INNOVATIVE TARGETS AND THE IMPORTANCE OF GENOMICS IN THE DIAGNOSIS OF PCOS

Abstract

Polycystic ovarian syndrome (PCOS) is a multifaceted endocrine condition that affects many thousands of people around the world. Due to its variability and complex makeup, PCOS presents difficulties in both diagnosis and therapy. Omics technologies have recently become important resources for comprehending the mechanisms behind and enhancing PCOS therapy. The aim of this review is to examine how omics methods, including genomics, transcriptomics, proteomics, and metabolomics, can be used to diagnose and treat PCOS. Studies that were published between 2010 and 2023 were included after a thorough review of the literature was undertaken. These studies explored between 500 and 10,000 cases globally, with varying numbers of people taking part in each study. Researchers from many nations, notably the US, China, India, Australia, and European countries, have made important contributions to the area, demonstrating PCOS's widespread effects. Furthermore, PCOS patients now have access to more individualized therapy options through omics-based studies. Pharmacogenomic studies have honed therapeutic outcomes by assisting in the prediction of individual drug responses and the selection of medications. Epigenomic studies have also brought attention to the influence of external factors on PCOS progression and identified possible epigenetic targets.

Keywords : PCOS, Omics, diagnostic

Authors

Monisha Anandan
Faculty of Pharmacy
Dr. M.G.R Educational and Research Institute
Velappanchavadi, Chennai
India.

Ranjitha S
Faculty of Pharmacy
Dr. M.G.R Educational and Research Institute
Velappanchavadi, Chennai
India.

Divya R
Faculty of Pharmacy
Dr. M.G.R Educational and Research Institute
Velappanchavadi, Chennai
India.

I. INTROCUTION

A widespread hormonal condition known as polycystic ovarian syndrome (PCOS) affects 4% to 20% of women worldwide who are of reproductive age [1]. The term of PCOS varies depending on the context because there are currently no standards for diagnosing PCOS [2]. A menstrual cycle that is irregular or protracted, an elevated testosterone level, and frequently obesity are clinical symptoms of PCOS [3, 4]. There are several well-known common causes for PCOS, including genetic, hormonal, and environmental variables, yet its exact etiology is not entirely understood [5]. Women with a history of low birth weight, poor vitamin D levels, a parent who has had PCOS, childhood overweight, or adult obesity have all been linked to an increased chance of developing the condition later in life [6]. PCOS affects between 1.14 to 11.04% of adolescent females worldwide, with 0.76% of cases being diagnosed and 0.56% going undiagnosed. One in 200 teenage girls between the ages of 15 and 19 had PCOS that was officially diagnosed. In reality, there are more than likely 2 out of every 200 adolescent females who have PCOS [7, 8].

The "Omics" innovations develop a promising method for the research of PCOS disease since they attempt to examine the worldwide and dynamic genetic changes in various healthy and diseased conditions. Omics technologies have grown significantly in recent years, notably next-generation sequencing (NGS) and mass spectrometry. The study of genomics has moved quickly towards quantitative and high-throughput methods. Integrating genomes, proteomics, metabolomics, and transcriptomics, known as multi-omics," plays an important role in tailoring drugs to disease [9]. Investigators in the medical field have been able to compile an enormous amount of so-called omics datasets due to the leverage of high-throughput innovations in biotechnology [10]. Early breakthroughs in the application of omics technology to unravel disease and facilitate the development of drugs give rise to the competence that a great number of more efficient diagnostic tools and therapies adapted to the unique biological, environmental, and dietary aspects of every person will be created. Personalized medicine, or more lately, precision medicine, are terms that are frequently used to describe the widespread use of such testing and diagnostics [11, 12]. Numerous potential genes associated with PCOS susceptibility have been identified through genomic investigations, giving insight into the genetic underpinnings of the condition [13]. Transcriptomics investigations have discovered irregular sequences of gene expression in PCOS, shedding light on the underlying molecular mechanisms. Finding new drugs to treat and diagnose diseases depends heavily on research into proteins. The metabolic profile of persons with PCOS is altered by the metabolomics analysis. The complexes between genes, proteins, and metabolic factors in PCOS were treated using a multi-omics analysis [14]. These multi-omics datasets can be used to uncover important biological processes and create prediction models for individualized PCOS diagnosis and therapy. Systems biology tools, like as network modeling and artificial intelligence, can take advantage of this [15].

II. MATERIALS AND METHODS

In view of treatment of PCOS, herein, we discuss the Clinical Symptoms and Signs, Etiology, Pathophysiology, Diagnostic and Management of PCOS, Omics Technology (Genomics) by search in the Pub Med, Google Scholar, and Science Direct databases.

III. CLINICAL SYMPTOMS AND SIGNS

The PCOS complicated disorder known as have a number of additional conditions that damage one's health. Women with PCOS frequently have more than 13 cysts in their ovaries, each measuring 8 mm in diameter, which makes them infertile. Acne and hirsutism are caused by hormonal imbalances that result in higher androgen levels. Obesity and diabetes are caused by insulin resistance. An irregular menstrual cycle, sadness, anxiety, and sleep apnea are all caused by this disorder [16].

IV. ETIOLOGY

This disorder has genetic and environmental causes, which make up its etiology. PCOS risk factors include infection, nutritional changes, and lifestyle modifications. Annovulation is brought on by a spike in testosterone levels brought on by insulin resistance that disrupts ovarian function. It also depends on the FSH and LH levels. In addition to all of them, the family history of PCOS is a significant influence [17]. PCOS is caused by a genetic variation or mutation in the sequence of nucleotides that affects a gene's ability to carry out transcription. PCOS is mostly caused by gene mutations in the androgen receptor, lutenizing hormone receptor, and follicular stimulating hormone receptor, which result in ovarian malfunction. Additionally, there is an increased risk when insulin and testosterone levels are raised. This causes insulin resistance by stimulating visceral adipose tissue to produce free fatty acids [18].

V. PATHOPHYSIOLOGY

A challenging endocrine condition that affects women of reproductive age is PCOS. It is characterized by hormonal imbalances, metabolic abnormalities, and the accumulation of many cysts in the ovaries [19]. Although the precise etiology of PCOS is unknown, it is thought to be the result of a combination of hereditary and environmental factors. PCOS is caused by an aberrant feedback process in the ovaries, pituitary gland, and hypothalamus. FSH and LH, the gonadotropin-releasing hormones, are dysregulated in the pituitary [20]. Follicle formation and ovulation are impacted by the hormones LH and FSH, which increase and decrease in people with PCOS due to the ovaries' increased synthesis of androgen. High levels of androgen (testosterone) in females are a hallmark of PCOS. The natural menstrual cycle is affected by this imbalance, which also causes additional symptoms to emerge. This is caused by a folliculogenic process that is malfunctioning, in which the ovarian follicles do not develop to release eggs during ovulation, leading to the development of tiny cysts in the ovary [21]. The metabolic abnormalities associated with PCOS include insulin resistance, obesity, abnormal lipid profiles, etc. It is believed that these abnormalities interact and lead to PCOS. The lack of insulin responsiveness in women with PCOS causes insulin resistance. The insulin hormone controls blood glucose levels. When there is insulin resistance, the pancreases produce more insulin, which raises the blood level of insulin and prompts the ovaries to release testosterone. Insulin resistance has an impact on glucose metabolism, which is important for lipid metabolism. The breakdown of stored fat decreased due to the increased lipid synthesis in the liver. Due to the accumulating fat, obesity develops [22, 23].

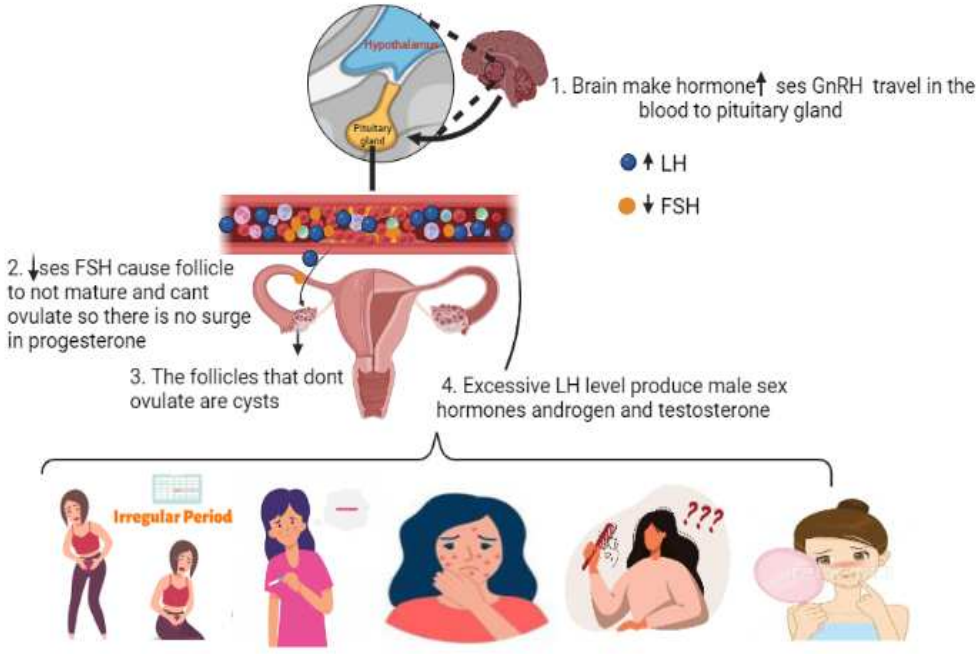

Figure 1: Pathophysiology of PCOS

VI. DIAGNOSTIC AND MANAGEMENT OF PCOS

It is a common condition characterized by a combination of various signs and symptoms. While I can provide you with a general diagnostic overview, it's important to consult a healthcare professional for an accurate diagnosis, as they will consider your specific medical history, conduct physical examinations, and perform appropriate tests. Nonetheless, here are some common diagnostic criteria and considerations for PCOS.Menstrual irregularities or menstrual absence are one of the main signs of PCOS [24]. Menstrual periods that are much longer than the typical 28-day cycle or cycles that vary in length every month are referred to by this term. PCOS frequently results in an overabundance of male hormones in a woman's body. Hirsutism (excessive hair growth in places like the forehead, chest area, or the back), pimples, and baldness that follow the male pattern are symptoms of hyperandrogenism [25, 26]. Ovaries with many tiny cysts, or follicles, may be seen on ultrasound during a patient with polycystic ovaries. These cysts often have a diameter of less than 10 mm and look as a "string of pearls" on the ultrasound imaging. It's important to rule out any other conditions that might be causing the symptoms you're experiencing [27]. At times, the signs and symptoms of PCOS might be confused with conditions including thyroid abnormalities, hyperprolactinemia, and certain illnesses of the adrenal glands [28]. The Rotterdam criteria, which need the presence of at least two of the three symptoms—irregular or nonexistent menstrual periods, symptoms of hyperandrogenism, and polycystic ovaries on ultrasound—can be used by medical practitioners to confirm a diagnosis of PCOS. Testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone-binding globulin (SHBG) levels can also be determined by blood testing. To evaluate the metabolic components of PCOS, studies of lipid profiles and glucose tolerance may be advised. Always look for medical advice from a qualified practitioner [29].

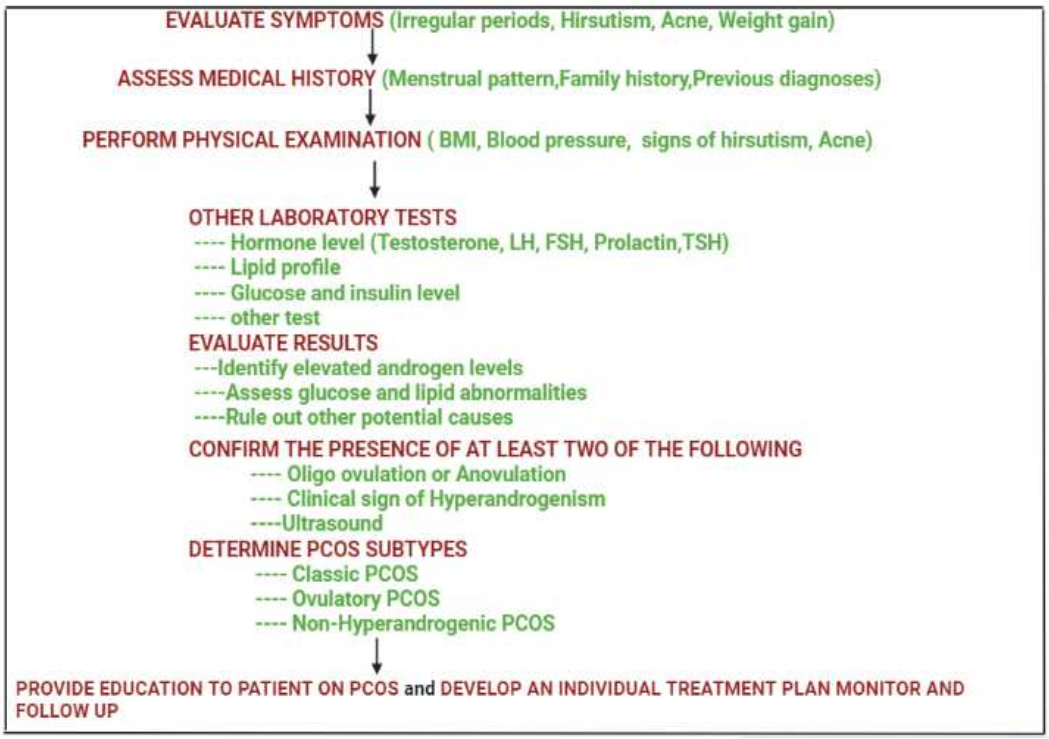

Figure 2: Diagnostic management of PCOS

VII. OMICS TECHNOLOGY

1. **Genomics:** There is evidence that PCOS is a composite disease with a hereditary component. Numerous studies have been conducted on genes; however, no reliable replication or significant associations have been found for any of the PCOS. There is increasing proof that the cause of PCOS is influenced by hormone release, IR, and obesity. Genomic research has since focused on cytokine, adipokine, vitamin D, and hormone SNP associations with PCOS. Numerous genes have been linked to the growth of PCOS. Genes linked to PCOS risks were discovered through a genomics investigation. Numerous gene loci related to PCOS were found by genome-wide association studies [30]. Genomic technology helps us understand how genes are expressed. By comparing an erroneous gene expression to that of a healthy, normal person, numerous researchers were able to identify the problem. This knowledge aids in our comprehension of the molecular basis of PCOS [31].By identifying the primary important genes and pathways that contribute to hormone function and dysregulation, among other things, researchers using genomics can discover a treatment for various diseases. Understanding these molecular pathways expands knowledge on possible targets for treating illnesses that enhance the success of development [32]. Genomic technology enables the creation of personalized disease management strategies. A doctor can customize a patient's medication by evaluating the patient's gene sequence and any abnormalities. Additionally, genomic information is used to build precision therapy that targets the genetic and molecular anomalies linked to PCOS. The list below includes the genes linked to PCOS that have a right away effect on the ovaries [33].

2. Function of Various Gene in PCOS

- **Genes Responsible in Ovarian and Adrenal Steroid genesis:** According to Several Studies, Genes CYP11 are involved in Ovarian and Adrenal steroidogenesis. An enzyme that is required for the conversion of cholesterol to progesterone, which is a rate-limiting step in the conversion process, is encoded by the CYP11a gene. An improper anabolism of steroid production caused by an alteration in the CYP21 gene, which is responsible for the production of the hormone steroids, causes PCOS. Pregnenolone and progesterone are transformed into 17-hydroxypregnenolone and 17-hydroxyprogesterone by the gene CYP21. On chromosomal 15q21.2, the CYP19 gene produces aromatase p450, which is required for the production of estrogen [34, 35, and 36].

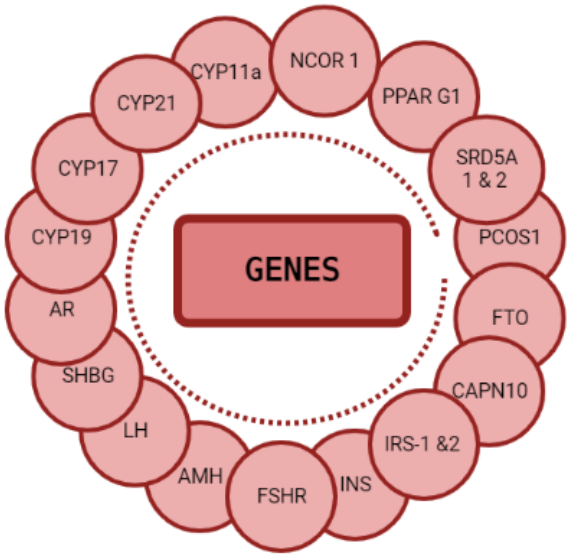

Figure 3: Role of genes in PCOS

- **Genes involved in Sex Hormone:** PCOS is brought on by structural flaws and mutations in the androgen receptor gene AR. When the AR gene on the X chromosome is inactive, this results in a rise in androgen hormone synthesis, which disrupts the molecular process. Hormone-Sex Binding Globulin Genes create proteins that attach to androgens, mostly with the help of estrogens and testosterone, to regulate the body's amount of sex hormones. Compared to normal women, PCOS women have decreased SHBG concentrations. This has mostly been ascribed to a negative impact of hyperinsulinemia on the production of SHBG [37, 38].

- **Genes Involved in GnRH Regulation:** The LH hormone raises androgen levels, which is followed by a fall in the negative feedback process. FSH has a significant indirect function by reducing androgen conversion to oestrogen and escalating the level of excess androgen in the ovaries. These genes produce LH, and PCOS patients have been found to have a point mutation (Trp8Arg and Ilg15Thr) in a gene that produces the B subunit.The AMH gene, which has five ends in all, codes for an amino acid and the variant of that protein that causes impotence. GWAS and

complete exome sequencing revealed numerous AMH gene variations as potent PCOS predictors. The 14 exons that make up FSHR, which is found on the "p" arm of chromosome 2, code for the g protein-coupled receptor needed for gonad development. PCOS is brought on by a gene mutation that disturbs protein structure and hormone levels [39, 40].

- **Insulin Regulation and Secretion-Related Genes:** Gene pathway dysregulation, including mutations in genes like INSR, IRS-1, and GLUT4, is linked to PCOS. The pathway involving phosphoinositide 3-kinase/protein kinase B causes the insulin gene, which plays a significant role in androgen synthesis, to become active in PCOS theca cells. Increased insulin levels boost androgen production similarly to how LH does. At 11p15.5, the INS gene is positioned within the IGF-II and tyrosine hydroxylase genes. The VNTR is located in the 5′ not translated region [41]. VNTR polymorphism controls the pace of INS and IGF-II expression. Numerous research were undertaken to look for a link between fertility problems and PCOS in obese women.The insulin binds to and activates the receptor in insulin receptor substrate proteins. By doing this, the INS receptor gets phosphorylated to become IRS-1, and IRS-2 is then employed to generate a calcium-dependent cysteine protease in a subsequent step [42]. Calpain 10 is the gene that prevents the metabolism and production of insulin. PCOS is brought on by a low amount of insulin; hence, a calpain 10 mutation also brings on PCOS. Alpha-ketoglutarate, a metabolic enzyme, is encoded by the FTO genome. The gene is found on chromosome 16's "q" arm. The gene has been linked to both obesity and type 2 diabetes.PCOS1 is located on chromosome 19p13.2. The PCO gene, which is sometimes known as a susceptibility area on chromosome 19, is truly a gene. SRD5A2 is located on chromosome 2 at the position 2p23.1 and has been linked to PCOS and hirsutism risk [43].

VIII. CONCLUSION

To sum up, omics technologies have fundamentally changed the understanding of PCOS by delivering in-depth molecular insights and prospective diagnostic and treatment approaches. Further advances in omics studies, in addition to clinical data integration, will lead to enhanced accuracy in diagnosis, patient categorization, and personalized PCOS care, eventually leading to greater results and a higher standard of living for those with the condition. Additionally, omics technologies present the possibility for non-invasive diagnostic techniques, such as the examination of circulating biomarkers in blood or urine samples, enabling the early detection and follow-up of PCOS. They also show potential for discovering new therapeutic targets and creating focused interventions, such as individualized pharmaceutical treatments, lifestyle changes, and approaches to precision medicine that are catered to particular patients.

REFERENCE

[1] March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod*. 2010;25(2):544–551. doi:10.1093/humrep/dep399

[2] Wiencek JR, McCartney CR, Chang AY, Straseski JA, Auchus RJ, Woodworth A. Challenges in the assessment and diagnosis of polycystic ovary syndrome. *Clin Chem*. 2019;65(3):370–377. doi:10.1373/clinchem.2017.284331

[3] Barbieri RL, Ehrmann DA. Clinical manifestations of polycystic ovary syndrome in adults. *UpToDate*. 2014;17.
[4] Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl*. 2006;29(1):278–285. doi:10.1111/j.1365-2605.2005.00623.
[5] Aarestrup J, Pedersen DC, Thomas PE, et al. Birth weight, Childhood body mass index, height and growth, and risk of polycystic ovary syndrome. *Obes Facts*. 2021;14(3):283–290. doi:10.1159/000515294
[6] Begum GS, Shariff A, Ayman G, Mohammad B, Housam R, Khaled N. Assessment of risk factors for development of polycystic ovarian syndrome. *Diabetes*. 2017;1:2.
[7] Christensen SB, Black MH, Smith N, et al. Prevalence of polycystic ovary syndrome in adolescents. *Fertil Steril*. 2013;100(2):470-477. doi:10.1016/j.fertnstert.2013.04.001
[8] Naz MSG, Tehrani FR, Majd HA, et al. The prevalence of polycystic ovary syndrome in adolescents: a systematic review and meta-analysis. *Int J Reprod Biomed*. 2019;17(8):533-542. doi:10.18502/ijrm.v17i8.4818
[9] Reilly MP, Bornfeldt KE. Integrative Multiomics approaches for discovery of new drug targets for cardiovascular disease. Circulation. 2021; 143 (25):2471–2474.
[10] Plenge RM, Scolnick EM, Altshuler D. Validating therapeutic targets through human genetics. Nat Rev Drug Discov. 2013 Aug;12(8):581-94. doi: 10.1038/nrd4051. Epub 2013 Jul 19. PMID: 23868113.
[11] Nimmesgern E, Benediktsson I, Norstedt I. Personalized Medicine in Europe. Clin Transl Sci. 2017 Mar;10(2):61-63. doi: 10.1111/cts.12446. Epub 2017 Mar 3. PMID: 28083940; PMCID: PMC5355974.
[12] Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med. 2015 Feb 26;372(9):793-5. doi: 10.1056/NEJMp1500523. Epub 2015 Jan 30. PMID: 25635347; PMCID: PMC5101938.
[13] R.S. Legro, D. Driscoll, J.F. Strauss, J. Fox, A. Dunaif, Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome, Proc. Natl. Acad. Sci. USA 95 (25) (1998) 14956–14960, https://doi.org/10.1073/pnas.95.25.14956.
[14] Li J, Chen H, Gou M, Tian C, Wang H, Song X, Keefe DL, Bai X, Liu L. Molecular Features of Polycystic Ovary Syndrome Revealed by Transcriptome Analysis of Oocytes and Cumulus Cells. Front Cell Dev Biol. 2021 Sep 6;9:735684. doi: 10.3389/fcell.2021.735684. PMID: 34552933; PMCID: PMC8450412.
[15] Pei, Chang-Zhu, Lan Jin, and Kwang-Hyun Baek. "Pathogenetic analysis of polycystic ovary syndrome from the perspective of omics." *Biomedicine & Pharmacotherapy* 142 (2021): 112031.
[16] Arentz, Susan, et al. "Perceptions and experiences of lifestyle interventions in women with polycystic ovary syndrome (PCOS), as a management strategy for symptoms of PCOS." *BMC women's health* 21.1 (2021): 1-8.
[17] Goodarzi, Mark O., et al. "Polycystic ovary syndrome: etiology, pathogenesis and diagnosis." *Nature reviews endocrinology* 7.4 (2011): 219-231.
[18] Chapman, John C., et al. "The estrogen-injected female mouse: new insight into the etiology of PCOS." *Reproductive Biology and Endocrinology* 7 (2009): 1-11.
[19] Shaaban, Zahra, et al. "Pathophysiological mechanisms of gonadotropins–and steroid hormones–related genes in etiology of polycystic ovary syndrome." *Iranian journal of basic medical sciences* 22.1 (2019): 3.
[20] Shaaban, Z., Khoradmehr, A., Shirazi, M. R. J., & Tamadon, A. (2019). Pathophysiological mechanisms of gonadotropins–and steroid hormones–related genes in etiology of polycystic ovary syndrome. *Iranian journal of basic medical sciences*, *22*(1), 3.
[21] Balen, Adam. "The pathophysiology of polycystic ovary syndrome: trying to understand PCOS and its endocrinology." *Best practice & research clinical obstetrics & gynaecology* 18.5 (2004): 685-706.
[22] Adams, Judith M., et al. "Polycystic ovarian morphology with regular ovulatory cycles: insights into the pathophysiology of polycystic ovarian syndrome." *The Journal of Clinical Endocrinology & Metabolism* 89.9 (2004): 4343-4350.
[23] Garg, Deepika, and Reshef Tal. "The role of AMH in the pathophysiology of polycystic ovarian syndrome." *Reproductive biomedicine online* 33.1 (2016): 15-28.
[24] Sirmans, Susan M., and Kristen A. Pate. "Epidemiology, diagnosis, and management of polycystic ovary syndrome." *Clinical epidemiology* (2013): 1-13.
[25] Trent, Maria, and Catherine M. Gordon. "Diagnosis and management of polycystic ovary syndrome in adolescents." *Pediatrics* 145.Supplement_2 (2020): S210-S218.
[26] Ding, Tao, et al. "Diagnosis and management of polycystic ovary syndrome in the UK (2004–2014): a retrospective cohort study." *BMJ open* 6.7 (2016): e012461.

[27] Al Wattar, Bassel H., et al. "Clinical practice guidelines on the diagnosis and management of polycystic ovary syndrome: a systematic review and quality assessment study." *The Journal of Clinical Endocrinology & Metabolism* 106.8 (2021): 2436-2446.
[28] Kyrou, Ioannis, Martin O. Weickert, and Harpal Singh Randeva. "Diagnosis and management of polycystic ovary syndrome (PCOS)." *Endocrinology and diabetes: Case studies, questions and commentaries* (2015): 99-113.
[29] Kriedt, Kathiuska J., Ali Alchami, and Melanie C. Davies. "PCOS: diagnosis and management of related infertility." *Obstetrics, Gynaecology & Reproductive Medicine* 29.1 (2019): 1-5.
[30] Escobar-Morreale, Héctor F., Manuel Luque-Ramírez, and José L. San Millán. "The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome." *Endocrine reviews* 26.2 (2005): 251-282.
[31] Teede, Helena J., et al. "Translation and implementation of the Australian led PCOS guideline: clinical summary and translation resources from the International Evidence based Guideline for the Assessment and management of polycystic ovary syndrome." *Medical Journal of Australia* 209 (2018): S3-S8.
[32] Babu, Achsha, and Gnanasambandan Ramanathan. "Multi-omics insights and therapeutic implications in polycystic ovary syndrome: a review." *Functional & Integrative Genomics* 23.2 (2023): 1-21.
[33] Rani, Shikha, and Piyush Chandna. "Multiomics Analysis–Based Biomarkers in Diagnosis of Polycystic Ovary Syndrome." *Reproductive Sciences* 30.1 (2023): 1-27.
[34] harani, N.; Waterworth, D.M.; Batty, S.; White, D.; Gilling-Smith, C.; Conway, G.S.; McCarthy, M.; Franks, S.; Williamson, R. Association of the steroid synthesis gene Cyp11a with polycystic ovary syndrome and hyperandrogenism. *Hum. Mol. Genet.* 1997, *6*, 397–402.
[35] Diamanti-Kandarakis, E.; Bartzis, M.; Bergiele, A.T.; Tsianateli, T.C.; Kouli, C.R. Microsatellite polymorphism (tttta)n at −528 base pairs of gene CYP11α influences hyperandrogenemia in patients with polycystic ovary syndrome. *Fertil. Steril.* 2000, *73*, 735–741.
[36] Pusalkar, M.; Meherji, P.; Gokral, J.; Chinnaraj, S.; Maitra, A. CYP11A1 and CYP17 promoter polymorphisms associate with hyperandrogenemia in polycystic ovary syndrome. *Fertil. Steril.* 2009, *92*, 653–659. [Google Scholar] [CrossRef]
[37] Zhang, C.-W.; Zhang, X.-L.; Xia, Y.-J.; Cao, Y.-X.; Wang, W.-J.; Xu, P.; Che, Y.-N.; Wu, X.-K.; Yi, L.; Gao, Q.; et al. Association between polymorphisms of the CYP11A1 gene and polycystic ovary syndrome in Chinese women. *Mol. Biol. Rep.* 2012, *39*, 8379–8385.
[38] Franks, Stephen, Neda Gharani, and Mark McCarthy. "Candidate genes in polycystic ovary syndrome." *Human Reproduction Update* 7.4 (2001): 405-410.
[39] Urbanek, Margrit, et al. "Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin." *Proceedings of the national academy of sciences* 96.15 (1999): 8573-8578.
[40] Panda, Pritam Kumar, et al. "Genetics of PCOS: A systematic bioinformatics approach to unveil the proteins responsible for PCOS." *Genomics data* 8 (2016): 52-60.
[41] Ewens, Kathryn G., et al. "Family-based analysis of candidate genes for polycystic ovary syndrome." *The Journal of Clinical Endocrinology & Metabolism* 95.5 (2010): 2306-2315.
[42] Chaudhary, Hiral, et al. "The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis." *Journal of ovarian research* 14 (2021): 1-21.
[43] Lee, Eung-Ji, et al. "A novel single nucleotide polymorphism of INSR gene for polycystic ovary syndrome." *Fertility and sterility* 89.5 (2008): 1213-1220.

