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REVIEW ARTICLE

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Polycystic ovary syndrome revisited: An interactions network approach

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Abstract

Background: The polycystic ovary syndrome (PCOS) has genetic, epigenetic, metabolic and reproductive aspects, while its complex pathophysiology has not been conclusively deciphered.

Aim: The goal of this research was to screen the gene/gene products associated with PCOS and to predict any possible interactions with the highest possible fidelity.

Materials and Methods: STRING v10.5 database and a confidence level of 0.7 were used.

Results: A highly interconnected network of 48 nodes was created, where insulin (INS) appears to be the major hub. INS upstream and downstream defects were analysed and revealed that only the kisspeptin- and glucagon-coding genes were upstream of INS.

Conclusion: A metabolic dominance was inferred and discussed herein with its implications in puberty, obesity, infertility and cardiovascular function. This study, thus, may contribute to the resolution of a scientific conflict between the USA and EU definitions of the syndrome and/or provide a new P4 medicine approach.

KEYWORDS

insulin, interactome, P4 medicine, polycystic ovary syndrome, systems medicine, thermoregulation

1 | INTRODUCTION

The polycystic ovary syndrome (PCOS) has genetic, epigenetic, metabolic and reproductive aspects.[1-3] The World Health Organization reported an overall prevalence of 3.4% (almost 116 million in 2012) among women worldwide.[4] Yet, the actual prevalence ranges from 2% to 20%, depending on the diagnostic criteria employed, the ethnicity and the screening method used to identify ovulatory and/or androgen dysfunction. The clinical manifestations include ovarian physiology and morphology features, high androgen levels and/or oligo-anovulation.

The diagnostic criteria vary depending upon three definitions:

- the National Institutes of Health-NIH (1990): oligo-anovulation, androgen excess, exclusion of other disorders characterized by menstrual irregularity and hyperandrogenaemia.[5]
- the Rotterdam consensus-Rott (2003): oligo/un-ovulation, excess androgen activity, polycystic ovaries by ultrasound.[6-8]
- the Androgen Excess PCOS Society-AES (2006): oligo/un-ovulation, polycystic ovaries by ultrasound, excess

androgen activity and exclusion of other disorders characterized by menstrual irregularity and hyperandrogenaemia.[6]

The management of PCOS is primarily focused on lifestyle interventions (ie body weight control and physical activity increase) and symptomatic therapies, rather than administration of medications correcting the aetiology of the syndrome. Kisspeptin is currently being investigated for a potential role in the pathophysiology of PCOS, and/or as a potential treatment.[9]

The goal of this research was to screen for gene/gene products associated with PCOS and to unravel any possible interactions with high confidence. This could help in the recognition of novel pathophysiologic and/or therapeutic regimens and could contribute to the resolution of current definitional controversies.

2 | METHODS

The biomedical literature was searched for genes or gene products implicated in PCOS. The workflow is presented in a chart (Figure 1). The potential associations among them were investigated by employing STRING (Search Tool for Retrieval of Interacting Genes/Proteins) v10.5,[10] a database of both known and predicted direct (physical) and indirect (functional) interactions among genes or proteins, derived from diverse resources, including automated literature mining, gene co-expression, biological and biochemical pathways, experimental studies and computationally predicted gene/protein associations. In this study, a high confidence interaction score (ie indicative of the reliability of the interactions) above 0.7 was chosen. The word cloud was created on a logarithmic scale, with the Word Cloud Generator (https://www.jasondavies.com/wordcloud/).

3 | RESULTS

In total, 58 PCOS-related gene/gene products were retrieved (Table 1). Of those, 48 molecules form a highly interconnected network (Figure 2), leading to the suggestion that they interact among themselves, either physically or functionally. The generated network is dense, with an average node degree of 6.82. It includes 10 enzymes, 9 receptors, 7 transcriptional regulators, 12 hormones, 5 cytokines, 2 growth and/or differentiation factors, 2 signal-transducing adaptor proteins (STAPs) and 1 transporter (Table 1).

A word cloud was generated, where the molecules with the greater number of interactions are indicated by a larger word size (Figure 3). The main hub was insulin (INS) with 23 nodes.

4 | DISCUSSION

Complex human chronic diseases, such as obesity and type II diabetes mellitus, have been mapped, leading to a better insight of their genetic and epigenetic traits. Unlike the progress observed in the understanding of these diseases, PCOS complexity is poorly elucidated at this time. There are numerous potential reasons: a. heterogeneity in the phenotype, b. complex nature of the syndrome, c. variable diagnostic criteria, d. underpowered, small samples in many gene-association publications and e. inconsistent or nonreplicated associations. Large populations are critical for every epidemiological investigation, and in genetic epidemiological studies, this condition is a *sine qua non*. In the PCOS case, small samples may compromise homogeneity consensus. Accordingly, studies examining entire candidate genes are preferred to gene variant investigations, which might not be replicable. All above prerequisites may be addressed by genome-wide association studies, unique diagnostic criteria, meta-analyses and interactions networks.

Our PCOS interactome presented herein includes all candidate genes that exhibit high confidence and are implicated in various phenotypes. Summarizing and interpreting the literature, the network construction profits from large population studies, as well from translational research outcomes leading to 'predicted associations'. It surpasses the diagnostic criteria limitation by targeting strongly reliable interactions. INS was the major hub in the constructed PCOS interactome, and the discussion below is based on this premise.

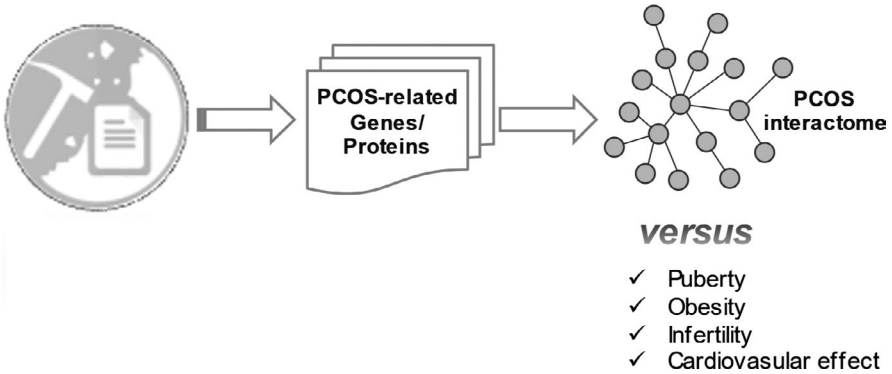

FIGURE 1 Flow chart of work

TABLE 1 Genes included in the PCOS interactome

References	Symbol	Description	Gene ID
19,25	ACE	Angiotensin I–converting enzyme (peptidyl-dipeptidase A)1	1636
19,25	ACE2	Angiotensin I–converting enzyme (peptidyl-dipeptidase A) 2	59272
11	AGT	Angiotensinogen	183
18,22	AKR1C3	Aldo-keto reductase family 1 member C3	8644
30,34	AMH	Anti-Mullerian hormone	268
30,34,45	AMHR2	Anti-Mullerian hormone receptor type 2	269
5,6,28,31,35	AR	Androgen receptor	367
25,52	AVP	Arginine vasopressin	551
9	BMP15	Bone morphogenetic protein 15	9210
9	C9orf3	Chromosome 9 open reading frame 3	84909
9	CAPN10	Calpain 10	823
19	CCK	Cholecystokinin	885
25	CGA	Glycoprotein hormones, alpha polypeptide	1081
18,22,37-39	CYP17A1	Cytochrome P450 family 17 subfamily A member 1	1586
18,22	CYP19A1	Cytochrome P450 family 19 subfamily A member 1	1588
18,22	CYP21A2	Cytochrome P450 family 21 subfamily A member 2	1589
9	FANCC	Fanconi anaemia complementation group C	2176
5,6	FSHB	Follicle-stimulating hormone beta subunit	2488
25	FTO	FTO, alpha-ketoglutarate dependent dioxygenase	79068
19	G6PC	Glucose-6-phosphatase	14377
46	GATA4	GATA binding protein 4	2626
12,19,25	GCG	Glucagon	2641
9	GDF9	Growth differentiation factor 9	2661
5,6,36	GHSR	Growth hormone secretagogue receptor	51738
5,6,13,14,53	GNRH1	Gonadotropin-releasing hormone 1	2693
5,6	GNRHR	Gonadotropin-releasing hormone receptor	2796
9,29,31,37-39,47-49	IGF1	Insulin-like growth factor 1	3087
9,31,47-49	IGF2	Insulin-like growth factor 2	3479
19,23,24,44	IL1A	Interleukin 1 alpha	3481
19,23,24,44	IL1B	Interleukin 1 beta	3552
19,23,24,44	IL6	Interleukin 6	3553
19,23,24,28,44	IL8	Interleukin 8	3569
1,9,19-21	INS	Insulin	3576
9,19	INSR	Insulin receptor	3630
19	IRS1	Insulin receptor substrate 1	3659
19	IRS2	Insulin receptor substrate 2	3667
9,12-21	KISS1	KiSS-1 metastasis-suppressor	3739
11	KISS1R	KISS1 receptor	3814
19,25,56-58	LEP	Leptin	11103
5,6,27	LHR	Luteinizing hormone receptor	3952
25	NCOR1	Nuclear receptor corepressor 1	252969
19	PPARG	Peroxisome proliferator-activated receptor gamma	9611

(Continues)

TABLE 1 (Continued)

References	Symbol	Description	Gene ID
19	SERPINE 1	Serpin family E member 1	10111
9,31,32	SHBG	Sex hormone binding globulin	9869
31,32	SULTA1	Sulfotransferase family 2A member 1	6648
48	TCF7L2	Transcription factor 7 like 2	6822
19,23-25,50,53-55	TNF	Tumor necrosis factor	63892
32	VDR	Vitamin D receptor	10155
47	YAP1	Yes-associated protein 1	7515

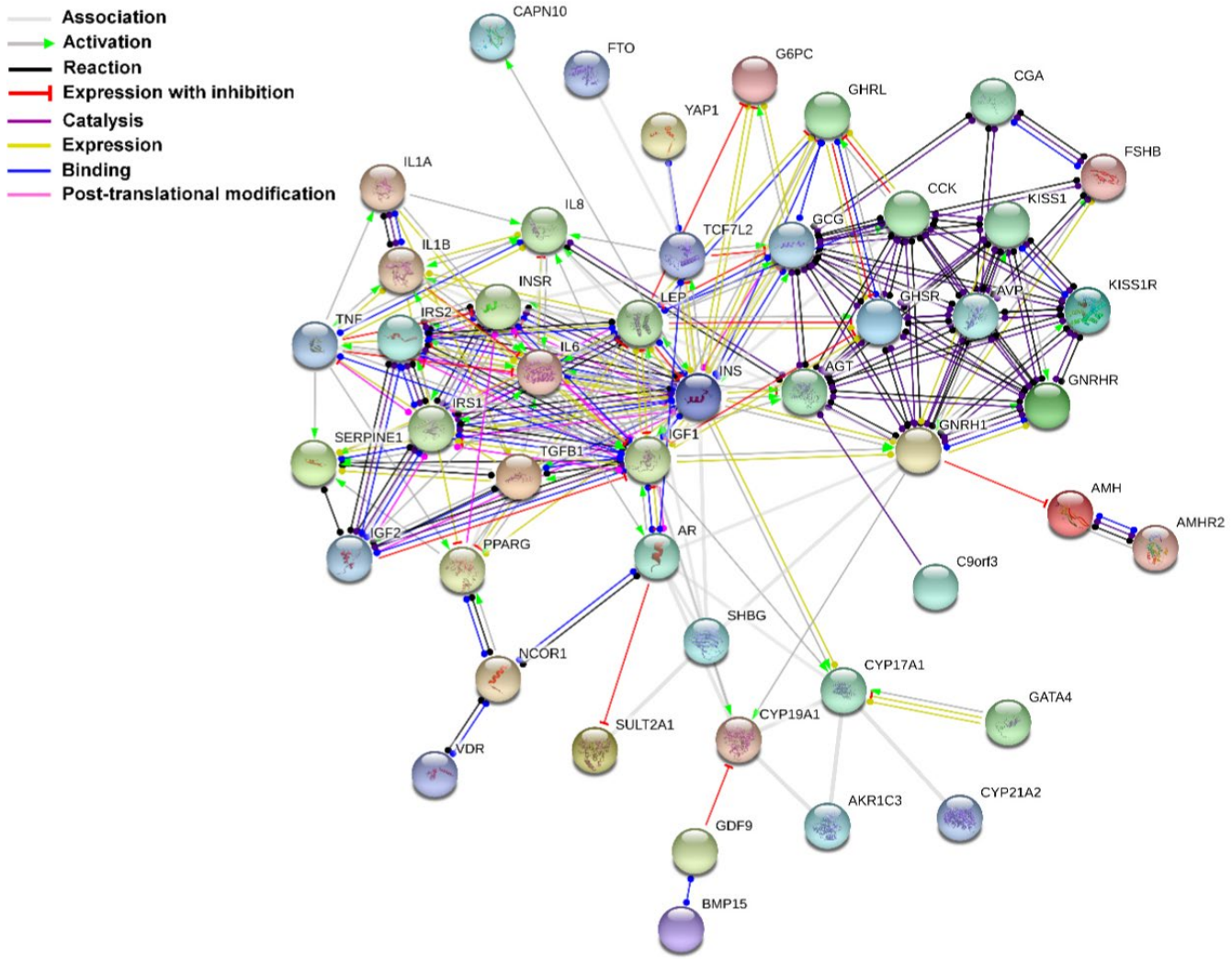

FIGURE 2 Network of the interactions among the gene/gene products listed in Table 1. The connecting lines denote the predicted mode of action with an interaction score higher than 0.7

Both human KISS1 and its cognate receptor, the G protein-coupled receptor KISS1R,[11] are predicted to be associated with glucagon (GCG), angiotensinogen (AGT), arginine vasopressin (AVP), growth hormone secretagogue receptor (GHSR), cholecystokinin (CCK), gonadotropin-releasing hormone 1 (GNRH1) and gonadotropin-releasing hormone receptor (GNRHR) (Figure 2). In particular, GCG was demonstrated to directly upregulate *KISS1* in murine hepatocytes.[12] Moreover, translational research results have shown that KISS1 enhances GNRH1 neuronal excitability in mice,[13] while it was regulated directly by the KiSS1 hexadecapeptide (KiSS1-16) in the preoptic area of the scombroid fish.[14] Of importance, GCG, AGT, AVP, GHSR, CCK and GNRH1 are associated directly with INS, a major hub in the network with a total of 23 interactions (Figures 2 and 3). Insulin is suggested to interact with components of the

FIGURE 3 Word cloud of the molecular interactions presented in Figure 2. The larger the word size, the higher the number of interactions

immune system, including the inflammatory cytokines IL-1β, IL-6, IL-8 and tumour necrosis factor-α (TNF), as well as the transforming growth factor β1 (TGF-B1) (Figure 2).

4.1 | Puberty and PCOS

Kisspeptin mediates gonadotropin-releasing hormone release at puberty initiation.[15] As the main activator of GnRH neurons, it also mediates sex steroid feedback regulation and metabolic pathways at different developmental stages throughout the lifespan.[16] Its role in puberty onset is pivotal, while its role in PCOS has gained great interest lately. Importantly, kisspeptin stimulates GnRH pulsatile secretion during ovulation.[17] Plasma kisspeptin levels were negatively correlated with insulin resistance and circulating free androgens in women with PCOS.[18]

Contrary to these findings, Jeon et al [2013] and Yilmaz et al [2014][19-21] suggested kisspeptin as a specific marker of hyperandrogenism in PCOS, as they observed increased kisspeptin levels in normal weight and obese PCOS women, unlike hyperandrogonaemic non-PCOS controls. Importantly, these observations were independent of insulin resistance.

4.2 | Obesity and PCOS

Kiss1 is implicated in obesity and diabetes mellitus—entities that may be included in PCOS phenotypic variations. On the other hand, insulin is not only a major hub for the entire obesidome, but also for the genetic obesity interactome.[22] Other common nodes within the obesity interactions network are the gut hormones LEP, GHRL, genes encoding inflammation markers, as PPARG, TNF and IL-6. Rotter et al(2003)[23] demonstrated that the proinflammatory cytokines IL-6, IL-8 and TNF caused insulin resistance in human subcutaneous adipose cells. 'Proinflammatory' genotypes, including polymorphisms of genes coding for TNF, IL-6 and IL-6 receptor, have been associated with PCOS.[24]

The PCOS network and the obesidome share metabolic markers, such as INS, CCK, AGT, AVP, FTO and GCG. In the PCOS interactome, LEP, a gut hormone related to body weight, interacts with PPARG, GCG, INS, INSR, IRS-1, IRS-2, IGF-1, GHRL, IL-6, IL-8, IL-1B, TNF, CCK, GHSR, SERPINE1, G6PC, HBG and GNRH1.[19] Most of these connections are common with ANS inflammation–induced obesity and genetic obesity,[25] whereas interactions predicted in the obesidome need to be further validated and studied in PCOS populations. Accordingly, GHRL interacts with GHSR, LEP, INS, GCG, CCK and IGF1.

4.3 | Infertility and PCOS interactome

Infertility is not a *sine qua non* consequence of PCOS. Nevertheless, it is a common clinical phenotypic symptom of the syndrome. Furthermore, it is well established that some degree of hypogonadism is common in poorly controlled diabetes, but this may be a result of the stress that accompanies this state.[26,27] Our network revealed that INS is the major hub of the disease and all interacting reproduction-related peptides are INS downstream.

Translational research has indicated that AR suppresses *GNRH1* gene activation.[28] In addition, GNRH1 was shown to increase the release of IGF-1 in bovine anterior pituitary cells.[29] Cimino and colleagues (2016) have also suggested that AMH increases the excitability of GNRH1 neurons and hormone release in mice.[30] Thus, the androgen receptor is connected to INS through IGF1 produced in the liver, another highly connected node in our network. IGF-1 exhibits anabolic function, while it is connected to several hormones and hormone receptors, including GNRH1 and AR, and the sex hormone–binding globulin (SHBG), which binds to the two sex steroid hormones, androgens and oestrogens,[31] as well as the growth hormone secretagogue receptor (GHSR). The latter in its turn binds ghrelin, thereby playing an important mediating role in several aspects of energy homeostasis[32] and regulation of body weight, fat mass and food uptake.[33] In particular, IGF-1 was shown to amplify GNRH1 responsiveness in the murine ovary,[34] to induce AR activity,[35] and to negatively regulate GHSR expression in the rat pituitary gland.[36] IGF-1 also regulates the activity of sex-differentiated liver cytochrome P450 enzymes in male rats.[18,22]

4.4 | Cardiovascular effects and PCOS

PCOS patients are at an increased risk of developing cardiovascular diseases (CVD).[37-39] Recently, a large cohort study investigating whether the risk profile of PCOS translates into a greater likelihood of developing CVD and whether this persists along the human lifespan was published.[40] The study involved 60 574 women receiving assisted reproduction treatment, that is in vitro fertilization (IVF), from 1994 to 2015. Of those, 6,149 (10.2%) were diagnosed with PCOS. The researchers used medical records to examine the life course of these women for nine years. During this follow-up period, 2,925 (4.8%) women developed CVD. Overall, women with PCOS were at 19% higher CVD risk than their non-PCOS peers. When clustered into age groups, women with PCOS aged >50 years had a CVD risk similar to their non-PCOS peers, unlike the 30- to 40-year-old cluster, where the CVD risk of PCOS patients was higher. Although we consider the latter effect limited, as the healthy comparison group was rather small, we confirm our network structure: kisspeptin and glucagon act upstream of INS, whereas other reproduction-related molecules act downstream of it.

The cytochrome P450 family 19 A member 1 (CYP19A1) interacted with GNRH1. Moreover, two family members of cytochrome P450, CYP17A1 and CYP19A1, as well as the serpin family E member 1 (SERPINE1)—a negative regulator of fibrinolysis responsible for the controlled degradation of blood clots—were also included in the network.[41-43]

AGT is predicted to interact directly with IL-6, IL-8 and TGF-B1. Of note, angiotensin II, which is derived from the precursor molecule AGT, was found to increase the expression of the pleiotropic proinflammatory cytokines IL-6 and IL-8 in rat vascular smooth muscle cells.[28,44] GNRH1 interacted with 15 molecules (Figures 2 and 3), including insulin-like growth factor 1 (IGF-1), the androgen receptor (AR), the sex hormone–binding globulin (SHBG), the cytochrome P450 family 19 subfamily A member 1 (CYP19A1), the anti-Mullerian hormone (AMH) and calpain 10 (CAPN10).

The clinical role of elevated AMH is under discussion, as it characterizes adolescent and adult PCOS women, as well as the daughters of PCOS women.[45] It has been also reported by De Leo and colleagues that "elevated mid-gestation maternal T levels predict high AMH levels in the adolescent daughters".[45] Accordingly, high AMH levels have been correlated to follicular growth arrest and hyperandrogenaemia.

Our network predicted GATA4, YAP1 and TCF7L2 as contributors to PCOS. GATA4 is essential for cardiac function during development and in adult life. It might contribute to the interpretation of the findings in the daughters of women with PCOS.[46] The potential involvement of YAP1 transcription factor—implicated in metabolism—has been confirmed in Chinese women in a recent meta-analysis,[47] while TCF7L2 has been associated to glucose intolerance, thus, the cardiometabolic syndrome,[48] which is prevalent in the PCOS population.

The IGF-1 system seems to be connected to a relatively large number of molecules, a total of 18 (Figures 2 and 3), including IGF-2, the insulin receptor (INSR), the insulin receptor substrates IRS-1 and IRS-2 and glucagon, consistent with a role of IGF-1 in mediating insulin-associated effects.

Clinical studies have reported that the inositol system, which underlies insulin resistance, is involved in lean PCOS subjects.[49] Furthermore, IGF-1 has been linked to the immune-related molecules IL-1β, IL-6, IL-8, TGF-β1 and TNF. Of note, IGF-1 was shown to increase the expression of proinflammatory cytokines,[50] as well as to be regulated by them.[51] In addition, IGF-1 has been linked to several hormones and hormone receptors, including GNRH1, AR and the sex hormone–binding globulin (SHBG), which binds both testosterone and estradiol.[31] The above-stated interactions support the observation that PCOS patients are at greater risk of developing cardiometabolic syndrome and, hence, cardiovascular diseases.[37-39]

Moreover, the constructed interactome includes genes/gene products associated to thermoregulation, such as AVP,[52] IL-1, IL-6, TNF[53-55] and leptin,[56-58] and enhances the need for future research on sympathetic and vagal innervation and their involvement in the development and persistence of the syndrome.[59] Of note, our work is limited to the investigation of wild-type genes.

5 | CONCLUSIONS

We hereby used the interactions network tool to revisit the pathophysiology of PCOS, as the genetic architecture of PCOS variant phenotypes has been inconclusively studied. This interactome revealed INS as a main hub, whose downstream defects include inflammatory, metabolic and reproductive pathways, whereas upstream signals extended to metabolic and autonomic nervous system functions. Thus, the pathophysiologic 'defects' of PCOS may act either downstream or upstream of INS. Notably, only glucagon and kisspeptin activations occur prior to INS production. The findings suggest that NIH and AES criteria might be more appropriate than the Rotterdam ones. Also, this signalosome shows that treatments such as contraceptive pills and clomiphene might be of limited effectiveness, whereas insulin and/or sympathetic/parasympathetic control changes might be more important. We conclude that management of PCOS defect 'remedies' need to be revisited and reweighted.

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Authors have no conflict of interest to declare.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

DISCLOSURE

Preliminary results have been presented in the meeting of the European Society of Pediatric Endocrinology (ESPE) in 2018.

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REFERENCES

1. Condorelli RA, Calogero AE, Di Mauro M, La Vignera S. PCOS and diabetes mellitus: from insulin resistance to altered beta pancreatic function, a link in evolution. *Gynecol Endocrinol*. 2017;33(9):665-667.
2. Abbott DH, Rogers J, Dumesic DA, Levine JE. Naturally occurring and experimentally induced rhesus macaque models for polycystic ovary syndrome: translational gateways to clinical application. *Med Sci*. 2019;7(12):107.
3. Mohamed-Hussein ZA, Harun S. Construction of a polycystic ovarian syndrome (PCOS) pathway based on the interactions of PCOS-related proteins retrieved from bibliomic data. *Theor Biol Med Model*. 2009;6(1):18.
4. Kabel AM. Polycystic ovarian syndrome: insights into pathogenesis, diagnosis, prognosis, pharmacological and non-pharmacological treatment. *Pharmacol Rep*. 2016;1(103):E1-E5.
5. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome;towards a rational approach. In: Dunaif A, Givens JR, Haseltine F, (Eds). *Polycystic ovary syndrome*. Boston, MA: Blackwell Scientific. 1992. pp. 377-384.
6. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med*. 2010;8:41.
7. Aziz R. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. *J Clin Endocrinol Metab*. 2006;91(3):781-785.
8. Revised 2003 consensus on diagnostic criteria and longterm health risks related to polycystic ovary syndrome (PCOS). The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. *Hum Reprod*. 2004;19(1):41-47.
9. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reproductive Biol Endocrinol*. 2016;14(1):38.
10. Szklarczyk D, Morris J H, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res*. 2017;45(D1):D362-D368.
11. Cho SG, Li D, Tan K, Siwko SK, Liu M. KiSS1 and its G-protein-coupled receptor GPR54 in cancer development and metastasis. *Cancer Metastasis Rev*. 2012;31(3–4):585-591.
12. Song WJ, Mondal P, Wolfe A, et al. Glucagon regulates hepatic kisspeptin to impair insulin secretion. *Cell Metab*. 2014;19(4):667-681.
13. Han SK, Gottsch ML, Lee KJ, et al. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *Journal Neurosci*. 2005;25(49):11349-11356.
14. Ohga H, Adachi H, Kitano H, Yamaguchi A, Matsuyama M. Kiss1 hexadecapeptide directly regulates gonadotropin-releasing hormone 1 in the scombroid fish, chub mackerel. *Biol Reprod*. 2017;96(2):376-388.
15. Cortés ME, Carrera B, Rioseco H, Pablo del Río J, Vigil P. The role of kisspeptin in the onset of puberty and in the ovulatory mechanism: a mini-review. *J Pediatr Adolesc Gynecol*. 2015;28(5):286-291.
16. Pielecka-Fortuna J, Chu Z, Moenter SM. Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. *Endocrinology*. 2008;149(4):1979-1986.
17. Meczekalski B, Katulski K, Podfigurna-Stopa A, Czyzyk A, Genazzani AD. Spontaneous endogenous pulsatile release of kisspeptin is temporally coupled with luteinizing hormone in healthy women. *Fertil Steril*. 2016;105(5):1345-1350.
18. Panidis D, Rousso D, Koliakos G, et al. Plasma metastin levels are negatively correlated with insulin resistance and free androgens in women with polycystic ovary syndrome. *Fertil Steril*. 2006;85(6):1778-1783.
19. Jeon YE, Lee KE, Jung JA, et al. Kisspeptin, leptin, and retinol-binding protein 4 in women with polycystic ovary syndrome. *Gynecol Obstet Invest*. 2013;75(4):268-274.
20. Yilmaz SA, Kerimoglu OS, Pekin AT, et al. Metastin levels in relation with hormonal and metabolic profile in patients with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2014;180:56-60.
21. Schöfl C, Horn R, Schill T, Schlösser HW, Müller MJ, Brabant G. Circulating ghrelin levels in patients with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2002;87(10):4607-4610.
22. Rasmussen E, Ask B, Finnstrom N, Skottner-Lundin A, Rane A. Insulin-like growth factor 1 (IGF-I) effects on sex-specific cytochrome P450 enzymes in normal and hypophysectomised male rats. *Biochem Pharmacol*. 1998;56(4):459-466.
23. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J Biol Chem*. 2003;278(46):45777-45784.
24. Franks S, McCarthy M. Genetics of ovarian disorders: polycystic ovary syndrome. *Rev Endocr Metab Disord*. 2004;5:69-76.
25. Geronikolou SA, Pavlopoulou A, Cokkinos D, Chrousos G. Interactome of obesity: obesidome : genetic obesity, stress induced obesity, pathogenic obesity interaction. *Adv Exp Med Biol*. 2017;987:233-241.
26. Castellano JM, Navarro VM, Fernández-Fernández R, et al. Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. *Diabetes*. 2006;55(9):2602-2610.
27. Martini AC, Fernández-Fernández R, Tovar S, et al. Comparative analysis of the effects of ghrelin and unacylated ghrelin on luteinizing hormone secretion in male rats. *Endocrinology*. 2006;147:2374-2382.
28. Kim HY, Kang YJ, Song IH, Choi HC, Kim HS. Upregulation of interleukin-8/CXCL8 in vascular smooth muscle cells from spontaneously hypertensive rats. *Hypertens Res*. 2008;31(3):515-523.

29. Hashizume T, Kumahara A, Fujino M, Okada K. Insulin-like growth factor I enhances gonadotropin-releasing hormone-stimulated luteinizing hormone release from bovine anterior pituitary cells. *Anim Reprod Sci*. 2002;70(1–2):13-21.
30. Cimino I, Casoni F, Liu X, et al. Novel role for anti-Müllerian hormone in the regulation of GnRH neuron excitability and hormone secretion. *Nat Commun*. 2016;7:10055.
31. Hammond GL. Diverse roles for sex hormone-binding globulin in reproduction. *Biol Reprod*. 2011;85(3):431-441.
32. Pazos Y, Casanueva FF, Camina JP. Basic aspects of ghrelin action. *Vitam Horm*. 2008;77:89-119.
33. Stevanovic D, Trajkovic V, Müller-Lühlhoff S, et al. Ghrelin-induced food intake and adiposity depend on central mTORC1/S6K1 signaling. *Mol Cell Endocrinol*. 2013;381(1–2):280-290.
34. Zhou J, Kumar TR, Matzuk MM. Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary. *Mol Endocrinol*. 1997;11(13):1924-1933.
35. Fan W, Yanase T, Morinaga H, et al. Insulin-like growth factor 1/insulin signaling activates androgen signaling through direct interactions of Foxo1 with androgen receptor. *J Biol Chem*. 2007;282(10):7329-7338.
36. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Oikawa S. Insulin-like growth factor-I down-regulates ghrelin receptor (growth hormone secretagogue receptor) expression in the rat pituitary. *Regul Pept*. 2005;127(1–3):203-206.
37. Ramezani Tehrani F, Amiri M, Behboudi-Gandevani S, Bidhendi-Yarandi R, Carmina E. Cardiovascular events among reproductive and menopausal age women with polycystic ovary syndrome: a systematic review and meta-analysis. *Gynecol Endocrinol*. 2020;36(1):12-23.
38. Zhao L, Zhu Z, Lou H, et al. Polycystic ovary syndrome (PCOS) and the risk of coronary heart disease (CHD): a meta-analysis. *Oncotarget*. 2016;7(23):33715-33721.
39. Mumusoglu S, Yildiz BO. Metabolic syndrome during menopause. *Curr Vasc Pharmacol*. 2019;17(6):595-603.
40. Oliver-Williams C, Vassard D, Pinborg A, Schmidt L. Risk of cardiovascular disease for women with polycystic ovary syndrome: results from a national Danish registry cohort study. *Eur J Prev Cardiol*. 2020:2047487320939674. https://doi.org/10.1177/2047487320939674
41. Lee MH, Vosburgh E, Anderson K, McDonagh J. Deficiency of plasma plasminogen activator inhibitor 1 results in hyperfibrinolytic bleeding. *Blood*. 1993;81(9):2357-2362.
42. Fay WP, Parker AC, Condrey LR, Shapiro AD. Human plasminogen activator inhibitor-1 (PAI-1) deficiency: characterization of a large kindred with a null mutation in the PAI-1 gene. *Blood*. 1997;90(1):204-208.
43. Jankun J, Aleem AM, Selman SH, et al. Highly stable plasminogen activator inhibitor type one (VLHL PAI-1) protects fibrin clots from tissue plasminogen activator-mediated fibrinolysis. *Int J Mol Med*. 2007;20(5):683-687.
44. Funakoshi Y, Ichiki T, Ito K, Takeshita A. Induction of interleukin-6 expression by angiotensin II in rat vascular smooth muscle cells. *Hypertension*. 1999;34(1):118-125.
45. De Leo V, Musacchio MC, Cappelli V, Massaro G, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol*. 2016;14:38.
46. Wood JR, Ho CK, Nelson-Degrave VL, McAllister JM, Strauss JF. 3rd The molecular signature of polycystic ovary syndrome (PCOS) theca cells defined by gene expression profiling. *J Reprod Immunol*. 2004;63(1):51-60.
47. Li T, Zhao H, Zhao X, et al. Identification of YAP1 as a novel susceptibility gene for polycystic ovary syndrome. *J Med Genet*. 2012;49(4):254-257.
48. Biyasheva A, Legro RS, Dunaif A, Urbanek M. Evidence for association between polycystic ovary syndrome (PCOS) and TCF7L2 and glucose intolerance in women with PCOS and TCF7L2. *J Clin Endocrinol Metab*. 2009;94(7):2617-2625.
49. Baillargeon JP, Nestler JE, Ostlund RE, Apridonidze T, Diamanti-Kandarakis E. Greek hyperinsulinemic women, with or without polycystic ovary syndrome, display altered inositols metabolism. *Hum Reprod*. 2008;23(6):1439-1446.
50. O'Connor JC, McCusker RH, Strle K, Johnson RW, Dantzer R, Kelley KW. Regulation of IGF-I function by proinflammatory cytokines: at the interface of immunology and endocrinology. *Cell Immunol*. 2008;252(1–2):91-110.
51. Kim H, Moon SY, Sohn MY, Lee WJ. Insulin-Like growth factor-1 increases the expression of inflammatory biomarkers and sebum production in cultured sebocytes. *Ann Dermatol*. 2017;29(1):20-25.
52. Mooradian AD, Morley GK, McGeachie R, Lundgren S, Morley JE. Spontaneous periodic hypothermia. *Neurology*. 1984;34(1):79-82.
53. Nisoli E, Briscini L, Giordano A, et al. Tumor necrosis factor alpha mediates apoptosis of brown adipocytes and defective brown adipocyte function in obesity. *PNAS*. 2000;97(14):8033-8038.
54. Ely BR, Francisco MA, Halliwill JR, et al. Heat therapy reduces sympathetic activity and improves cardiovascular risk profile in women who are obese with polycystic ovary syndrome. *Am J Physiol Regul Integr Comp Physiol*. 2019;317(5):R630-R640.
55. Cartmell T, Southgate T, Rees GS, Castro MG, Lowenstein PR, Luheshi GN. Interleukin-1 mediates a rapid inflammatory response after injection of adenoviral vectors into the brain. *J Neurosci*. 1999;19(4):1517-1523.
56. Rozhavskaya-Arena M, Lee DW, Leinung MC, Grasso P. Design of a synthetic leptin agonist: effects on energy balance, glucose homeostasis, and thermoregulation. *Endocrinology*. 2000;141(7):2501-2507.
57. Salkeld BD, MacAulay JC, Ball RW, Cannon JG. Modulation of body temperature, interleukin-6 and leptin by oral contraceptive use. *NeuroImmunoModulation*. 2001;9(6):319-325.
58. Fischer AW, Hoefig CS, Abreu-Vieira G, et al. Leptin raises defended body temperature without activating thermogenesis.W *Cell Rep*. 2016;14(7):1621-1631.
59. Vieyra-Valdez E, Linares-Culebro R, Rosas-Gavilán G, Ramírez-Hernández D, Domínguez-Casalá R, Morales-Ledesma L. Roles of the cholinergic system and vagal innervation in the regulation of GnRH secretion and ovulation: experimental evidence. *Brain Res Bull*. 2020;165:129-138.

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