Insertion/deletion of angiotensin-converting enzyme gene polymorphism as a marker of polycystic ovary syndrome (PCOS) development in South Indian cohort women

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Abstract. Polycystic ovary syndrome (PCOS) is an endocrine/metabolic disease because of the elevated levels of androgen which could lead to anovulation. The angiotensin-converting-enzyme (*ACE*) is a zinc metallopeptidase that converts angiotensin I to angiotensin II. *ACE* is bound to the plasma membrane and expressed in many tissues such as ovarian tissues. The renin-angiotensin system (RAS) help the production of angiotensin, angiotensinogen, and *ACE*. Angiotensin II plays a major role in ovulation, steroidogenesis, follicular atresia and hyperandrogenic syndromes such as PCOS. This study aimed to determine the association of *ACE* polymorphism in PCOS to analyze the distribution allele frequency of insertion or deletion variation in PCOS patients of the South Indian cohort. A total of 430 women with PCOS confirmed based on the Rotterdam criteria and 300 age and sex-matched control samples were studied. PCR technique was used to determine the frequency of polymorphism in the *ACE* gene. The genotyping distribution of II, DD and ID in PCOS was 4.56%, 30.23%, and 65.11%, respectively, whereas the control group showed 30%, 20%, and 50% for II, DD and ID, respectively. The deletion (D) allele frequency was 62.79% and insertion (I) allele was 37.2 % in PCOS patients, whereas in the control group, it was 45% and 55% for D and I alleles, respectively. This study concludes that the distribution of deletion (D) allele frequency of *ACE* could be considered as a genetic marker for PCOS in the South Indian cohort.

Keywords: *ACE* Gene, Cohort, PCOS, PCR

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine/metabolic disease because of the elevated levels of androgen that could lead to anovulation in 5–10% women of reproductive age (Brassard *et al.*, 2008). Several genes involved in insulin secretion, energy metabolism, steroid biosynthesis, and gonadotropin action have shown altered expression in PCOS (Prapas *et al.*, 2009). Although the role of genetic factors is strongly supported in the development of PCOS (Goodarzi *et al.*, 2006), the genetic pathogenesis of

PCOS is not well established at the molecular level. Since there is no gold standard diagnosis for PCOS, three different assessment criteria have been used, i.e. the National Institutes of Health criteria (Zawadzki and Dunaif, 1992), the Rotterdam criteria (Rotterdam, 2004) and the Androgen-Excess PCOS criteria (Azziz *et al.*, 2009).

Ovarian renin-angiotensin system (RAS) has been reported to associate with PCOS and hyperandrogenism (Jaatinen *et al.*, 1995; Palumbo

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et al., 1993). The follicular fluid of ovary has 100-fold of prorenin level compared with normal plasma levels (Glorioso *et al.*, 1986). The increasing level of prorenin during ovulation is directly associated with a total number of mature oocyte follicles (Itskovitz, 1987). The angiotensin-converting-enzyme (*ACE*) is a main effector molecule of the RAS, converting angiotensin I to angiotensin II. This enzyme is involved in the ovulation process, maturation of the oocyte, steroidogenesis, and development of corpus luteum (Yoshimura, 1997). The angiotensin I-converting enzyme, encoded by the *ACE* gene, was found in chromosome 17 and locus q23 and has 26 exons and 25 introns (Sayed-Tabatabaei *et al.*, 2006). The concentration of the angiotensin I-converting enzyme was determined by the insertion or deletion polymorphism of the *ACE* gene (Elshamaa *et al.*, 2011; Rigat *et al.*, 1990) reported that individual variation in plasma concentration of angiotensin I-converting enzyme was dependent on the insertion (I)/deletion (D) polymorphism in 16th intron of the *ACE* gene. DD or DI genotypes were common in PCOS women (Ozegowska *et al.*, 2016; Ozegowska *et al.*, 2019). Homozygous deletion (D/D) has the highest level of angiotensin I-converting enzyme whereas homozygous insertion (I/I) gene type has the lowest level of the enzyme. Heterozygous allele (I/D) for an individual, however, has a moderate level of the angiotensin I-converting enzyme. Hence, this study aimed to determine the polymorphism association *ACE* gene with PCOS patients of South Indian Cohort.

MATERIALS AND METHODS

Study population

The study includes PCOS patients (n=430) and controls (n=300) from a native South Indian population were recruited from Sandhya Hospital, Vellore, and have been approved by the Institutional Human Ethical Committee, VIT, Vellore, India. The PCOS was defined by the Rotterdam PCOS consensus criteria (Rotterdam, 2004). The control samples were recruited according to the 2003 Rotterdam Criteria by excluding the diagnosis of PCOS and having a

normal cycle (b32 days). All individuals in the control group were healthy female and reproductively fit. All samples were collected after getting informed consent from donors. Data collection sheet included information such as personal profile including age, weight and height (Body mass index; BMI) and hormonal profile including Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL), Triiodothyronine (T3), Thyroxine (T4) and Thyroid-stimulating hormone (TSH). The blood test for screening hormone levels was taken on the second day of the menstrual period.

Determination of angiotensin-converting-enzyme (ACE) genotype

The genomic DNA from 2 ml of venous blood was extracted according to Abilash *et al.* (2016) and stored at -20°C deep freezer. The quality of DNA was checked by using Agarose gel electrophoresis. Amplification of *ACE* gene was carried using forward primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and reverse primer 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' and performed in Eppendorf PCR Machine using a 20 µl PCR mixture contained 10 pmol from each primer, 5µl water and 10 µl master mix (Ampliqon). DNA sample (3 µl) was amplified for 35 cycles with an initial denaturation for 5 minutes at 95°C followed by denaturation at 95°C for 45 seconds, annealing at 59°C for 45 seconds, an extension for 1 minute at 72°C and final extension for 10 minutes at 72°C which resulted in a 490bp product. The PCR products were run on 2% agarose gel electrophoresis containing 5 µl ethidium bromide (50 µg/µl) and were visualized using a UV Transilluminator. The results were photographed by gel documentation system and analyzed using the gel analysis software.

RESULTS

The clinical characteristics, namely age and BMI, were evaluated among PCOS and control groups. The differences between these two groups, however, were not statistically significant ($p>0.05$) (Table 1). When tested the hormonal profiles (FSH, LH, PRL, T3, T4, TSH) of each group, the differences were statistically significant ($p<0.05$)

(Table1). Our PCR results showed that an intense band of 190 bp was detected with deletion (D) allele and 490 bp with insertion (I) allele, whereas heterozygous (I/D) individual exhibited two bands (490 and 190 bp) (Figure 1).

The frequency distribution of *ACE* gene I/D genotype polymorphism frequency distribution in PCOS patients and controls were analyzed (Table 2). There was a statistically significant ($p < 0.05$) difference in the genotype distribution and allele frequencies between the PCOS patients and control groups. The genotyping distribution in PCOS for II, DD and ID among PCOS patients was 4.56%, 30.23%, and 65.11%, respectively, whereas the control group exhibited 30%, 20% and 50% for II, DD and ID, respectively. The deletion allele (D) frequency in PCOS was 62.79% and insertion allele (I) was 37.2%. In the control group, the deletion allele (D) frequency and

insertion allele (I) was 45 and 55%, respectively (Table 3).

Figure 1. *ACE* gene I/D polymorphism of PCOS using PCR-RFLP analysis.

L: 100 bp DNA Ladder, Lane 1: ID (Heterozygous), Lane 2: ID (Heterozygous), Lane 3: DD (Homozygous), and Lane 4: ID (Heterozygous).

Table 1. Clinical characteristics of PCOS patients and controls.

Individual features	Controls	Patients	P value
Age (year)	24.83±2.57	24.19±4.12	>0.05
BMI (kg/m ²)	27.23±5.11	28.42±6.73	>0.05
FSH (mIU/ml)	16.99±2.74	6.17±2.02	<0.0001
LH (IU/ml)	9.72±3.74	16.90±3.99	<0.0001
PRL (ng/ml)	27.85±19.09	18.41±5.96	<0.05
T3 (ng/dl)	157.71±26.15	130.47±35.40	<0.05
T4 (ng/dl)	8.00±3.16	9.39±2.59	<0.05
TSH (mIU/ml)	2.41±0.83	3.10±1.73	<0.05

Table 2. The frequency of the *ACE* gene I/D polymorphism genotypes between PCOS patients and controls.

Genotype	Controls	Patients	P value	OR	95% CL
II (HOMO)	90	20			
DD (HOMO)	60	130	< 0.0001	9.75	5.497, 17.29
ID (HETERO)	150	280	< 0.0001	8.4	4.976, 14.18
D Allele	135	270	< 0.0001	9	5.314, 15.24
I Allele	165	160	< 0.0001	4.364	2.565, 7.423

Table 3. The percentage of genotypes and allele frequencies between PCOS patients and controls.

Genotypes	Distribution of genotypes			Allele frequencies	
	II(Homo)	DD(Homo)	ID(Hetero)	D Allele (%)	I Allele (%)
Patients	4.65%	30.23%	65.11%	63.00%	37.00%
Controls	30.00%	20.00%	50.00%	45.00%	55.00%

DISCUSSION

In this study, we could not find any specific association of *ACE* gene polymorphism and clinical features between PCOS patients and control group. However, based on probability, this study supports the association of D allele in the *ACE* gene I/D polymorphism and may be more common over PCOS patients of the South Indian cohort. Our study was in agreement with the study carried by Deepak *et al.* (2013), whereby the authors reported that the genotype distribution of DD, ID and II in PCOS patients was 39, 37, and 24%, respectively, and it was 31, 51, and 18% for the genotype distribution of DD, ID and II, respectively, in the control group. The authors also concluded that the DD genotype of *ACE* I/D gene polymorphism in an individual has predisposition and ID genotype has protective towards PCOS. Individuals with D allele of a single or double dose may develop early symptoms of PCOS and significantly associated with acanthosis (Deepika *et al.*, 2013).

Another study led by Jia *et al.* (2013) who used a more precise estimation to analyze the association between I/D polymorphism of the *ACE* gene and risk of PCOS. The author found that no significant association was observed in the overall population. However, in subgroup analyses stratified by ethnicity, a significant association was observed in Caucasians but not in Asians (Jia *et al.*, 2013). Koika *et al.* (2012) found that there was a significant increase in the frequency of DI genotype in Greek origin when analyzing the *ACE* gene I/D polymorphism in the PCOS. They also found an association of II genotype with insulin resistance (Koika *et al.*, 2012). For Turkey women with PCOS, Bayram *et al.* (2011) found that their D allele frequency and homozygous DD were significantly higher than controls. The PCOS have 56% DD, 24% ID and 20% II genotypes compared to 28% DD, 47% ID, and 25% II, respectively, in controls (Bayram *et al.*, 2011).

CONCLUSION

Our study indicated that a strong association of D allele in the *ACE* gene I/D polymorphism could be the reason for causing the development of PCOS in women of South Indian origin. To

validate our data, further research by including other geographical areas of a country is needed since the variation in the ethnic background may also play a major role in gene pools.

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