Acta Scientific Biotechnology

Volume 5 Issue 2 April 2024

Review Article

Genetic Polymorphic Associations of Anti-Mullerian Hormone and Luteinizing Hormone/Chorionic Gonadotropin Receptor in Women Suffering from Polycystic Ovarian Syndrome: Insights from South Indian Population

Brindha G Sankaran[1]*, Manimekalai K[1], Mukul M Barwant[2] and Mudasir A Dar[3]

[1]*Assistant Professor, Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore, India*

[2]*Department of Botany, Sanjivani Arts, Commerce and Science College Kopargaon, Maharashtra India*

[3]*Biofuels Institute, School of Environment and Safety Engineering, Jiangsu University, Zhenjiang, China*

***Corresponding Author:** Brindha G Sankaran, Assistant Professor, Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore, India.

Received: April 25, 2024

Published: April 30, 2024

Abstract

Polycystic ovarian syndrome (PCOS) presents a multifaceted clinical picture, characterized by hyperandrogenism, ovarian dysfunction, and metabolic disturbances, including insulin resistance and dyslipidemia. Within this complex landscape, anti-Müllerian hormone (AMH) emerges as a key player, reflecting ovarian reserve and serving as a prognostic marker for fertility. This study aimed to elucidate the phenotypic and genotypic spectrum of AMH (rs10407022) and LHCGR (rs13405728) in PCOS among South Indian women, leveraging a cohort of 600 subjects diagnosed according to Rotterdam criteria. Hardy-Weinberg Equilibrium analysis facilitated gene-gene interaction exploration. Our investigation revealed a noteworthy presence of the TT allele variant of AMH rs10407022 in 18 individuals within the PCOS cohort. Furthermore, LHCGR rs13405728 exhibited four instances of the CC allele variant. These findings underscore the significance of AMH and LHCGR gene polymorphisms in PCOS pathogenesis and folliculogenesis among South Indian women, offering insights into the genetic underpinnings of this complex endocrine disorder.

Keywords: PCOS; AMH; LHCGR; Folliculogenesis Allelic Frequency; Single Nucleotide Polymorphism

Introduction

Polycystic Ovarian Syndrome (PCOS) is multifaceted disorders which are characterized by several factors such as elevated androgen levels, menstrual irregularity, and presence of small cyst on one or both ovaries. PCOS can be defined as genetic predisposition in the women and problems are influenced by lifestyle modifications and environmental factors. The principal component to diagnose PCOS is by morphological characteristics, biochemical (elevated androgen levels) and clinical hyperandrogenism (acne, hirsutism and androgen alopecia) which leads to inhibition of development of follicles, an ovulation and menstrual changes [1]. The diagnostic criteria for PCOS has been described based on three different definitions provided by the National Institutes of Health/National Institute of Child Health and Human Disease (NIH/NICHD), 2003 Rotterdam Consensus raised by European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) or the 'Rotterdam Criteria' and criteria raised by Androgen Excess and PCOS Society (AES) [2]. Ovaries perform two principal functions such as progressive development and release of mature oocytes for fertilization and synthesize steroid hormone required for follicle release, menstrual cycles. The ovaries produce oocytes which are responsible for fertilization and major hormones secreted are oestrogen and progesterone which plays main role in menstrual cycle. Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus, a part of the brain while circulating in the blood it releases two important hormones namely FSH and LH [3]. Several environmental factors, such as prenatal exposure to androgens and weight gain, have been associated as factors leading to the origin of PCOS; genetic factors may give a high susceptibility to PCOS. Research on PCOS in India carried out

in handiness samples stated that an occurrence of 3.7% to 22.5% and also along with 9.13% to 36% prevalence in adolescents [4]. The good clinical practice recommendations (GCPR) are a determination to deliver a framework for addressing problems relates to the administration of PCOS in India [5]. PCOS has a strong genetic association in which several candidate genes have been involved in pathways based on etiology and association of PCOS. Any change in nucleotide may cause a defect in the transcription activity of the gene that leads to PCOS, mostly genes that encode receptors such as androgen, luteinizing hormone, follicular stimulating hormone, and leptin. Single nucleotide polymorphisms (SNPs) present in the coding region play a major role in reproductive outcome as they are involved in hormone receptor synthesis, transportation of molecules or enzymes. Functional candidate genes in PCOS affect steroid biosynthesis, gonadotropin secretion and regulation, chronic inflammation and influence insulin action and secretion [6]. The hormone, AMH is a homodimeric glycoprotein, which inhibits the growth of Mullerian ducts in male embryos. The AMH gene is located on chromosome 19 (small arm) and consists of 5 exons,produced as a pro-hormone that secretes the transforming growth factor and exerts its biological effects, creating a downstream signaling pathway [7]. The AMH genes play an important role in the path physiology of PCOS by the folliculogenesis control mechanism. The number of antral follicles correlates with anti-Mullerian hormones that serve as a reliable marker in humans. High serum AMH levels are directly correlated with increased testosterone and/or luteinizing hormone (LH) concentrations in women with PCOS, as well as with altered oocytes maturation and low embryo quality. Genetic studies have shown that AMH gene receptors are associated with the reproductive function of women, including infertility, and play an important role in PCOS [8]. The LH plays primary role in the synthesis of the progesterone, androgens steroid hormones and oestrogen grounded on genetic level function of LH gene binding to the LHCGR (luteinizing hormone choriogonadotropin receptor). The chorionic gonadotropin (hCG) and luteinizing hormone of LHCGR is essential for reproduction which includes pregnancy steroidgenesis and ovulation. Irregular secretion of LH levels fallouts in hyperandrogenism and these outcomes in alteration of androgens to oestrogen, which prolongs the menstrual cycle. As progesterone secretion increases, LH secretion decreaseswith feedback regulation; if the ova is not fertilized, the secretion of progesterone also beginsto decrease. Without progesterone, the endometrium cannot be preserved and starts to sloughduring menstruation. The FSH secretion begins to increase (as oestrogen and progesteronereduce) and the cycle begins again. Thus, all those cellular and structural changes occur in amenstrual cycle are tightly regulated by the respective hormonal changes. Altered secretionscan results in normal reproductive physiology and may results in irregular menstrual cycle and associated fertility related conditions. The research is mainly focused to determine single nucleotide polymorphism involved in folliculogenesis and steroidgenesis pathway which pave the way to determine the impact of clinical and genetic polymorphic association of Anti-Mullerian hormone (AMH-rs10407022) and Luteinizing hormone/chorionicgonadotropin receptor (LHCGR- rs13405728) factors on PCOS of South Indian population and to deliver the society about the impact of genetic polymorphism and their role involved in metabolic and biochemical pathway as well to pave way towards the scientific and medical community in treating the PCOS affected woman in best ways. The etiology of the syndrome is multifactorial and yet it has become important to develop awareness on PCOS among women about associated genes covering the molecular aspects and genetic variations along with regulation of genes. The impact of the syndrome on an individual varies significantly depending on several factors – the severity of the co-morbidity and the length of life taken into account

Materials and Methods

The study was performed with approval of the Institutional Human Ethics Committee obtained at PSG Institute of Medical Science and Research July 2017 (Approval no: 17/243). The PCOS diagnosed women with an age group of 18–35 years were taken for investigation.

Sample collection and questionnaire

In present study 600 subjects were enlisted, which includes 300 PCOS diagnosed women and 300 normal control subjects of South Indian population. This is the first and most evident preliminary polymorphic study carried out among South Indian population to confirm the polymorphic prototype for single nucleotide polymorphism for AMH (rs10407022) and (LHCGR- rs13405728).

Blood sample collection

Nearly 5ml of blood sample were pinched from the median cubital vein from eachand every woman on third day of the cycle or progesterone persuaded cycle into a synthetic pyrogen free disposable syringe. Then the blood sample was transferred into a syn-

thetic tube and left for a 20mins to allow it to coagulate. From both patient and control subject's blood samples were obtained for DNA analysis. The genomic DNA was extracted from whole peripheral samples collected.

Single Nucleotide Polymorphism Selection. The SNP is well-defined as a simple and excellent form of DNA variation such as alteration in single nucleotide can be also conversion or Trans version form in genome contemporary in more than1% of populace. The SNPs in an aspirant gene, and assumed as accountable for the diversity amongst populations, genome evolution, ancestral traitssuch as twisted hair, inter individual modifications in drug response, and alter an accompanying danger for multifaceted diseases such as diabetes, obesity, cancer and hypertension. There are several candidate genes involved in PCOS development, but polymorphic pattern of AMH-Anti Mullerian Hormone (rs10407022), LHCGR-Luteinizing hormone/chorionic gonadotropin receptor (rs13405728) were selected to check the association of polymorphism in PCOS. The polymorphic associations of these two genes among PCOS have been reportedamong Chinese population and this is the first study carried out to check the significance of association among Indian population (South) by Restriction Fragment Length Polymorphism (RFLP) and ARMS PCR method respectively.

Gene	SNP receptor	Chromosome	Gene loci	Genotype	Method
AMH	rs10407022	19	Exon 2&3	G/T	PCR-RFLP
LHCGR	rs13405728	19	Exon 1&5	C/T, A/G	Allele specific PCR

Table A: Showing the showing the location of the AMH and LHCGR on the chromosome.

Designing of primers

Primers for the gene were designed using NCBI Primer BLAST (http://www.ncbi.nlm.nih.gov/tools/primerblast/) for the AMH gene sequence. The PCR primer sequences were designed by selecting FASTA sequence from NCBI database, after designing the forward and reverse primers. The restriction enzyme was selected by using Bio edit software -BtsCI- enzyme recognition site GGATG nn'. The specificity of primer is very significant in intensifying a choice of curiosity in genome. Primer specificity was partitioned by using in silico PCR device in UCSC genome web browser (https://genome.ucsc.edu/cgi-bin/hgPcr/) (Kent *et al.,* 2002) and NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Ye *et al.,* 2012). The primer sequences exploited for genotyping of single nucleotide polymorphism were enumerated. All PCR reactions were achieved in 20 µL and by mean of 2X PCR master mix (4mM MgCl2), forward and reverse primers and DNA prototype and nucleasefree water.

- **Forward Primer**: 5′- TTTGAGAAGGCCACTCTGCCT -3′
- **Reverse Primer:** 5′- TTCCTCCAGGTGTAGGACCA -3′

Restriction digestion completed in an entire volume of 30 µL reaction by means of SNP embattled restriction enzyme and discerning buffer and nurtured at37 ºC for 2 hours and the reaction was detained by warmth inactivation at 65 ºC for 20 min.After incubation, digested products were resolved by Polyacrylamide gel electrophoresis (PAGE) on the size of digested products and stained with ethidium bromide to viewunder gel documentation for interpretation of bands.

Primers for LHCGR gene

AS-PCR is a technical procedure in the parallel method to conventional PCR, in which two groups of reactions were achieved to differentiate alleles by means of a primer elongation with an allele specific primer anticipate intensifying the target loci (Newton *et al.,* 1989). In AS-PCR, primers intended with a discrepancy in 3 prime locations to upsurge the productivity and specificity of this PCR. Allele-specific primers are premeditated to tie, at their 3' ends, the two variations (A/B) at a single nucleotide polymorphism.

- **LH-outer forward primer:** 5′- GAGATTCGTTGCTGAGAAGCAAGGAAG -3′
- **LH outer reverse primer:** 5′- CTCCGGGGGTCATTTGCCAAAT -3′
- **LHT forward primer**: 5′- CCATAATGCAGCCATTTGTT -3′
- **LHC Reverse primer**: 5′- AGAAGAGGCACATGTTGG -3′

ARMS –PCR based method was followed in which DNA fragment was amplified with specific primers. The obtained PCR products were established by 2% agarose gel electrophoresis.

DNA sequencing

DNA sequencing is the gilded standard technique in single nucleotide polymorphism or mutation detection. To authorize the genotype consequence from RFLP, PCR products of the randomly selected samples of AMH gene SNPs wereunswervingly sequenced using Applied Biosystems DNA analyzer. Sequencing of PCR outcomes comprises three dissimilar steps, for instance (i) cycle sequencing (ii) refinement of sequencing products and (iii) investigating the sequencing PCR products. Sequencing technique is asymmetric PCR, exploiting single primer only.

Statistical analysis

Statistical examination was achieved by graphical sort of Logistic regressionanalysis [9]. Experiential and predictable genotype frequencies for respective SNP were premeditated in both controls and PCOS samples discretely. Allele magnitudes were considered and verified for SNP parting from Hardy-Weinberg equilibrium (HWE) by $\chi 2$ test with one degree of freedom.

Results

Clinical, biochemical and hormonal presentation of PCOS

A total of 600 women inclusive of control and PCOS diagnosed were subjected for analysis. The phenotypic information was obtained through a structured questionnaire. The mean age of PCOS and control women were 25.07 and 25.74 with probability of 0.520. The distribution of body mass index was categorized as underweight below 18.5Kg/m^2, ideal 18.5-24.9 Kg/m^2, overweight 25-29.9 Kg/m^2 and obesity above30 Kg/m^2 and their respective percentages were 8%, 34%, 39%, 19% in PCOS and 5%,49%, 38%, 8% in control women (Table 1, Figure 1).

Distribution of BMI in PCOS and Control Women				
BMI	Control/PCOS			
	Control	Control (%)	Case	Case (%)
<18.5 (Underweight)	16	5%	25	8%
18.5-24.9 (Ideal weight)	147	49%	103	34%
25-29.9 (Over weight)	113	38%	114	39%
>30 (obesity)	24	8%	58	19%

Table 1: Distribution of Body Mass Index between PCOS and Control Women.

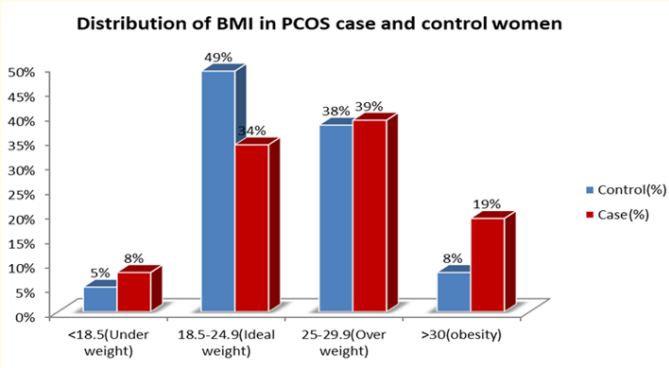

Figure 1: Graphical representation of BMI-PCOS and control groups.

A person with a BMI of 30 kg/m^2 or higher is measured obese in accordance with the world health organization [10]. Obesity is accountedto be connected with different diseases like cardiovascular, metabolic syndrome.The close relation between PCOS and obesity, the occurrence of obesity in women withPCOS is extremely changeable depends on age, and ethnicity in the general population [11].

Polymorphism and association of selected gene towards pathogenesis of PCOS

Genotyping study of AMH and LHCGR polymorphism

The blood samples were taken from both PCOS and non-PCOS subjects. According to the results observed by electrophoresis of allele specific PCR and RFLP method, the genotype of every individual for AMH (rs10407022) and LHCGR (rs13405728) gene polymorphism were determined. The results of the allele and genotype frequency, the differences in genotype distribution, and p value for each polymorphism are tabulated. Distribution of different allelic forms and their corresponding values of significance are enumerated. For LHCGR (rs13405728) gene –(TT) homozygous cut size -571bp+221bp, heterozygous (CT)- 571bp+387bp+221bp and change of allele (CC)-571 bp+387bp. Genotyping for AMH gene rs10407022 polymorphism was performed by the PCR-restriction fragment length polymorphism (RFLP) assay using enzyme BtsCI and the recognition site- GGATG and samples are loaded on polyacrylamide gel electrophoresis (PAGE), total base pair size -560bp and uncut (change of allele)-560 bp (TT), (CC)-293bp and (CT)-560bp +293bp.

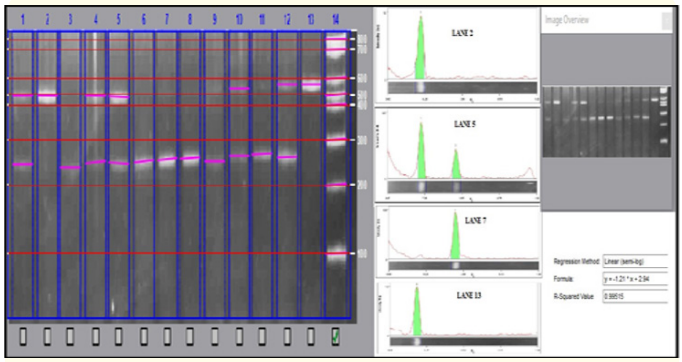

Figure 2: RFLP –Restriction Fragment Length Polymorphism of AMH gene rs10407022-Gel Images. Polyacrylamide gel electrophoresis stained with Etbr, the bands were viewed under gel documentation and change of allele uncut enzyme (560bp) (TT) was obtained in lane 2, lane 5 and lane 7 bands were heterozygous (CT) and homozygous (CC).

Results of AMH gene in PCOS case and control women				
	Control/PCOS case			
Results of AMH gene	Control	Control (%)	Case	Case (%)
CC	162	61%	136	45%
CT	138	39%	146	48%
TT	0	0%	18	6%

Table 2: Results of AMH gene in PCOS case and control women.

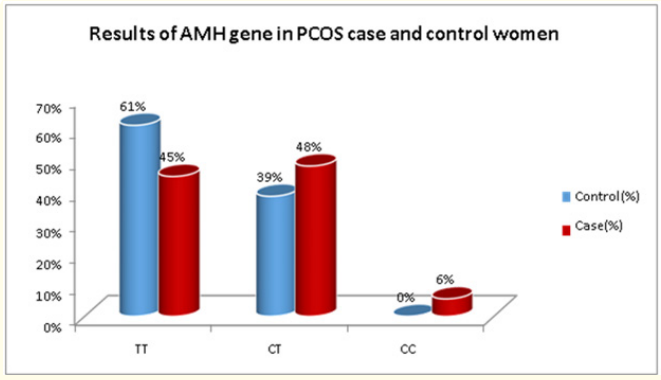

Figure 3: Graphical representation of AMH gene PCOS and control subjects.

H0: There is no association between Results of AMH gene and Control/PCOS case groups.

H1: There is association between Results of AMH gene and Control/PCOS case groups. The Chi-square Analysis was performed which results the chi-square value, degrees of freedom and the p-value. Therefore, χ^2=27.73, df=2, p-value=0.000. p-value< 0.05 was considered to be statistically significant.

Amplification refractory mutation system (ARMS) of LHCGR gene rs13405728- Gel Images Interpretation

The amplified products of both control and patient samples were loaded on 2.5% agarose gel and bands were viewed on chemiluminesence gel documentation system to recognize the cut size and based on the band size the amplified products were categorized - homozygous cut size -571bp+221bp, heterozygous (CT)-571bp+387bp+221bp and change of allele (CC)-571bp+387bp.

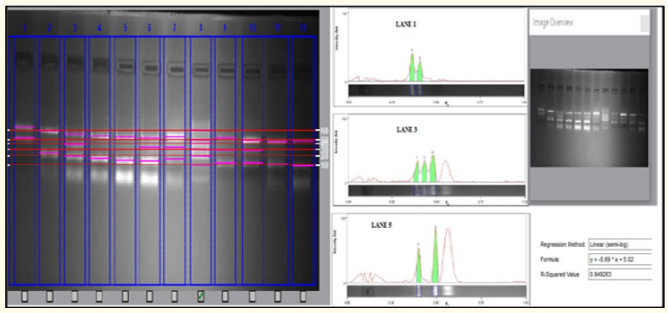

Figure 4

Results of LHCGR gene in PCOS case and control women				
	Control/PCOS case			
Results of LHCGR gene	Control	Control (%)	Case	Case (%)
TT	226	85%	217	71%
CT	74	17%	79	27%
CC	0	0%	4	2%

Table 3: The results of the LHCGR Gene in PCOS case and control women.

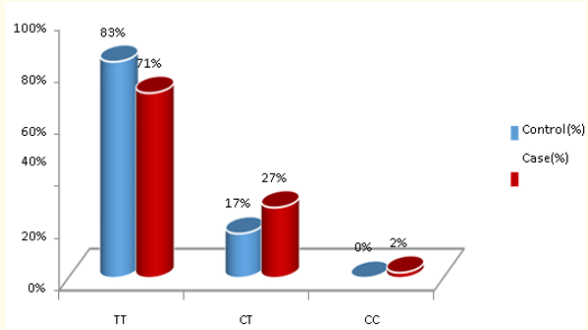

Figure 5: Graphical representation of LHCGR gene PCOS and control subjects.

Based on null and alternate hypothesis

- H0: There is no association between Results of LHCGR gene and Control/PCOS case groups.
- H1: There is association between Results of LHCGR gene and Control/PCOS case groups. Significantly proved that Change of allele (CC) the percentage obtained was about 2%. By using SPSS statistical software, 6% of women are prone to have PCOS among300 patients, 48% and 39% are heterozygous representing patients and control subjects.

SNP	Restriction Enzyme	Genotype and Ingested Band Size (bp)		
AMH rs10407022	BtsCI (Buffer mastermix)	T/T560	C/T293	C/C 560+293
LHCGR rs13405728	-	C/C 571 and 387	C/T 571, 221 and 387	T/T 571 and 221

Table 4: Genotypes of SNP Relates on Restriction Enzyme Ingestion Outlines of PCR Products.

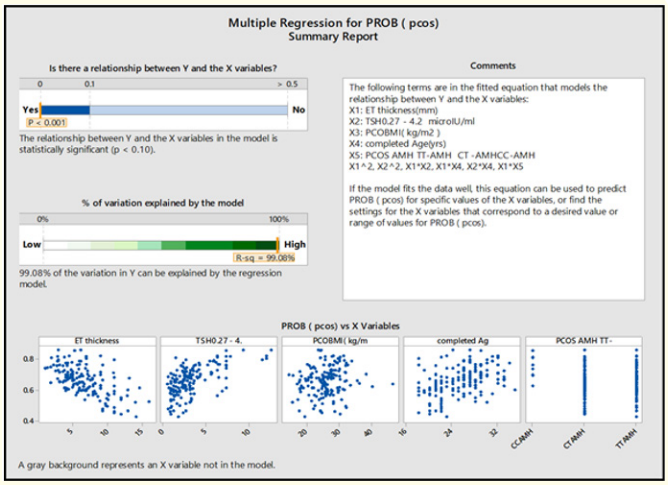

Figure 6: Multiple Regression–Probability Test Combined Effect of AMH.

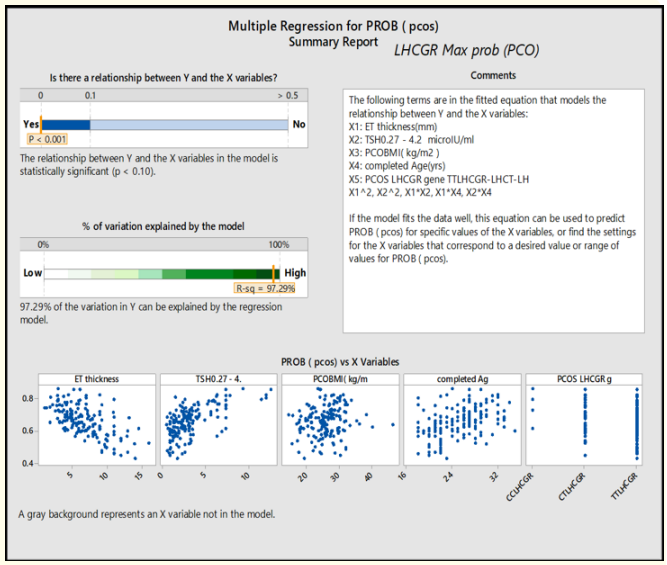

Figure 7: Multiple regression–probability test combined effect of LHCGR hormone.

The predictors that have been identified as determinants for probability of PCOS explained significantly 99% of the variation (R^2-99). Endometrial thickness inversely related and thyroid stimulating hormone was directly related to PCOS. This wasinterlinked with life style habitat, change of food habits from conventional to convenience habitat.

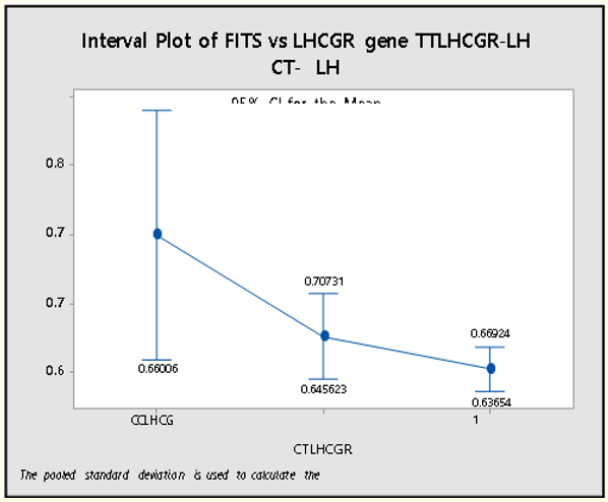

Figure 8: Gene-gene interaction analysis.

Multiple Regression–Probability Test Combined Effect ofLH-CGR Hormone

The predictors that have been identified as determinants for probability of PCOS explained significantly 97% of the variation (R^2-97.29%). This was proved by regression model, statistically significant ($p < 0.10$), and the effect of combinatorial factors with genotype frequency fitted the equation and hence was proved.

From the gene analysis method LHCGR proved that it was significantly prone to the onset of PCOS as it plays major role in steroid genesis pathway in female reproductive system and among 300 samples 4 of them have severe significance of PCOS, the linearity of graph clearly depicts the correlation of gene and hormone, inwhich one patient were 27.29 Kg and prone to obese and one patient falls under weight category.

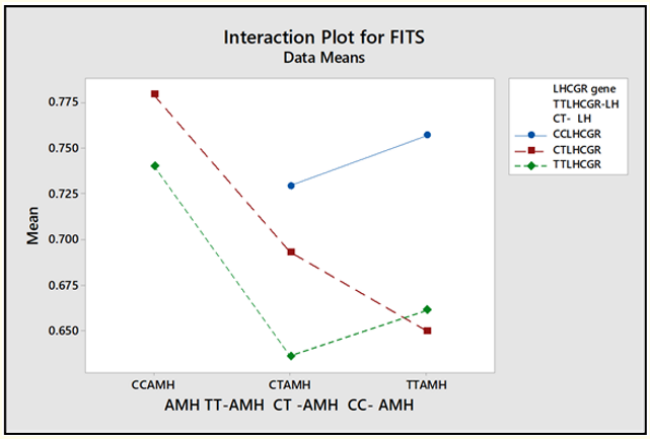

Figure 9: Graphical interpretation of AMH-Comparison between AMH and its gene.

The AMH gene is involved in follicular development of women, 18 patients were found to have change in gene sequence out of 300 patients, which was found to be more critical for women. Out of 18 cases, 11 patients had high TSH value than normal value. From the graph it was proved that hormone and gene play major part in human reproduction in which the mean value was found to 0.750 of (TT) compared with thyroid stimulating hormone of patients. Positive value (blue and red color) indicates synergistic epistasis between two loci and blue color represents independence high degree of synergy.

Discussion

The root of PCOS is still uncertain due to numerous factors implicated in the disease; though, it has been experiential that diverse genetic and environmental factors, such as genetic discrepancy, diverse regulation of genes, biological, metabolic and signalingpathways, may contribute to the pathogenesis of this disease [14]. The measurement of the clinical and hormonal profiles exposed that the biochemical and hormonal variations play a very vital role in womenand any modification of those variables agitate numerous signaling pathways and in turn interrupt the biological coordination. As the obese circumstance plays a serious role in fertility, and PCOS women inclined to be overweight due to the metabolic feature playing a role, it was proposed to evaluate and appraise the anthropometric distinctiveness of the PCOS and non-PCOS women. Present study coincides with another study in which the fecundity of obese women is lesser in contrast to that of women with normal body mass, and ovulation chaos is more recurrent in the obese women [15]. In spiteof the close relation between PCOS and obesity, the occurrence of obesity in women withPCOS is extremely changeable depends on age, and ethnicity in the general population 16. Genetic modification in LHCGR-rs13405728 and AMH-rs10407022 are established to be connected with impaired reproductive functions and that direct toinfertility in both sexes. In present study, PCR analysis of LHCGR-rsrs13405728 and AMH-rs10407022 SNPs exhibited the occurrence of change of allele as (C/C (04) for rs13405728, T/T (18) for rs10407022 among PCOS diagnosed women. In previous study, it was demonstrated that genes concerned in immunopathologicalresponse play a role in the PCOS expansion, or been concerned in the enlargement of PCOS [17]. The inaugural investigation delineating the genotype and allele frequencies of LHCGR SNP rs13429458, as revealed by GWAS, among PCOS-afflicted and healthy individuals of the Hui ethnic group, posited a potential association between these SNPs and PCOS within specific ethnic populations, suggesting a potential role for genetic variation in the etiology of PCOS [18].

LHCGR is the receptor for two glycoprotein which encodes LH and human chorionicgonadotropin (hCG). LH hormone stimulates ovarian theca cells to produce testosterone. According to GWAS between PCOS and healthy Hui ethnic people researchers reported in their findings, LHCGR SNP rs13425728 in the PCOS cases were higher than control group and the levels of T, TG and LDL were statistical different between rs13425728 genotypes, as well hypoth-

esized that SNPs were associated with PCOS in particular ethnics and genetic variation may play a role in the pathogenesis of PCOS [19].

Present study deals withthe genetic predisposition by representing for the first time an alliance between AMHrs10407022 and LHrs13405728 on the occurrence of PCOS in South Indian women. The outcome emphasizes the significance of this genetic polymorphism and its involvement in direction to the hazard of PCOS and connected fertility and other pregnancy related disorders. Genotype existence among PCOS subjects and non-PCOS controls were evaluated by Fisher exact test. $P < 0.05$ was measured statistically significant. The existing genetic studies put forth a strapping familial constituent and PCOS is measured a polygenic trait, which may be an upshot of the communication of susceptible and suspicious genomic variants and ecological aspects all through the prenatal or postnatal life. Lifestyle behavior like diet and exercise interferences were found to improve altitudes of follicle stimulating hormone, sex hormone strapping globulin, total testosterone, androstenedione, free androgen manifestation, and Ferriman-Gallwey scores in individuals with PCOS. Reducing body weight and exercise are significant factors accounted to progress the stipulation of menstrual destruction, and infertility was perceived in overweight women with PCOS. Thus, the two genes contribute to steroidgenesis and folliculogenesis in female reproductive system, which contribute to the greater risk of PCOS.

Conclusion

Polycystic Ovary Syndrome (PCOS) is acknowledged to be categorized by metabolic disarray in which peripheral insulin resistance and hyperinsulinemia are innermost characteristics. In our study results were indicative of a positive relationship between AMHrs10407022 and LHrs13405728 and PCOS in the South Indian population, Coimbatore, Tamilnadu. The single nucleotide polymorphism AMHrs10407022 and LHrs13405728 were established to illustrate an organization in PCOS inhabitants with insulin resistance in contrast to the control women. Polymorphism of rs10407022 and rs13405728 gene along with mutant homozygous (C/C) 4 and 18 allele in PCOS patients confirmed a stronger association and its involvement in PCOS women along with insulin resistance. SNP of Luteinizing and Anti-mull rein genes, rs10407022 and rs13405728 appears to hold a defensive role over PCOS. The mutant genotypes of these SNPsrobustly inclined BMI, height, weight, age, insulin resistance, endometrium thickness and ovarian volume in PCOS women. In this research study, the polymorphism of AMH rs10407022 change of allele (TT) was found to be 18 among 300 women, first work to interpret the results. For LH rs13405728 significantly results were observed in which 04 (CC) change of allele in South Indian women, (TT) homozygous 217 and (CT) heterozygous 79 were interpretedIn conclusion, our study suggested that the polymorphism of AMH and LH gene play a vital role in the occurrence in women with PCOS and associated with folliculogenesis and hyperandrogenism which would be interpreted with insulin resistance.

Declaration of Competing Interest

The authors declare that they have no conflict of interest with the contents of this article.

Acknowledgements

This work was funded by the National Natural Science Foundation of China under grant number 32250410285, and Foreign Expert Program, Ministry of Science and Technology (MoST) of China under grant number WGXZ2023020L.

Bibliography

1. Taghavi M and Fatemi S. "Macroprolactinemia accompany in patients with hyperandrogenemic symptoms". *Iranian Journal of Endocrinology and Metabolism* 10 (2008): 273-276.

2. Mohammad M B and Seghinsara AM. "Polycystic ovary syndrome (PCOS) diagnostic criteria, and AMH". *Asian Pacific Journal of Cancer Prevention: APJCP* 18.1 (2017): 17.

3. Hiam D., *et al*. "The genetics of polycystic ovary syndrome: an overview of candidate gene systematic reviews and genome-wide association studies". *Journal of Clinical Medicine* 8.10 (2019): 1606.

4. Malik S., *et al*. "Management of polycystic ovary syndrome in India". *Fertility Science and Research* 1.1 (2014): 23.

5. Rehman R., *et al*. "Interplay of leptin with obesity and polycystic ovarian syndrome'. *Life Science Journal* 12.4 (2022): 83-89.

6. Day F., *et al*. "Large-scale genome-wide meta-analysis of polycystic ovary syndrome suggests shared genetic architecture for different diagnosis criteria". *PLoS Genetics* 14.12 (2018): e1007813.

7. Wang F., *et al*. "The role of AMH and its receptor SNP in the pathogenesis of PCOS". *Molecular and Cellular Endocrinology* 439 (2017): 363-368.

8. Yang Y., *et al*. "Association of polymorphisms of interleukin-18 gene promoter region with polycystic ovary syndrome in chinese population". *Reproductive Biology and Endocrinology* 8.1 (2010): 1-7.

9. Essah PA., *et al*. "The metabolic syndrome in polycystic ovary syndrome". *Clinical Obstetrics and Gynecology* 50.1 (2007): 205-225.

10. Khalil Gerges N and Albiomy Khalefa A. "Serum nesfatin-1 levels in experimentally-induced polycystic ovary in lean and obese albino rats". *Al-Azhar Medical Journal* 45.4 (2016): 885-900.

11. Saddick SY. "Identifying genes associated with the development of human polycystic ovary syndrome". *Saudi Journal of Biological Sciences* 27.5 (2020): 1271-1279.

12. Haase M and Willenberg HS. "Hyperandrogenämie". *DMW-Deutsche Medizinische Wochenschrift* 138.49 (2013): 2509-2511.

13. Shen W., *et al*. "Calpain-10 genetic polymorphisms and polycystic ovary syndrome risk: a meta-analysis and meta-regression". *Gene* 531.2 (2013): 426-434.

14. Corbin K D., *et al*. "Obesity in type 1 diabetes: pathophysiology, clinical impact, and mechanisms". *Endocrine Reviews* 39.5 (2018): 629-663.

15. Giles LC., *et al*. "Growth trajectories in early childhood, their relationship with antenatal and postnatal factors, and development of obesity by age 9 years: results from an Australian birth cohort study". *International Journal of Obesity* 39.7 (2015): 1049-1056.

16. Lim SS., *et al*. "Lifestyle changes in women with polycystic ovary syndrome". C*ochrane Database of Systematic Reviews* 3 (2019).

17. Knebel B., *et al*. "Increased low grade inflammatory serum markers in patients with polycystic ovary syndrome (PCOS) and their relationship to PPARγ gene variants". *Experimental and Clinical Endocrinology and Diabetes* 116.8 (2008): 481-486.

18. Ha L., *et al*. "Association study between polycystic ovarian syndrome and the susceptibility genes polymorphisms in Hui Chinese women". *PLoS One* 10.5 (2015).

19. Zou J., *et al*. "Association of luteinizing hormone/choriogonadotropin receptor gene polymorphisms with polycystic ovary syndrome risk: a meta-analysis". *Gynecological Endocrinology* 35.1 (2019): 81-85.