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# Molecular Aspects of Polycystic Ovarian Syndrome in Female Population in Karnataka at the Southwestern Region of India

**Soumya V Menon[1]*, Nirmalya Bhattacharya[2] and Surajit Sasmal[3]**

[1]*Department of Chemistry and Biochemistry, School of Sciences, India*

[2]*School of Tropical Medicine, Calcutta Medical College, India*

[3]*Prestige Tech Park, Verse Innovation Pvt Ltd, India*

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***Corresponding author:** Soumya V Menon, Department of Chemistry and Biochemistry, School of Sciences, India

E-mail: sweetsou_02@yahoo.com

**Abstract**

Polycystic ovarian syndrome (PCOS) is predominant in the urban population of females in Karnataka. Much information is not available in the disorder occurrence and prevalence in and around Karnataka especially, Bangalore. Various reasons contribute to the disease mainly improper lifestyle, obesity, diabetes etc. In the current study we have focused on isolation of genomic DNA, amplification, and sequencing of DNA from both PCOS females and control. The study also identified amplification and identification of SNPs associated with this disorder such as rs13405728 and rs13479428. Personalized medicine and specific cure are still unexplored for PCOS due to its multifactorial symptoms and occurrence. Based on the SNP identification, elucidation of genetic mechanisms of PCOS can be revealed and hence it can pave way for improved diagnosis and treatment.

**Keywords:** Genomic DNA; Insulin Tolerance; Obesity; Polycystic Ovarian Syndrome; Single Nucleotide Polymorphism

**Abbreviations:** PCOS: Polycystic Ovary Syndrome; AR: Androgen Receptor; LH: Leutinizing Hormone; PCR: Polymerase Chain Reaction; IRS: Insulin Receptor Substrate

## Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder affecting 5-10% of women of reproductive age. It generally manifests with oligo/anovulatory cycles, hirsutism, and polycystic ovaries, together with a considerable prevalance of insulin resistance. Although the aetiology of the syndrome is not completely understood yet, PCOS is considered a multifactorial disorder with various genetic, endocrine, and environmental abnormalities. Moreover, PCOS patients have a higher risk of metabolic and cardiovascular diseases and their related mobidity, if compared to the general population [1]. Polycystic Ovary Syndrome (PCOS) is a common heterogenous endocrine disorder associated with hyperandrogenism, hirsutism, obesity, insulin resistance, and an approximately 7-fold increased risk of type 2 diabetes mellitus. It is a leading cause of female infertility. The prevalence of PCOS among reproductive-age women has been estimated at 4%-12%. Multiple biochemical pathways have been implicated in the pathogenesis of PCOS.

Several genes from these pathways have been tested include genes involved in steroid hormone biosynthesis and metabolism (StAR, CYP11, CYP17) obesity, energy regulation and insulin secretion and many others. Most women with PCOS, both obese and lean, have a degree of insulin resistance. Polymorphic alleles of both IRS-1 and IRS-2 (insulin receptor substrate 1-2) alone or in combination, may have a functional impact on the insulin-resistant component PCOS. PCOS appears to be associated with the absence of the four-repeat-units allele in a polymorphic region of pentanucleotide (TTTTA) n repeats with in CYP11A gene, which encodes cytochrome P450scc. [2] Hyperandrogenism is currently thought to be central to the pathogenesis of polycystic ovarian syndrome (PCOS), a common endocrine disorder in premenopausal women characterized by irregular menstruation and anovulatory infertility. All androgens act through the x-linked androgen receptor (AR), the n-terminal domain of which contains a polyglutamine tract encoded by a highly polymorphic CAG trinucleotide repeat tract. Recently, variations

in the CAG microsatellite tract, while remaining within the normal polymorphic range (11-38 CAGs), have been inversely correlated with receptor activity.

Insulin resistance is associated with hyperandrogenemia more than to irregular menus in women with PCOS. The menus irregularities can be due to insulin sensitivity and other obesity related factors (Lego et al., 2002).

The difference in ethnicity plays an important role in the pathogenesis of disease concerned, it is seen that Mexican American women with PCOS are much more insulin resistant than the white ones, thus a single type of screening test cannot be applied to all the ethnic groups as every individual shows difference in symptoms and have a different lifestyle. [3] Another study of PCOS in different ethnic groups concluded with the fact that IGT and type II diabetes mellitus are prevalent at similar rates irrespective of their age and weight. Also, it is difficult to identify a significant number of women with PCOS just with the databases of diabetic patients.

Above all the facts, proper evaluation of insulin sensitivity and other metabolic parameters are prerequisite for a better diagnosis of PCOS [4]. Some researchers suggest that IR in PCOS patients is not generally a result of mutations in the INSR gene. (Ja et al. 1996) also the mathematical indices should not be the only parameter to conclude with as different populations may show different results with respect to IR. (Diamanti-Kandarakis et al. 2004). In the current study we aim to do the preliminary screening of PCOD female population and determine the various clinical parameters of pcos and control samples. In the study undertaken we have also tried to identify SNP from genomic DNA and then compare between PCOS patient and control female population.

## Materials and Methodology

### Blood sample collection from control and PCOD patients

Blood samples were collected from ten different females (5 control; 5 PCOD) belonging to different regions of Karnataka and proceeded further for clinical and molecular studies. Study consent was obtained from all the patients and control females prior to the study.

### Isolation of DNA

Take the initial volume 1ml of blood in a clean 15 ml centrifuged tube. Then RBC lysis was performed where 3ml of RBC lysis buffer (R075) was used and mixed well the tube contents intermittently by inverting several times during incubation. The tubes were centrifuged at 2,000 xg (= 5000 rpm) for 5 minutes at room temperature (15- 25°c) and discarded the supernatant containing the lysed RBC cell carefully without disturbing the white pellet. The tubes were vortexed vigorously so as to resuspend the white blood cell completely. Wbc lysis buffer(1ml) is used here to resuspend wbc and pipette up and down to lyse the cell. solution should become viscous. then cool the sample at room temperature (15- 25°c) for future processing. Then the proteins were precipitated where, 60 µl PBP buffer is used to cell lysate, mix by vertexing for 30 sec at high speed and incubated on ice for 5 minutes. DNA precipitation is obtained by transferring the 15 ml supernatant centrifuge tube, containing 2ml of 100% isopropanol. ensure that no protein pellet gets transferred along the supernatant. Mix by inverting the tube 40 -50 times gently.

Again, centrifuge for 3 minutes at room temperature. A small white pellet of DNA will be visible. then discard the supernatant and dry the pellet by adding 2 ml of 70% ethanol, inverting the tube for a few times. Centrifuge at 2000 x for 2 minutes at room temperature (15-25°c). carefully pour off the ethanol. the pellet may be very loose at the point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 11 -12 for second ethanol wash. Then we will invert the tube on paper towel and air dry the pellet for 10-15 minutes. The add 200 µl of elution buffer and vortex for 1 minutes to dissolve the DNA pellet properly. Incubate the tube at 65°c for 1 hour and at room temperature (15-25°c) overnight to rehydrate the DNA. Gently shake the tube several times intermittently during the incubation to dissolve the DNA completely.

### Genomic DNA isolation

**Table 1:** Polymerase chain reaction (PCR) of isolated DNA from all samples.

| Sr. No. | Components | Amounts to be Added (µl) | Final Concentration (µm) |
|---|---|---|---|
| 1 | Hi-SYBr master mix | 10 | 1x |
| 2 | Upstream primer | 0.2-2.0 | 0.1-1.0 |
| 3 | Downstream primer | 0.2-2.0 | 0.1-1.0 |
| 4 | Template DNA | 1-5 | <250ng |
| 5 | Biology grade water for per | Up to 20 | |

5ml blood was drawn from 10 female participants (5 PCOS patients & 5 controls) in Karnataka after taking written consent from them. Genomic DNA was isolated from the participants' blood using the genomic DNA isolation kit from HIMEDIA. 5µl of the amplified purified genomic DNA was loaded on an 2% agarose gel and visualized in an UV transilluminator Our aim was to identify mainly two single nucleotide polymorphisms: i.e., rs13405728 and rs13479428 (Table 1). PCR reaction was continued for 30-40


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cycles involving initial denaturation, denaturation, annealing and extension.

**Identification by agarose gel electrophoresis**

The PCR reaction was analysed in an 1% gel in 1 x TAE Buffer. After separation by agarose gel electrophoresis, the pcr fragments was gel curl with a clean sharp cutter. The gel slice was weighed, and 3 volume of gel extraction buffer was added (100 mg - 100µl) and incubated at 55°c for 5-10 minutes with intermittent mixing till agaroe was solubilized completely. Then, 1 gel volume of isopropanol was added and mixed. This solution was passed to a spin column. The DNA bound column was washed with the wash buffer and the DNA was eluted using the 20µl of the elution buffer and observed in agarose gel under gel doc.

**Sanger sequencing**

After this isolated PCR products was sent for sanger sequencing, EUROFINS. Though the process is under progress.

## Results

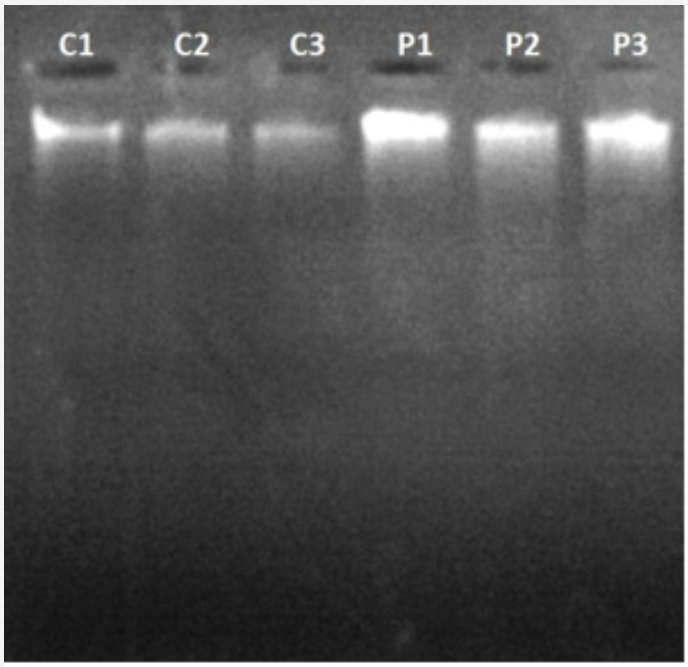


**Figure 1:** Column purified Genomic DNA.

After the isolation of genomic DNA, we performed PCR at the appropriate condition. The desired gene product of 230 base pair was obtained. (Figure 1) depicts the PCR products obtained from the samples. The PCR products of the participants were purified from the agarose gel using gel purification kit. From our study we could identify the two PCOS sample amplified DNA (S4, S5) collected from Dharwad region and these sample's pcr products varied from the control sample collected from same region as shown in (Figure 1). Until now the relationship between leutinizing hormone (LH) and PCOS as not defined clearly. LH is very important hormone which promotes the secretion of androgens by ovarian theca cells.

Discovery of SNP as new markers of human genome opened new methods to demonstrate genetic association of candidate genes to complex disorders (Liu et al. 2012). We could successfully identify the two snps in pcos samples and relate them to various factors related to disease. The change or mutation in these snps may be due to the various factors like genetic, hormonal, and even metabolic changes occurring in female population with PCOS. Replication studies have demonstrated that variants at several of these loci also confer risk for pcos women. The strongest loci in pcos women for DENND1A is THADA, with additional association in loci containing the LHCGR AND FSHR, YAP1 and RAB5/SUOX.

The next variants are to determine the direct cause of PCOS and functional studies in pathophysiology borne out by these loci and variants will include mapping to determine the causal variant and gene, phenotype studies associate with particular features of pcos. To examine a possible role of fibrillins in PCOS, particularly FBN3 is tagged and done the functional SNP analysis using DNA from PCOS patient and control. No SNP showed significant association with pcos SNP and alleles of mist SNPs showed identical population frequencies between pcos and control. Also, no significant difference in micro satellite was observed. The results of isolated genomic DNA are depicted in (Figure 1).


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## Discussion and Conclusion

The blood samples were taken from different regions of Karnataka such as from Dharward and others from southern region of Bangalore and compared with different control patient. The current study was aimed to study the role of Leutinizing hormone (LH) with the polycystic ovarian syndrome (PCOS). The study focused on ten samples i.e., controls and PCOS samples collected from various hospitals/infertility clinics in and around Karnataka. The written consent was obtained from all the patients and the control population. The best results were obtained for the samples collected from Dharwad region of Karnataka compared to samples obtained from Bangalore. We were successful in identifying the two snps functioning in PCOS samples and comparing it with the controls. We isolated the genomic DNA, amplified it and also sequenced it for further evaluation. Similar results were obtained in [5] in another case study in PCOS.

Another study with similar SNPs reported by Xia et al. (2019). SNP rs13405728, rs12478601, and family history of diabetes mellitus (DM) were found to be influencing factors for the occurrence of PCOS. The significant association between increased risk of PCOS and rs12478601 has already been validated before by [6]. As a previous genome-wide association study demonstrated, the SNP variants rs12478601 and rs13405728 in the THADA, DENND1A as well as LHCGR genes were found to be independently correlated with PCOS. Moreover, [7] reported that a family history of DM is able to affect the phenotype of PCOS, which is in consistency with our study. Two single-nucleotide polymorphisms, rs12470652 c.872A>G/p.Asn291Ser and rs2293275 c.935G>A/p.Ser312Asn, in the LHCGR gene has to be screened in healthy Bulgarian women. Asn291Ser minor allele G was found in 7.5% of healthy women and 6.8% of those with PCOS. rs13405728 was studied among north Chinese women and reported that the SNP was associated with PCOS and have role in the pathogenesis and phenotypic character of PCOS. rs2293275 was studied among Sardinian women, using polymerase chain reaction (PCR)-restriction fragment length polymorphism, and reported that rs2293275 was associated with increased risk of PCOS in patients who carry at least one copy of polymorphism and found that in homozygous mutant patients, the risk of PCOS increases 2.7-fold compared to homozygous wild-type patients (Figure 2). Among Egyptian population, 2 studies showed the association between rs2293275 and risk for PCOS using the PCR-restriction fragment length polymorphism genotyping technique. Thathapudi et al. reported that rs2293275 was strongly associated with PCOS risk among Indian women (3.36-fold) and suggested to use this SNP as a molecular marker to identify Indian women at risk. Genotype-phenotype associations were studied and revealed that the frequency of infertility cases in the G/A (40.0%) genotype was more than A/A (7.75%) and G/G (14.8%). Different PCOS genetic variants may influence ovarian response and are linked to female infertility [8].

**Figure 2:** PCR amplified SNPs.

In previous reports it was indicated that these two SNPs (rs13429458 and rs12478601) of THADA genes are associated with PCOS [9]. In another study by [10], investigated the polymorphisms of the THADA gene in some Iranian women population with PCOS for the first time and it was found that rs12478601 is significantly associated with increased susceptibility to PCOS, while rs13429458 was not associated with PCOS. Similarly, [11] identified no correlation between rs13429458 and risk of PCOS. Due to the increased levels of androgen hormone, hyperandrogenism followed by hirsutism,


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acne, androgenic alopecia was observed. Other manifestations like weight gain, menstrual irregularities, acanthosis nigricans, and insulin resistance are also done by increased androgen excess as reported by [12].

The main lacuna in this area of research is that many studies have not been done in genetics and mechanisms of PCOS, and that was the main challenge in the current study. Based on our results, we could relate the role of LH genetics in the pathophysiology of PCOS. Further detailed study is required in this area to explore more possibilities for research in PCOS. Based on the glucose estimation data (previous study), it was concluded that glucose intolerance or imbalance also can be a cause of PCOS. Increased blood glucose level indicated the insulin resistance in PCOS patients compared to the control. This further suggests that obesity and food habits can lead to gestational diabetes, other complications, hyperandrogenism etc. Therefore, early diagnosis is very crucial in such types of syndrome. In future, other than the clinical diagnosis, computational tools may be helpful in finding the cause of this syndrome using various structural aspects. The genes and proteins responsible for PCOS can be subjected to computational modeling using various bioinformatics tools. This may ultimately pave the way in understanding the genetics, metabolic mechanisms and other hormonal and etiological factors related to polycystic ovarian syndrome [13-16].

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