AN UPDATE ON POLYCYSTIC OVARY SYNDROME (PCOS): DIAGNOSIS, RISKS, ETIOLOGY, AND TREATMENT OPTIONS REVISITED

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Declaration

It is hereby declared that

1. The thesis submitted is our original work while completing the degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.

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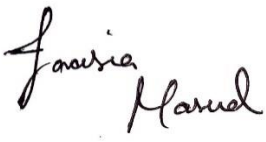

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Abstract

Polycystic Ovary Syndrome or PCOS is the most common endocrine disorder in women of reproductive age, which is still incurable. Despite its prevalence, little is known about its etiology. In this review article, the up-to-date diagnostic features and parameters recommended on the grounds of evidence-based data and veteran consensus are explored. The ambiguity and insufficiency of data when diagnosing adolescent women have been put under special focus. We look at some of the most recent research done to establish relationships between different gene polymorphisms with PCOS in various populations along with the underestimated impact of environmental factors like endocrine-disrupting chemicals (EDC) on the reproductive health of these women. Furthermore, the article concludes with existing treatment options and the scopes for advancement in the near future. Through multiple randomized controlled studies and clinical trials conducted over the years, various therapies based on their efficacy have been considered as a form of potential treatment and are described in this article. Common therapies ranging from metformin to newly found alternatives based on vitamin D and gut microbiota could shine some light and guidance towards a permanent cure of this metabolic syndrome in the future.

Keywords: PCOS, Polycystic Ovary Syndrome, genetic association, endocrine disorder

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List of Acronyms

Acronym	Full Form
AE-PCOS	Androgen Excess and Polycystic Ovary Syndrome Society
AFC	Antral Follicle Count
ASRM	American Society for Reproductive Medicine
ESHRE	European Society of Human Reproduction and Radiology
IGT	Impaired Glucose Tolerance
EDC	Endocrine-disrupting Chemical
FAI	Free Androgen Index
FNPO	Follicle Number Per Ovary
fT	Free testosterone
mFG	Modified Ferriman-Gallwey score
NIH	National Institute of Health
OSA	Obstructive Sleep Apnea
PCOS	Polycystic Ovary Syndrome
PCOM	Polycystic Ovary Morphology
SHBG	Sex hormone-binding globulin
T2D	Type 2 Diabetes
TT	Total testosterone

1. Introduction

Polycystic Ovarian Syndrome (PCOS) is a widely prevalent metabolic and endocrine disorder diagnosed in reproductive-aged females. The disease is distinguished by the presence and degree of 3 major features: *irregular menstruation, hyperandrogenism, and polycystic ovarian morphology (PCOM)* (1). The prevalence of PCOS is known to be around 5-20%, depending on the varying definitions used (2). Despite the many advances and adaptations in developing the diagnostic criteria and interpreting the pathophysiology of the condition, PCOS remains to be a less understood disorder in terms of criteria for uniform diagnosis and treatment (3). The multifaceted effects of the disease are spread across the lifetime of a woman beginning from conception and extending across the years following menopause (4). A majority of the studies related to PCOS are made in an effort to develop timely and efficient diagnosis particularly for adolescents, effective treatment and management of comorbidities associated with PCOS that gravely impact the quality of life, and a homogenous protocol that can be implemented by healthcare officials (5). In this review, the diagnostic procedures and other screening protocols mentioned are centered around the most recent international evidence-based guidelines for PCOS. Furthermore, the article goes into detail about the diagnosis of PCOS in adults together with the challenges faced in diagnosing adolescent girls. This results from a lack of age-specific guidelines that in turn is a consequence of a lack of scientific investigation. Additionally, the causal links, both genetic and environmental, are summarized with a brief insight into the pathogenesis of PCOS. Lastly, the current state of the treatment is looked at and the new options with considerable potential are discussed.

The incentive to work with PCOS stemmed from the understanding that a certain percentage of women are still being misdiagnosed or left undiagnosed due to unawareness and to some extent,

misunderstanding. While working on this paper (which serves as a foundation for future, in-depth laboratory work), it was made clear that many countries, especially Bangladesh, are yet to take PCOS seriously. This was demonstrated through the lack of research in these geographical regions. Thus, putting forth the true situation about the condition, we believe, would help narrow the chasm in recognition and pave way for better answers.

2. Diagnosis of Polycystic Ovary Syndrome

2.1. Introduction to different diagnostic criteria

PCOS is a recurrent endocrinopathy prevalent in approximately 8-13% (varying across different populations) of the women belonging to the reproductive age bracket (6-9). Despite its frequency, guidance regarding implementation of the diagnostic procedures for detection of PCOS is rather obscure and inconsistent among the health professionals. Up to 70% of these women are known to remain undiagnosed (8). This further adds to the problem of unestablished etiology or origin of PCOS. However, over the past decades, the characteristic traits observed in women with PCOS have been studied and analyzed eventually resulting into the development of 3 different diagnostic criteria based on the hallmarks of PCOS.

The **3 distinctive features** of this endocrinologic condition namely include *ovulatory dysfunction, hyperandrogenism* and *polycystic ovarian morphology (PCOM)* in accordance with the consensus based international guidelines (10-12). PCOS usually manifests in the form of hirsutism, oligo-anovulation, amenorrhea or irregular menstrual cycle, and sterility (13). Owing to its heterogenic characteristics there are **3 classifications of diagnostic criteria** that were established over time. The criteria are listed as follows:

1. **National Institute of Health (NIH) Criteria** – It was established in 1990 based on the agreement of a veteran panel and the first kind of diagnostic criteria to be ever generated. The NIH criteria authorized 2 definite features to be representative of PCOS: (i) signs of hyperandrogenism (clinical or biochemical) (ii) oligo-anovulation or oligomenorrhea (10). This criterion was later revised in 2012, where the third determinant, Polycystic Ovarian Morphology (PCOM), was integrated so as to conform to the Rotterdam criteria (14).
2. **Rotterdam Criteria** – It was proposed in 2003 by the European Society of Human Reproduction and Radiology (ESHRE) and American Society for Reproductive Medicine (ASRM) in which a third determinant of PCOS was added, that is a polycystic ovarian morphology (PCOM) upon ultra-sonogram imaging. The Rotterdam criteria signified the presence of any two among the three factors to be present in a PCOS diagnosis. Diagnosis using this criterion allows further classification into 4 different phenotypes from A to D (11).
    - Phenotype A – Hyperandrogenism + Ovulatory Dysfunction + PCOM
    - Phenotype B – Hyperandrogenism + Ovulatory Dysfunction
    - Phenotype C – Hyperandrogenism + PCOM
    - Phenotype D – Ovulatory Dysfunction + PCOM

The Rotterdam criteria is widely used by the gynecologists, obstetricians and other healthcare personnel; it was also adapted by the International PCOS guideline presented in 2018 and other guidelines (15, 16). Furthermore, this criterion was also suggested by the NIH evidence-based methodology PCOS workshop held in 2012, alongside phenotype identification in all researches (14).

3. **AE-PCOS Criteria** – It was put forward in 2006 by the Androgen Excess and PCOS Society. The third criteria suggested the presence of hyperandrogenism along with any one or two of the remaining determinants (ovulatory dysfunction and/or PCOM) for PCOS diagnosis (6).

In short, the 3 diagnostic criteria previously mentioned necessitates the levels of the classical features of PCOS (hyperandrogenism, ovulatory dysfunction and PCOM) to be assessed for diagnosis of the condition. However, it is worth noting that diagnosis through any one of the above mentioned 3 criteria will only be conclusive of the condition provided that other endocrine disorders such as hyperprolactinemia, thyroid disease, Non-classical Congenital Adrenal Hyperplasia (NCAH), Cushing's syndrome/disease, hypogonadotropic hypogonadism or androgen producing tumors which exhibit similar manifestations (clinical/biochemical/morphological) as that of PCOS are ruled out (14, 15). For instance, (NCAH) that manifests in the form of hirsutism or irregular menstruation can be tested for by measurement of 17-hydroxyprogesterone (17 OHP) levels with an additional ACTH stimulation test in borderline cases (17). Similarly, hyperprolactinemia can be detected if a prolactin level exceeding the threshold of 500μg/L is found that exhibits symptoms of galactorrhea and irregular periods (18). Thyroid diseases can be ruled out by calculating levels of the thyroid stimulating hormone (TSH) (14). Cushing's syndrome, on the other hand, is a relatively serious condition which displays features of obesity, high blood pressure and amenorrhea. It is associated with over secretion of cortisol, thus, an overnight dexamethasone suppression test or midnight salivary cortisol test will assist in distinguishing this condition from PCOS (19).

2.2. Diagnostic features in adults

Data from a recent meta-analysis and systemic review revealed a clear picture of the overall prevalence of PCOS based on the 3 available diagnostic features where ovulatory dysfunction, hirsutism, biochemical hyperandrogenism and PCOM was found to be in 12%, 13%, 11% and 28% women respectively (9). The major diagnostic features observed in women with PCOS in a spectrum of degrees are discussed in the succeeding parts of the paper.

(i) Ovulatory Dysfunction

A staggering proportion of approximately 75% of PCOS individuals are known to have ovulatory dysfunction (20). It is described as a state of irregular menstrual cycle (14). In a standard ovulation cycle, menstruation is known to begin by the $24^{th}/25^{th}$ day (21). In an adult, irregular menstrual cycle refers to <21 or >35 days which is equivalent to having 8 menstrual cycles per year or lower (22). However, this threshold may vary correspondingly, as the reproductive age progresses. Continued irregular menstruation is indicative of anovulation or oligo-anovulation which can later aggravate to a PCOS condition (14).

Contrastingly, regular menstruation reflecting normal ovulatory cycles has also been noticed in women with PCOS (23). The phenomenon is known as *subclinical ovulatory dysfunction* and maybe explained by the presence of hyperandrogenism. This clearly poses a challenge in the diagnosis of PCOS (24). On the other hand, serum progesterone level above 5 ng/ml (during day 21-24 of the cycle or the luteal phase of the menstrual cycle) may function as a confirmatory test for ovulation in women with PCOM or hirsutism (25).

(ii) Hyperandrogenism

Excessive serum androgen level is another salient feature of PCOS as stated by the Rotterdam criteria. A significant proportion (about 60-100%) of PCOS-afflicted women maybe be suffering

from hyperandrogenism (clinical and/or biochemical) (15). Hyperandrogenism in PCOS women may either be assessed by their clinical signs or through biochemical tests.

(a) Clinical hyperandrogenism

Clinical hyperandrogenism observed in the form of hirsutism, acne or alopecia is usually representative of low to average levels of androgen excess(26).

- **Hirsutism** appears more frequently than the remaining two in about 80% of people with hyperandrogenism and it refers to the visually detectable growth of terminal hair (hair that can grow beyond 5mm, if untroubled) (25). The extent of hirsutism is evaluated via the modified Ferriman-Gallwey (mFG) score (27). This involves visual evaluation of *9 areas* (chin, chest, upper lips, upper arms, thighs, upper and lower abdomen and back) of the body with a score ranging anywhere in between 0 to 4 (28, 29). The scores are then presented in the form of a graphical image. The state of hirsutism is conclusive if the total mFG score is ranged within ≥4-6; this range was extended to a score of 8, to adjust for variation in ethnicities (30, 31).
- **Comedonal acne** is a widespread issue among women, especially adolescent girls. It was estimated that around 40% of the acne incidence can be traced back to a susceptible PCOS condition (32). Although acne can be linked with biochemical hyperandrogenism, there are no specialized measuring tool to assess this condition as of now (26).
- **Alopecia** is the least common feature among the three. Only 22% of women displaying male-like hair loss was discovered during PCOS diagnosis (32). Many factors other than hyperandrogenemia may be associated with alopecia. The Ludwig scale is a tool of

measurement for hair loss around the scalp that rates the degree of hair loss from grade I to III upon visual assessment (1).

The subjective variability, racial/ethnic differences and the existence of a condition named *idiopathic hirsutism* (hirsutism devoid of hyperandrogenism) play a major role in determining the clinical signs of PCOS diagnosis and thus require more well-defined data (33).

(b) Biochemical hyperandrogenism

Women are assessed for signs of biochemical hyperandrogenism when the clinical signs of hyperandrogenism are obscure (12). This diagnostic criterion relies on one of the characteristic traits of PCOS involving elevated serum androgen level. About 60-80% of women with PCOS are known to exhibit features of biochemical hyperandrogenism (6). According to evidence-based recommendation, the condition can be detected through measurement of total testosterone (TT), Free Androgen Index (FAI), calculated free testosterone (fT) and/or calculated bioavailable testosterone (12). Previous data evaluation suggests serum free testosterone to be the most sensitive parameter for detecting biochemical hyperandrogenism out of others like total testosterone, dehydroepiandrosterone sulphate (DHEAS), or androstenedione (A4). The latter two have been observed to be elevated in women with PCOS and is particularly useful when high testosterone level is not detected. Furthermore, DHEAS and A4 facilitate the exclusion of other hyperandrogenic conditions (15, 26). Then again, Free Androgen Index (FAI) is another indirect means of evaluating the free testosterone level; it is the ratio of the total testosterone and Sex Hormone Binding Globulin (SHBG) multiplied by 100 (34).

Total circulating or free testosterone levels can be measured using high quality assays like extraction/chromatography immunoassays and Liquid Chromatography-Mass Spectrometry

(LCMS)/ mass spectrometry. Other automated direct assays include enzyme-linked immune sorbent assay (ELISA), chemiluminescence assay (CLIA) or radioimmunoassay (RIA); however, these assays exhibit reduced sensitivity and therefore provide imprecise results (35-37). Cut-off values vary widely within laboratories and the type of method used. Normal thresholds maybe derived from a healthy population of women (12). To conclude, the lack of evidence-based cut-off values, androgen of interest and consensus regarding use of assessment technique constitute the major areas of uncertainty in biochemical hyperandrogenism evaluation.

(iii) Polycystic Ovarian Morphology (PCOM)

PCOM is recognized as the most widely used feature in the diagnosis of PCOS. It was first included in the Rotterdam Criteria as the third feature of PCOS in 2003 (11). This diagnostic criterion is much debatable owing to the lack of homogeneity in the results regarding its implementation, and its exclusivity of application for women above the gynecological age of 8 years, therefore it has been deemed a non-essential feature in the presence of remaining two criteria (15).

The process of ovulation is ceased due to follicular arrest in adult women with PCOS. The minute follicles resemble cyst-like structures on a transvaginal ultrasonography (38-42). Initially, (as authorized in the Rotterdam criteria) the cut-off values for identifying a polycystic morphology was a value of 12 or more Follicle Number Per Ovary (FNPO), measuring between the size of 2-9mm or alternatively, an ovary with a volume of 10 cm^3 on transvaginal scan (26). The FNPO value which is a key parameter was later updated in conformity with the technological advancements that enabled more magnified imaging. When using high resolution transducer frequency of 8 MHz or more, an FNPO value of 20 or more of the same sized follicles (2-9mm) and ovary volume of 10 cm^3 for adult women was recommended by an international evidence-based PCOS guideline consensus held in 2018 (15). The ovarian volume plays a significant role

when it is difficult to determine FNPO/ Antral Follicle Count (AFC) due to technical complications for imaging, as in the case of transabdominal ultrasound (26).

Transvaginal ultrasound is the recommended approach for examining FNPO suggestive of a polycystic ovary, however it is to be used only in sexually active women (12). Automatic antral follicle count under 3D scan has shown more accurate results as opposed to the 2D grid system method, nonetheless information regarding this is still sparse and hence not yet recommended for routine purposes (43-49). Other ultrasound metrics include ovarian stroma and ovarian blood flow; studies with respect to these parameters are minimal hence no cut-off values are available for clinical use (12).

(iv) Anti-Mullerian Hormone (AMH) as an alternative diagnostic feature for PCOS

Considering the ambiguity about the efficacy of ultrasound as a diagnostic tool Anti-Mullerian Hormone (AMH) has been suggested as an alternative for detection of PCOM (15). An upward trend of AMH levels in correlation with the number of antral follicles in PCOS was observed in women with PCOS. This is due to the fact that AMH is produced by the granulosa cells in ovarian follicles (namely pre-antral and antral) which are elevated in PCOS (50-52). Despite its potential as a valuable diagnostic tool, AMH is still not authorized as a part of routine PCOS diagnosis owing to the inadequate standardization of cut-off values and heterogeneity among the studies (12, 53).

2.3. Diagnostic challenges in adolescents

Much of the already existing grey areas in the diagnosis of PCOS for females of all ages can be attributed to its unspecified etiology heterogeneity, the lack of evidence-based cut-off values for the diagnostic features and clearly defined technology that can be universalized for the most

accurate results (54). Furthermore, a distinct set of diagnostic criteria for adolescents is essential since the existing guidelines mostly comply with features relevant to adults (cystic acne, irregular menstruation and PCOM). Owing to the lack of specialized guidelines for adolescents the NIH criteria was recommended by the endocrine society clinical guideline (currently retired) for women of this age group but was not backed up by sufficient evidence-based records. The paediatric society then recommended the presence of hyperandrogenism and persistent oligo-anovulatory cycles for detection of PCOS in adolescents. The application of these guidelines for adolescents may lead to over or under diagnosis since the manifestation of these features in young girls is also a result of normal pubertal development stemming from an underdeveloped hypothalamic-pituitary-ovarian (HPO) axis (55). At the outset, adolescence period has been defined to be the age frame within 10 and 19 years by the World Health Organization. Alternatively, young women within the gynecological age of 8 years were also taken into account for PCOS studies aimed towards adolescents (56).

A conclusive PCOS condition cannot be diagnosed without the concurrent existence of both menstrual irregularity and hyperandrogenism in PCOS. It is also important to acknowledge a state of "at risk" category to be followed up by further age specific diagnosis for PCOS as a means to avoid over or under diagnosis young women (56, 57).

(i) Problems with defining ovulatory dysfunction in adolescence

Diagnosis of PCOS has been recommended to women with persistent irregular menstruation, however, defining irregular menstruation that is reflective of ovulatory dysfunction in adolescents is much of a challenge in itself (58). Definition of irregular menstruation in accordance with the gynecological age is tabulated in Figure **01** (56).

Type of Irregular Period	Definition
Primary amenorrhea	Absence of menses or period (usually till age 16 or 3 years after thelarche)
Secondary amenorrhea	No periods for more than 90 days after menarche
Oligomenorrhea	
• <1 year after menarche	Any irregularity is part of pubertal transition
• >1 but <3 year after menarche	Less than 4 cycles a year
• >3 years after menarche	Less than 8 cycles a year

Figure 01: *Definitions of irregular menstruation according to reproductive age.*

Irregular menstruation is a common observation in young adults after menarche which can sometimes extend as long as 5 years post-menarche, therefore presence of this physiological event cannot be considered a prerequisite for PCOS diagnosis until 2 years succeeding menarche (59-61). However, continued oligomenorrhea even before the two-year threshold after menarche indicate an "at risk" status of PCOS in young adults (55).

Determining concurrent anovulation in adolescents is yet another challenge since about 85% of the cycles are known to be anovulatory in the first year following menarche, which shows a downward trend with the number of years post menarche being 59% and 25% in the 2nd and 3rd year respectively (60). Anovulation can be confirmed by serum progesterone level measurement as like in adults (14). Lastly, the variation in age of menarche among females further complicate the assessment and identification of ovulatory dysfunction (62).

(ii) Adolescent physiological aspects mimicking hyperandrogenic conditions

As mentioned previously, besides persistent irregular menstruation androgen excess is a valuable indication of PCOS in adolescents which may present itself as visible clinical signs (hirsutism, severe acne and/or rarely alopecia) or elevated serum androgen level.

(a) Clinical hyperandrogenism

Acne is a common condition in adolescents and therefore not a definitive diagnostic criterion for PCOS unless accompanied by other features. However, this may be indicative of hyperandrogenism if the degree of acne ranges between moderate to severe and is not responsive towards topical dermatologic therapy (58, 63).

Alopecia in adolescents is still not well understood (64).

Although hirsutism has been linked with hyperandrogenism when in association with menstrual irregularity, the presence of various confounding factors such as, genetic and ethnic variations deem it to be a less prominent feature in diagnosing a hyperandrogenic status (29, 65, 66). Moreover, the modified Ferriman-Galwey scoring system may not accurately assess the degree of hirsutism in adolescents since it was structured using data from adult Caucasian women (67-69).

(b) Biochemical hyperandrogenism

Assessment of biochemical hyperandrogenism has its own set of complications due to lack of standardization, technical difficulties, interference with other steroid hormones and effect of SHBG on testosterone level, all of which are irrespective of a woman's age. Despite the physiological effect of puberty leading to a rise in the testosterone levels, the same determinants (free and/or total testosterone) are used to gauge androgen excess in adolescents. These methods are limited by lack of specifically designed studies for adolescents and well-adjusted thresholds.

(iii) Controversies regarding PCOM (Polycystic Ovarian Morphology) in adolescents

According to the International guidelines on PCOM, transabdominal ultrasound is not recommended for use as a diagnostic criterion until at least 8 years post menarche, mainly due to the high prevalence of characteristic follicular increase in young adults, and an enlarged ovarian

volume during this period (12, 70-73). The implementation of adult thresholds adjusted for the transvaginal route may lead to over-diagnosis of PCOM in adolescents, hence PCOM in adolescents is not a reliable diagnosis of PCOS (74).

3. Risks associated with PCOS

PCOS has been associated with potential risk of cardiovascular and cerebrovascular events, type 2 diabetes mellitus (T2D), impaired glucose tolerance (IGT), pregnancy-linked complications, gestational diabetes, venous thromboembolism, and endometrial cancer (1). Many of these metabolic and reproductive conditions stem out from an intrinsic feature of PCOS- insulin resistance (IR) (75-77).

3.1. Cardiometabolic events

Cardiometabolic impacts of PCOS shown on **Table 01** are largely linked with dysglycemia which is a result of the insulin resistant characteristic of PCOS. Insulin resistance or consequential hyperinsulinemia in turn is a state heavily influenced by the hyperandrogenic mechanism of the PCOS pathophysiology (77). Despite the paucity of comparative studies concerning CVD risk factors with and without PCOS, it is noteworthy that the cardiometabolic conditions are prominent in PCOS which pose a risk of developing cardiovascular diseases (CVD). According to clinical consensus recommendation by the PCOS guideline group (2018), manifestation of the cardiometabolic risk factors such as Impaired Glucose Tolerance (IGT), dyslipidemia, hypertension, smoking, obesity, other metabolic syndrome, and a sedentary lifestyle in PCOS women allocates them in the vulnerable category (15).

Risk factors	Association and screening recommendations for factors
IGT/T2D	The prevalence of IGT and T2D in PCOS affected women has been observed to be independent of but made worse with BMI, according to past meta-analyses. This can be traced back to the correlation of PCOS with dysglycemia which refers to the aberrant glucose level in blood. Thus, estimation of the glycemic status of women with PCOS has been recommended by the International Evidence-based Guideline (2018) at a frequency of 1-3 years depending on the presence of other diabetic confounding factors. In addition to that, screening for T2D has been suggested by all consensus recommendations (Endocrine Society, International Evidence-based Guideline in Australia as well as Androgen Excess and PCOS society) (61, 78). The method of screening however is still undecided among Oral Glucose Tolerance Test (OGTT), fasting glucose, and HbA1c test (15).
Dyslipidemia	Dyslipidemia is a recurring CVD risk factor identified in women with PCOS. A significant proportion of women (70%) with PCOS were known to exhibit dyslipidemia in a past report (79). A meta-analysis of 30 studies demonstrated higher levels of lipids in women with PCOS (age <45) particularly HDL, LDL, non HDL-C, LDL-C, and TG. Moreover, TG and HDL-C were substantially higher in the obese stratum indicating a possible link of PCOS with obesity (80, 81). The latest guidelines suggest women of all ages diagnosed to undertake a lipid profile (12).
Hypertension	The association of hypertension and PCOS is rather complex and influenced by many other factors. The inconsistency between the studies necessitates the requirement of more investigation (67, 82-84). However, the recent international evidence-based guideline recommends annual blood pressure measurement considering the significance of hypertension in the cardiovascular events (12).
Obesity	Despite its frequency in women with PCOS, there is surprisingly no solid evidence of their causal relationship. Obesity has been linked to some of the severe PCOS manifestations, including CVD (85-87). Therefore, regular monitoring of body weight has been suggested by the most recent guideline (12).
Other risks	Sympathetic nervous system dysfunction, chronic inflammation, oxidative stress, and vitamin D deficiency are emerging risk factors of PCOS paving the path for further research (77).

Table 01: *A chart showing the relationship between PCOS and cardiometabolic risk factors and their suggested screening protocols.*

3.2. Cardiovascular Diseases

The combined effect of a low number of studies and the relatively young population of women in them restricts a concluding relationship between PCOS and CVD and therefore calls for more

research. Nonetheless, the importance of screening for CVDs in PCOS women have been acknowledged. Quantitative research on the clinical manifestations of CVD is insufficient despite researches based on the sub-clinical CVD (15, 77).

3.3. Fertility related complications in women with PCOS

PCOS comes with lifelong repercussions for women as stated earlier. Gestational complications associated with PCOS are gradually being recognized; some of which include preeclampsia, gestational diabetes, pregnancy-induced hypertension, and even miscarriage (88). It is estimated that much of the pregnancy related inconveniences are partly ramifications of the already existing metabolic and endocrine effects of PCOS in women well ahead of pregnancy such as, hyperandrogenism and increased BMI (89). Manifestation of gestational diabetes mellitus (GDM) in expecting women with relatively larger weight was observed to be alarming than their counterparts of lower weight. However, it is worth noting some of these complications are also influenced by other independent factors such as age, obesity, ongoing fertility treatment, and ethnicity (88). Apart from this, several studies have also reported atypical newborn anthropometrics in children of women with PCOS (90-92).

Although screening for GDM and hypertension in women with PCOS (pre-conception and antenatal) have been mentioned in the recommended guidelines, other avenues of pre-conception and antenatal screening for PCOS are still underdeveloped as they are not supported by enough evidence (15, 88).

3.4. Endometrial Cancer

Malignancies associated with PCOS are rather indirect and stem from PCOS-induced infertility in women. Of these, endometrial cancer has been acknowledged to have an association with PCOS (93). Although its occurrence is multifactorial and influenced by other morbidities (T2D, obesity,

infertility and the administered specific PCOS treatment methods), it has shown to rise by 2-6 times in women with PCOS. This may be attributed to the anovulatory cycles where the endometrium is exposed to a continued flux of estrogen (94-96). Despite the correlation between the two, routine screening for endometrial cancer has not yet been recommended; however awareness on this issue is encouraged (15).

3.5. Obstructive Sleep Apnea (OSA)

OSA is a chronic sleeping disorder accompanied by disruptive upper airway function and consequential hypoxia and erratic sleeping pattern (97). It has also been linked with modified heart rate and sympathetic activity, and altered blood pressure ultimately extending towards more severe outcomes such as, CVD and hypertension (98-100). A positive correlation was demonstrated through several systemic reviews and meta-analyses (101). The evaluation of this condition is based on qualitative analysis and a screening tool involving the Berlin Questionnaire. As of present, treatment for OSA only involves management of distinctive patient symptoms without therapeutic remedy of the linked metabolic diseases (12).

4. Etiology of Polycystic Ovary Syndrome

The current literature emphasizes on the role of genetics in PCOS. There are many genes that have been said to directly or indirectly contribute to the progression of the disease. But till date, no penetrant gene has been identified (102). Studies conducted in multiple families show low penetrance linked with hormonal/environmental factors or other co-variants. Many studies have suggested that PCOS is a polygenic, multifactorial disorder. Single genes, gene-gene interactions, and gene-environment interactions have been reported to pave way for the development of the

disease (102). This part of the article will review the current genetic understanding of the disease along with some of the environmental determinants explored latter in the paper.

To understand the roles of these genes better, it helps to take a glance at some of the aspects of PCOS pathogenesis, the ovaries, and the hormonal metabolism. The ovaries are the primary reproductive organ that release eggs meant to be fertilized by sperms. They also produce estrogen and progesterone which help regulate the monthly menstrual cycle; and, also tiny amounts of male hormone, testosterone, one of the 5 kinds of androgen. FSH and LH are gonadotropin hormones released by the pituitary gland in response to the secretion of GnRH by the hypothalamus. These two control ovulation; FSH primarily stimulates the growth of follicles into proper eggs while LH triggers the release of these eggs. Their hormonal interplay in the body is illustrated in **Figure 02**. PCOS is a syndrome (or, a group of symptoms) that interferes with ovaries and ovulation, in brief. PCOS predominantly has 3 features: irregular/missed periods, high levels of androgen and cysts which are fluid-filled sacs in the ovaries. These sacs are essentially immature follicles that never see an ovulation. Lack of ovulation disturbs the hormonal harmony in the body. On top of this, raised androgen levels disrupt the monthly cycles. The underlying justification behind upsetting hormonal balance has been pointed towards genetic alterations, environmental determinants and epistatic changes.

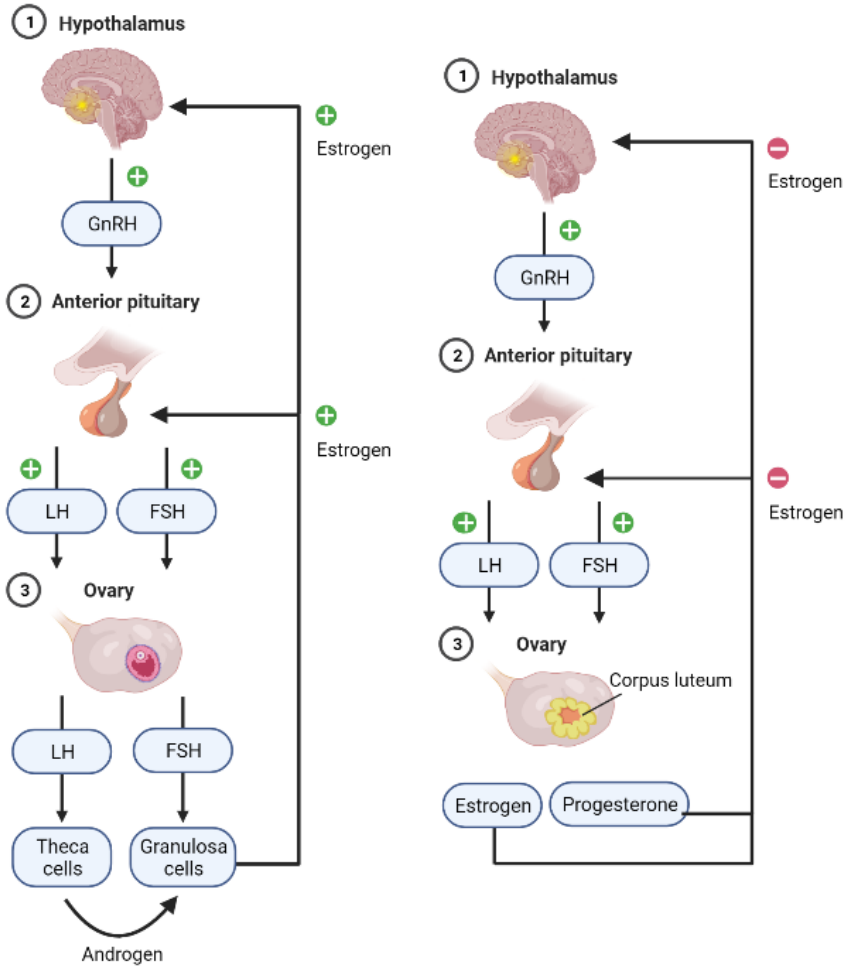

Figure 02: *The hormonal cycle in the female body illustrated with the positive and negative feedback mechanisms. The diagram on the left shows a state prior to ovulation and the right after ovulation.*

Biosynthesis of hormones in the ovary in brief

In a secondary follicle, thecal and granulosa cells work in conjunction to produce estrogen, progesterone, and testosterone. The process has been outlined in **Figure 02.** There are 5 types of androgen: dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), androstenedione, and testosterone. LH and FSH secreted by the pituitary gland activate these cells. The thecal cells express LH receptors for LH to bind. Granulosa cells, on the other hand, bind with FSH. When activated by LH, thecal cells increase their absorption of LDLs from the bloodstream. The cholesterol is then used in the synthesis of steroids, like progesterone.

Progesterone is then enzymatically converted in a series of steps into androgens. Due to lack of aromatase enzymes, thecal cells are unable to produce estrogen independently. Thus, the androgens diffuse into the blood and into the granulosa cells where it successfully gets converted into estrogen via aromatase. This estrogen later enters the blood. In a positive feedback mechanism, the hypothalamus is stimulated consequently, the characteristic LH surge in the menstrual cycle is seen.

The granulosa cells too have LH receptors but are unable to pick up LDLs from the blood; LDLs cannot move past the basal membrane easily. When the follicle ruptures during ovulation, the membrane is destroyed hence enabling LDL absorption and progesterone production. However, the cells do lack enzymes needed to convert progesterone into androgens. Thus, majority of the progesterone diffuses into the blood which explains the rapid rise in its level post-ovulation. After ovulation, both cells produce progesterone and to a lesser extent, androgens.

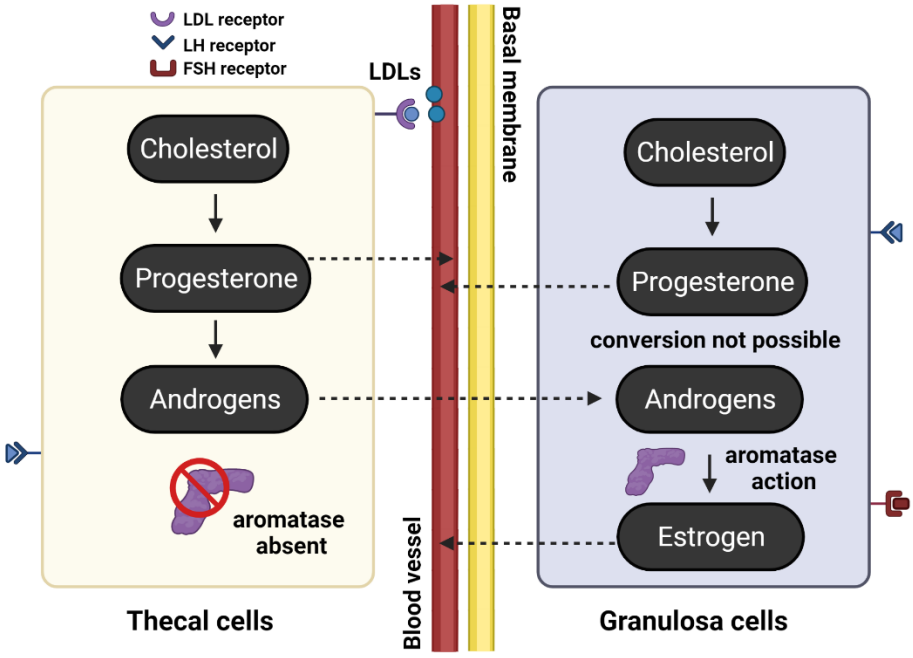

Figure 03: *The biosynthesis of androgen and estrogen inside the ovary.*

Cholesterol is the precursor of all steroid hormones that can be classed into 3 categories: glucocorticoids, mineralocorticoids, and gonadocorticoids (or sex hormones). Sex steroids are mainly androgens, estrogens, and progesterone. All steroid hormones are hydrophobic and require a protein carrier when being transported in blood. These are albumin, corticosteroid-binding globulin, and sex hormone-binding globulin. The cholesterol is 27-carbon compound that undergoes a multi-step process and gets shortened and hydroxylated eventually. The series of conversion is shown briefly in **Figure 04**. It is these enzymes (for example, cytochrome p450 members) involved here that are targeted by studies; polymorphisms in their coding region can ultimately affect hormonal metabolism and lead to hyperandrogenism. The idea is that some defect in the hormonal pathway causes the classic characteristics of PCOS; these avenues are probable areas for research. Numerous studies on the relationship between gene polymorphisms and PCOS have already been carried out and will soon be discussed.

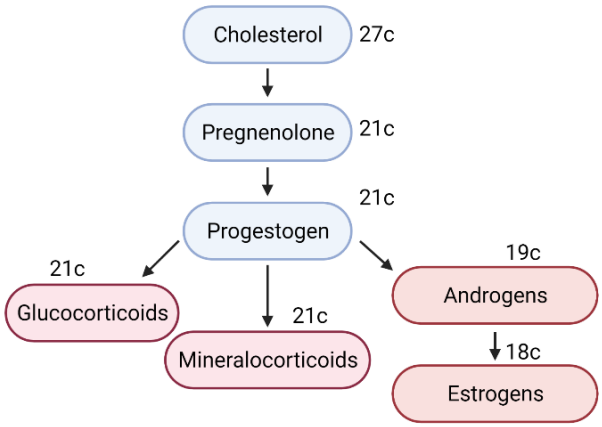

Figure 04: *Summary of steroidogenesis with the end-products shown.*

While ovaries are generally considered as the main source of androgens, the adrenal glands also contribute. In fact, increased adrenal androgens (DHEA and DHEAS) are consistent with 20-30% of the PCOS population (103)- a phenomenon called *adrenal hyperandrogenism*.

Hormonal association in PCOS

Hormones play a crucial role in the normal functioning of the ovary and the regulation of menstruation. If hormonal disturbances persist, the ovary's function is interrupted leading to the formation of a cyst inside of its sac (104). PCOS patients exhibit an imbalance in levels of Gonadotropin-releasing Hormone (GnRH), Follicular Stimulating Hormone (FSH), Luteinizing Hormone (LH), androgen, and prolactin (105). The progression of PCOS and its severity rise with an increasing level of insulin and testosterone. Hyperinsulinemia is known to affect ovarian theca cells inflating androgen concentration (106). Then again, elevated androgen levels trigger Visceral Adipose Tissue (VAT) that is responsible for the production of Free Fatty Acids (FFA) which in turn elicits insulin resistance (107). Due to insulin resistance and its consequent outcome of elevated levels of insulin, androgen levels rise which leads to anovulation (7).

To support the hormonal association with PCOS, a cross-sectional study using Pakistani women, healthy and affected, were clinically examined. Blood samples were drawn from individuals and hormonal analysis was carried out using immunoradiometric assay and radioimmunoassay. Their findings stated that FSH, LH and prolactin levels, and BMI were higher in PCOS cases. Current diagnosis of PCOS involves looking at FSH, LH and androgen levels (108). We know that raised LH levels result in higher androgen levels which gives rise to PCOS among other reasons.

4.1. The genetic connection

There are a number of genes involved in the etiology of Polycystic Ovarian Syndrome. As of present, there are 3 databases manually curated and published: $PCOSKB_{R2}$ (2020), PCOSBase (2017) and PCOSDB (2016) (109-111). PCOSDB had been inaccessible at the time of writing. The three databases are compared in **Table 02**.

Attributes	PCOSKB$_{R2}$	PCOSBase	PCOSDB
Official Name	PolyCystic Ovary Syndrome KnowledgeBase	PolyCystic Ovary Syndrome Base	PolyCystic Ovary Syndrome Database
Database URL	http://www.pcoskb.bicnirrh.res.in/	http://pcosbase.org/	http://www.pcosdb.net
Overall Content	533 genes, 145 SNPs, 29 miRNAs, 1150 pathways, and 1237 PCOS-associated diseases. More 4023 genes identified from microarray expression studies on PCOS included.	8185 PCOS-related proteins, 7936 domains, 1004 pathways and 1928 PCOS-associated diseases, 29 disease classes and 91 tissues.	208 gene, 427 molecular alterations including detailed annotations, 46 associated phenotypes.

Table 02: *A brief comparison of the 3 PCOS databases published so far.*

As per PCOSKB $_{R2}$, there are 241 genes and 114 SNPs are closely involved in PCOS (109). Changes like polymorphisms negatively affect the transcriptional activity of the gene ultimately leading to PCOS. Now, the genes suitable for PCOS study are those that code for receptors of hormones like androgen, LH, FSH and even, leptin (112). Genes namely AR, CAPN10, FTO, FSHR, cytochrome family P450, and insulin gene have been widely discussed.

We will discuss some of genes commonly involved in ovarian and adrenal steroidogenesis, in steroid hormones, in gonadotropin action and regulation, and insulin action and secretion as well as a few notables and the epigenetics of PCOS. **Table 03** details the research, on these groups of genes.

SL.	Category	Gene	Year	Sample Size and ethnicity/location	Findings/Conclusion	Key Methods	Comments	Ref.
1	**Involved in ovarian and adrenal steroidogenesis**	**CYP11A**	2014	267 cases v. 275 controls "South Indian"	15 different alleles identified with repeats ranging from 2-16. Repeats greater than 8 were thrice more likely to be susceptible to PCOS and have been found comparatively more in the patients. CYP11A1 **(ttta)n** repeat polymorphism is likely to be potential molecular marker for PCOS diagnosis.	DNA extracted from blood and genotyped by PCR-PAGE		(113)
2			2014	1236 cases v. 1306 controls "Asian and Caucasian" Argentina, China, Greece, India, Spain, UK	Positive association between CYP11A1 **(tttta)n** repeat polymorphism and PCOS.	Meta-analysis of 9 studies published between 2000-2010		(114)
3			2014	1571 cases v. 1918 controls "Asian and Caucasian" China, India, Greece, Spain, Turkey, UK, USA	CYP11A1 microsatellite **(ttta)n** repeat polymorphism (along with CYP1A1 MspI) showed significant association with PCOS risk in Caucasian population.	Meta-analysis of 13 studies published between 2000-2010		(115)
4			2012	314 cases v. 314 controls "Chinese"	SNP rs4077582 in CYP11A1 was found to be strongly associated with PCOS susceptibility. No association was observed in rs11632698.	PCR-RFLP	Assessed the association of SNPs rs4077582 and rs11632698 in CYP11A1 with PCOS.	(116)
5		**CYP21**	2013	197 patients	The study looked into 14 molecular defects of the CYP21A2 gene and concluded that its contribution to PCOS is unsubstantiated.	Allele-specific PCR	Study investigated the contribution of CYP21A2 heterozygous	(117)

							mutations to PCOS pathogenesis	
6			2010	50 cases v. 60 controls "Italian"	The data suggested lack of association between CYP21 V281L polymorphism and PCOS.	PCR-RFLP		(118)
7			2005	114 cases v.95 controls "Non-Hispanic White"	CYP21 mutations found to play a limited role in the development of PCOS.		Prospective case-control study	(119)
8			2000	n<50	CYP21 V281L mutations seemingly manifested PCOS-like phenotype.			(120)
9		**CYP17**	2021	394 cases v. 306 controls "Kashmiri"	Mutant genotype has been found to be associated with hyperandrogenism.	PCR-RFLP	T/C polymorphism in 5'UTR of CYP17 gene was analysed to find its connection to hyperandrogenism and PCOS	(121)
10			2021	204 cases v. 100 controls "Pakistani"	rs743572 polymorphism significantly associated with PCOS.	PCR-RFLP	5'UTR region (MspA1) of CYP17 gene was analysed	(122)
11			2019	50 cases v. 109 controls "Kurdish" West Iran	Data suggested positive link of CYP17 T-34C polymorphism with PCOS risk.	PCR-RFLP; Chemiluminescent method for hormone measurements		(123)
12			2018	250 cases v. 250 controls North India	Data suggested that − 34T>C polymorphism in CYP17A1 is associated with PCOS in North Indian females.	PCR-RFLP; lipid profile via biochemical analyzer		(124)
13			2012	287 cases v. 187 controls	The gene has been suggested as a non-major risk factor.	SNP genotyping and	4 SNPs of CYP17 analyzed	(125)

				"Caucasian"		haplotype determination		
14		**CYP19**	2020	204 cases v. 100 controls "Pakistani"	The polymorphism, rs2414096 (genotype GA) of CYP 19 gene was found to be considerably associated with PCOS vulnerability.	PCR-RFLP	The study looked at both CYP17 and CYP19 genes	(122)
15			2019	50 cases v. 109 controls "Kurdish" West Iran	No statistical significance found in the association of CYP19A1 with PCOS risk.	PCR-RFLP; Chemiluminescent method for hormone measurements		(123)
16			2018	250 cases v. 250 controls North India	Variations of CYP19A1 were not statistically relevant with PCOS.			(124)
17			2017	70 cases v. 70 controls Iran	The study concluded that SNP rs 2414096 in CYP19 is likely to play a role in the development of PCOS in Iranian women.	PCR-RFLP; enzyme digestion with HSP92II		(126)
18			2014	62 cases v. 60 controls	Polymorphism of rs 2414096 in CYP19 was found to associated with the pathogenesis of PCOS.	PCR-RFLP; statistical analysis by SPSS		(127)
19	**Involved in steroid hormone effects**	**AR**	2013	114 cases v. 1409 controls Diverse ethnicities	CAG variants in AR gene were found to be unassociated with PCOS risk while they may be related to the T levels in PCOS.	Meta-analysis of 11 studies published between 2000-2012		(128)
20		**SHBG**	2020	1660 cases v. 1312 controls	rs6259 and rs727428 polymorphisms in SHBG are not associated with PCOS susceptibility.	Meta-analysis of 7 studies published between 2007-2019		(129)
21	**Involved in insulin secretion and action**	**CAPN10**	2017	169 cases v. 169 controls South India	No association of rs2975766, rs7607759 with PCOS.	RT-PCR	The study looked at other gene polymorphisms too.	(130)

22			2014	127 cases v. 150 controls Tunisia	UCSNP-43 (rs3792267), UCSNP-19 (rs3842570), and UCSNP-63 (rs5030952) were investigated along with their haplotypes. No significant association, except one with obese PCOS subjects, was found.	PCR-RFLP		(131)
23			2014	668 cases v. 200 controls "Caucasian Greek"	No correlation of CAPN 10 polymorphism (UCSNP-43) with the incidence of PCOS. Additionally, the gene polymorphism could not be associated with any biochemical, clinical, hormonal or ovarian features of PCOS.	Primer extension; MALDI-TOF mass spectrometry		(132)
24			2013	2123 cases v. 3612 controls	9 common SNPs were examined. UCSNP 19/63/44 likely to be associated with increased PCOS risk among Asians. No statistically significant association with UCSNP-22, UCSNP-43, UCSNP-45, UCSNP-56, UCSNP-58, and UCSNP-110 polymorphisms.	Meta-analysis of 14 studies published between 2002-2013	The review is very thorough and helpful.	(133)
25			2009	88 cases "Brazilian"	Data provides evidence that UCSNP-43 may play a role in PCOS while UCSNP-19 and UCSNP-63 remained unassociated with phenotypic traits in PCOS.			(134)
26			2008	50 cases v. 70 controls "Chilean"	Data suggests contribution of UCSNP-43 polymorphism to PCOS in Chilean women.	PCR-RFLP	The study looked at UCSNP 19 and 63 as well.	(135)
27		**IRS**	2016	2975 cases v. 3011 controls "Asian and Caucasian"	The findings suggested IRS-1 Gly972Arg polymorphism to be associated with PCOS in the Caucasian ethnicity, and IRS-2 Gly1057Asp polymorphism to be correlated with PCOS in the Asians.	A meta-analysis of 28 studies published between 2001-2014		(136)
28			2013	150 cases v. 175 controls Croatia	Data did not support an association between Gly792Arg IRS-1 (along with VNTR INS, C/T INSR) polymorphisms and			(137)

					PCOS. Nor did it find any correlation with insulin resistance in Croatian women with PCOS.			
29		**INSR**	2016	2975 cases v. 3011 controls "Asian and Caucasian"	The INSR polymorphism, His 1058 C/T was not found to be associated in PCOS development.	A meta-analysis of 28 studies published between 2001-2014		(136)
30			2015	17460 cases v. 23845 controls	The meta-analytical data suggested no significant correlation between rs1799817/rs2059806 SNPs and PCOS susceptibility. On the other hand, it was concluded rs2059807 could be a promising SNP involved in the susceptibility of PCOS.	A meta-analysis of 12 studies published between 1994-2013		(138)
31	**Involved in gonadotropin action and regulation**	**AMH**	2020	383 cases v. 433 controls "Chinese"	15 rare but known AMH variants were identified along with 7 novel heterozygous variants. Researchers conclude that AMH can play a role in PCOS development.	Sanger sequencing		(139)
32			2019	608 case v. 142 controls	The AMH signaling cascade was deduced as a key player in PCOS etiology. Variants have been identified.	Targeted sequencing	Regions of AMH and AMHR2 were looked at.	(140)
33			2017	643 case v. 153 controls	Rare, genetic variants of AMH related to PCOS identified.	Targeted sequencing	Replication of whole genome sequencing	(141)
34			2017	2042 cases v. 1071 controls	Meta-analytical data showed no association of AMH (or AMHR2) with heightened risk of PCOS.	A meta-analysis of 5 studies published between 2002-2013		(142)
35		**FSHR**	2021	130 cases Iran	FSHR polymorphisms Ala307Thr and Asn680Ser concluded to be statistically associated with PCOS women.	PCR followed by sequencing		(143)
36			2020	1882 cases v. 708 controls "Chinese"	rs2300441 found to be a primary contributor.	-	GWAS	(144)
37			2018	93 cases v. 52 controls	Significant difference found in FSH and LH levels in PCOS	PCR-RFLP;		(145)

				"Kurdish" Northern Iraq	patients with different genotypes of Ala307Thr polymorphism. No relationship established between polymorphism and PCOS.	Eam1105I enzymatic digestion		
38			2017	377 women v. 388 controls "Korean"	Findings suggested significant association between FSHR gene polymorphisms (Thr307Ala or Asn680Ser) and PCOS.	RT-PCR		(146)
39			2014	1760 cases v. 4521 controls "Asian and Caucasian" China, Italy, Korea, Netherlands, Turkey, UK	Results showed no association between Thr307Ala and Asn680Ser polymorphisms of FSHR with PCOS susceptibility.	A meta-analysis of 10 studies published between 1999-2013		(147)
40			2014	215 cases v. 205 controls "Han Chinese" North China	The Ala307Thr and Ser680Asn polymorphisms of FSHR are not related to PCOS in Han ethnic Chinese women.	PCR-RFLP; direct sequencing	Another study in North China with slightly more PCOS cases has found similar results (148)	(149)
41		**LHCGR**	2015	203 cases v. 211 controls "Bahraini Arab"	First study to suggest association of LHCGR polymorphisms (rs7371084, rs4953616) with PCOS. The study also added a strong association of FSHR (rs11692782) as well.	RT-PCR		(150)
42	**Other genes**	**FTO**	2019	55 cases v. 110 controls "Srilankan"	FTO gene rs9939609 polymorphism was significantly more common among PCOS subjects.	Allele-specific real-time quantitative PCR (AS-qPCR)		(151)

Table 03: *A rundown on the associations of different polymorphisms with PCOS found by most recent studies. Red text means negative association or no association, orange text implies a weak link to PCOS and green text indicates strong correlation with PCOS.*

Table 03 focuses on some of the recent and unique studies that have been carried out. The genes discussed are merely a fraction of the entire set of genes that are likely to play a role in PCOS pathogenesis. However, it begs the question what makes a gene suitable or a target for investigation. For example, cytochromes P450 are a superfamily of enzymes that play a major role in steroid conversion; it aids in the conversion of androgen into estrogen. Any defect in this pathway will cease the conversion (152). The human genome is known to include 18 CYP families, each having a sub-family of their own (153). The CYP families are CYP1-8, CYP11, CYP17, CYP19-21, CYP24, CYP26-27, CYP39, CYP46, and CP51.The aromatase genes that are frequently reported in PCOS databases are CYP11A1, CYP11B2, CYP17A1, CYP19A1, CYP1A1, CYP21A2, CYP3A7 (154). Thus, any abnormality in this gene family can potentially lead to PCOS. Aside from the studies condensed in the table, similar investigations have taken place earlier, most of which have been included in the meta-analytical reviews mentioned. Similarly, elevated androgen levels have been commonly seen in PCOS cases. Thus, the genes that are usually targeted are somehow connected with androgen, its receptor, or its metabolism. Any defect or polymorphism in their coding regions could lead to an explanation for the increased androgen levels. Examples of these genes are CYP1A1, CYP11A, CYP17, CAPN10, INSR, SHBG and many others. The genes summarized in **Table 03**, when studied, will reveal some form of a connection that could potentially pave way for PCOS pathogenesis. In this way, possible avenues for genes to be involved in the defective mechanism of PCOS are identified and assessed. Genetic links can be sought anywhere as long as it is relevant to PCOS; the genes that code for enzymes involved in the metabolism of different hormones, the genes responsible behind insulin action and so on. Insulin is a key player in the production of androgen by the theca cells. Like LH, higher level of insulin enhances the androgen synthesis.

In conclusion, the databases mentioned earlier can be a useful starting point in the quest to finding susceptible genes. The pattern in studies that aim to establish an association between different gene polymorphisms and PCOS is that every study narrows its subjects to a certain geographical location or ethnicity, collects blood sample and analyzes the DNA in whichever way feasible. This process lead to many individual studies with inconsistent findings; some suggest a genetic link to PCOS while others disagree with the same conclusion. Numerous studies have been carried out till date in an attempt to find novel polymorphisms or support existing data. Systematic reviews and meta-analyses come in very useful too. Additionally, strong emphasis on the outcomes of GWAS have been placed. However, at the end, all papers demand larger-scale studies to be undertaken. Often these research look at multiple genes together. Perhaps, other than looking at the genes and how they may correlate with PCOS or subjects' biochemical profile, researchers can start looking at the environmental risk factors the subjects could have been exposed to simultaneously. PCOS is said to be a multifactorial, polygenic complex disease. To fully elucidate its etiology, all factors in play should be investigated as much as possible.

4.2. Environmental determinants of PCOS

We have already discussed the genetic susceptibility associated with PCOS. With this in mind, it is likely that the environment is an active player in the expression of PCOS-related genes (155, 156). Some evidence already supports the fact that environmental toxins play a role in disrupting reproductive health. But the research linking these to the development of PCOS is very limited. These environmental risk factors can eventually trigger or aggravate PCOS pathology (157). In this section of the review, we briefly discuss the environmental determinants, especially the endocrine disruptors, that are potentially involved in the etiology and modulation of PCOS.

(i) Prenatal exposure

Individuals can be exposed to environmental risks during prenatal or postnatal periods of life. According to experimental studies, intrauterine exposure to excess androgens/glucocorticoids at critical phases of fetal development may lead to PCOS symptoms and can determine the phenotypic expression in adulthood (156). One way to explain this would be that intrauterine growth restriction (IUGR) can cause increased prenatal exposure of androgens and glucocorticoids which could possibly induce PCOS programming in the fetus (156, 158). It goes without saying that studies concerning prenatal exposure has its own implementation-related difficulties.

(ii) Postnatal exposure

Evidence to investigate into potential prenatal risk factors for humans is lacking. However, there is an increasing amount of research being carried out to study the effects of postnatal exposure. Obesity and low physical activity are harmful lifestyle factors that are targeted in the disease management. Obesity has been found to exacerbate the metabolic and ovulatory dysfunction in PCOS (159, 160). On the other side, weight loss restores ovulation and improves hyperandrogenism (161, 162). Moreover, phenotypical variations between ethnicities suggest that cultural factors play a stronger role in the metabolic consequences than previously thought.

One such postnatal exposure is environmental toxins. Environmental toxins are chemical pollutants present in the environment that enter living organisms via inhalation, ingestion, or absorption through skin/mucous membranes ultimately having a detrimental impact on them (163). An emerging body of evidence is pointing to the lasting effects of environmental toxins on the human reproductive health (164-166). Common pollutants include mercury, lead, pesticides, CFCs and so forth. Nevertheless, when it comes to PCOS, a certain group of chemicals known as endocrine-disrupting chemicals (EDCs) have gained special interest and is the main focus in this

section of the review. They have been proposed in its etiology as they have the ability to interfere with the hormone system. Some compounds have been described on **Table 04** and are accompanied with examples of their uses in our daily lives. It has been estimated that of all synthetic chemicals, about 1,000 of them are likely to exhibit endocrine-acting properties (167); these compounds can be categorized under groups such as, phthalates, xenoestrogens and so forth. The compounds are a heterogenic group of molecules that interfere with steroid hormone synthesis as well as interact with hormone receptors (168). As a consequence of their lipophilic structure, they have a tendency to bio-accumulate in the adipose tissue. Humans being at the end of the food chain become the most exposed to these toxins. Apart from adipose tissue, endocrine disruptors have been detected in amniotic fluid (169), milk (170), serum (171), and urine (172).

BPA or Bisphenol A is an endocrine disruptor. BPA is produced globally in abundance; production exceeds 6 billion pounds every year (173). It is said to be a xenoestrogen, a chemical that mimics natural estrogen, owing to its phenolic structure which enables it to bind to estrogen receptors (ER). Higher serum level of BPA has been found among PCOS women when compared with non-PCOS women (174). Animal studies show an association between neonatal BPA exposure and PCOS-like symptoms (175). To make matters worse, it has been shown that BPA directly stimulates synthesis of androgens in ovarian theca-interstitial cells (176). Furthermore, correlation between testosterone and BPA levels have been seen in the serum of women with PCOS (174). Data from rat studies show BPA is capable of increasing testosterone production in theca-interstitial cells and decreasing estradiol formation in granulosa cells. These effects can be explained via some form of upregulation induced by BPA on the key genes involved in ovarian steroidogenesis- CYP17A1, CYP11A1 (177) and downregulation of CYP19A1 (178). BPA is also

known to interact with sex-hormone binding globulin whose gene is another PCOS candidate gene (179).

A study in 2019 focusing on adolescents looked at BPA levels in 62 girls with PCOS and 33 control subjects in the age range, 12-18 years (180). High-performance liquid chromatography was employed to measure urinary BPA concentrations. The adolescent patients demonstrated a markedly greater BPA levels (case: 15.89 μg/g vs control: 7.30 μg/g creatinine). A similar study using serum BPA from adolescents has found similar results too (181). On the contrary, a study focusing on a number of EDCs failed to establish an association of urinary BPA level with PCOS in relatively older women (18-45 years) (182). There are many other studies available linking specific groups of EDC with PCOS. For instance, two case-control studies delved in to find the correlation between concentrations of certain EDCs and PCOS (182, 183). Both studies revealed a significantly higher serum levels of per-fluorinated compounds in PCOS women as compared to control subjects. It is to be noted no causal relationship was proven. Similar to the genetic links, the ultimate findings of the studies are inconsistent. While it is true EDCs negatively impact the reproductive health of humans, the mechanism as to how these chemicals upset hormonal balance or interfere with their receptors is yet to be elucidated. A few common ones are listed on **Table 04** (167, 184, 185).

Endocrine disruptor	Use
Bisphenol A (BPA)	In epoxy resins found in many plastic products including food storage containers.
Dioxins	By-product of herbicide manufacture and paper bleaching, released during burning of waste and wildfires.
Parabens	Cosmetics, personal care products.
Per- and polyfluoroalkyl chemicals (PFAS)	Non-stick cookware, waterproof clothing, food packaging.
Phthalates	Cosmetics, children's toys, food packaging.

Polybrominated diphenyl ethers (PBDE)	Flame retardants.
Polychlorinated biphenyls (PCB)	Electrical equipment like transformers, hydraulic fluids, lubricants etc.
Triclosan	Anti-microbial and personal care products.

Table 04: *A list of common endocrine-disrupting chemicals (EDCs) accompanied with their uses.*

5. Treatment options for PCOS

The remedy for this particular endocrinopathy has been on lookout ever since the gravity of its impact on the female health was realized. The common symptoms for this particular disorder are irregular menstrual flow, hirsutism, weight gain, ovarian cysts, insulin resistance, hyperandrogenism which when not evaluated can manifest to T2D, cardiovascular disease, hyperinsulinemia, and infertility.

There is no permanent cure for this particular disease. Treatment is adjusted in an individualized manner and then administered to help tackle the symptoms and allow the patient lead a less cumbersome life.

5.1. Current Therapies

(i) Metformin

For decades, metformin has been used to induce ovulation as well as fight against insulin resistance, a salient feature detected in women battling PCOS. Metformin belongs to a class of drugs called biguanide and are mostly prescribed to individuals with T2D. Through various experimental evaluations conducted over the years, metformin has proven to be beneficial by increasing the overall glucose uptake in the body that leads to improved insulin sensitivity, reduction in serum androgen level, and proper regulation of menstrual cycle (186, 187). However, its mode of action remains unclear when it comes to it being used exclusively or in combination with other drugs while handling complications like infertility or clinical live birth rate. According

to a cohort study conducted by a hospital in Riyadh, Saudi Arabia it was notified that metformin did not demonstrate much significant result when it came to it being used as a co-treatment for improving pregnancy rate in woman seeking IVF (188). Based on another systemic analysis conducted (189) metformin was found to be successful in eradicating the risk of ovarian hyper stimulation syndrome for pregnant woman but nonetheless it had no association with clinical live birth rate. On the other hand Sun et al. (190) reported that linking metformin with drugs like clomiphene citrate was better at reforming infertility and ovulation rate but at the same time. Kar et al. (191) found no difference in their combined effect. All these evidences imply the fact that the potency of this drug is debatable and requires competent systemic analysis in order to procure better result.

(ii) Spironolactone

Most notable effects of spironolactone involve reduction of androgen level, improvement of hirsutism and acne when used as a form of treatment (192). An aldosterone antagonist predominantly used as a diuretic (193), the basic concept behind its complex mode of action involves blocking androgenic receptors, partly obstructing adrenal steroidogenesis, and blocking 5α reductase thereby increasing the level of SHBG (sex hormone binding globulin) protein (194).Unlike metformin the dosage needs to be considered and supplemented in a precise manner to avoid health issues; this is why spironolactone is suggested to be taken with an oral contraceptive whilst avoiding pregnancy so as to not promote complications like, feminization of male fetus (194, 195). Other side effects found to be involved with high doses were hypokalemia and menstrual disturbances (196). According to a study conducted by C. Sabbadin et al. (194) regarding the effects of spironolactone on estradiol levels and intermenstrual bleeding on 30 individuals with normal BMI, it was concluded that certain individuals did exhibit intermenstrual bleeding as a

form of side effect due to lower estradiol levels and endometrial thickness. But then again, it was also detected that those individuals who displayed this effect had pre-treatment estradiol values lesser than those individuals that did not face this issue at all. For this reason, this particular proposition requires further assessment in order to provide confirmatory evidence. Based on another study conducted by Zulian et al. (197) this drug has significantly proven to improve glucose and metabolic lipid profile when it was experimented on 25 PCOS patients over a course of 12 months. This medication still continues to be prescribed when it comes down to tackling hormonal imbalance and disease management.

Some of the other commonly prescribed drugs are listed on **Table 05** with reference to the metabolic problems they target, their dosages and side-effects.

5.2. Newly Emerging Therapies

There are various therapies in consideration with the hopes of being implemented in near future.

(i) Statins

Statin is an inhibitor and performs its function by impeding 3-hydroxy-3-methylglutaryl coenzyme reductase (HMG-CoA), a rate-determining enzyme involved in cholesterol synthesis, thereby halting its conversion to mevalonate. Atorvastatin specifically has shown significant outcome when it comes to the reduction of insulin resistance and hyperandrogenism in a 12-week study. The study showed increased levels on vitamin D in PCOS women when compared to control women; headaches were seen as side-effects. Another study conducted in 2013 validated that while atorvastatin improved lipid profile and chronic inflammation, it did not do much in case of insulin sensitivity. Despite its promising result, more clinical studies need to be conducted it order to determine its efficacy (198, 199).

(ii) Surgical Method

Procedures like ovarian drilling and bariatric surgery have been considered for treating PCOS despite the risks of reduced ovarian cells and decreased fertility these methods carry (193). Even though laparoscopic ovary drilling has shown to improve ovulation and reduce androgens, various clinical trials conducted over the years were somewhat inconsistent and failed to provide concrete evidence to sustain as a form of treatment. On the other hand, bariatric surgery does promote excessive weight loss for obese individuals leading to improved ovulation and reduced risk of T2D (200, 201).

(iii) Inositol

The most commonly described forms of inositol are myo-inositol and d-chiro-inositol. These sugar molecules function as messenger that mostly up-regulate glucose intake and synthesis. Myo-inositol has been used as a form of supplement in PCOS women and has proven to improve insulin sensitivity and menstrual cycles as well as cause lesser gastric issues as compared to metformin. However, a Cochrane systemic review could not display any promising role of inositol when it was tested on 1472 sub-fertile PCOS females (198, 199, 201).

(iv) Vitamin D

Few studies are backing up the fact that vitamin D deficiency plays a role when it comes to insulin resistance thereby being connected to the pathogenesis of PCOS. When a dosage was given it did ameliorate insulin resistance (195) nonetheless its role still remains contradictory based off many RCTs (randomized controlled trials) conducted previously. According to a double-blind, randomized, placebo-controlled trial executed by Trummer et al. (202) at a medical university in Austria, the no significant results were achieved in attesting the effects of vitamin D on different metabolic parameters. While on the other hand a similar trial conducted by Gupta et al. (203)

showed vitamin D supplement to enhance menstrual cycle, ovulatory dysfunction, and fasting blood sugar level.

(v) Gut microbiota

There have been widespread speculations regarding the alterations in gut microbiota being related to the metabolic syndromes of PCOS or vice versa. There are some key features that has been reported to provide a better understanding of this hypothesized linkage.

Studies have come forward with contradictory result regarding the imbalance between the gut microbial diversities (α, β) being caused by either gut microbiota partaking in the regulation of sex hormone leading to the dysbiosis of it or circulating sex hormones alone alters the gut microbiota.

Other than being observed in obese patients, PCOS also gives rise to the aspect of gene encoding pro-inflammatory cytokines like TNF-α (Tumor Necrosis Factor) and IL-6 (Interleukin 6) being associated with the triggering of the immune system and causing intestinal permeability has been pinpointed, that leads to the elevation of LPS (lipopolysaccharide) which ends up causing inflammation by pooling the endotoxin in the blood circulation hindering the metabolic system ultimately leading to ovarian inflammation too. This particular theory has been related to the imbalance of microbial composition (204).

Probiotics stood out and was chosen as a form of treatment in order to deal with gut microbial related issues, based off the benefits it comes along with. It has displayed anti-inflammatory activities and healthy regulation of gut flora. Based on randomized trials conducted it has also shown to alleviate insulin resistance overall keeping the lipid profile balanced. According to a study initiated by Jing Xue et al. (205) where the efficacy of Inulin(probiotic) and Metformin was assessed by targeting gut microbiota in PCOS mice. The result did provide promising evidence

when it came down to reducing pro inflammatory cytokines through a surge of anti-inflammatory cytokines in turn decreasing ovarian inflammation (204, 205).

Metabolic Syndrome	Treatment	Dosage	Side-effect	Ref.
Insulin Resistance	Metformin	500 mg (starting) Over a course of 1-2 weeks	Nausea, bloating	(206)
Menstrual Irregularity	Oral Contraceptive Pill	20-35µg	Weight gain	(207, 208)
Hirsutism	Cyproterone Acetate	50-100 mg (alone) Ethinylestradiol (combined) 20-50 µg	Headaches, breast tenderness	(207)
Infertility	Clomiphene Citrate	50-150 mg for 5 days	Nausea, mood swings	(207, 209)
Hyperandrogenism	Spironolactone, Flutamide	Spironolactone 100 mg Flutamide 500 mg+OCP	Spironolactone- Fatigue, menstrual irregularity (in high dosages) Flutamide-hepatoxicity (if not maintained properly)	(207, 208)

Table 05: *Treatment options for various symptoms listed along with the administration dosage and side-effects.*

6. Conclusion

PCOS looks different for everyone to some extent. It is currently incurable and continues way beyond the child-bearing age or post-menopause. Research points towards strong genetic implication but the dots are yet to be connected to give us the full picture. It is likely that once etiological grounds are explicated diagnostics, treatment, and disease management will be subject to change dramatically. We have come a long way from 1935 when it was first officially described by American gynecologists, Irvin F Stein, Sr. and Michael L Leventhal. With advancements made

in diagnostic technology, it has been easier to treat patients yet genetic, ethnic etc. variations pose difficulty in instituting universal “laws” of the disease. In spite of progress, accounts of dissatisfaction related to diagnosis are still significant. The gaps in the field of PCOS diagnosis can be attributed to a plethora of causes which include the heterogeneity of the condition itself, discrepant use of the diagnostic criteria and tools, vagueness in the assessment of the salient features, and due to lack of clarity in adolescent diagnosis. Knowledge around these discrepancies and a multidisciplinary intervention must be adapted to reduce delayed and poor diagnosis of PCOS in women. It is also worth mentioning that all the guidelines developed to date are predominantly based upon consensus veteran opinion, clearly indicating the need for generation of evidence-based data. The development of PCOS has a strong genetic component. Results from twin studies and familial clustering grounds a strong genetic basis for PCOS; having a mother or a sister with PCOS increases the risk of developing PCOS by 30-50%. Following the analysis of the studies conducted on different gene polymorphisms, it can be said that some of these polymorphisms could potentially serve as biomarkers for diagnosis and prognosis of PCOS. Given that there are many studies linking genes with PCOS pathogenesis, it is essential that this research gets extrapolated into projects with much larger sample sizes. PCOS phenotype does vary with ethnicity, geographical region, and probably socioeconomic status. Thus this would further add to the complex polygenic nature of PCOS. The importance of GWAS is already established in discovering new locus for susceptibility. The next step would be to link the genetic factors in question with other determinants and the patient history to establish whether PCOS is indeed a polygenic disease and what genes can truly be called key triggers.

Researchers are continually trying to dig deep to get to the root cause behind this complex metabolic syndrome. With new discoveries flow in tougher challenges and it all comes down to

selecting an effective solution through various trial and errors. The above-mentioned therapies do give assuring outcomes but large scale, properly structured and funded trials/studies need to be carried out so that this particular disorder can be demystified even more whilst taking contributive factors like ethnicity, environmental exposures, familial history and more into account. Keeping the diverse outcomes of PCOS in mind, it can be suggested that a team comprising of a physician, a gynecologist, an endocrinologist, and a reproductive medicine specialist will help these patients manage their lives much better.

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