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An Update on the Genetics of Polycystic Ovary Syndrome

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Abstract

PCOS is a common endocrinopathy among women of reproductive age, with a worldwide prevalence of 8 to 13%, depending on the criteria used for diagnosis. It is characterized by a constellation of features, including oligo/anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology. PCOS is one of the common causes of female infertility. It is also associated with metabolic derangements, including obesity, insulin resistance, and compensatory hyperinsulinemia, which increase the likelihood of developing type 2 diabetes mellitus. Despite extensive research, the etiology of PCOS remains largely unknown. It seems likely that the hypothalamic-pituitary-ovarian axis dysfunction, partial folliculogenesis arrest, insulin resistance, and ovarian and adrenal androgen secretion may play a role in the pathogenesis of PCOS. Familial clustering of the cases of PCOS points to a genetic component linked with it. The initial genetic studies suggest an autosomal dominant pattern of inheritance of the disorder in some families; however, most studies support multifactorial origin. Since PCOS is a complex trait, the typical form of inheritance of PCOS follows a non-Mendelian pattern and involves complex genetic mechanisms. Studies involving linkage and association have suggested a connection between genetic variations and the risk of developing PCOS in certain families or populations. Through genome-wide association studies and next-generation sequencing techniques, several candidate genes have been identified that play a role in the etiopathogenesis of the disorder. Pathogenic variants of various genes such as *INSR, IRS1, GHRL, LDLR, MC4R, ADIPOQ, UCP1, UCP2, UCP3, FTO, PCSK9, FBN3, NEIL2, FDFT1, PCSK9, CYP11, CYP17, CYP21, HSD17, STAR, POR, AKR1C3, AMH, AMHR2, INHBA, AR, SHBG, LHR, FSHR, FSH β, SRD5A, GATA4, THADA, YAP1, ERBB2, DENND1A, FEM1B, FDFT1, NEIL2, TCF7L2,* etc. in some PCOS cases are linked as underlying etiologic associations. This review aims to provide insight into the current genetic knowledge about PCOS. Discovering the genetic factors and pathways involved in the disorder will help us better comprehend the underlying mechanisms of the disorder.

Keywords: Etiopathogenesis, Genetic Variants, GWAS, Next-Generation Sequencing, Polycystic Ovary Syndrome, Whole Exome Sequencing

1. Introduction

PCOS is a heterogeneous endocrine disorder characterized by a combination of symptoms, including hyperandrogenism (HA; clinical/biochemical), ovarian dysfunction (OD; oligo/anovulation), and/or polycystic ovarian morphology (PCOM; polycystic/enlarged), provided that other possible diagnoses such as hyperprolactinemia, non-classical adrenal hyperplasia, and thyroid disorders have been ruled out[1]. The prevalence of PCOS ranges from 8 to 13% depending on the population studied and definitions used[2,3]. PCOS is associated with notable metabolic and reproductive features, including a greater likelihood of developing Type 2 Diabetes Mellitus (T2DM) at a younger age, impaired glucose tolerance, insulin resistance, Cardiovascular Disease (CVD), subfertility and an increased risk of experiencing symptoms related to depression and anxiety[4,5].

Due to the numerous potential diagnostic approaches, treatment options, and often contradictory recommendations, a global consortium was established to thoroughly analyze the evidence and develop evidence-based guidelines for diagnosing and managing

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PCOS which were published in 2018[6,7]. Of the 175 recommendations in the international guidelines, only 31 were classified as evidence-based. The current consensus in PCOS diagnosis is the Rotterdam criteria with NIH 2012 phenotypic classifications, i.e., A, B, C, and D. Phenotype A requires the presence of all three features i.e., HA+OD+PCOM, phenotype B requires HA+OD, phenotype C is diagnosed as HA+PCOM, and phenotype D manifests as OD+PCOM.

Notably, mapping the susceptibility loci for PCOS has been done through Genome-Wide Association Studies (GWAS), which identified multiple candidate genes for PCOS. These genes include those associated with ovarian and adrenal androgen biosynthesis (*StAR, CYP21, CYP11, CYP17, CYP19*), insulin resistance (*INSR, IRS2*), and reproductive hormones and their receptors (*LHCGR, FSHR, AMH, AMHR2*), pointing at the possible role of genetic mechanisms in the pathogenesis of PCOS[8-13].

The estimated heritability of PCOS ranges between 70% to 80% as reported in the twin studies[14]. However, the common susceptibility loci identified through GWAS account for only a tiny proportion of the total heritability of PCOS[15]. The presence of rare variants with considerable biological effects that are difficult to identify through contemporary GWAS has been proposed to explain the missing heritability of the disorder[16]. With high-throughput sequencing techniques, the simultaneous mapping of the genomic regions has made identifying pathogenic and disease-causing variants possible[17].

PCOS affects all aspects of reproductive and neuroendocrine physiology, but the exact pathophysiology of the condition is yet to be ascertained. This paper will review the current evidence for the leading causes of PCOS along with the candidate genes associated with the different pathways. An update on the heritability, inheritance, and status of PCOS genetics will also be provided.

2. Inheritance of PCOS

It is well-known that inherited genetic factors strongly influence PCOS. That PCOS could have a genetic susceptibility was first suggested in the studies carried out in the 1960s, when families with more than one PCOS-affected woman were reported. These studies observed that the genetic susceptibility for PCOS is different among the members of the same family[18-22]. This observation was crucial because the mode of inheritance of the disorder was unknown. Familial clustering of reproductive features of the syndrome was found among the relatives of the affected women[23-28]. The menstrual and hyperandrogenic characteristics are common among female siblings and their mothers, affecting up to ~40% of reproductive-age sisters.

In contrast, hyperandrogenic symptoms appear in male siblings in the form of baldness[23,25,26]. The phenotypic similarities between hyperandrogenism and metabolic syndromes, such as insulin resistance, were previously observed[29,30]. In the non-Hispanic white women, independent and additive defects in insulin action were associated with PCOS[31]. Further investigations revealed the presence of insulin receptor mutations in PCOS women[32,33]. It has also been observed that insulin resistance and β-cell dysfunction markers are elevated among male children who inherit genetic variations related to PCOS from their affected mothers[34]. The same study reported elevated levels of circulating AMH in the daughters of women with PCOS, regardless of whether they inherited genetic factors associated with PCOS, suggesting that genetic and epigenetic factors govern PCOS. Occasionally, the prevalence of PCOS among identical twins has also been reported. A 50% incidence of PCOS has been observed among 34 twin pairs studied in an Australian study, which, owing to a high discordance in sonographic ovarian imaging among twins, suggested a complex inheritance pathway and the critical role of environmental factors in the genetic transmission mechanism of PCOS[35]. The initial genetic investigations suggest that PCOS has an autosomal dominant inheritance pattern, suggesting that the disorder is passed down through either sex[25,36]. However, these findings were constrained by a small sample size and a failure to examine all the relatives. Further studies have, however, revealed that PCOS has a multigene origin[35].

3. Heritability

Heritability is defined as the proportion of variation in a trait attributable to genetic differences between individuals[37]. Genetic studies are more informative for traits or diseases with higher heritability. Twin studies are believed to be a good starting point in understanding a trait's heritability because twins share a common environmental milieu during their developmental stages[38]. The easiest way to assess the heritability of a trait is by comparing the correlations of traits between pairs of Monozygotic (MZ)

twins, who share identical genetic material, to those of dizygotic twins, who share 50% of their genetic material. The heritability of PCOS has been estimated from studies conducted in various populations (ethnic groups, twins, and families with affected women). In 2006, Vink and colleagues approximated the heritability of PCOS in an extensive study of Dutch twins[14]. Their diagnostic criteria included women who presented with fewer than nine menstrual cycles in a year, along with hirsutism or acne. The results showed that MZ twins having PCOS defined by these criteria had a 0.71 heritability, whereas it was 0.38 in DZ twins. The overall heritability was estimated at 0.79 using a standard pathway model accounting for oligomenorrhoea, acne, and hirsutism[14].

Individual hormonal components have been shown to be highly heritable through family-based studies. The heritability of testosterone, for example, has been observed to be 0.26 to 0.50 in women[26,39-41]. A heritability rate of 0.44 has been suggested for dehydroepiandrosterone sulfate (DHEAS) when correlated with PCOS probands and their sisters[42]. For SHBG, the heritability estimates are 0.56 to 0.63[26]. The heritability of metabolic factors such as insulin resistance and BMI has also been observed among sisters of women with PCOS[26].

4. Current Status of PCOS Genetics: From Pathophysiology to Genes

Stein and Leventhal, in the 1930s, described the association between polycystic ovarian morphology, infertility, and menstrual disturbances[43]. Many patients experienced restoration of regular menstrual cycles through the surgical procedure of ovarian wedge resection. This finding indicated that ovarian dysfunction plays a vital role in the development of the disorder. We now understand that PCOS is a complex disorder with varying etiology, resulting in intricate pathophysiology and intrinsic mechanisms (Figure 1). These mechanisms interact and perpetuate the clinical manifestations of PCOS, such as hyperandrogenism, Polycystic Ovarian Morphology (PCOM), and ovulatory dysfunction. The syndrome is further complicated by insulin resistance, aggravated by the accumulation and malfunction of adipose tissue related to hyperandrogenism, leading to lipotoxicity and oxidative stress[44]. Numerous genetic studies have mainly aimed at tracing the genes involved in essential pathways associated with PCOS (Figure 2). We review below the various gonadotropic, steroidogenic, and metabolic dysfunctions and their associated genes with PCOS.

4.1 Gonadotropic Derangements

Under normal circumstances, several hormones, most importantly FSH, influence the maturation of immature oocytes, while the Luteinizing Hormone (LH) stimulation is essential for ovulation and final maturation. Increased Gonadotropin-Releasing Hormone (GnRH) pulse frequency is a neuroendocrine abnormality frequently observed in PCOS, which further leads to increased pulse frequency of LH while simultaneously suppressing FSH release[45]. As a result, the circulating LH/FSH ratio is increased and is reported to be more prevalent among lean PCOS women compared to obese women with PCOS[46,47]. The finding that women with PCOS experience heightened LH pulses and increased daytime LH pulse secretion at an early stage of puberty suggests abnormalities in the pulsatile release of GnRH may be responsible for developing PCOS, at least in some women[48]. Hypersecretion of androgens in theca cells of ovarian follicles is due to increased LH/FSH ratio and resistance to FSH in the ovaries.

Consequently, follicular development is impaired. Impaired follicular development reduces the progesterone-mediated inhibition of GnRH pulse frequency, further promoting the development of PCOS. The usual negative feedback actions of estradiol and progesterone on the hypothalamic GnRH pulse generator are inhibited by excess testosterone, which renders the hypothalamus insensitive to the inhibitory actions of progesterone and estrogen. Testosterone reduces the responsiveness of the hypothalamic GnRH pulse generator to the usual feedback effects of estradiol and progesterone, which are meant to decrease the pulse frequency[49]. It has been noted that elevated levels of LH stimulate excess androgen synthesis by decreasing the aromatization of testosterone to estrogen in the theca cells of ovaries[50,51].

The LHCGR gene is located on the short arm of chromosome 2p16.3 and encodes for both the luteinizing hormone receptor and chorionic gonadotropin hormone receptor[52]. It is expressed in the granulosa cells or the preovulatory follicles in the ovary and plays an essential role in ovulation by transducing luteinizing hormone-mediated signals[53]. Loss-of-function mutations in LHCGR can lead to elevated levels of LH, menstrual irregularities,

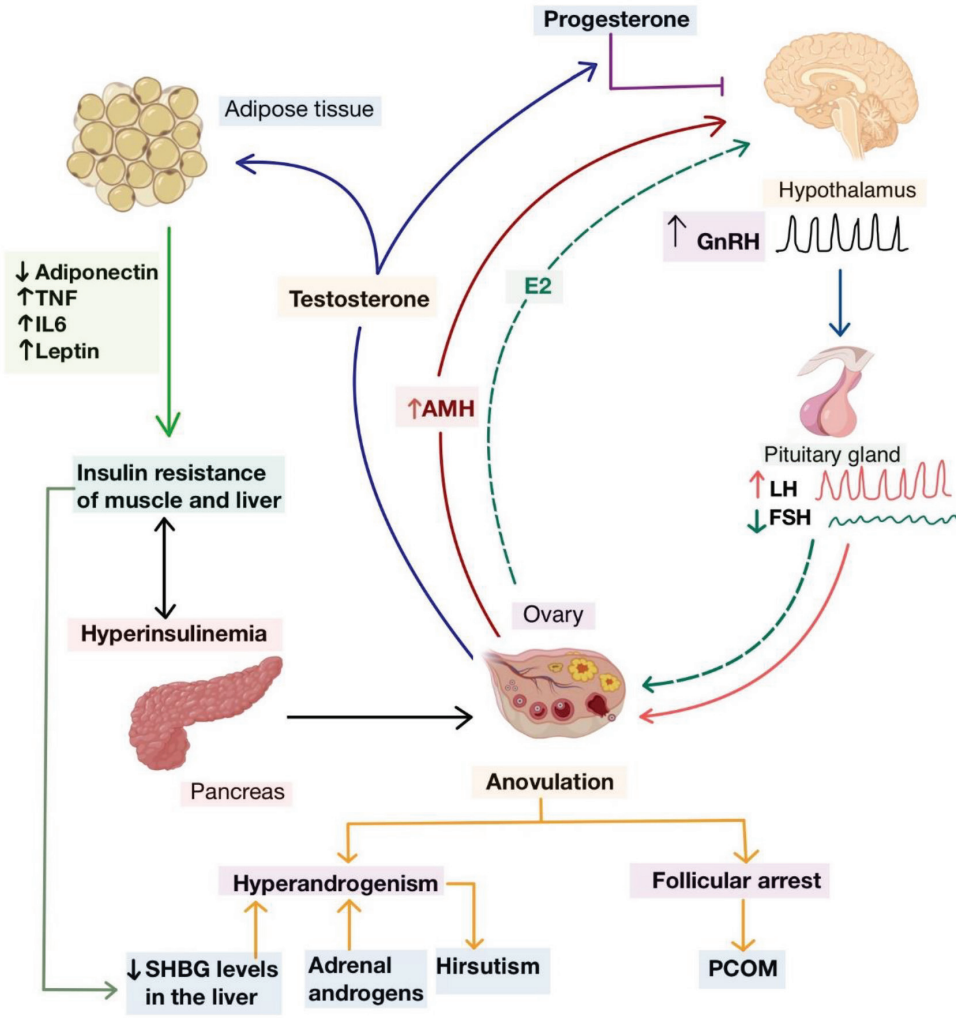

Figure 1. The proposed pathophysiology and features of PCOS.

The central abnormality in PCOS is disturbed pulsatility of gonadotropin-releasing hormone (GnRH) which occurs from the hypothalamus, consequently leading to excess ovarian androgen secretion and ovarian dysfunction. This hypersecretion of LH is a result of perturbed inhibition of progesterone on GnRH secretion. The follicles are more resistant to the effects of FSH in PCOS women, despite the levels of FSH being normal. Hyperinsulinemia and insulin resistance further contribute to hyperandrogenic state in PCOS. Adiposity may be caused by a vicious cycle where hyperandrogenism promotes the growth of abdominal fat, which in turn encourages further androgen production from the ovaries and/or adrenal glands.

LH (luteinizing hormone); FSH (follicle stimulating hormone); AMH (anti-Mullerian hormone); PCOM (polycystic ovary morphology); E2 (estradiol); GnRH (gonadotropin releasing hormone); SHBG (sex hormone-binding globulin); IL6 (interleukin 6); TNF (tumor necrosis factor)

and infertility in women. On the contrary, activating mutations in LHCGR can result in hyperandrogenism[54]. The 2p16.3 region containing LHCGR loci was found to be associated with PCOS risk in a GWAS conducted in Han Chinese and European populations[55,56]. In women of Chinese ethnicity, the LHCGR rs13405728 SNP showed an association with PCOS. However, the association of the same SNP with the disorder was not observed in European populations[55,57-59]. An SNP located nearby in exon 10 (rs13405728) was studied in the Sardinian population and was reported to be linked with the risk of developing PCOS[60]. Recently, the mutant genotype (rs2293275) was found to have a 1.7-fold risk of developing PCOS in the Indian population[61]. The data from these genomic studies indicate that LHCGR is a potential candidate gene for developing PCOS.

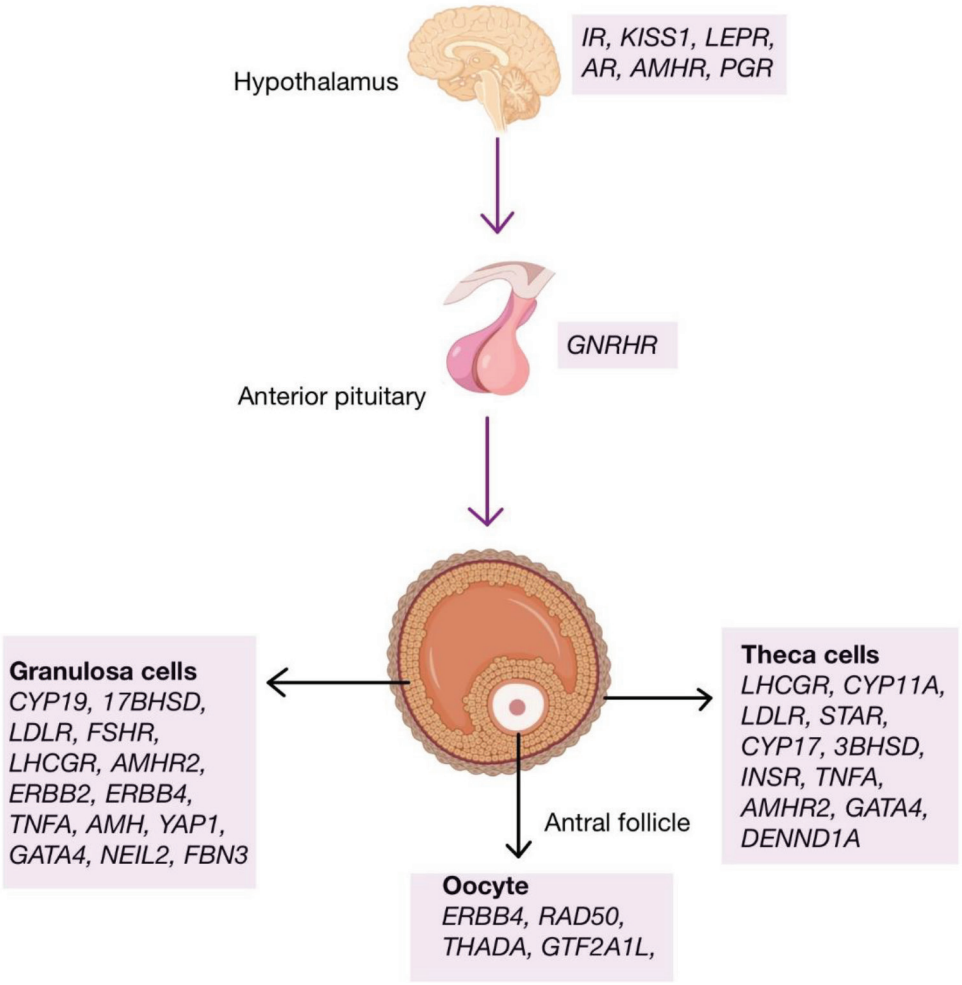

Figure 2. Genes related to the reproductive hormone and receptors, GnRH pulsatility, androgen biosynthesis and action and folliculogenesis, identified through GWAS, candidate-gene studies and next-generation sequencing. Genetic variations in these genes may lead to the development of PCOS.

The Follicle-Stimulating Hormone Receptor (FSHR) on chromosome 2p21 belongs to the G protein-coupled receptor[62]. Its primary expression is in granulosa cells of the ovaries. FSHR plays a crucial role in oogenesis, follicle development, and gametogenesis. Upon binding with FSH, FSHR stimulates follicle development and proliferation of granulosa cells[63]. The mutations inhibiting FSHR gene expression result in hypogonadotropic hypogonadism leading to follicle development arrest at the preantral stage[64]. GWAS studies in Han Chinese and European populations have reported an association between FSHR polymorphism and PCOS[10,56,65].

Additionally, the association of two variants in exon 10 of the FSHR gene Thr307Ala and Asn680Ser have been found with PCOS[66-69]. In a meta-analysis, however, the Asn680Ser was strongly associated with PCOS, whereas Thr307Ala failed to show any association[70]. A pathogenic intronic variant was recently identified in a whole-exome sequencing study conducted in an Indian cohort[71]. Similarly, a heterozygous variant in FSHR (p.Arg283Trp) was observed in a recent study on South American PCOS women[72] (Table 1). There remain significant gaps in the knowledge of how the FSHR variants might be causing the PCOS phenotype; however, considering the polymorphism studies, the FSHR gene can be considered a risk factor for PCOS.

The GnRH receptor (GnRHR) is a G-protein coupled receptor present in the anterior pituitary's gonadotroph membrane and other tissues like the ovary, placenta, breast, and cancerous tissues[73,74]. Upon binding with GnRH, GnRHR activates the phosphatidylinositol-Ca^{2+} second messenger system, which affects LH and FSH

Table 1. Current Next-Generation Sequencing (NGS) studies in PCOS

Reference	Ethnicity	Study Type	Year	Sequencing technique	Gene	Variant	Zygosity	ACMG classification
(9)	European	Population-based study	2017	WGS	AMH	T143I; P270S; exon2/3 splice site	NA	NA
				Targeted sequencing		V12G; A24T; P46A; R91H; T99S; T143I; P151S; A156T; Q185E; splicing (ex2/3); R194H; P270S; P284S; D288E; R302Q; Q325R; P352S; P362S; P366L; A372V A385V; H506Q; A519V; V553L	Heterozygous	
(107)	European	Population-based study	2019	Targeted sequencing	AMH	P30S; R548Q	Heterozygous	NA
(204)	Han Chinese	Population-based study	2021	WES	Androgen receptor (AR)	p.V3M; p.Q72R; p.S158L; p.S176R; and p.G396R	Heterozygous	NA
(235)	Han Chinese	Population-based study	2022	WES	ESR1	A207T	Heterozygous	NA
(72)	South America	Population-based study	2022	WES and targeted sequencing	LMNA	Arg249Gln; Gly638Arg; Arg644Cys	Heterozygous Heterozygous Heterozygous	Pathogenic VOUS VOUS
					FSHR	Arg283Trp	Heterozygous	VOUS
					PIK3R1	Leu26Phe	Heterozygous	Likely Pathogenic
					GATA4	Arg265Cys	Heterozygous	VOUS
					NR5A1	Pro129Leu	Heterozygous	VOUS
					INSR	Tyr1190His	Heterozygous	Likely pathogenic
					BMP15	Arg68Trp	Heterozygous	VOUS
					DLK1	Gly199Alafs*11	Heterozygous	Likely Pathogenic
(209)	Indian	Population-based study	2022	WES	CYP21A2, and) and	p.Ala392Thr p.Gln319Ter and p.I143N	Heterozygous	Pathogenic Pathogenic VOUS
					StAR	p.Arg53 Leu	Heterozygous	VOUS
					AKR1C3	p.Phe205Val	Heterozygous	VOUS
					P450 oxidoreductase	p.Val334Ile p.Val251Met	Heterozygous	VOUS
					HSD17B6	p.Gly40Ser	Heterozygous	VOUS

Table 1. Continued...

Reference	Ethnicity	Study Type	Year	Sequencing technique	Gene	Variant	Zygosity	ACMG classification
(78)	Israel	Familial study	2017	WES	GNRHR	Q106R	Homozygous in three sisters Heterozygous in the parents	NA
(237)	Turkey	Familial study	2022	WES	FBN3	c.4823A>G c.4498G>A	Heterozygous	VOUS
					FN1	c.1802C>T	Heterozygous	VOUS
(71)	India	Familial study	2023	WES	DFFB	p.Arg196Lys	NA	Pathogenic
					FSHR	intron	NA	Pathogenic
					HLA-B	p.Ala223Val	NA	Likely Pathogenic
					HLA-C	p.Ala330Val p.Thr329Ala p.Val319Ala	NA	Likely Pathogenic Likely Pathogenic Likely Pathogenic
					HLA-DRB1	p.Ser66Tyr p.Ala169Thr	NA	Pathogenic Pathogenic
					MUC12	p.Arg1880His	NA	Likely Pathogenic
					GATA4	424-425KTAYEX	NA	Pathogenic
					CYC1	203-?	NA	Pathogenic
					MYL6B	p.Thr101Ile	NA	Likely Pathogenic
					ATP5EP2	p.Val22Ala	NA	Likely Pathogenic
					IGHD	p.Ala406Thr	NA	Pathogenic
					ALDAO	p.Leu333LeuMetAlaLeu	NA	Pathogenic
					ACTG1	?-42	NA	Pathogenic
					GPX4	60-?	NA	Pathogenic
					INSR	Intron	NA	Pathogenic

Table 1. Continued...

Reference	Ethnicity	Study Type	Year	Sequencing technique	Gene	Variant	Zygosity	ACMG classification
					NWD1	p.Ile78Val	NA	Likely Pathogenic
					UBC	S190P	NA	Likely Pathogenic
					SCARB1	V135I	NA	Likely Pathogenic
					PABPC3	P191T	NA	Likely Pathogenic
					KIF23	-	NA	Likely Pathogenic
					CALR	234-?	NA	Likely Pathogenic
(236)	Japanese	Population-based study	2023	WES	GSTO2	Pro16Thr Asp46Tyr His49Leu Thr77Ile Ala206Val	Heterozygous	NA

VOUS (variant of uncertain significance); WGS (whole genome sequencing); WES (whole exome sequencing); NA (not available)

synthesis and secretion[75]. The molecular analysis of GnRHR in patients with PCOS showed that it is unlikely for a mutation in GnRHR to be responsible for the development of PCOS[76]. It has been documented that there is a crosstalk between GnRH signaling and the release of Thyroid-Stimulating Hormone (TSH), insulin resistance, and insulin signaling in PCOS patients[77]. A comprehensive analysis was conducted on a consanguineal family with three sisters diagnosed with PCOS using whole exome sequencing[78]. The study confirmed a variant in the *GNRHR* gene which encodes the gonadotropin-releasing hormone receptor, belonging to the G-protein coupled receptor family present at the surface of pituitary gonadotrope cells. All family members were subjected to Sanger sequencing, confirming that the p.Q106R variants in the *GNRHR* gene were homozygous in the three affected sisters and heterozygous in parents (healthy carriers).

4.2 Ovarian Follicular Arrest

The disrupted coordination and interaction between LH, FSH, AMH and Insulin-Like Growth Factors (IGF1), and enzymes involved in androgen conversion, among others, contribute to oligo-ovulation (irregular ovulation) or anovulation (absence of ovulation) in PCOS[79]. In PCOS, the regular selection of a dominant follicle that proceeds to ovulation in each menstrual cycle is hampered due to insufficient FSH secretion and local inhibition of FSH action[80]. Follicular FSH resistance may be due to other regulators of FSH action within the ovary. One of these regulators is the increased levels of AMH in PCOS, which reduces the FSH sensitivity of individual ovarian follicles[81] and prevents the conversion of androgen to estrogen by inhibiting aromatase activity, thereby contributing to hyperandrogenism. Genetic variants in the FSH molecule or its receptor might account for differences in FSH sensitivity between patients with PCOS and healthy controls[82].

Anti-Mullerian Hormone (AMH) belongs to the TGF-β superfamily, exerting its effects through the AMHR2 receptors and through Type I receptors ALK1/ACVR1, ALK3/BMPR1A, or ALK6/BMPR1B, and the SMAD1, SMAD5, and SMAD8 proteins, which are shared between AMH and Bone Morphogenetic Proteins (BMP)[83]. In females, AMH is expressed by the ovaries' granulosa cells, which regulate folliculogenesis[83]. Elevated circulating levels of AMH arise due to increased maturing follicles and the increased synthesis of AMH per follicle[84]. This is particularly true for women with anovulatory PCOS, where reduced AMH levels in small follicles may promote the recruitment of additional small primordial follicles[85] However, hypersecretion of AMH in granulosa cells of more mature small antral follicles could subsequently inhibit further follicular growth by impeding FSH and aromatase action[81,86]. As a result, in women with anovulatory PCOS, the levels of circulating FSH, although low-to-normal, may not be sufficient to overcome the suppression of aromatase activity by AMH in the antral follicles[87]. Notably, the expression of the AMH receptor, AMHR2, on the neurons of the hypothalamic Gonadotropin-Releasing Hormone (GnRH) has been reported in mice and humans[88]. It has been reported that AMH exerts positive feedback on GnRH neuronal firing in mice[88,89]. In this way, AMH can exert extra-gonadal actions, leading to the development of PCOS[88,89].

Serum AMH is utilized in clinical practice as a biomarker of the growing pool of follicles[90,91], which directly indicates the size of the primordial follicle pool[92,93]. Thus, AMH is also a reliable biomarker of ovarian reserve[94-96]. Of note, serum levels of AMH are increased in women with PCOS[97-99]. Interestingly, PCOS women show 2- to 4-fold elevated levels of AMH in both the serum and the follicular fluid[100]. It has been suggested that the increase in the levels of AMH occurs both due to the increase in the number of small antral follicles, which show the highest expression of AMH, and its receptor (AMHR2) by their granulosa cells[101-102]. The abnormal regulation of several hormones in PCOS also plays a crucial role in the overexpression of AMH and its receptor. The overexpression of AMH and AMHR2 can partially be due to androgen excess, one of the most prominent diagnostic features of PCOS, as many studies have reported a positive correlation between the elevated levels of androgens and AMH[103-105]. Although it has been suspected for a long time now that overexpression of AMH and AMHR2 are involved in the pathophysiology of PCOS, however, studies investigating the single nucleotide polymorphisms in the AMH gene have found AMH Ile49Ser and AMHR2-482A>G polymorphisms which reduce the bioactivity of the enzymes and are associated with PCOS[106].

Two studies on the candidate genes for PCOS were conducted on *AMH* and *AMHR2*[9,107]. The studies focused on rare variants (with a minor allele frequency of less than 1%) in PCOS cases and used AMH-mediated luciferase assay to measure their functional impact. Women with PCOS often have higher levels of AMH, a crucial factor in developing follicles[108-110]. The study found 37 rare variants

specific to PCOS that significantly reduced the signaling activity of AMH. These variants were linked to PCOS at the population level. In all cases where PCOS was present alongside these functional variants, the affected individuals were carriers of the variant in only one of their gene copies. Five of these AMH variants that had a functional impact had been previously identified in men with a rare condition called Persistent Mullerian Duct Syndrome (PMDS), in which men have both Mullerian and Wolffian duct-derived reproductive organs[111]. It has been seen that men with AMH mutations have low or undetectable levels of AMH[112]. In PCOS, however, the lack of inhibition of CYP17 by AMH could be a contributing factor[113]. Thus, AMH variants that weakened the signaling ability caused a significant reduction in the inhibition of CYP17α1 expression compared to the wild-type AMH. About 6.7% of PCOS cases in these groups carried at least one of the rare variants in the AMH/AMHR2 genes[9,107]. While higher AMH levels are a more common characteristic of PCOS, the studies of rare variants suggest that the role of AMH in PCOS is more complex than previously believed and may differ among women with the disorder. The precise way weakened AMH signaling contributes to PCOS needs more research to fully understand.

4.3 Hyperandrogenism

The levels of testosterone and/or androstenedione and/or dihydrotestosterone are elevated in the serum of women with PCOS[114,115]. Androgen excess arises mainly due to increased androgen synthesis by the theca cells of ovaries, which show an upregulated expression of various genes involved in steroid biosynthesis[116]. Overexpression of DENND1A, which is a candidate gene for PCOS, has been observed in the theca cells obtained from patients with PCOS[116]. Additionally, theca cells show increased expression of the CYP17A1 gene, which encodes a rate-limiting enzyme in androgen biosynthesis. This leads to an increased conversion of progesterone precursor to androgens[116]. The theca cells obtained from PCOS patients show more responsiveness regarding hyperandrogenism to insulin and LH compared to the theca cells isolated from control women[117]. Hyperinsulinemia also contributes to androgen excess in PCOS patients by reducing the synthesis of SHBG in the liver, thus, leading to increased levels of free testosterone in circulation[118].

Although the ovaries are the primary source of androgen secretion in PCOS women, about 20% to 30% of patients show adrenal androgen excess, which points to adrenocortical hyperfunction[117]. This is because the adrenal glands also share the steroidogenic enzymes in the theca cells[1,3,120]. It has also been observed that PCOS women show enhanced adrenal sensitivity to ACTH[121]. One of the consequences of androgen excess in PCOS is hirsutism. The effect exerted by testosterone and dihydrotestosterone through their action on androgen receptors stimulates ornithine decarboxylase synthesis in the hair follicle, leading to polyamine synthesis. Polyamines are essential for cellular proliferation in the hair follicle.

The StAR gene is located on chromosome 8p11.2 and encodes a protein that transports cholesterol from the outer to the inner mitochondrial membrane in the first step of steroidogenesis[122-124]. In women with PCOS, theca cells of the follicles showed increased expression of StAR, indicating hyperstimulation of these cells and excess androgen production[125]. However, granulosa cells did not show any change in the expression of StAR, suggesting that increased responsiveness of granulosa cells to LH may contribute to arrested follicle development[125]. Some studies have reported no changes in StAR expression in PCOS ovaries compared to healthy ovaries, and no correlation between StAR Single Nucleotide Polymorphisms (SNPs) and PCOS was found in Caucasian[126] and Iranian women[127]. In contrast, research led by Jahromi and colleagues has reported increased expression of the StAR gene in prenatally androgenized rat model and hypomethylation at the promoter region of StAR[128,129].

Furthermore, a recent study has reported significantly increased expression of StAR gene in PCOS rat models compared to the control group[130]. The study also showed a positive correlation between StAR gene expression and serum testosterone levels[130]. These studies indicate that alterations in the steroidogenesis pathway after exposure to excess androgen could be due to changes in the expression pattern of the StAR gene.

The gene for sex-hormone globulin is located on chromosome 17p13.1[131]. The hepatocytes mainly produce SHBG and have a strong affinity for binding with androgens[132]. It is responsible for regulating the levels of sex hormones in the bloodstream and controlling the access of target tissues to androgens[133]. Women with PCOS often have high levels of androgens, insulin resistance, and hyperinsulinemia, inhibiting the liver's production and release of SHBG[134]. According to research, low levels

of SHBG in women with PCOS can cause symptoms of hyperandrogenism like access hair growth, acne, male-pattern baldness, and virilization[135-138].

Additionally, certain genetic variations in the SHBG gene can affect the levels of SHBG in circulation and may play a role in the development of PCOS[139,140]. Studies have found two new mutations in the coding region of the SHBG gene[141]. One of these mutations affects glycosylation, while the other leads to truncated synthesis of SHBG protein. These mutations result in low SHBG levels and increased free testosterone levels in circulation. SHBG gene can, thus, be considered as a candidate gene playing a crucial role in the development of PCOS.

The CYP21 (P450c21) gene is located on chromosome 6p21.3[142]. The 21-hydroxylase enzyme encoded by this gene plays a crucial role in converting C21 steroids like progesterone and 21-hydroxyprogesterone into 11-deoxycorticosterone and 11-deoxycortisol[143]. The adrenal cortex is the leading site where CYP21 is expressed, and it is crucial for synthesizing specific adrenal steroids like cortisol, corticosterone, and aldosterone[144,145]. The prevalence of heterozygosity of mutations in the CYP21A2 gene among women with PCOS has been extensively studied. However, the results have been contradictory. In a study by Witchel *et al.*[146], the prevalence of heterozygous mutations was 35.2% among adolescents with hirsutism and/or irregular menses and 6% among healthy controls.

Similarly, a study conducted by Escobar-Morreale *et al.*[147] showed that 13.3% of the women with ovarian hyperandrogenemia were carriers of CYP21A2 mutations. In comparison, only 7.7% of the healthy women were heterozygous for the same mutations. Additionally, in a later study by Witchel *et al.*, 33% of the women in the hyperandrogenic group and 7% in the control group exhibited heterozygosity in mutations in CYP21A2 gene[148]. Decreased or absence of 21-hydroxylase enzyme is the cause of non-classical adrenal hyperplasia. Some evidence suggests that a single mutation in the CYP21 gene can result in increased adrenal androgens without developing a complete CAH phenotype[149]. While there is a possibility of overlapping symptoms between CAH and PCOS, it is that CYP21 has some contribution to the pathogenesis of PCOS.

The CYP11A1 gene belongs to a group of enzymes from the cytochrome P450 family located on chromosome 15q24.1[150]. These mono-oxygenase enzymes are expressed on the inner mitochondrial membrane and play a crucial role in steroid biosynthesis, cholesterol metabolism, and drug metabolism[151]. One of the essential functions of these enzymes is the conversion of cholesterol to pregnenolone, which is the first and rate-limiting step in synthesizing steroid hormones[152]. The expression of the CYP11 gene is found in ovaries, kidneys, breasts, testes, and bladder[153]. Based on the linkage review, there is a strong association between the pentanucleotide repeat polymorphism (TTTTA)n in the CYP11A 5'-UTR and hirsute PCOS patients[154]. However, the association between these pentanucleotide repeats and PCOS susceptibility varies among ethnic groups. A recent meta-analysis in the Caucasian population found a clear link between the microsatellite repeat polymorphism and an increased risk of PCOS[155]. The allele variants of CYP11A and its polymorphism related to serum testosterone levels might be associated with androgen excess and hyperandrogenemia[156]. The meta-analysis results showed a link between PCOS and a particular pentanucleotide repeat polymorphism in the promoter region of the CYP11A gene[157]. The studies also indicate that this gene is also associated with hirsutism but not with ovulatory dysfunction, suggesting that it plays a significant role in developing hirsutism in PCOS. Given the importance of this gene in the steroidogenesis in the ovary, all the studies suggest that the CYP11A gene may be a possible genetic biomarker that plays a significant role in the development of PCOS.

The CYP17A1 gene, located on chromosome 10q24.3, encodes for an enzyme belonging to the cytochrome P450 superfamily. They are essential in steroid biosynthesis, cholesterol metabolism, and drug metabolism[158]. The P450c17 enzyme has both 17-hydroxylase and 17-lyase activities, which catalyzes the conversion of pregnenolone to 17-hydroxypregnenolone and progesterone to 17-hydroxyprogesterone. It cleaves the 17-20 bonds to produce C19 steroids dehydroepiandrosterone and androstenedione[159]. The CYP17 gene is mainly expressed in the theca cells of the ovaries, which are the site of androgen production[160,162]. The expression of the CYP17 gene and the enzyme activity is increased in the theca cells, in addition to the transactivation of the CYP17 promoter in women with PCOS[162,163].

Furthermore, some studies have reported reduced stability of CYP17 mRNA in PCOS patients[164]. Mutations and polymorphisms in this gene have been reported and linked with PCOS[165-168]. Thus, CYP17 is believed to play a significant role in developing hyperandrogenic phenotype and insulin resistance in PCOS women[169,170].

The CYP19 gene is located on chromosome 15q21.1[171,172] and is essential for converting C19 androgens, androstenedione, and testosterone to the C18 estrogen, estrone, and estradiol[173,174]. The granulosa cells of pre-ovulatory follicles in the ovaries are the primary sites of the expression of CYP19 gene[175]. A reduction in aromatase enzyme activity has been reported in patients with hyperandrogenism in various studies[176,177]. In addition, a deficiency of aromatase enzyme has been observed in both lean and obese women with PCOS. Hypermethylation of the promoter region reduces the expression of the CYP19 enzyme and the overall activity of the aromatase enzyme in PCOS[178]. Significant association of CYP19 rs2414096 has been observed with reduced aromatase activity, increased levels of estradiol compared to testosterone, androgen excess, and PCOS development in African, Caucasian, American, Indian, Iraqi, Iranian, Chinese, and Egyptian populations[179-184]. Furthermore, a tetranucleotide repeat polymorphism (TTTA)n in the CYP19 gene has been linked to inhibition of aromatase activity leading to hyperandrogenism, increased testosterone levels, and high LH: FSH ratio in PCOS women[183,185,186]. Thus, the association of decreased activity of aromatase enzyme and its association with hyperandrogenism suggests a pivotal role of the CYP19 gene in the pathogenesis of PCOS.

Androgen receptor (AR) codes for the androgen receptor and facilitates the effect of androgens. The AR gene is located on the X chromosome and at Xq11-12 consisting of 11 exons. It has been reported to carry a genetic polymorphism in exon one characterized by a CAG trinucleotide repeat encoding polyglutamine residues[187]. Elevated androgen levels have been linked to impairments in follicle development, menstrual irregularities, anovulation, and formation of microcysts in the ovaries[1,188]. Studies in experimental models have suggested that exposure to intrauterine androgens can lead to developing PCOS later in life[189]. Recently, inhibition of AR expression in mouse models has been shown to ameliorate PCOS-like traits[190]. AR has been identified in the theca interna cells of preantral follicles, granulosa cells of preantral and antral follicles, and in both theca and granulosa cells of dominant follicles[191]. The genetic polymorphism in exon one of the AR gene, characterized by CAG repeats, indicates a possible correlation between AR activity and PCOS[192]. In Chinese and Caucasian populations, a higher frequency of short AR CAG repeats among PCOS women may contribute to the onset of the disorder[193,194]. In addition, this polymorphism results in upregulation of AR and increased androgen sensitivity in PCOS women[195,196]. However, no such association has been observed in Indian, Korean, Slovene, and Croatian populations[197-199]. Androgen excess not only has phenotypic manifestations such as hirsutism and acne, but it also has a role in the over-recruitment of follicles, which prevents selection of dominant follicles, ultimately leading to anovulation[200]. Reports have suggested that androgen regulation in the ovarian follicle depends on the follicular phase[201]. A study by Walters and colleagues indicated that in mice with granulosa cell-specific AR knockout, there was a considerable extension of estrous cycles and a decrease in the number of offspring[202]. AR receptor knockout causes damage to the hypothalamic-pituitary-gonadal (HPG) axis, resulting in impaired follicular development[203]. In a study by Tian *et al*., a total of five heterozygous missense mutations (p.V3M, p.Q72R, p.S158L, p.S176R, and p.G396R) in the androgen receptor genes were observed in five out of 258 patients[204]. It was also found that the patients with the pathogenic mutations in the *AR* gene also had significantly lower estrogen levels on the day they received human chorionic gonadotropin injection[204]. These findings indicate that AR-mediated actions play a critical role in the pathogenesis of PCOS.

GATA binding protein 4 (GATA4) is a transcriptional factor that binds to the GATA motifs in the promoter regions of genes to control their expression and cell differentiation. GATA4 was identified as a potential gene associated with PCOS in a Caucasian population through GWAS11. However, a meta-analysis of the Caucasian population revealed that the association of GATA4 with PCOS showed great variance12. It was observed that the association was stronger when the patients were diagnosed according to NIH criteria as compared to the Rotterdam criteria12. On the contrary, a subsequent GWAS that examined the variant associations with each criterion failed to replicate these findings13. Several studies have demonstrated the crucial role of GATA4 in ovarian follicle development after selectively knocking out the GATA4 gene in granulosa cells[205,206]. GATA4 also plays an essential role in ovarian steroidogenesis by regulating the expression of StAR and CYP19 genes[207,208]. Recently, a whole exome sequencing study reported a rare variant of uncertain significance (Arg265Cys) in the GATA4 gene in a PCOS with abnormal adrenal steroidogenesis[72].

Our whole exome sequencing to identify rare, pathogenic variants in 51 unrelated PCOS patients

reported eight heterozygous exonic variants in genes involved in steroid hormone biosynthesis[209]. These included CYP21A2 (p.Ala392Thr, p.Gln319Ter, and p.I143N), StAR (p.Arg53 Leu), AKR1C3 (p.Phe205Val), P450 oxidoreductase (p.Val334Ile and p.Val251Met) and HSD17B6 (p.Gly40Ser) genes, of which two were pathogenic. Five variants were identified as having uncertain significance in 8 out of 51 patients[209]. The identified variants were predicted to cause protein destabilisation, thus likely contributing to the pathogenesis of PCOS. Some variants showed significant differences between PCOS patients and the population database.

4.4 Insulin Resistance and Hyperinsulinemia

Under normal circumstances, there is a reduction in insulin sensitivity and an increase in insulin secretion, which is needed to maintain a constant hyperbolic relationship[210]. Women with PCOS may have an increase in their basal insulin secretion[211]. However, their insulin response to an insulin load is generally insufficient, leading to a lower Disposition Index than control women of the same age and a Body Mass Index (BMI)[211-213]. Therefore, even though women with PCOS experience hyperinsulinemia, they exhibit a relative pancreatic β-cells dysfunction[118]. In addition, women with PCOS show reduced removal of insulin by the liver, which further contributes to elevated insulin levels[214-215].

The molecular mechanisms responsible for insulin resistance in PCOS are distinct from those in other common conditions characterized by insulin-resistant states such as obesity and type 2 diabetes (T2DM). Specifically in muscle tissue, an increase in serine phosphorylation is observed in the insulin receptor and Insulin Receptor Substrate (IRS1)[213], which leads to compromised insulin signaling and function[213,216]. PCOS women also show abnormalities in insulin function in the adipose tissues and adipocytes, although the nature of these abnormalities may vary[217,218].

The IRS2 gene, on chromosome 13q34, encodes for the Insulin Receptor Substrate protein (IRS2), a signaling molecule located in the cytoplasm comprising 1354 amino acids. IRS2 acts as a molecular adaptor that regulates the function of pancreatic islets β-cells and peripheral glucose metabolism by facilitating the action of insulin, Insulin-Like Growth Factor 1 (IGF-1), and other cytokines[219]. It has been observed that women with PCOS, regardless of their BMI, may present with insulin resistance and hyperinsulinemia[220]. A meta-analysis showed that the IRS1Gly972Arg polymorphism (rs1801278) was associated with PCOS in women of Caucasian ethnicity, while the IRS2 Gly1057Asp polymorphism (rs1805097) was linked with PCOS in women of Asian ethnicity[221]. Furthermore, polymorphisms in the IRS2 gene contributed to developing type-2 diabetes and other metabolic conditions, including obesity and PCOS[222]. Another meta-analysis showed that the A allele of Gly972Arg posed a significantly increased risk of PCOS compared with the G allele[223]. A case-control study by Pablo *et al.* found an association between genetic variants in the IRS2 gene and variable insulin response to different fatty acids and glucose metabolism[224]. A recent study reported that IRS polymorphism rs1865434 may be a risk factor in the pathogenesis of PCOS[225].

The insulin receptor (INSR) gene comprises 22 exons on chromosome 19[226]. The knockout of the INSR gene in mice leads to extreme insulin resistance[227]. The significance of insulin signaling in PCOS is demonstrated by HAIR-AN syndrome (hyperandrogenism, insulin resistance, and acanthosis nigricans), a subset of PCOS characterized by extreme insulin resistance[228]. Insulin resistance plays an essential role in the up-regulation of LH secretion by the pituitary, androgen excess due to increased testosterone production by the theca cells, and increased activity of P450scc in granulosa cells, which disturb follicular development and leads to PCOS[220]. It has been speculated that INSR may be a risk factor for PCOS, as accumulating data has shown an association between INSR gene polymorphisms and PCOS[229]. Numerous studies have investigated the relationship between polymorphisms in the INSR gene and PCOS[230,231]. Although no association was found between INSR polymorphisms and PCOS in a meta-analysis, two genome-wide association studies in 2011 reported a positive correlation between INSR polymorphisms and PCOS[8,232,233]. The findings from another meta-analysis indicated no significant association between the SNPs rs1799817 or rs2059806 and the onset of PCOS. However, the SNP rs2059807 could be a risk factor for PCOS[234]. These studies point to the INSR gene being a potential candidate for the pathogenesis of PCOS. Recently, a pathogenic intronic variant in the INSR gene was reported in a study from India employed whole-exome sequencing[71,72]. Additionally, a heterozygous likely pathogenic variant (Tyr1190His) was reported by Crespo *et al.* in a PCOS patient with severe insulin resistance[72].

Hence, a limited number of genes, including *FSHR, LHCGR, AMH, AMHR, CYP11, CYP21, CYP19, StAR, GNRHR, INSR,* and *IRS*, have been consistently replicated

in a good number of populations and yielded significant association with PCOS.

5. Conclusion

Advances in understanding the genetic basis of PCOS have furthered our knowledge of the etiology of the disorder. Various candidate genes associated with PCOS that affect the reproductive and metabolic pathways are identified using genetic studies. An additional area of further exploration is the functional analysis of pathogenic variants and their relevance to biology in predicting response to standard treatments for PCOS women. These are also crucial molecular markers for pharmacogenomic studies. There is a need for studies that may encompass genomics, cell biology, and clinical research for conveying this knowledge to the clinical practice.

6. References

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