anovulation of various etiologies (27). It is accepted that PCOS is a multifactorial disease with polygenic nature, and this heterogeneity complicates the effort to investigate additional genetic components of its pathogenesis. A well-characterized cohort is fundamental in order to elucidate any pathogenetic component of PCOS and maybe an explanation for the contradictory results in several studies regarding PCOS.

The frequency of the ACE I/D gene polymorphism in women with PCOS has been investigated previously by

<table>
<thead>
<tr>
<th></th>
<th>DD (n (%))</th>
<th>DI (n (%))</th>
<th>II (n (%))</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS (801)</td>
<td>313 (39.1)</td>
<td>395 (49.3)</td>
<td>93 (11.6)</td>
<td>0.035</td>
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<tr>
<td>Group A (592)</td>
<td>242 (40.9)</td>
<td>285 (48.1)</td>
<td>65 (11)</td>
<td>0.039</td>
</tr>
<tr>
<td>Group B (129)</td>
<td>40 (31)</td>
<td>75 (58.1)</td>
<td>14 (10.9)</td>
<td>0.010</td>
</tr>
<tr>
<td>Group C (92)</td>
<td>31 (38.8)</td>
<td>35 (43.8)</td>
<td>14 (17.5)</td>
<td>0.939</td>
</tr>
<tr>
<td>Group A+B (721)</td>
<td>282 (39.1)</td>
<td>360 (49.9)</td>
<td>79 (11)</td>
<td>0.017</td>
</tr>
<tr>
<td>Controls (286)</td>
<td>109 (41.0)</td>
<td>112 (42.1)</td>
<td>45 (16.9)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Clinical characteristics of PCOS groups and controls. Group A presented biochemical hyperandrogenism, combined with and/or anovulation and/or polycystic ovarian morphology; Group B, clinical hyperandrogenism combined with anovulation and/or polycystic ovarian morphology; Group C, chronic anovulation and polycystic ovarian morphology; and Group A+B presented women with hyperandrogenism (the sum of women of Groups A and B).

<table>
<thead>
<tr>
<th></th>
<th>PCOS total</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>PCOS A+B</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>24.05±5.78*</td>
<td>23.64±5.43*</td>
<td>26.04±6.27*</td>
<td>23.91±6.82*</td>
<td>24.07±6.56*</td>
<td>31.15±5.66*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.59±7.07</td>
<td>26.63±6.96</td>
<td>26.75±7.42</td>
<td>26.02±7.35</td>
<td>26.65±7.04</td>
<td>27.47±7.28</td>
</tr>
<tr>
<td>Waist/hip</td>
<td>0.79±0.23</td>
<td>0.78±0.07</td>
<td>0.79±0.08</td>
<td>0.77±0.07</td>
<td>0.78±0.07</td>
<td>0.78±0.07</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.93±7.15*</td>
<td>5.87±7.17*</td>
<td>6.24±1.87</td>
<td>5.81±1.83*</td>
<td>5.94±1.74*</td>
<td>6.88±3.32</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>7.73±5.38*</td>
<td>8.27±5.75*</td>
<td>6.05±3.47</td>
<td>6.44±4.18</td>
<td>7.87±5.48*</td>
<td>5.59±2.88</td>
</tr>
<tr>
<td>R</td>
<td>1.75±0.84*</td>
<td>1.45±0.97*</td>
<td>1.01±0.69</td>
<td>1.18±0.77*</td>
<td>1.37±0.94</td>
<td>0.86±0.44</td>
</tr>
<tr>
<td>DHEAS (µg/l)</td>
<td>2990±1307*</td>
<td>3273±1297*</td>
<td>2257±968</td>
<td>2080±944</td>
<td>3091±1304*</td>
<td>1981±810</td>
</tr>
<tr>
<td>TESTO (ng/dl)</td>
<td>73.93±28.84*</td>
<td>85.02±24.61*</td>
<td>42.34±11.41</td>
<td>42.77±12.10</td>
<td>77.38±28.08*</td>
<td>40.34±11.26</td>
</tr>
<tr>
<td>Δ4 (ng/ml)</td>
<td>2.76±1.08*</td>
<td>3.03±1.16*</td>
<td>2.01±0.74*</td>
<td>2.04±0.70*</td>
<td>2.84±1.08*</td>
<td>1.70±0.47</td>
</tr>
<tr>
<td>17-OH (ng/ml)</td>
<td>1.14±0.57</td>
<td>1.20±0.58</td>
<td>0.88±0.39</td>
<td>1.03±0.62</td>
<td>1.15±0.56</td>
<td>0.79±0.41</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>43.00±25.83*</td>
<td>40.57±22.96*</td>
<td>49.12±34.02*</td>
<td>51.14±27.72</td>
<td>42.10±25.48*</td>
<td>58.36±31.88</td>
</tr>
<tr>
<td>FAI</td>
<td>8.24±6.66*</td>
<td>9.71±6.96*</td>
<td>3.97±2.40*</td>
<td>4.18±3.87</td>
<td>8.69±6.75*</td>
<td>3.05±1.73</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>12.38±10.98</td>
<td>12.28±10.84</td>
<td>12.21±10.05</td>
<td>13.33±13.23</td>
<td>12.27±10.70</td>
<td>11.17±13.28</td>
</tr>
<tr>
<td>GLU (mg/dl)</td>
<td>98.34±15.14</td>
<td>97.61±16.20</td>
<td>98.95±10.73</td>
<td>102.81±12.03</td>
<td>97.85±15.37</td>
<td>99.75±11.30</td>
</tr>
<tr>
<td>GLU/INS</td>
<td>12.10±8.34*</td>
<td>11.73±7.21*</td>
<td>12.60±9.03</td>
<td>14.07±13.35</td>
<td>11.88±7.56*</td>
<td>13.74±8.32</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.15±3.42</td>
<td>3.17±3.58</td>
<td>2.97±2.42</td>
<td>3.27±3.50</td>
<td>3.04±3.03</td>
<td>2.84±3.77</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.03±0.03</td>
<td>0.04±0.03</td>
<td>0.03±0.03</td>
<td>0.34±0.04</td>
<td>0.34±0.03</td>
<td>0.35±0.03</td>
</tr>
</tbody>
</table>

*P values between PCOS women and controls: *P<0.001; †P<0.01; ‡P<0.05.

...found no differences in the frequency of the ACE I/D gene polymorphism among PCOS women as the inclusion of milder forms of PCOS (namely those without hyperandrogenism) in the cohort studied dilated the impact of the more severe forms. Therefore, this trend appears to be so mild that it could only be demonstrated by including large numbers of properly selected probands, as in this study. Nevertheless, and in our cohort of PCOS women, women without hyperandrogenism do not demonstrate any difference in the genotypic distribution of the ACE I/D gene polymorphism compared with the ovulatory control group, indicating once again the need for a limited number of researchers. Karabulut et al. (28) found no differences in the frequency of the ACE I/D gene polymorphism between PCOS and the general population. This study involved a limited number of women with PCOS, while the diagnosis of the syndrome was based on the Rotterdam criteria. Sun et al. (29) also found no differences in the frequency of the ACE I/D gene polymorphism in PCOS, although the presence of DD genotype was associated with increased serum testosterone levels, both in women with PCOS and in healthy controls (although within normal limits). Both these studies could not depict an increased frequency of the ACE I/D gene polymorphism among PCOS women as the inclusion of milder forms of PCOS (namely those without hyperandrogenism) in the cohort studied dilated the impact of the more severe forms. Therefore, this trend appears to be so mild that it could only be demonstrated by including large numbers of properly selected probands, as in this study. Nevertheless, and in our cohort of PCOS women, women without hyperandrogenism do not demonstrate any difference in the genotypic distribution of the ACE I/D gene polymorphism compared with the ovulatory control group, indicating once again the need for...
a reconsideration of the Rotterdam criteria in the light of the newly proposed criteria by the Androgen Excess Society (30).

In our study, for PCOS women as a whole as well as for all different PCOS subgroups, no association of the ACE I/D gene polymorphism with serum androgens was detected. The vast majority of women PCOS included in this study were hyperandrogenic and therefore it might be difficult to access a genotypic correlation within a narrow variation of serum androgen concentrations. The increased frequency of the DI genotype in hyperandrogenic PCOS women does not necessarily imply its direct contribution to increased serum androgen levels as it might directly or indirectly contribute to androgen expression in the ovary. Previous data have shown the presence of a local ovine ovarian microenvironment with changes in angiotensin II concentration, not detected in peripheral plasma (31). It was previously shown that the increased local expression of ACE enzyme induces the production of androgens in both the ovarian granulosa cells and the adrenal cortex (11). The presence of the RAS in the ovary plays an important role in steroidogenesis, follicular development, and in the mechanism of atresia (11). The ovarian RAS can induce hyperandrogenism and contribute to the manifestation of PCOS. It is also known that baseline levels of renin are elevated in women with PCOS and correlate with serum androgen levels (32, 33). Prorenin production has been detected in the theca cells (34), while the cystic follicles in PCOS have demonstrated increased production of renin and angiotensin II from theca and granulosa cells, as occurs in normal atretic ovarian follicles (35). We could suggest that the role of I/D ACE polymorphism in the pathogenesis of PCOS might have resulted from the simultaneous presence of the D allele, which leads to local excess androgen production, and the I allele, which is implicated in IR that could lead to a 'hostile' ovarian microenvironment.

In our study, an association of the DI genotype with decreased levels of 17-OH progesterone, a precursor hormone in the biosynthesis of sex steroids, was also found. Low levels of 17-OH progesterone might indicate a higher rate of androgen production as well as estrogen biosynthesis, not detected in our study. The reason for this lack of evidence might be attributed to the inability to detect in serum subtle differences from a local, adrenal and ovarian, increased production and action of androgens. Therefore, it could be suggested that RAS contributes to ovarian and adrenal hyperandrogenism without a necessary reflection to the systemic levels of circulating androgens.

Significant and of particular interest is the association of II genotype of ACE polymorphism with IR, which plays an essential dual role of cause and effect in PCOS (36). The association of the ACE II genotype with IR has been further strengthened detecting an increased frequency of II genotype in the IR PCOS women.

In this study, the difference in HOMA-IR between PCOS women and controls did not reach statistical significance. This might be due to the potential effect of oral natural micronized progesterone administration on insulin sensitivity, as administration of this drug for 7 days in PCOS women has been shown to decrease HOMA-IR (37), as well as in younger PCOS women. The participation of ACE in the pathogenesis of IR has been demonstrated in various models both in vivo and in vitro. Shiuchi et al. (38, 39) have shown that the inhibition of ACE in diabetic mice improves IR and increases glucose uptake, and by blocking the receptor angiotensin II increases insulin sensitivity. It was also observed that inhibition of ACE in older adults has, as a consequence, increased the levels of serum concentration of IGF1 (40), which is involved in the physiological mechanism of insulin sensitivity.

In contrast to our observations, in the study by Celik et al. (41), it was found that the DD and not the II genotype is associated with IR in women with PCOS, although in a small cohort of patients. This is probably due to the great difference in size of the studied populations and to the different selection criteria of probands.

In agreement with our results is the observation of Ryan et al. (42) in overweight women that homozygosity for the D allele induced more sensitivity to insulin, while the I allele carriers showed greater IR. The same group subsequently described lower insulin

![Figure 3](https://www.eje-online.org)  
**Figure 3** Schematic representation of the I/D ACE polymorphism frequency among hyperandrogenic PCOS women with (IR) and without (NIR) insulin resistance. A statistically significant increase in the II genotype was noted in PCOS women with IR ($P = 0.016$) ($\chi^2$ test).

<table>
<thead>
<tr>
<th></th>
<th>DD (n (%))</th>
<th>DI (n (%))</th>
<th>II (n (%))</th>
<th>P (n (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS IR</td>
<td>86 (35.1)</td>
<td>122 (49.8)</td>
<td>37 (15.1)</td>
<td>0.016</td>
</tr>
<tr>
<td>PCOS NIR</td>
<td>166 (40.7)</td>
<td>209 (51.2)</td>
<td>33 (8.1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Frequency of I/D ACE polymorphism in PCOS women with (IR) and without (NIR) insulin resistance.
sensitivity in subjects with DD than DI and II genotypes in healthy subjects of both sex (43). An explanation of this discrepancy could be attributed to the involvement of probands of different sex in these studies. It was shown that there is a gender difference in the IGF1 axis incidence, and the association of the II genotype with IR. These data suggest that the role of I/D ACE polymorphism in the pathogenesis of PCOS might result from the simultaneous presence of the D allele, involved in the local increase of androgen production and the I allele, implicated in IR.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**References**


4 Carmina E & Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. *Journal of Clinical Endocrinology and Metabolism* 1999 84 1897–1899. (doi:10.1210/jc.84.6.1897)


6 Dunaf A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocrine Reviews* 1997 18 774–800. (doi:10.1210/er.18.6.774)


32 Morris RS, Wonq IL, Hatch IE, Gentschein E, Paulson RJ & Lobo RA. Prorenin is elevated in polycystic ovary syndrome and may reflect hyperandrogenism. *Fertility and Sterility* 1995 **64** 1109–1113.


39 Shiuchi T, Iwai M, Li HS, Wu L, Min LJ, Li JM, Okumura M, Cui TX & Horiuchi M. Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. *Hypertension* 2004 **43** 1003–1010. (doi:10.1161/01.HYP.0000125142.41703.64)


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