Chapter

Rare and Underappreciated Causes of Polycystic Ovarian Syndrome

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Abstract

While hyperinsulinemia is a common contributing mechanism in the pathogenesis of polycystic ovarian syndrome (PCOS), other mechanisms may give rise to or add to the effects of hyperinsulinemia, as well as other causes of hyperandrogenism, in the pathogenesis of PCOS. Such underappreciated causes may include autoimmune, insulin receptor mutations, mutations of post-receptor insulin signaling response elements, polymorphisms of LH, androgen, and estrogen signaling pathways, epigenetic alterations in hormonal signaling cascade response elements, infestations and infections with organisms capable of endocrine disruption by various mechanisms, as well as drugs and other chemicals which may be endocrine disruptors. In addition, alterations in the gut, oral, or vaginal biome may be associated with PCOS and insulin resistance and may, in some instances, have a role to play in its pathogenesis. In this chapter I plan to review what is known about these lesser-known causes of PCOS, in the hopes of alerting clinicians to consider them and stimulating investigators to better understand PCOS pathogenesis in general and, hopefully, develop more individualized, precision treatment and prevention strategies for the people in our care.

Keywords: PCOS, insulin resistance, biome, polymorphisms, epigenetic, endocrine disruptors, autoimmune, vitamin D

1. Introduction

Polycystic ovarian syndrome (PCOS) is believed to be the most common cause of infertility in reproductive age women [1]. Although men lack ovaries, there is also a syndrome called male PCOS featuring a similar set of cardiometabolic indicators and risks to that seen in female PCOS as well as early balding [2]. Most people with PCOS are insulin resistant/hyperinsulinemic [3]. When overweight or obesity is present in people with PCOS (as is the case in most people with this disorder) insulin resistance/hyperinsulinemia is exacerbated [3]. Several mutations are associated with an increased risk of PCOS [4]. Gene methylation and histone acetylation abnormalities as well as certain non-coding RNAs may also contribute to the expression of PCOS [5].

Autoimmune disease has been shown to play a role in some people with PCOS, severely insulin resistant diabetes, acanthosis nigricans, and systemic lupus

erythematosus with nephritis [6]. Besides these rare and dramatic examples of the Type B syndrome, there is some evidence that an autoimmune process triggered by low progesterone levels may be a trigger for PCOS in many women [7]. A similar phenotype without lupus or anti-insulin receptor antibodies, but with insulin receptor mutations or abnormal post-receptor signaling has also been described [6].

Parasitoses, which are especially common in the tropical and subtropical parts of the world (and becoming more common as a result of the climate crisis), can induce PCOS by virtue of their ability to synthesize steroid hormones, e.g., estradiol and 1,25-OH2-vitamin D3 from its precursor, 25-OH-vitamin D [8].

Alterations in the microbiome have been reported in people with PCOS which may play a causative role [9–11].

Endocrine disruptor chemicals (EDCs), which are ubiquitous and increasing in our environment, including certain drugs, may also contribute to the pathogenesis of PCOS [12, 13].

Epilepsy has been cited as a cause of PCOS, however, there is controversy as to whether the disorder itself, treatment with valproate, or both are responsible [14].

Many drugs are associated with an increase in insulin resistance (IR) which may be an initial step in PCOS pathogenesis. In addition, EDCs in our environment may initially cause IR or bind as agonists to estrogen or androgen receptors, eventually contributing to PCOS [15].

In the remainder of this chapter, I shall review what is known about these diverse contributing causes of PCOS in the hope that in so doing clinicians might explore these often-reversible factors in their patients. I further hope that such a review may point to common pathogenic pathways in many, if not all, people with PCOS. Finally, I hope that appreciation of the various causes of PCOS can lead to improved preventive strategies and individualized, precision treatment of people with PCOS.

2. Genetic predisposition to PCOS

The marked tendency of PCOS for familial clustering (made even more remarkable by the hypo-fertility of people with PCOS) has long supported the notion that PCOS has a genetic component. However, since families often share similar diets, lifestyles, and EDC exposures, twin studies using monozygotic twins raised in very different environments would be helpful in separating genetic and environmental effects. Although it was shown that the tetrachoric correlation for PCOS in monozygotic twin sisters is higher than for dizygotic twins or for non-twin sisters, each set of twins or sisters in this large study was brought up in the same family. Despite efforts of most parents to raise monozygotic twins as distinct individuals, they are apt to, nonetheless, share a more similar environment and set of experiences than less closely related siblings, leaving open the possibility that shared environment/experience contributes significantly to the correlation [16]. In addition to tetrachoric correlation, both univariate analysis and a trivariate genetic analysis of major findings occurring in women with PCOS suggested a strong genetic component of PCOS in this Dutch twin study by Vink and colleagues [16]. Other twin studies in people with PCOS have reached similar conclusions [17–20].

Genome-wide association studies (GWAS) have been helpful in identifying polymorphisms that are associated with an increased risk of PCOS development [21–24]. Nevertheless, only about 10% of the apparent heritability of PCOS to date can be explained by these associations, leading to speculation that various phenotypes

are associated with rare polymorphisms. Newer technologies e.g., gene and whole exome sequencing may clarify the contribution of rare polymorphisms to different phenotypes in the future [21].

Among the GWAS-identified candidate loci are DENND1A, LHCGR, INSR, FSHR, ZNF217, YAP1, INSR, RAB5B, and C9orf3 [22]. Polymorphisms found in DENND1A (P = .0002), THADA (P = .035), FSHR (P = .007), and INSR (P = .046) in Chinese women with PCOS were also strongly associated with PCOS in European women [24].

GWAS often fails to identify candidate loci in the mitochondrial portion of the genome [25]. Recent publications suggest that the mitochondria may play a pivotal role in PCOS pathogenesis, both genetically and epigenetically, given the essential mitochondrial role in cellular metabolism and IR. Recently Ye and colleagues reported that a 4977 base pair deletion in mitochondrial DNA detected in peripheral blood using multiplex probe-based qPCR was highly associated with PCOS in a logistic regression analysis [26]. In a study by Saeed and colleagues it was reported that most of the mitochondrial DNA mutations (80%) were limited to a 3157–3275 base region which is evolutionarily conserved and would be expected to change the secondary structure of mitochondrial transfer RNAs. As suspected, 6 mutations (A to G and/or T to C) altered the expected base pairing. Mitochondrial DNA copy numbers were also diminished in women with PCOS compared with controls [27]. Zeng et al. have reviewed the role of oxidative stress (OS) in people with PCOS [28]. They summarized much of what is currently known about the role of mitochondrial dysfunction in PCOS. Reduction of mitochondrial DNA copy number and mitochondrial mutations contribute to IR, metabolic syndrome, and disordered development of ovarian follicles through increased production of reactive oxygen species (ROS). Obesity plays a pivotal role in the pathogenesis of PCOS in most people, however, mitochondrial genome alterations related to PCOS with obesity are not yet well understood, underlining the need to investigate changes in the mitochondrial genome that are associated with obesity. External environmental factors may also disrupt mitochondrial function. Recent attention has focused on the effect of environmental factors e.g., cigarette smoke and bisphenol A on reproduction. Cigarette smoke has been reported to disrupt ovarian development; 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal (NNA), contained in third-hand smoke, reduced ovarian weight and follicle number in rats exposed to NNA for 30 days compared with controls and even had a serious negative effect on development of the offspring of NNA-exposed rats. These adverse reproductive effects of cigarette smoke seem to be due to mitochondrial dysfunction. NNA exposure causes ROS buildup by increasing superoxide dismutase (SOD) mRNA levels, inducing apoptosis. Benzo(a)pyrene (BaP), another component of cigarette smoke, causes massive mitochondrial ROS leakage/dysfunction, resulting in significant plasma membrane lipid peroxidation and disrupted ovum fertilization. Cigarette smoke also adversely effects the development of granulosa cells, which have an essential role in providing optimal amounts of the hormones and nutrients needed for follicular development.

3. Epigenetic contributions to PCOS

These include abnormalities of DNA methylation, histone acetylation, and downstream signal transduction abnormalities.

3.1 Abnormalities of DNA methylation and histone acetylation

Epigenome-wide association studies (EWASs) are helping in the discovery of environmentally mediated molecular changes in PCOS from disease pathogenesis to the discovery of epigenetic markers. Recent epigenetic studies offer persuasive evidence linking epigenetic regulation with PCOS etiology, presentation, clinical phenotypes, and comorbidities, which could potentially lead to improved disease prevention and management via precisely targeted strategies. Several pivotal biological pathways have been repeatedly reported by independent groups, supporting functional regulation by endocrine abnormalities and metabolic dysfunction in PCOS, while also suggesting an autoimmune component in the syndrome [29]. Increasing application of high-throughput sequencing technologies for epigenome analysis combined with evidence-based causal inference should facilitate precision PCOS prevention/treatment in the future.

Vázquez-Martínez et al. recently reviewed the topic of DNA methylation in women with PCOS [5]. Alterations in DNA methylation, histone acetylation and non-coding RNAs have been found in diverse tissues of women with PCOS. DNA methylation abnormalities appear in peripheral and umbilical cord blood, and in ovarian and fat tissue of women with PCOS, suggesting a pivotal role for these epigenetic modifications in the pathogenesis of this disorder. Possibly, these derangements in DNA methylation facilitate deregulation of gene expression involving inflammation, hormone biosynthesis and signaling, as well as glucose and lipid metabolism. The authors have compiled an extensive table of the tissues in which methylation abnormalities are encountered in women with PCOS indicating whether the involved DNA is hypo- or hypermethylated, the changes in gene expression, if any, related to the methylation variants, and any documented clinical/phenotypic expression resulting from these changes. Interestingly, both hypomethylation of some genes and hypermethylation of others may predispose to PCOS.

3.2 Epigenetic effects of hyperandrogenism

Qu and colleagues studied the effects of hyperandrogenism on the expression of histone deacetylase 3 (HDAC3), peroxisome proliferator-activated receptor gamma 1 (PPARG1), and nuclear corepressor 1 (NCOR1) genes in the granulosa cells of women with a hyperandrogenic form of PCOS, compared with women with non-hyperandrogenic PCOS, women without PCOS who had tubal infertility, and a rodent model of PCOS [30]. NCOR1 and HDAC3 mRNA expression was higher in the hyperandrogenic women than in normo-androgenemic women with PCOS and controls ($P < 0.05$). When all women were divided into successful and failed pregnancy subgroups, they found lower PPARG1 mRNA levels and higher NCOR1 and HDAC3 mRNA levels in the failed subgroup with hyperandrogenic PCOS ($P < 0.05$). Two hypermethylated CpG loci in the PPARG1 promoter and 5 hypomethylated CpG loci in the NCOR1 promoter were encountered only in the hyperandrogenic women with PCOS ($P < 0.01$–$P < 0.0005$). The acetylation levels of histone H3 at lysine 9 and p21 mRNA expression were low in human granulosa cells cultured with dihydrotestosterone in vitro ($P < 0.05$). A PCOS rodent model also displayed abnormal PPARG1, NCOR1, and HDAC3 mRNA expression and methylation alterations of PPARG1 and NCOR1, consistent with those found in women with hyperandrogenic PCOS. A strength of this study is the consistent effect of hyperandrogenism in the induction of epigenetic changes in PPARG1, NCOR1, and HDAC3 in granulosa cells in hyperandrogenic

women and rodents with PCOS as well as in vitro, which have a role in the ovarian dysfunction encountered in women with a hyperandrogenic PCOS phenotype.

4. Parasitosis as a cause of PCOS

When considering our genome and our epigenome we often lose sight of the fact that the organisms that live within us and on us, though having a different number of chromosomes than the cells we think of as human with somewhat different gene sequences, contribute to our total genome and epigenome. In sheer number, the cells of our biome far exceed the number of cells we think of as human. The character and density of their gene products profoundly influence our hormonal, metabolic, and immune milieu, and even our mood and personality. In the case of parasites, they are in turn hosts to biomes of their own.

As mentioned in the Introduction, we have published the case history of a woman who had PCOS associated with extensive neurocysticercosis [8]. She had refused standard treatment with albendazole for her parasitosis (which she presumably acquired in her native Mexico) because of fear of drug side effects that some of her affected friends had experienced. She had been referred to our clinic because of complaints of worsening hirsutism and amenorrhea x 2 years. She was 32 years old G1P1001. Diagnostic work-up fulfilled Rotterdam criteria for PCOS with amenorrhea, hirsutism, low sex hormone binding globulin, and an elevated LH/FSH ratio. Non-classic adrenal hyperplasia, pregnancy, and virilizing tumors were excluded by appropriate tests. Hypovitaminosis D was excluded by measurement of vitamin D metabolites, however, her serum 1,25(OH)2-vitamin D3 level was elevated. Treatment with lifestyle modification (weight loss diet, prescribed exercise), and gradually uptitrated doses of metformin to 2000 mg/day was associated with a gradual reduction in hirsutism and a return of menses, although still with oligomenorrhea. SHBG rose slightly and there was normalization of the LH/FSH ratio.

We wanted to know whether her extensive burden of neurocysticercosis was playing a role in the etiopathogenesis of her PCOS, perhaps by pressing on the GnRH cells of the hypothalamus, however, the neuroradiologist could find no evidence of anatomic hypothalamic involvement by the encysted parasites. We also considered the possibility that her elevated serum 1,25-(OH)2-vitamin D3 elevation was due to the formation of granuloma-like lesions around the encysted parasites with either the encysted parasites or the surrounding mononuclear cells synthesizing 1,25(OH)2-vitamin D3 in excess, as occurs in other granulomatous disorders like pulmonary sarcoidosis and tuberculosis. We also performed a literature search for associations between cysticercosis and PCOS. While we did not find any reports of such an association, we did learn that Taenia sp. prefer female to male hosts, and pregnant to non-gravid hosts [31–33]. It was later learned that Taenia sp. have steroidogenic enzymes and can synthesize steroid hormones e.g., estradiol [34–39]. As the cysticercosis burden increases, the host, whether female or male, will be further estrogenized, rendering the host milieu more favorable to the parasites. While PCOS is correctly considered a hyperandrogenic condition in most women, it is also important to remember that it is also a state of unopposed estrogen effect in anovulatory or oligo-ovulatory women. The sustained estrogen effect would be conducive to Taenia parasitization and increasing cysticercosis burden. In addition, Taenia sp. can metabolize the relatively weak androgen, androstenedione, to the more potent androgen, testosterone [34].

In searching further, we learned that the selective estrogen receptor modulator (SERM), tamoxifen, had successfully reduced cysticercosis burden in a murine model [40]. Since our patient continued to decline standard treatment for cysticercosis we offered her a trial of treatment with another SERM, raloxifene, which did not carry the risk of estrogenic endometrial stimulation reported with tamoxifen [41]. We thoroughly reviewed the article by Vargas-Villavicencio et al. with our patient and carefully explained that raloxifene was an approved and generally safe drug in the US for the treatment of post-menopausal osteoporosis/osteopenia, but not for neurocysticercosis. We explained that it was similar to, but distinct from and safer than the tamoxifen used in that article. We emphasized the importance of avoiding conception during the trial using abstinence or reliable barrier contraception as this was an FDA Category X drug (should not be used in pregnancy). We obtained her informed consent and initiated treatment with raloxifene at the standard dose for osteoporosis/osteopenia of 60 mg/day. When she returned to clinic, about 7 weeks after starting raloxifene, she related that she thought she might be pregnant and that, despite being forewarned, she had had unprotected intercourse on a few occasions. Pregnancy was confirmed by physical examination and serum HCG level, and she was counseled on her options. She elected to terminate her pregnancy. Following termination, a repeat brain MRI was performed. It was read by the same neuroradiologist who had read her baseline study. He was blinded regarding her treatment between the 2 studies. On the repeat study the total number of encysted lesions fell from 37 to 33, 10 lesions shrunk, 5 disappeared, 18 were unchanged, 4 enlarged and 1 new lesion appeared. Subsequently, after the patient belatedly agreed to and underwent standard treatment with albendazole and dexamethasone, serum 1,25-(OH)2-vitamin D3 fell from 81 to 41 pg/ml while 25-OH-vitamin D level only fell from 34 to 30 ng/ml. This reduction in calcitriol level occurred even though dexamethasone has been reported to increase the serum concentration of this metabolite [42].

This was the first case to be reported of human neurocysticercosis wherein modification of the hormonal milieu was associated with a reduction of cestode burden. The pregnancy on raloxifene, though unfortunate, supported the concept that neurocysticercosis contributed to the pathogenesis of her PCOS. Serum 1,25-(OH)2-vitamin D3 may ultimately prove to be a useful biomarker for assessing disease activity in neurocysticercosis, as it is in several other granulomatous disorders [43]. This report and the preclinical reports preceding it conceptually opened the field of biome contribution to endocrine disorders.

5. The Biome in the pathogenesis and maintenance of PCOS

Yurtdaş and Akdevelioğlu recently reviewed the literature on the gut biome and PCOS [44]. While genetic, neuroendocrine, epigenetic and metabolic factors are reported to contribute to the pathogenesis of PCOS, knowledge of the etiologies of the syndrome(s) remains incomplete. Recently, studies in humans and preclinical models have found associations between alterations in the gut microbiome and the metabolic/clinical features of PCOS.

It is theorized that gut dysbiosis could be a pathogenetic factor in PCOS. Accordingly, changing the gut microbiome using probiotics, prebiotics, and synbiotics as well as diet may serve as a new therapeutic modality for PCOS. Specific changes of the gut microbiome in women with PCOS are apparently associated with distinct PCOS phenotypes. Several recent studies indicate that IR, sex steroid concentrations,

and obesity alter the quantity, diversity and species composition of gut bacteria in women with PCOS (and vice versa).

Liang and colleagues studied gut biome dysbiosis in PCOS in association with obesity [45]. They recruited 8 obese women with PCOS, 9 lean women with PCOS, and 9 lean control women. Gut bacterial composition was assessed by PCR. Obese women with PCOS were found to have lower observed bacterial structural variants (SVs) and alpha diversity (a composite of different measurements that estimate diversity in a single sample) than the control group, higher beta diversity (a measure of the similarity/dissimilarity of 2 communities) than the lean PCOS group ($P < 0.05$), and lower abundances of genera (particularly butyrate producers). Regression analysis demonstrated that decreased abundances of several bacterial genera correlated with higher serum testosterone and impaired glucose tolerance. PCOS was associated with alterations in the gut microbiome population. Obesity appears to have a critical role in the development of a dysbiotic gut microbiome in women with PCOS.

Lindheim et al. studied associations between changes in the gut microbiome composition and gut barrier function and metabolic and reproductive abnormalities in women with PCOS [46]. Gut microbiome composition was assessed in stool samples from women with PCOS (n = 24) and healthy control women (n = 19) using 16S rRNA gene amplicon sequencing. Processing of data and microbiome analysis were performed in mothur and QIIME utilizing differing relative abundance cut-off points. Integrity of gut barrier function, inflammation, and endotoxemia were assessed using serum and stool indicators. Correlations with anthropometric, metabolic, and reproductive measures were then calculated. The stool microbiome of women with PCOS demonstrated lower bacterial species diversity and an altered phylogenetic mix compared with controls. The authors did not find significant differences in any bacterial taxa with a relative abundance>1%. Among rare bacterial taxa the relative abundance of those from the order ML615J-28 (phylum Tenericutes) and from the family S24-7 (phylum Bacteroidetes) was significantly lower and was associated with unfavorable reproductive parameters in women with PCOS. Women with PCOS showed alterations in some, but not all markers of gut barrier function and endotoxemia.

Women with PCOS had less species diversity and an altered phylogenetic mix in their stool microbiome, which was associated with certain adverse clinical parameters. Gut barrier malfunction and endotoxemia were not pivotal factors in these women, however, they may contribute to the particular phenotype seen in some people with PCOS.

Given the accumulating data that the gut biome population contributes to the etiopathogenesis of PCOS it seems intuitive that "normalizing" the gut biome in the most rapid way possible, fecal transplant from a "healthy" woman to a woman with PCOS, might effect the most rapid amelioration of the syndrome with the least risk. Such an approach has been dramatically successful in treating pseudomembranous colitis [47]. Although there are no human studies to date, a small study assessing fecal transplant to treat PCOS in a rodent model has been reported with encouraging results [48]. This same study also found amelioration of PCOS in the model with isolated Lactobacillus transplantation.

While a relatively short term improvement in the gut biome is usually sufficient to treat antibiotic dysbiosis-related conditions like pseudomembranous colitis, more chronic conditions, like PCOS, metabolic syndrome, Type 2 diabetes, and inflammatory bowel disease seem to require long term lifestyle changes e.g. shifting from a Western-style diet high in sucrose, animal fat, and animal protein to a prebiotic/probiotic rich,

lower calorie, phytonutrient-rich, mostly plant-based diet and an increased amount of regular exercise in order to sustain the improved gut biome and remission of the disorder being treated [49, 50]. Plant-based diets of this type are accompanied by reduced inflammation, less gut permeability, reduced generation of reactive oxygen species (ROS), and improved insulin sensitivity.

5.1 Drugs which alter the gut biome

Certain drugs, chiefly those used to treat obesity, prediabetes, and T2DM are known to alter the gut biome favorably, while others, the best known of which are antibiotics, may cause dysbiosis with unfavorable metabolic consequences [51–53]. Among the drugs with beneficial gut biome effects which explain at least part of their clinical actions are metformin, the alpha-glucosidase inhibitors, the GLP-1 receptor agonists, and the dual GLP-1/GLP-2 receptor agonist, tirzepatide.

5.2 Bariatric surgery and the gut biome

In addition to diet and drugs, bariatric (metabolic) surgery may affect the gut biome [54]. The taxonomic make-up of the gut bacterial microbiome is significantly affected by metabolic surgery. The most frequent alteration reported in most preclinical and human studies is a relative decline in abundance of Firmicutes with an increase in Bacteroidetes, Proteobacteria, and its class Gammaproteobacteria (order Enterobacteriales, family Enterobacteriaceae, genus Escherichia). Interestingly, the gut microbiome population differs substantially in rodents and humans. Proteobacteria increase after metabolic surgery due to a higher gut lumen pH and higher levels of dissolved oxygen that favor growth of facultative aerobic bacteria and inhibit growth of anaerobic bacteria. Reduction in stomach volume after bariatric surgery increases luminal gastric and distal gut pH, resulting in altered bacterial populations and overgrowth. More alkaline gut pH favors growth of *Akkermansia muciniphila*, *Escherichia coli*, and Bacteroides spp. which are species more typical of the oral microbiome. The greater bacterial diversity postoperatively includes increases in the phyla Verrucomicrobia and Fusobacteria, and a lower proportion of Actinobacteria. It is interesting that the use of metformin is also associated with an increased growth of Akkermansia [55].

5.3 Alterations in the vaginal biome are also associated with PCOS

While far more research has been reported on the contributions of the gut biome to the pathogenesis and maintenance of PCOS, recently the possible role of the vaginal biome in PCOS has come under scrutiny [10]. Hong and associates obtained vaginal swabs from 39 women with recently diagnosed PCOS and 40 women without PCOS and compared them using 16S rRNA gene sequencing in a case control study. Screening values for possible bacterial biomarkers of PCOS were analyzed by receiver operating characteristic (ROC) curve methodology. There were significant differences in the vaginal biome bacterial populations between the 2 groups. The vaginal bacterial species in the PCOS group were more diversified than those in the control group (Simpson index of the PCOS group vs. the control group: median 0.49 vs. 0.80, $P = .008$; Shannon index: median 1.07 vs. 0.44, $P = .003$; Chao1 index: median 85.12 vs. 66.13, $P < .001$). This is in marked contrast to what has been reported for the gut biome, which is less diverse in women with PCOS, obesity, and T2DM than in healthy

control women. Relative abundance of *Lactobacillus crispatus* in the stool samples of the women with PCOS was significantly lower than in healthy controls (P = .001), and relative abundance of Mycoplasma and Prevotella was higher than in healthy control women (P < .001, P = .002, respectively). Adjustments for BMI and vaginal cleanliness grade did not change these associations. Genus Mycoplasma may be a biomarker for PCOS screening, since ROC analysis showed that the area under the curve (AUC) for relative abundance of Mycoplasma was 0.958 (95% CI, 0.901–0.999).

5.4 The oral cavity biome and PCOS

The oral cavity biome has also been explored in terms of PCOS [11]. This study was designed to investigate the hypothesis that the concentrations of suspected periodontal pathogens in saliva and the host serum antibody response is elevated in women with PCOS, compared with healthy controls. In total, 125 women in 4 groups were studied: 45 with PCOS+healthy periodontium, 35 with PCOS+gingivitis, 25 systemically and periodontally healthy women, and 20 systemically healthy women with gingivitis.

Salivary concentrations of 7 suspected periodontal pathogens were analyzed by quantitative real-time PCR, while serum antibody titres were measured by ELISA. In women who had PCOS, salivary populations of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Streptococcus oralis* and *Tannerella forsythia* levels were higher than in matched systemically healthy women, especially when gingivitis was also present. PCOS was also associated with increased *P. gingivalis*, *Prevotella intermedia*, and *S. oralis* serum antibody titres if gingivitis was present. The most consistent effect appeared to be the increased population of and antibody response to *P. gingivalis*.

In my search I could not find any reports of associations of the skin, aural, or nasal/sinus biomes with PCOS.

Although newer technologies e.g., 16S rRNA are a giant step forward in our understanding of biome/systemic disorder interactions, it is important to understand that the study of biomes is still in its infancy. Our microbiomes include viruses, fungi, prions, protozoa, and sometimes parasites, and algae. Future research will doubtless uncover important associations between these organisms/pre-organisms and systemic disorders like PCOS.

6. Endocrine disrupting chemicals and PCOS

Endocrine disrupting chemicals (EDCs), both environmental and drug, appear to contribute to the etiopathogenesis of PCOS. This may occur via binding to sex hormone receptors or by causing IR/hyperinsulinemia; additional mechanisms are also possible.

Environmental EDCs-In our species increased serum bisphenol A (BPA) concentrations have been reported in teenagers and women with PCOS compared with reproductively healthy controls and these are positively correlated with androgen levels, suggesting a role for this chemical in the etiopathogenesis of PCOS, although causality is yet be established [56–60]. It is possible that embryonic/fetal exposure to certain EDCs permanently changes reproductive, neuro-endocrine, and metabolic regulation favoring PCOS development, in genetically predisposed people, or hastening and/or exacerbating the natural course of the disorder via lifelong exposure.

In pre-clinical studies, exposure of mothers to BPA changes postnatal development and sexual maturation in the offspring. Exposure to dibutyl phthalate and

di(2-ethylhexyl)phthalate during pregnancy results in polycystic ovaries and a hormonal profile similar to that seen in human PCOS. Androgenic EDCs, nicotine, and 3,4,4′-trichlorocarbanilide, all contribute to the creation of a concerning hyperandrogenic embryonic/fetal milieu. Prenatal EDC exposure may contribute to abnormal embryonic/fetal developmental programming and partially explain the wide variability in PCOS phenotype.

Research has mostly focused on the possible roles of the most widely distributed and studied environmental agents suspected of contributing to the etiopathogenesis of PCOS. Plasticizers, including BPA and phthalates, which are known EDCs, and advanced glycation end products (AGEs) are ubiquitous in our milieu; therefore, our attention should be focused on reducing such exposure. The timing of EDC exposure is critical for understanding the diversity and severity of adverse health consequences. Embryos/fetuses, infants, and young children are the most vulnerable groups. Prenatal EDC exposure that imitates some actions of endogenous hormones may contribute to abnormal fetal programming and, ultimately, result in PCOS and other adverse health consequences, possibly even trans-generationally. Acute or more protracted EDC exposure and dietary (mostly from Western type diets), as well as endogenously formed AGE exposure in different stages of the life cycle can alter the hormonal milieu and result in disruption of reproductive function. AGEs are proinflammatory molecules capable of interacting with cell membrane receptors and mediate triggering of proinflammatory signaling pathways and oxidative stress. These agents may also contribute to metabolic changes, e.g., obesity, IR, and the compensatory hyperinsulinemia that can create or worsen the PCOS phenotype and contribute to its complications, e.g., Type 2 diabetes and cardiovascular disease. Prediabetes and T2DM both result in hyperglycemia, leading to the formation of even more AGEs in a vicious cycle [61, 62].

Large population surveys find countless chemicals in our serum and tissues that did not even exist in our grandparents' generation [60] Sadly, regulatory agencies are losing the race to evaluate these compounds for safety before they are released into our environment.

7. Drugs which may contribute to the pathogenesis of PCOS

In addition to the EDCs which accidentally find their way into our bodies, many prescription drugs may also contribute to the etiopathogenesis and maintenance of PCOS [61]. Most of the drugs which contribute to causing PCOS do so by causing IR/hyperinsulinemia. In so doing they often contribute to causing other disorders associated with IR, including metabolic syndrome, T2DM, hypertension, gout, dyslipidemia, and congenital adrenal hyperplasia [62, 63]. Among these drugs are some of the beta-blockers, thiazides and related diuretics, like indapamide, some of the inhibitors of the renin-angiotensin system, nicotinic acid, the fluoroquinolones (which may also contribute by causing bacterial dysbiosis), protease inhibitors, nucleoside reverse transcriptase inhibitors, antipsychotic drugs, especially atypical antipsychotic drugs, divalproex, and high estrogen oral contraceptives.

8. Role of vitamin D in PCOS

The role of vitamin D and polymorphisms in its receptor have been the subject of considerable research, given that vitamin D deficiency has been associated with

IR [63–68]. Vitamin D has a physiologic role in female reproduction, which includes ovarian follicle development and luteinization, by regulating anti-Mullerian hormone (AMH) signaling, follicle-stimulating hormone (FSH) sensitivity, and progesterone biosynthesis in granulosa cells. Vitamin D also affects glucose homeostasis via diverse routes. The evidence for an important role for vitamin D on glucose metabolism includes: the presence of vitamin D receptors in pancreatic β-cells and skeletal muscle, the expression of 1-α-hydroxylase enzyme in these tissues which catalyzes the 1-α-hydroxylation of 25-hydroxy vitamin D (25(OH)D) to 1,25-dihydroxyvitamin D, as well as the presence of a vitamin D response element in the human insulin gene promoter region. About 67–85% of women with PCOS have vitamin D deficiency. While there is no significant difference in serum 25(OH) D concentrations between women with PCOS and controls, a high prevalence of vitamin D deficiency is reported to be associated with metabolic syndrome.

Hypovitaminosis D may worsen the signs and symptoms of PCOS, such as IR, ovulatory and menstrual perturbations, infertility, androgen excess, obesity and increased risk of cardiovascular disease. Many observational reports support a role for vitamin D in an inverse association between women's vitamin D status and metabolic disturbances in PCOS, however, it is difficult to reach a conclusion regarding causality because of contradictory findings from various individual studies and from a recent meta-analysis.

Supplementation of vitamin D reduces abnormally elevated serum AMH concentrations and raises serum anti-inflammatory soluble receptor for AGEs in women with both vitamin D-deficiency women and PCOS. Notably, vitamin D and calcium added to metformin in women with PCOS and vitamin D deficiency improves menstrual regularity and ovulatory rate.

Low serum 25(OH)D concentrations are significantly associated with IR in women with PCOS, leading to suggestions that genes regulating vitamin D metabolism could be candidate genes for PCOS susceptibility. Certain polymorphisms in the vitamin D receptor (VDR) gene including: Cdx2, Taq1, Bsm1, Apa1, and Fok1, have been reported to play an important regulatory role on insulin secretion and sensitivity in women with PCOS. The VDR Fok1 polymorphism was found to have a protective effect against the risk of Type 2 diabetes mellitus, while the Bsm1 polymorphism augmented the risk of Type 2 diabetes. The Apa1 polymorphism has been reported to reduce the risk of vitamin D deficiency [65].

A study was carried out in India, to investigate the association pattern of 4 VDR polymorphisms (Cdx2, Fok1, Apa1 and Taq1) with PCOS among Indian women. They reported a significant difference in genotype and allele frequency distributions of the Cdx2 polymorphism between women with PCOS and controls. A significantly higher frequency of the heterozygous GA genotype and the A allele of Cdx2 was encountered in control women when compared to those with PCOS ($P < 0.001$), suggesting that this single nucleotide polymorphism (SNP) affords some protection against PCOS development. Following adjustment for the covariates of BMI and age, the carriers of the GA genotype and the A allele remained relatively protected against PCOS development. No other significant associations were encountered between the remaining 3 VDR polymorphisms (Fok1, Apa1 and Taq1) and PCOS. They also investigated associations between VDR genotypes and some PCOS clinical/biochemical characteristics and reported that the Cdx2 genotypes were significantly associated with serum testosterone levels while the Fok1 polymorphism showed a significant association with infertility. In addition, the 2 haplotypes made up of 4 polymorphisms, ACCA and ACTA, were also significantly associated with PCOS risk [64].

In a group of Austrian women with PCOS, the VDR Cdx2 polymorphism was found to be associated with higher insulin sensitivity, and the Apa1 polymorphism was associated with lower serum testosterone concentrations. Nevertheless, other investigators did not report any significant differences in the VDR gene polymorphism frequencies between women with PCOS and controls [65].

In a study from Taiwan, it was found that the VDR 1a promoter polymorphisms were not associated with the risk of PCOS but were associated with serum 25(OH)D levels. This study also found that significantly lower serum 25(OH)D levels were seen in women who carried the heterozygous 1521CG/1012GA haplotype of the VDR 1a promoter polymorphisms in both women with PCOS and controls. However, metformin was only able to increase serum 25(OH)D concentrations in women with PCOS who carried the homozygous 1521G/1012A haplotype [65].

Even though several polymorphisms in the VDR gene have been implicated in the etiopathogenesis and presenting phenotype of PCOS, there is considerable heterogeneity in reports from both individual investigators and meta-analyses. Therefore, the role of these VDR gene polymorphisms in the pathogenesis of IR and PCOS remains controversial [65].

Future research with large, independent cohorts and with diverse ethnic populations may clarify whether the associations between vitamin D and PCOS are ethnicity-specific or have differing thresholds depending upon the influence of other individual genotypes in women with PCOS.

A recent reanalysis of data from the D2d trial by the original study authors, using a Cox proportional hazards model, concluded that daily vitamin D intake, sufficient to achieve and maintain a serum 25-(OHD) level ≥ 100 nmol/l, is a promising approach to reduce the risk of T2DM in adults with prediabetes, in contrast with their original conclusion, that vitamin D administration was not effective in the prevention of T2DM in those with prediabetes [67, 68].

9. Autoimmunity contributing to the etiopathogenesis of PCOS

In addition to the Type B syndrome of severe insulin resistance, acanthosis, SLE with nephritis, & PCOS discussed in the Introduction, several other autoimmune disorders are associated with PCOS [69, 70]. These include vitiligo, alopecia areata, and the autoimmune polyglandular syndrome. Autoimmune thyroid disease, especially autoimmune (Hashimoto's) thyroiditis, is about 3x more common in women with PCOS compared with controls [70]. Among the reasons cited for these associations are the sustained high estrogen/progesterone ratios in women with PCOS, which prenatally derail embryonic/fetal thymic development and disrupt thymic function as regards preservation of immune self-tolerance, vitamin D deficiency/insufficiency and VDR gene polymorphisms, as well as similarities in the gut biome in people with PCOS and autoimmune disorders, such as increase in those species causing more gut permeability and a reduction of overall bacterial species diversity. These biomic changes are also seen in obesity, metabolic syndrome, and T2DM. In addition, 3 genetic polymorphisms have been reported as predisposing to both PCOS and Hashimoto's thyroiditis. They are polymorphisms of the genes for gonadotropin releasing hormone receptor, fibrillin 3, regulating the activity of transforming growth factor-β and regulatory T cell levels, and CYP1B1 affecting estradiol hydroxylation.

10. PCOS resulting from insulinoma or nesidioblastosis

Murray and colleagues reported PCOS in association with an insulinoma, which resolved following successful removal of the tumor [71]. My literature search did not find any reports of nesidioblastosis-associated PCOS, however, it is predictable, given their chronic hyperinsulinemia, that such individuals will eventually be found.

11. Insulin resistance is not global in PCOS

While there is evident insulin resistance in people with PCOS in terms of carbohydrate, lipid, and uric acid metabolism, there is also evidence of normal or even increased insulin action in features such as hyperandrogenism, acanthosis nigricans, acrochordons, organomegaly, and visceral obesity.

There are 2 major signaling pathways through which insulin's actions are expressed: one signaling cascade is used to regulate intermediary metabolism while the other modulates growth and cell division as well as the hypothalamic/pituitary, gonadal and adrenocortical axes. Regulation of these 2 distinct cascades may be dissociated and data suggest that the activity of the signaling pathway which governs intermediary metabolism is decreased in people with PCOS, T2DM, metabolic syndrome, gout, and congenital adrenal hyperplasia, while the pathways modulating growth processes and mitoses is normal or even enhanced [72]. Most of the intermediary metabolism pathway is activated by insulin binding to its own receptor followed by phosphorylation of IRS-1 and IRS-2. Some of the pathway regulating growth and cell division is initiated by insulin binding to IGF-1 receptors. Even though insulin has greater affinity for its own receptor, when insulin levels are high its receptor is downregulated, limiting available binding sites, so that "excess" insulin will bind to the IGF-1 receptor as an agonist, mimicking the effects of growth hormone. When activation of the IGF-1 cascade is extreme it is sometimes referred to as pseudo acromegaly [73]. Studies show that insulin's signaling pathways normally regulate cell growth, metabolism and survival via activation of mitogen-activated protein kinases (MAPKs) and phosphotidylinositide-3-kinase (PI3K). Activation of PI-3K-associated with insulin receptor substrates-1 and -2 (IRS1, 2) and the subsequent Akt → Foxo1 phosphorylation cascade plays a pivotal role in regulating nutrient homeostasis and organ survival. Several mechanisms have been suggested as causes contributing to the development of IR and metabolic syndrome. These include genetic polymorphisms of proteins in the insulin signaling cascade, suboptimal fetal nutrition, and increased intra-abdominal fat. IR develops as the key player in a cluster of cardiovascular/metabolic dysfunctions we now recognize as metabolic syndrome, which may result in T2DM, a distinctive (Type IV, Fredrickson) dyslipidemia with high VLDL, low HDL, and normal-moderately elevated LDL, accelerated atherosclerosis, hypertension, or congenital adrenal hyperplasia depending on the genetic/epigenetic background of the person with IR including the genetic/epigenetic characteristics of our relevant biomes, vitamin D status, and the influence of drugs and environmental chemicals with endocrine disruptor effects. Inactivation of Akt and activation of Foxo1, via suppression of IRS1 and IRS2 in different tissues following hyperinsulinemia, metabolic inflammation, and overnutrition could be the mechanisms leading to metabolic syndrome in our species [74].

IR in women with PCOS seems to be associated with exaggerated serine residue phosphorylation of insulin receptor substrates. An enzyme extrinsic to the insulin receptors, quite possibly a serine/threonine kinase, causes this aberration and exemplifies a key mechanism for induction of human IR related to extrinsic factors regulating insulin receptor signaling. Serine phosphorylation seems to regulate the activity of P450c16, the pivotal regulatory enzyme in androgen biosynthesis. It is very possible that a single defect results in both IR and hyperandrogenism in some women with PCOS. This IR is selective, affecting glucose/lipid metabolism, but not cell division or growth [75].

12. Sleep disorders and PCOS

It has been reported that women with PCOS have significantly higher risk of obstructive sleep apnea (OSA). OSA severity is significantly correlated with plasma glucose and insulin levels and homeostasis model assessment for insulin resistance (HOMA-IR)-index in women with PCOS. It appears that the progressive worsening of PCOS results in OSA which, in turn, exacerbates the metabolic disturbances, such as IR, associated with this syndrome [76].

Clinic-based studies report that sleep disturbance and disorders such as OSA and excessive daytime sleepiness are more frequently encountered among women with PCOS. Data from the few published population-based studies is substantially concordant. Women with PCOS are mostly overweight/obese, however, this fact only partially explains their sleep problems as significant associations persist after adjusting for body mass index; sleep issues also occur in lean women with PCOS. There are several, likely bidirectional, pathways through which PCOS and sleep disturbances are associated. PCOS pathophysiology includes hyperandrogenemia, a unique form of IR, and possible changes in cortisol and melatonin secretion, plausibly reflecting hypothalamic-pituitary-adrenal dysfunction. Psychological/behavioral factors probably also play a role, such as anxiety and depression, tobacco use, alcohol use, and insufficient exercise which are also frequent among women with PCOS, likely in response to their symptoms. The effects of sleep disturbances on the health of women with PCOS is not completely understood, however, both PCOS and disordered sleep are associated with worsening long term cardiometabolic health and augmented T2DM risk. Immediate quality of life and long-term health status of women with PCOS will likely improve from timely diagnosis and comprehensive management of sleep disorders [77].

13. Light pollution as a contributing cause of PCOS

Several investigators have reported that exposure of rats to continuous light can induce PCOS; however, hyperandrogenism, a key feature of human PCOS, has not been reported previously. In Kang et al.'s article they reported that (a) body weight declined in female rats in continuous light conditions with both ovarian and uterine augmentation; (b) the estrous cycle in rats living in continuous light was disordered, and PCOS-like changes were noted accompanied by hair loss and lethargy; and (c) serum testosterone levels rose significantly in rats living in continuous light. Their results suggest that continuous light can lead to PCOS in female rats without the need for drugs. Poor sleep habits, faulty sleep hygiene, and light pollution may be important contributors to the pathogenesis of PCOS [78].

Dominoni and colleagues as well as others have described reproductive hardships in free-living wildlife associated with light pollution [79].

14. Noise pollution and reproduction

In addition, human-generated noise pollution has been implicated in reduced reproductive success in wildlife, although the mechanisms involved are not clear [80].

15. Undiagnosed non-classic adrenal hyperplasia (NCAH)

Based on my years in clinical practice and academia, I hope readers will indulge me in a personal gripe. When applying the Rotterdam criteria for the diagnosis of PCOS many clinicians ignore or only pay lip service to the exclusions which must be considered an essential part of these criteria. These include thyroid disease, Cushing's syndrome, androgen-secreting neoplasia, hyperprolactinemia, and non-classical congenital adrenal hyperplasia. In my referral practice I found, in reviewing the referral or the written or electronic medical records of patients referred to me for PCOS treatment, that these conditions, especially NCAH had very seldom been excluded by the referring colleague. In the PCOS research literature many investigators do not mention exclusion of these disorders in their PCOS cohorts. In many other articles a single morning unstimulated serum 17-OH-progesterone is proffered as excluding NCAH. The best articles offer a cosyntropin-stimulated 17-OH-progesterone to exclude this diagnosis. In my readings I have not yet encountered a study where NCAH was thoroughly excluded with genetic testing for 21-hydroxylase deficiency as well as cosyntropin stimulation of 17-OH-progesterone, 17-OH-pregnenolone, 11-deoxycortisol, deoxycorticosterone, corticosterone, and 18-OH-corticosterone. Thus, without fully testing for NCAH, most of us have the impression that PCOS is very common and NCAH, except in high-risk ethnic groups is very rare. This is concerning because NCAH and PCOS are often phenotypically identical. However, since therapies aimed at decreasing IR, normalizing the menstrual cycle, reducing androgen secretion or expression, and inducing ovulation are often able to ameliorate both conditions the real-world consequences of misdiagnosis of PCOS may not be as grave as we might expect [63]. Carbunaru and colleagues have reported that the common, non-classic or phenotypic form of 3-beta-ol dehydrogenase deficiency (3-beta-HSD) controlling the adrenal/ovarian synthesis of this enzyme is not associated with an exonic polymorphism, but is associated with IR, hyperandrogenism, and a PCOS phenotype, which in severe forms is called Hyperandrogenism, Insulin Resistance-Acanthosis Nigricans (HAIR-AN) syndrome [81]. It is possible, that a polymorphism may exist in the promoter region of the gene, as has been reported in a group of Brazilian women with non-classic 21-hydroxylase deficiency [82]. Alternatively, several epigenetic modifications could be downregulating the expression of the gene.

16. Lipodystrophies as a cause of PCOS

Lipodystrophies are associated with PCOS due to insulin resistance, which is intrinsic to the lipodystrophies [83, 84].

17. Conclusions

In this chapter I have tried to highlight truly rare contributing causes of PCOS, like insulinomas, as well as showcasing causes that are not particularly rare, but are very rarely considered in clinical practice. The latter include: biomic alterations, epigenetic disturbances such as disordered DNA methylation and/or histone acetylation, and EDCs, including many drugs which contribute to IR. In addition, I have described some very rarely reported causes, like cysticercosis, which, given its extensive global endemicity, will likely turn out to be much more common causes of PCOS than is currently recognized. In exploring this topic, I hope that I have shed some light on common pathways by which these diverse agents might contribute to the etiopathogenesis and maintenance of PCOS, mostly by causing IR/hyperinsulinemia, hyperandrogenism, chronic inflammation, or unopposed estrogenic effects. It is hoped that clinicians will consider these causes more often when evaluating their patients and considering treatments. In so doing, it is likely that better treatment results can be achieved. It is already possible for individual clinicians and their patients to achieve much with interventions such as lower calorie, plant-based diets, supplementation with pre- and probiotics, exercise, ensuring adequate vitamin D status, and choosing drugs with favorable effects on the biome. In addition, patients once educated, may be able to improve their therapeutic outcomes by minimizing their exposure to EDCs in plastics, self-care products, and household products. Major improvements in outcomes may result from efforts at the community, regional, national, and international levels to improve diets, increase exercise, and reduce our exposure to EDCs, light and noise pollution. Attention to sleep hygiene by patients and providers may further reduce the burden of PCOS, metabolic syndrome, resistant hypertension, and T2DM. Fecal transplantation may jump start amelioration of PCOS, provided it is followed with sustained lifestyle changes including plant-based diets, exercise, and possibly pre- and probiotic supplementation. Looking toward the future, the experience we have gained in developing mRNA vaccines against COVID-19 might be applied to develop mRNA "vaccines" against gene products whose overabundance is contributing to PCOS. A fragment of the mRNA could be used to synthesize a fragment of the peptide different enough from the native protein to provoke an adaptive immune response.

Our understanding of the biome and of epigenetics is still in its infancy. As more is learned the opportunities for precision prevention and treatment will increase.

Conflict of interest

The author declares no conflict of interest.

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