CHAPTER

27

Genetic and Environmental Factors in the Etiology of Polycystic Ovary Syndrome

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Abbreviations

5-αR	5-α reductase
5-HT	5-hydroxytestosterone
AGE	advanced glycation end product
AR	androgen receptor gene
BMI	body mass index
BPA	Bisphenol A
CARDIA	coronary artery risk development in young adults
CRP	C-reactive protein
DEHP	di (2-ethylhexyl) phthalate
EDCs	endocrine-disrupting chemicals
EPHX1	epoxide hydrolase 1 gene
FBN3	fibrillin 3 gene
FMT	fecal microbiota transplantation
FSH	follicle-stimulating hormone
FSHB	follicle stimulating hormone beta polypeptide gene
FSHR	follicle stimulating hormone receptor gene
FTO	fat mass and obesity-associated gene
GLO-1	glyoxalase 1
GLUT4	glucose transporter type 4
GWAS	genome-wide association study
hCG	human chorionic gonadotrophin
HOMA IR	homeostasis model of insulin resistance
HPA	hypothalamo-pituitary adrenal
INSR	insulin receptor gene
LH	luteinizing hormone
LHCGR	LH/choriogonadotrophin receptor gene
LMNA	lamin A/C gene
MC4R	melanocortin receptor 4 gene
MG	methylglyoxal
miRNA	micro-RNA
NF-κB	nuclear kappa B
NIH	National Institutes of Health
OR	odds ratio
OSA	obstructive sleep apnea
PCO	polycystic ovarian
PCOS	polycystic ovary syndrome
PCR	polymerase chain reaction
PFAAs	perfluoroalkyl acids
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PPARα	peroxisome proliferator-activated receptor alpha
RAGE	receptor for advanced glycation end products
SES	socioeconomic status
SHBG	sex hormone-binding globulin
SNP	single-nucleotide polymorphism
sRAGE	soluble receptor for advanced glycation end products
T2D	type 2 diabetes mellitus
TCC	triclocarban
VNTR	variable number of tandem repeats
VO_2 max	maximal aerobic capacity
XCI	X-chromosome inactivation

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common female endocrinopathy, with a prevalence of between 6% and 10% in reproductive-age women [1]. PCOS can manifest at any stage of reproductive life in women, but often develops during adolescence [2]. PCOS is characterized phenotypically with cardinal reproductive features (including primarily oligo-amenorrhea and impaired fertility), symptoms of hyperandrogenism (hirsutism, acne, and androgenic alopecia), and/or raised serum androgens such as testosterone [2]. In addition to reproductive and hyperandrogenic features, PCOS is also a metabolic disorder associated with insulin resistance and heightened risk for the development of type 2 diabetes mellitus (T2D) [3], impaired glucose tolerance, dyslipidaemia, nonalcoholic fatty liver disease [4], and obstructive sleep apnea (OSA) [5]. Obesity in women with PCOS greatly increases the risk of insulin resistance and other dysmetabolic features [2,6].

PCOS has a complex etiology, our understanding of which is incomplete. There are a number of factors that make PCOS an inherently difficult condition to study. These include the following:

- Heterogeneous clinical features, including ethnic and regional differences
- Subfertility, hindering transgenerational studies

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- A condition that only affects women of reproductive age
- The confounding effect of obesity, making it difficult to determine which features are specific to PCOS and which are simply obesity dependent
- Inherent ethical difficulties regarding research studies in adolescent girls (when PCOS most often becomes manifest)
- Lack of a clear and incontrovertible diagnostic test
- Existence of multiple diagnostic criteria and lack of global consensus regarding diagnostic features
- Nonspecific clinical features that are mimicked by numerous other conditions (such as Cushing Syndrome, congenital adrenal hyperplasia, and hyperprolactinaemia), with potential for misdiagnosis
- Confusion regarding nomenclature [with polycystic ovarian (PCO) morphology being confused for PCOS]

Despite these inherent problems, it is essential that we strive to improve our understanding and insight into the complex etiology of PCOS. There are three main reasons why this is important. The first is that we currently lack effective treatment strategies for this common condition. Our existing therapies are empirical and directed to management of symptoms. What we really need to develop are therapies that address the underlying pathogenic factors of this condition, and this will require a clear understanding of etiology. Although it could be argued that weight loss provides such a solution, until we have effective and widespread weight-loss therapies, this technique will remain only partially effective. The second reason is to provide insight into preventive management. If we know the factors that lead to development of PCOS, we can develop strategies to intervene and prevent or modify this sequence of events. In the future, such preventive strategies are likely to include genetic (or epigenetic) manipulations and other biotechnology that, for example, may reduce exposure to environmental factors, or modify our gut bacteria, for example. The third reason is that a clear understanding of etiology will enable effective screening and diagnostic tests to be developed. Such tests will enable more efficient diagnosis and improve our ability to preempt future development of PCOS in girls who are most at risk.

In this chapter, our current understanding of the etiology of PCOS will be discussed in Sections "Genetic Factors," "Epigenetic Modifications," and "Environmental Factors." In each section, the main hypotheses, with supporting evidence, will be discussed. The implications of such evidence for our current understanding of the etiology and management of PCOS will be discussed, and future directions suggested.

GENETIC FACTORS

PCOS has a significant genetic basis [7]. In a large Dutch twin study, the concordance of symptoms of PCOS was twice as high in monozygotic twins (70%) than in dizygotic twins [8]. It is likely that a number of genes are implicated in the development of PCOS, i.e., it is an "oligogenic" condition [9].

Results of the many candidate gene studies (including those inadequately powered) have yielded few, if any, conclusive results, and genome-wide association studies (GWAS) in PCOS, are in the early stages so that the underlying genetic architecture of this complex condition remains largely elusive. Furthermore, the genetic variants thought to underlie PCOS development that have been identified from such studies, account only for a small proportion of its heritability, leaving the vast majority of PCOS heritability largely unaccounted for. It is possible that epigenetic modifications (discussed in the next section) may explain some of this missing heritability. It is also possible that genetic contributors to weight gain [obesity itself being heritable [10]] contribute to the missing heritability of PCOS.

In this section, the main genetic studies in PCOS reported on to date will be discussed in Sections "GWAS" and "Candidate Gene Studies."

Genome-Wide Association Studies

One advantage of the genome-wide association study (GWAS) is that it does not rely upon any a priori understanding of etiology. This is particularly relevant in a condition such as PCOS, with a complex and varied phenotype. Furthermore, in a GWAS, the entire genome is screened, rather than just one of the 20,000 possible genes as occurs in the candidate gene approach. Such an approach has the potential to shed novel insights into pathogenesis through identification of hitherto unknown and unknowable gene variant associations. However, due to the number of gene variants assessed in a GWAS, the level of significance for association is much stricter than that in the candidate gene approach, and this requires enrolment of typically many thousands of subjects to achieve adequate power. This is particularly relevant for a condition such as PCOS in which the effect size of each individual gene variant is likely to be relatively low. Such studies inevitably require multicentre and multinational collaborations to achieve meaningful results.

The GWAS has risen to prominence in recent years, not just for studying genetics of PCOS but in the study of a plethora of human diseases. Within the field of PCOS, most of the GWAS to date have focused on subjects of

Chinese or European origin. The main results from these reported studies are discussed later:

Chinese-Ancestry Studies

GWAS in PCOS based on Chinese-ancestry populations have shown association of PCOS with variants within eleven single-nucleotide polymorphisms (SNPs). These include 2p16.3, 2p21, and 9q33.3 [11,12]. Hayes and colleagues demonstrated nominal replication of seven of these SNPs in a European-ancestry population (including SNPs within *LH/CGR* and *FSHR* which are gonadotrophin receptor genes) [13].

European-Ancestry Studies

One European-ancestry-based study included >980 women with PCOS [diagnosed based on NIH criteria for PCOS [14]] and >2900 population controls, with replication in >1700 PCOS cases and >1200 control women and further replications in the top variants within each region with a *P*-value $<5\times10^{-6}$ [13]. From this study, three loci were associated with PCOS at genome-wide significant threshold: 11p14.1 (*FSHB/ARL14EP* locus), 8p32.1 (*GATA4/NEIL2* locus), and 9q22.32 (*c9orf3/FANCC* locus). One of these loci, 9q22.32 had previously been identified to associate with PCOS in a GWAS based on women of Chinese ancestry [12]. The other two loci (11p14.1 and 8p32.1) were novel and had not been identified in any prior GWAS in PCOS. *FSHB* encodes follicle-stimulating hormone (FSH) beta polypeptide, a component of glycoprotein hormones including luteinizing hormone (LH) [15]. Furthermore, the lead SNP at the 11p14.1 *FSHB/ARL14EP* locus (rs11031006) associates strongly with LH levels [13]. It seems plausible therefore that the association of PCOS with variants within *FSHB* as demonstrated in this GWAS [13] is mediated via effects on LH [13]. With regard to *GATA4* (8p32.1 locus), this is known to encode a zinc-finger transcription factor that is implicated in the transcriptional control of genes involved in steroidogenesis and regulation of gonadal development [13]. *GATA4* is also expressed in ovarian follicles [16]. There are a number of potential mechanisms whereby variants within *GATA4* may influence the etiology and development of PCOS.

A further reported GWAS in PCOS that include women of European ancestry identified six signals for PCOS at genome-wide significance [17]. These signals occurred in or in close proximity to a number of genes: *ERBB4/HER4* (encoding epidermal growth factor receptors), *FSHB*, *YAP1*, *THADA*, *RAD50*, and *KRR1* [17].

Across all of the GWAS (both of Chinese- and European ancestry) reported on to date, there are a total of 16 replicated loci with genome-wide significance for association with PCOS [11–13,18–20]. As outlined earlier, however, each locus only has a small effect size on PCOS susceptibility, and collectively only a tiny proportion of PCOS heritability is accounted for by these 16 loci [13]. However, despite this, the GWAS data reported to date across Chinese- and European-ancestry populations have provided important insight into etiology of PCOS. These are summarized as follows:

- The implication of genes affecting gonadotrophin secretion suggests that these may play a significant role in the development of PCOS [13]. These, in turn, may drive anovulation and hyperandrogenism that characterize PCOS [13].
- The importance of gonadotrophin *action* in the development of PCOS. Gene variants that modulate gonadotrophin action at the LH and FSH receptors (encoded by *LHCGR* and *FSHR*, respectively) have been implicated [13].
- A possible genetic role for the control of steroidogenesis through variants within *GATA4* [13].
- Finally, the reported GWAS provide some insight into the evolutionary development of PCOS, with significant commonality between data from both European- and Chinese-ancestry cohorts, together with other gene variants that appear to be specific to each population. Further GWAS in more diverse populations, including African ancestry will be required to further explore the evolutionary genetic origins of PCOS, and how this mirrors the spread of our species, Homo sapiens out of Africa and around the globe. Such data will provide important insights into the underlying ethnic and regional heterogeneity that typifies PCOS.

GWAS studies are useful for providing genetic clues to etiology. However, understanding of etiology requires further functional studies, to decipher the cellular role of the genetic variants identified, and how these may impact on physiology and on reproductive and metabolic function in PCOS. Already, functional studies based on GWAS data from PCOS have been reported. These include the roles of *DENND1A*, *LHCGR*, *INSR*, *RAB5B* and various adapter proteins. Such studies have focused on the genetic regulation of theca cell androgen biosynthesis [21]. Further functional studies will be reported in the future, and these will provide deeper insight into the etiology of PCOS based on GWAS data.

Candidate Gene Studies

Most of the genetics studies on PCOS reported in the literature to date have employed a candidate gene approach: variants in more than 100 genes have been studied for association with PCOS using such an approach [22].

However, few of these studies have satisfied the rigorous criteria that are demanded to assess the true significance of any positive findings. These include attention to (1) stratification of the populations (cases and controls should be as homogeneous as possible); (2) adequate sized cohorts of cases and controls; (3) correction of any positive "hits" in candidate loci for multiple comparisons; and (4) most importantly, replication in other, independent series of cases and controls. Nevertheless, it is useful to review data from the main studies:

Steroid Production and Metabolism

A key step in the steroid pathway is the cleavage of the P450 side chain, which is catalyzed by a rate-limiting enzyme called P450 11a1 (encoded by the gene *CYP11A*). The literature on variants within *CYP11A* and susceptibility for development of PCOS is controversial [7]. However, some of our best evidence comes from one of the largest studies on *CYP11A* variants based on >370 UK-based PCOS cases, >330 population controls, and >1500 women from the Northern Finland Birth Cohort of 1966 (NFBC1966). Association of PCOS with promoter allele 4 of *CYP11A* was demonstrated, although no association with promoter allele 5 [23]. Replication in other populations though is required to validate these findings.

Hyperandrogenism in PCOS is influenced by 5α-reductase activity, which increases conversion of testosterone into dihydrotestosterone (a potent androgen), and also enhances breakdown of cortisol, which in turn drives adrenal androgen production through reduced negative feedback at the level of the pituitary corticotrophin cells [9]. 5α-Reductase isoforms are encoded by *SRD5A1* and *SRD5A2*. It was demonstrated in one study on 287 women with PCOS and 187 controls that haplotypes within *SRD5A1* and *SRD5A2* associate with PCOS [24] and a variant within *SRD5A2* (with *reduced* activity of 5α-reductase), associated with *protection* against development of PCOS [24]. Although this study contained relatively few subjects and lacks replication in other independent series, further (nongenetic) evidence to support a potential role for variants within *SRD5A1* and *SRD5A2* in the development of PCOS comes from urinary steroid profile data in 178 UK-based women with PCOS and 100 body mass index (BMI)-matched controls, in which women with PCOS were shown to have increased 5α-reductase enzyme activities and adrenocortical drive [25].

Obesity

Our own group published the first report of association of PCOS with a genome sequence variant (rs9939609 within the *FTO* gene) from 463 UK-based PCOS cases vs >1300 female population controls, with odds ratio (OR) 1.30 per minor allele copy [26]. Subsequently, association of another *FTO* variant (rs1421085) was also demonstrated within a subgroup of central European women with PCOS who also had obesity or metabolic syndrome [27]. Variants within *FTO* and also the melanocortin receptor 4 gene (*MC4R*, another gene known to be implicated in obesity) were also shown to associate with obesity in women with PCOS (although not with PCOS per se) [28]. At least in obese women with PCOS, therefore, there appears to be some consistency between the candidate gene studies that have reported on variants within *FTO* and PCOS in independent series.

Beta-Cell Function

Some candidate gene studies in PCOS have focused on T2D susceptibility gene variants implicated in control of beta-cell function (insulin secretion), such as *TCF7L2* (encoding transcription factor 7-like 2) and *KCNJ11* (encoding Kir6.2, part of the inwardly rectifying potassium channel within the beta-cell membrane) [7,29]. In a UK-based study of variants within *TCF7L2*, our own group showed no association either with PCOS or androgen levels [30]. In a further large UK-based study on the E23K variant within *KCNJ11*, our group also showed no association with PCOS or androgen levels [31]. Finally, no evidence was shown to associate the variable number of tandem repeats (VNTR) region of the insulin gene (that regulates insulin gene transcription and therefore insulin production and has also been shown to associate with T2D) with PCOS in a large UK-based study [32]. Based on these data and those from existing GWAS, an important role of the beta cell in the development of PCOS seems unlikely.

Ovarian Folliculogenesis

Oligo-amenorrhea, anovulation, and impaired fertility are cardinal features of PCOS [33]. In a screen of 37 candidate genes, it was shown that 19p13.2 on chromosome 19 is strongly associated with susceptibility for development of PCOS [22,34]. Within 19p13.2, a dinucleotide-repeat polymorphism called D19S884 is known to map in close proximity to two genes of interest: intron 55 of *FBN3* (the fibrillin 3 gene) and *INSR* (the insulin receptor gene). This polymorphism may influence the function of *FBN3* (the third member of the fibrillin extracellular matrix protein family). It has been hypothesized that this in turn could impair follicle development in PCOS [22,34]. The function of *INSR* (and therefore insulin resistance) may also be influenced by this polymorphism.

Gaining a clear understanding of genetic susceptibility to PCOS will provide much insight into its etiology and also provide a foundation on which to develop novel therapeutic strategies. The emergence of the GWAS has heralded a new era in the search for the genetic origins of PCOS. The GWAS is a much more powerful means of studying genetic susceptibility than the candidate gene approach, and one that has potential to identify

unexpected and novel pathogenic pathways and therapeutic strategies. It is important that further GWAS in PCOS are conducted within larger and more diverse populations (including those of African ancestry), to gain further insight into the genetic and evolutionary origins of PCOS. However, alternative mechanisms of heritability should also be explored. Unfortunately, GWAS are blind to these alternative mechanisms of heritability. One such mechanism, and one which has attracted heightened interest in recent years, is that of "epigenetic modification." It is this mechanism that we will explore in the next section of this chapter.

EPIGENETIC MODIFICATIONS

The heritability of PCOS is analogous to matter in the universe. In this analogy, the baryonic matter that we can see (which accounts for <5% of the matter in the universe) is like the DNA variants that we see associate with PCOS in GWAS. However, just like the vast majority of matter in the universe is dark and "missing" (which holds our galaxy together but which is unaccounted for and we cannot see), so too the vast majority of the heritability of PCOS is currently unaccounted for, and we have not been able to "see" these predominant heritability factors lurking within the genetic data of standard GWAS and candidate gene studies.

PCOS is highly heritable [8]. Evidence from both GWAS and candidate gene studies have identified a number of gene variants that are associated with PCOS but despite the tens of thousands of PCOS cases and controls from diverse populations reported on in GWAS on PCOS to date, very little of the heritability of PCOS has been identified (<1% for each of the three PCOS loci identified by Hayes and colleagues [13], for example). Indeed, no gene variants identified to date have been shown to play a major role in the development of PCOS [35]. Where is all the vast majority of missing heritability for PCOS hiding? One theory is that *epigenetic* modifications, resulting from environmental factors, could play an important role in the heritable development of PCOS [35]. In support of this notion, it is noteworthy that epigenetic changes have been shown to contribute to other conditions that have complex etiology, including T2D, depression, and some cancers [35], and a similar scenario of "missing heritability" has been observed following analysis of GWAS for other complex diseases [36]. It is quite possible, indeed likely therefore, that epigenetic factors play an important role in the heritability of complex diseases such as PCOS.

Epigenetic modifications, by definition, are changes in gene *expression* that lead to phenotypic changes. However, these changes do not change the DNA sequence itself [35]. Importantly though, these epigenetic changes can affect both somatic cells and gametes, and therefore can influence future generations [35]. This is the mechanism whereby epigenetic changes are thought to influence heritability of PCOS: through inducible changes in the expression of genes within gametes, such gene-expression changes can be inherited by offspring without affecting the structure of the DNA at all. These epigenetic modifications usually include histone modifications or DNA hypo- or hypermethylation [35]. Furthermore, regulation of gene expression is known to be influenced by micro-RNA (miRNA). Aberrant miRNA expression is known to contribute to development of a number of human diseases [35,37]. The interplay between miRNA and epigenetic modifications is complex, with histone and methylation modifications of DNA being modulated by miRNA [35]. Further complexity occurs through miRNA expression itself being transcriptionally modulated by epigenetic mechanisms (such as promoter methylation or histone acetylation) [38].

There is currently a lack of published data to support a potential role of epigenetic factors as contributors to the heritability of PCOS. Given its complexity, epigenomics is a difficult field of study. In one study on 20 women with PCOS vs 20 BMI- and age-matched controls, there were no significant differences noted in global methylation patterns [39]. In a further study, genome-wide site-specific methylation analysis from visceral adipose tissue was performed in an animal model of PCOS in which both infant and adult monkeys were subjected to androgen exposure [35]. Differentially methylated loci were identified in those androgenized monkeys (including gene variants implicated in the transforming growth factor-β signaling) [35]. However, associations of these candidate gene methylation profiles have not been identified in PCOS to date [35].

A number of studies have attempted to identify epigenetic factors in PCOS. These are outlined later:

Hypermethylation

Although methylation pattern within the promoter region of the follistatin gene in PCOS was shown to be equivocal [40], hypermethylation of the lamin A/C gene (*LMNA*) was shown to associate with insulin resistance in PCOS in one study [41]. Furthermore, hyperandrogenic women with PCOS were noted to have significant genetic alterations of methylated PPAR-γ1 and NCOR1 and acetylated HDAC3 [42]. Such epigenetic changes have been hypothesized to contribute toward ovarian dysfunction and even pregnancy outcomes and mediate the effects of excessive androgen exposure within the ovarian granulosa cells of women with PCOS [35].

Hypomethylation

In one study on women with PCOS vs controls, hypomethylation within the promoter region of the LH/choriogonadotrophin receptor (*LHCGR*) gene was demonstrated, with resultant increased LHCGR transcription [35,43]. In another study, hypomethylation of the promoter region of the epoxide hydrolase 1 (*EPHX1*) gene was shown in PCOS [44]. *EPHX1* encodes a protein that plays an important role in the female reproductive system [45].

Differential Methylation

Genome-wide methylated DNA immunoprecipitation showed that 79 genes were differentially methylated in insulin-resistant vs noninsulin-resistant PCOS [46]. There were 40 genes (including those implicated in immune response and cancer pathways), which had different methylation patterns in PCOS vs controls [46].

X-Chromosome Inactivation

Epigenetic studies on the androgen receptor (AR) gene in PCOS have focused on the CAG repeat sequence within exon 1 [47]. Due to the location of the *AR* gene on the X-chromosome, epigenetic studies have focused on preferential activation of the short or long allele [given the known effects of shorter AR CAG repeat sequence on enhanced sensitivity of the AR, and implications for PCOS development [35]]. In one study, preferential activation of the short CAG allele with nonrandom X-chromosome inactivation (XCI) pattern was demonstrated in PCOS cases [35,47]. CAG microsatellite repeat sequence within the AR and XCI was also shown to affect FSH and LH expression in one study [48]. Another study showed no significant association between XCI and PCOS [49]. However, in a subset of women with PCOS, nonrandom XCI was demonstrated, and in these women the shorter CAG allele was preferentially active [49]. In a study on 88 sisters of women with PCOS, the same pattern of XCI occurred more frequently in sisters with PCOS than in those sisters who did not have PCOS [50]. In another study, preferential activation of the *AR* allele with a shorter CAG repeat occurred more frequently in women with PCOS (54.3%) vs control women (46.2%) [49]. In the same study, in those women who had preferential activation of an *AR* allele that was shorter than the median CAG repeat length, there was a significant, twofold increased OR of PCOS status [49]. These observations raise the possibility that epigenetic effects on the *AR* gene, through preferential short CAG allele expression, may pertain in at least a subset of women with PCOS.

Micro-RNA

MiRNA are small and noncoding single-stranded (20–24 nucleotides in length) regulatory RNA molecules [35]. MiRNAs influence mRNA translation through degradation of transcripts [35]. A subset of miRNAs (epi-miRNA) is thought to target the epigenetic machinery itself, such as DNA methyltransferases, histone deacetylases, and polycomb repressive complex genes [35]. In this way, epi-miRNA may influence expression of genes that are controlled by epigenetic factors [35]. MiRNAs may influence pathways implicated in the development of PCOS that include cell communication, insulin signaling, beta-cell function, reproduction, carbohydrate metabolism, steroid synthesis, and cellular growth [35]. Some of the studies on miRNA in PCOS are outlined later:

- *DENND1A* is a susceptibility gene for development of PCOS [51], which encodes a miR-601 [52]. *DENND1A* and its associated miR-601 could represent overlap between genetic and epigenetic factors that influence the development of PCOS [35].
- In one study on serum miRNA profiles, increased expression was noted for 8 miRNAs, 3 of which were useful as PCOS biomarkers [53]. MiR-222 was shown to positively correlate with serum insulin, and miR-146a was shown to negatively correlate with serum testosterone in PCOS [53]. It has been shown that ovarian follicles miR-221 and miR-222 expression is repressed by androgens [54], and that miR-222 expression is decreased in PCOS cumulus granulosa cells [55]. Indeed, of all identified miRNAs, miR-222 has perhaps been most consistently associated with PCOS [35].
- Studies on follicular fluid miRNA profiling in PCOS identified 29 miRNAs that were differentially expressed in PCOS compared with healthy fertile oocyte donors: hsa-miR-9, -18b, -32, -34c, and -135a were significantly upregulated in PCOS [56]. A further study demonstrated differences in miRNA levels in follicular fluid from women with PCOS compared with control women, with highly expressed miRNAs in PCOS including hsa-miR-483-5p, -674-3p, -191, -193b, -320, -520c-3p, -24, -132, -146a, -222, and -1290 [35,57]. Of these, only miR-132 and -320 were downregulated in PCOS [35,57]. Another study showed upregulation of miR-320 in PCOS follicular fluid [58].
- In a study on primary adipocytes from women with PCOS, miR-93 expression was shown to be inversely correlated with its suspected target, glucose transporter type 4 (GLUT4) [59].

Given the potential for epigenetic modifications to influence gametes, it is quite possible, indeed likely, that at least some of the heritability of PCOS that has hitherto

been unaccounted for from existing GWAS, relates to methylation and histone modifications of DNA and the effects of miRNAs. By definition, such epigenetic modifications would not be detectable on standard GWAS, given that epigenetic modifications do not affect the DNA structure itself. It also seems likely that the development of PCOS, in at least some women, may have been influenced through environmentally induced epigenetic modifications of somatic cells, such as XCI, for example. What is clear from this brief overview, however, is the relative lack of reported epigenomic studies in PCOS and of a clear and consistent understanding of the pathogenic mechanisms, whereby epigenomic effects on genomic transcription and translation influence the development and phenotypic expression of PCOS. Epigenomics, by its nature, is a highly complex field and we may never fully understand or appreciate the myriad ways in which epigenetic modifications may influence gene transcription and translation, and how these influence development of PCOS. The fact that epigenetic modifications are impermanent, and subject to dynamic changes according to environmental and other epigenetic factors, only adds to its astonishing complexity.

To study epigenetic modifications in the etiology of PCOS effectively will require both cross-continental and cross-disciplinary collaborations between geneticists and colleagues from life sciences. Genome-wide studies that focus on both expression profiles, and how these associate with both methylation and histone modifications of DNA particularly around promoter regions, and miRNA expression will be required. Furthermore, given the heterogeneity of PCOS, the environmental contributors to epigenetic effects, and the dynamic nature of epigenetic modifications, a large and diverse population of women with PCOS from multiple ethnicities and environments will be desirable. This will be a Herculean task, but one that is necessary if we are to truly understand and gain insight from the etiology of PCOS. Such renewed insight will shed light on novel pathogenic pathways and therapeutic targets for PCOS. Furthermore, identification of key miRNAs in the epigenetic etiology of PCOS may also enable new screening tests for PCOS to be developed.

A summary of the main genetic and epigenetic features of PCOS is shown in Fig. 1.

ENVIRONMENTAL FACTORS

Having explored the genetic and epigenetic contributors to the etiology of PCOS, in Sections "Genetic Factors" and "Epigenetic Modifications," we will consider the environmental contributors to the development of PCOS. It is clear that PCOS is a genetic condition, the development of which is likely influenced by epigenetic factors. However, it is also clear that genetics and epigenetics alone cannot account solely for the development of PCOS in most women. Weight gain often coincides with manifestation of PCOS in those women genetically predisposed to its development [2]. In recent years, there has been a plethora of evidence to implicate numerous and varied potential environmental contributors to the development of PCOS. In this section, we will explore some of the main hypotheses and associated evidence, including the "fetal programming hypothesis," environmental toxins, dietary-related weight gain, gut microbiota, lack of exercise and physical activity, socioeconomic status, and advanced glycation end products. Supporting evidence for each hypothesis will be explored, including novel management strategies and future directions. Before exploring each of these hypotheses, however, it is important to emphasize that conclusive data to support an important role for most of these environmental candidates in the development of PCOS are still lacking, and that this field should be a focus for future research.

FETAL PROGRAMMING HYPOTHESIS

It has been reported that adolescent girls who develop PCOS have lower birth weights than control adolescent girls without PCOS [60]. Furthermore, other studies have reported that nutritional impairment during fetal development could play a role in future development of PCOS [61]. These observations suggest that intrauterine environmental factors are important for future development of PCOS. Further human observational and animal-based studies have led to a "fetal programming hypothesis" for the development of PCOS. Essentially, this hypothesis states that the intrauterine environment is contributory to future development of PCOS. There are two main mechanisms by which such a mechanism is hypothesized to occur: (i) intrauterine nutritional restriction results in changes to insulin levels and insulin resistance within the fetus, in addition to possible epigenetic changes (methylation of some genes), and (ii) exposure of the fetus to excess androgens results in "programming" and changes to differentiation of developing tissues [62]. It is hypothesized that each of these mechanisms can predispose the future development of PCOS in the affected fetus. Although the fetal programming hypothesis remains speculative, there are compelling data that are entirely consistent with such a hypothesis. The main relevant studies are discussed later:

Animal-Based Studies

Rat studies have shown that exposure to elevated testosterone levels early in intrauterine life results in

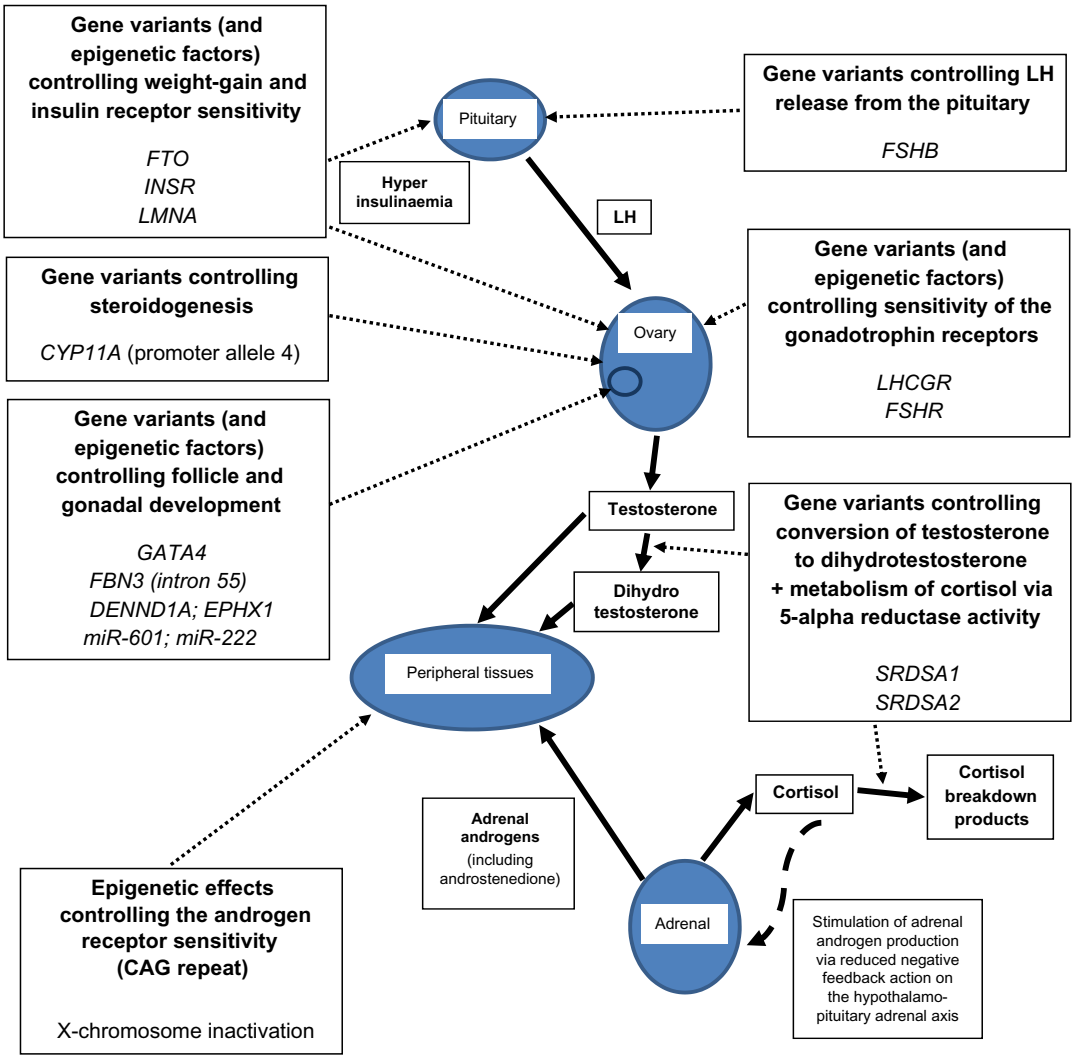

FIG. 1 Summary of the main genetic and epigenetic features of PCOS. *LH*, luteinizing hormone; *PCOS*, polycystic ovary syndrome.

polycystic ovaries and anovulatory sterility in their offspring [62]. Studies on female offspring from prenatally androgenized rhesus monkeys (maternal androgenization occurring in the early phase of gestation) demonstrated many features of PCOS that included: irregular ovulatory menstrual cycles; ovarian hyperandrogenism; enlarged polyfollicular ovaries; LH hypersecretion; visceral adiposity; insulin resistance; increased risk for development of T2D; and dyslipidemia [62,63]. When pregnant monkeys were exposed to androgen excess during late gestation, their female offspring also manifested an adult PCOS-like phenotype, but without abnormalities in LH and insulin sensitivity [62,63]. It was also noted that prenatally androgenized monkeys had elevated levels of androstenedione at birth, and adrenal androgens increase during early life [62].

Other changes in prenatally androgenized monkeys include increased number of primary, preantral, and small antral follicles, accelerated granulosa cell proliferation, transcription of genes within the granulosa cells (including *FSHR* and *IGF-1*, the gene encoding the IGF-1 receptor), enhanced 5α-reductase activity, and reduced aromatase activity [62,64]. Interestingly, prenatal exposure to excessive testosterone in both rodent and sheep studies results in reduced birth weight of offspring [62]. In humans, impairment of placental aromatization (with associated maternal testosterone excess) is also associated with low infant birth weight, a mechanism possibly mediated via effects of raised maternal testosterone levels on impairment of placental function with subsequent reduced fetal growth [62].

Human Observational Studies

There is some evidence reported in the literature to support the notion that exposure of human female fetuses to high androgen levels in utero can predispose to future

development of PCOS [62]. It has been observed that female fetuses with in utero exposure to elevated androgen levels from congenital adrenal hyperplasia (21-hydroxylase deficiency) and congenital virilizing tumors develop features of PCOS later in life despite therapies to lower androgen levels administered postnatally [62]. Furthermore, women with hyperandrogenic conditions such as rare defects within the *CYP19* gene (which encodes aromatase, an enzyme that converts testosterone to oestradiol) and mutations affecting the gene encoding sex hormone-binding globulin (SHBG) have been reported to have given birth to girls who subsequently develop PCOS later in life [62]. Exposure of pregnant mothers to androgen-like chemicals such as Bisphenol A (BPA) has also been associated with subsequent development of PCOS in their female offspring [62,65]. These observational studies of female fetal in utero exposure to raised androgens (either fetal or maternal origin), and subsequent development of PCOS, are consistent with the fetal programming hypothesis for the development of PCOS.

The examples of human studies outlined earlier result from rare and abnormally high in utero exposure to androgens. Under normal circumstances, placental aromatase activity protects the developing fetus from exposure to elevated androgens [62]. Such placental aromatase activity is limited and can be exceeded when androgen levels (of either fetal or maternal origin) become very elevated, such as in the cases outlined earlier. A cardinal feature of PCOS is hyperandrogenemia [2]. Importantly, however, serum androgen levels in PCOS are usually only moderately elevated and seldom become severely elevated. The female fetus would be expected to be protected from exposure to the typical moderate elevation of maternal androgens that occurs in mothers with PCOS, through high circulating concentrations of SHBG and by the activity of placental aromatase. So, although pregnant mothers with PCOS appear to have significant elevations in their androgen levels compared with healthy pregnant women [62], the fetus should be protected from exposure to the high androgen environment. Is it possible that, at least in some women with PCOS, placental aromatase activity is defective?

It has been shown that insulin inhibits aromatase activity in human cytotrophoblasts [62]. Given the association of PCOS with insulin resistance and secondary hyperinsulinemia, it is possible that in pregnant women with PCOS, unusually elevated insulin levels inhibit placental aromatase activity which in turn results in exposure of the developing fetus to elevated androgens of maternal origin. Such a hypothesis is supported by observations of hirsutism and ovarian cysts in female offspring of pregnant mothers with diabetes mellitus on insulin therapy, in addition to elevated testosterone and human chorionic gonadotrophin (hCG) levels in the amniotic fluid from such pregnancies [62,66].

Delivery of small-for-gestational age newborns is commoner in mothers with PCOS compared with control mothers [62]. It has been proposed that prenatal exposure to elevated (maternal origin) androgens from mothers with PCOS may contribute toward low birth weight of their offspring [62]. Furthermore, low birth weight of female fetuses has been shown to associate with later development of hyperandrogenism, insulin resistance, and dyslipidemia—features of PCOS—during subsequent adolescence [62]. Interestingly, male offspring of mothers with PCOS also have an increased risk of developing insulin resistance, T2D, dyslipidemia, and pancreatic defects later in life [62].

In addition to in utero exposure to elevated androgen levels, intrauterine nutritional insufficiency may result in reduced fetal insulin secretion (resulting from reduced availability of nutrients to the fetus) and compensatory insulin resistance within target tissues (as a protective response to inadequate fetal nutrient availability) [62]. It has been demonstrated that babies born with intrauterine growth retardation have an increased risk of developing metabolic dysfunction later in life, possibly through epigenetic modifications [62,65]. It has been proposed that such female babies are also at increased risk of developing PCOS later in life [62].

What are we to make of the fetal programming hypothesis for the development of PCOS? Although the data are compelling, this hypothesis is largely based on observational studies, with a current lack of clear evidence to demonstrate causality or directionality of effects, at least in humans. However, given the observational evidence outlined earlier from both animal studies and human observations, it is tantalizing to speculate that in at least some women who develop PCOS, prior fetal programming from either intrauterine exposure to elevated androgens or intrauterine nutritional deficiencies may pertain. It is important to emphasize, however, that the source of excess androgen for such a hypothesis to be plausible is likely to be the fetal ovary [67] and unlikely to be of maternal origin: despite the cases alluded to above, women with very high androgen levels in pregnancy (such as from an androgen-secreting tumor) often do not have androgenized offspring, and there is scant evidence to support a role for placental aromatase deficiency in mothers with PCOS. Finally, healthy nutritional advice should be provided, and avoidance of nutritional deficiencies implemented in all pregnant women.

ENVIRONMENTAL TOXINS

Given the data outlined earlier showing effects of prenatal androgen exposure on future risk of development of

clinical and biochemical features of PCOS [67], it is important to consider other potential environmental contributors to prenatal androgen exposure. One candidate, and one which is both topical and timely to discuss, is the group of endocrine-disrupting chemicals (EDCs). In our modern-day environment, we are all exposed daily to a multitude of EDCs that originate from an eclectic mix of materials that include plastics, soaps, toys, carpets, clothes, and fuel. EDCs can get into the body in a variety of ways that include intravenously (through intravenous administration of fluids and drugs) and more commonly via the oral route [68]. Furthermore, EDCs are known to have the potential to accumulate in the food chain, thereby facilitating human exposure to EDCs through food. It is recognized that many EDCs have the ability to pass through the placenta and accumulate within the amniotic fluid, thereby leading to exposure of the developing fetus [68]. It is important therefore to consider potential adverse effects of EDCs on fetal development, and specifically for the purposes of this chapter, on future development of PCOS. In the following section, evidence supporting a potential role for individual EDCs as environmental factors in the etiology of PCOS will be discussed.

Triclocarban

Triclocarban (TCC) also known as 3,4,4′-trichlorocarbanilide is found in everyday materials such as plastics, toys, clothing, and carpets [68,69]. The relevance of TCC to this discussion is that TCC is known to enhance the effects of testosterone on the AR through increased transcriptional activity [70], thereby mimicking the effects of hyperandrogenemia. In one study, it was demonstrated that TCC was detected in plasma from human umbilical cord [71]. The ubiquity of TCC in the human environment, the clear effects of TCC on enhancing andogenicity, and the detection of TCC in human umbilical cord in an urban environment promote TCC as a potential environmental contributor to prenatal androgenization of the human female fetus. TCC could therefore play a role in the future development of PCOS. However, on the basis of current evidence, such a role is speculative.

Bisphenol A

BPA is an estrogenic monomer used in polycarbonate plastics [68]. In one study, serum levels of BPA were shown to be significantly higher in those women with PCOS vs control women [65]. BPA has been shown to associate with androgens (including testosterone) in women with and without PCOS and to demonstrate estrogenic receptor binding [65]. Furthermore, BPA has also been detected in fetal serum and amniotic fluid confirming its passage through the placental barrier [72]. Although rodent studies have shown that BPA can influence prenatal programming and reproductive function, the effect of human prenatal exposure on future risk of PCOS development is unknown [68]. Human adult studies do not prove a causal effect of BPA on PCOS development. Overall, the existing data on BPA in women with PCOS are unconvincing: a definitive and prospective epidemiological study on human prenatal exposure to BPA and future risk of development of PCOS has yet to be performed.

Phthalates

Phthalates, used to soften plastics, are found in numerous products that include food packaging, toiletries, and intravenous tubing [73]. Based on detection of phthalates in amniotic fluid, these EDCs are known to cross the placenta [74]. Rodent studies have demonstrated an association of prenatal exposure to dibutyl phthalate and reproductive problems in female offspring [68]. Another EDC in this class called di (2-ethylhexyl) phthalate (DEHP) has been shown to be associated with increased ovarian weights in female offspring of mice that had prenatal exposure to this chemical [75]. Furthermore, exposure to DEHP in adult female rats has been shown to result in suppression of ovulation and polycystic ovarian morphology [76], typical features of PCOS. Interestingly, human studies on phthalates seem to show that serum testosterone levels in female fetuses and infants are *inversely* proportional to maternal serum levels of phthalates [68]. In humans, phthalates appear to have antiandrogenic effects [68]. Indeed, in one human study, it was even shown that in utero exposure to phthalates was associated with a *reduced* risk of developing PCOS in female offspring [77].

The rodent and human data regarding the reproductive effects of phthalates appear to lack consistency. Regardless of the mechanisms involved, however, it appears that both prenatal and adult exposure have the potential to interfere with normal reproductive function. The translation of the available data, however, toward a unified and well-defined hypothesis of the effects of phthalates as an environmental contributor of PCOS development is far from clear. One thing that does seem clear, however, is that phthalate exposure is unlikely ever to be recommended as a preventive strategy for PCOS development!

Perfluoroalkyl Acids

Perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)

are used widely in commercial products [68]. Through effects on peroxisome proliferator-activated receptor alpha (PPARα) activity, such chemicals are known to have the potential to influence serum concentrations of testosterone [78]. In the Avon Longitudinal Study of Parents and Children (ALSPAC), prenatal serum concentrations of PFAAs were measured in pregnant mothers, and these levels were compared with total testosterone concentrations from their daughters at the age of 15 years [78]. A real strength of this study is its prospective design. For those daughters who were born to mothers with PFOS levels in the upper concentration tertile compared to those born to mothers with levels in the lower concentration tertile, total testosterone concentrations were 0.18 nmol/L higher [78]. The number of subjects in this study is quite low ($n = 72$) [78]. Furthermore, these data do not prove causality but do suggest an association between maternal exposure to PFOS and subsequent level of testosterone in 15-year-old daughters. Given the importance of androgenicity in the pathogenesis of PCOS, it remains possible that certain PFAAs such as PFOS may play a role as a prenatal environmental contributor to the future development of PCOS in some girls.

Nicotine

Nicotine has pleiotropic endocrine effects on both mothers who smoke and on fetal reproductive development. An association between smoking and levels of free testosterone in women with PCOS was demonstrated in one study [79]. There are numerous fetal endocrine effects that have been shown to associate with smoking in pregnancy. These include dysregulation of cytochrome P450, increased fetal estrogen levels, and changes in the expression of FSH receptors [68,80]. A rodent study also showed that testosterone levels in female offspring were increased following maternal exposure to nicotine during the prenatal period [81]. A potential role of maternal smoking and prenatal nicotine exposure as an environmental contributor to the future development of PCOS in female offspring remains speculative. However, given the global prevalence of maternal smoking [82], further epidemiological data on maternal smoking and future risk of PCOS development in female offspring would be interesting to explore. Regardless of risk for PCOS development, however, the health risks of smoking in pregnancy are legion [83] and clearly outweigh any potential risk of nicotine on future development of PCOS as a rationale to support smoking cessation in pregnancy as an important public health message.

One of the challenges of research in this field is the bewildering array of EDCs that we are all exposed to in our daily environments. Those outlined here represent a tiny proportion of the multitude of chemicals in our environments, all of which might have potential to act as an EDC and cross the placental barrier, thereby resulting in potential for prenatal fetal exposure. It would be unrealistic to study every environmental chemical, and in our view there will always be a degree of uncertainty regarding the longer-term effects of EDCs. A further challenge is that epidemiological and observational studies usually only provide associations, and often lack clear evidence for causality. It is therefore very difficult to make clear conclusions regarding the role of EDCs in pathogenesis of disease including PCOS on the basis of current reported data. Experimental rodent studies may go some way to overcome this problem, but as highlighted in the data outlined earlier, humans and rodents do not always seem to respond to EDCs in the same ways, thereby limiting this approach somewhat. An optimistic and perhaps naïve view of our future environment is that novel materials will be developed that avoid release of EDCs into their surrounding environments, thereby reducing and ultimately eliminating our exposure to EDCs. A more realistic view is that EDCs are here to stay, and we need to accept that our future exposure to them is inevitable. EDCs are ubiquitous in our environment. Given the uncertainty in this field and the emphasis on prenatal exposure to EDCs, a pragmatic approach may be for pregnant women to limit their exposure to EDCs. However, this advice may be difficult to implement given the ubiquity of EDCs in our daily environment.

DIETARY-RELATED WEIGHT GAIN

Perhaps the most important environmental factor implicated in the etiology of PCOS is weight gain and obesity. Any discussion of obesity as an "environmental" factor, however, should be tempered with an understanding and appreciation that BMI itself is a heritable trait [26]. Therefore, obesity as an entity cannot be considered as an entirely environmental trait, but rather one that manifests from both genetic and environmental origins. Indeed, some of the heritability of PCOS is likely to be mediated through gene variants implicated in control of fat mass. Notwithstanding this important caveat, it is important to consider the effects of obesity on the development of PCOS, the mechanisms involved, and the implications for weight loss through lifestyle (primarily dietary) change.

The association of PCOS with obesity is clear from epidemiological data, which reveal that between 38% and 88% of women with PCOS are either overweight or obese [2,84]. Recently, data published from the Northern Finland Birth Cohort 1966 have shed invaluable insight into the important role of weight gain, especially during early adulthood, in the emergence of PCOS [85], and the heightened risk of developing T2D in women with PCOS

[86]. The important role of fat mass as an etiological driver of the expression of PCOS is further highlighted by observation that modest weight loss of just 5% in obese women with PCOS often improves the classical reproductive, hyperandrogenic, and cardiometabolic features of this condition [87,88]. Our current understanding of the etiology of PCOS based on existing evidence is that PCOS usually manifests in women who are genetically predisposed to this condition, who subsequently gain weight (often during puberty) [2].

The effects of weight gain on the development of PCOS (including its reproductive, hyperandrogenic, and cardiometabolic facets) are believed to be mediated primarily through effects on insulin resistance and associated secondary hyperinsulinemia [2]. Insulin resistance is known to affect between 50% and 90% of women with PCOS. Furthermore, severity of insulin resistance in PCOS usually exceeds that which typifies age- and BMI-matched control women without PCOS [89]. Insulin resistance results in secondary hyperinsulinemia, which has pleiotropic effects on the reproductive axis in PCOS. This includes enhancement of testosterone production from ovarian theca cells through cogonadotrophic effects of insulin, interacting synergistically with LH [90,91], via activation of CYP17 [P450c17α], a key enzyme in ovarian androgen biosynthesis [92]. Hyperinsulinemia also has suppressive effects on SHBG production from the liver, resulting in enhanced hyperandrogenic effects from increased free androgen index [2]. The hyperandrogenic effects of hyperinsulinemia in PCOS may be heightened through possible effects on enhancement of LH pulse amplitude in pituitary tissue (demonstrated in rodent models) [93] and stimulation of adrenal androgen production through enhanced adrenal P450c17α activity [92]. In addition to hyperinsulinemia being implicated in the development of hyperandrogenism in PCOS, there are also important effects of hyperinsulinemia on ovulation and menstrual cyclicity in this condition. Insulin is known to impair folliculogenesis through inhibition of preantral follicle development [2]. Arrested preantral follicle development underlies subfertility and oligoamenorrhea in PCOS. The direct effects of secondary hyperinsulinemia (resulting from increased insulin resistance) on both enhancement of hyperandrogenemia and arrest of antral follicle development in PCOS are thought to mediate the effects of weight gain on the hyperandrogenic and reproductive features of this condition [2].

The mediated effects of weight gain induced insulin resistance and secondary hyperinsulinemia on the etiology of PCOS actually extend even further than that outlined earlier. Steroid metabolism is also known to be influenced by hyperinsulinemia in PCOS [2,94]. In the largest study to date on urinary steroid profiles in women with PCOS ($n=178$) vs BMI-matched control women ($n=100$), increased 5-α reductase (5-αR) activity was demonstrated in women with PCOS compared with control women [25]. It was also shown in both the groups that increased fat mass associates with enhanced 5-αR activity [25]. Such an effect results in increased adrenal androgen production from enhanced hypothalamopituitary adrenal (HPA) axis stimulation (from increased breakdown of cortisol and reduced negative feedback on the HPA axis) [25]. Furthermore, increased conversion of testosterone to 5-hydroxytestosterone (5-HT) also enhances hyperandrogenism given that 5-HT is a more potent androgen than testosterone [25]. Therefore, an important mechanism that mediates the effects of weight gain on hyperandrogenism in PCOS is thought to implicate enhanced 5-αR activity, which in turn is likely influenced by hyperinsulinemia.

The effects of weight gain on adipokine release in PCOS may further influence the phenotypic expression of this condition [94]. Adiponectin has received lots of focus in the PCOS literature to date, with >30 studies published to date. It was demonstrated in a large meta-analysis on >3400 subjects that compared with controls and following adjustment for differences in BMI, women with PCOS have lower levels of serum adiponectin [95]. It is known that adiponectin inhibits production of androgens from ovarian theca cells [96]. It follows therefore that suppressed levels of adiponectin may facilitate the effects of hyperinsulinemia on enhancement of ovarian androgen production [94]. Given the known effects of adiponectin on insulin sensitivity, lower adiponectin levels in PCOS probably also contribute toward insulin resistance that is characteristic in women with this condition [95].

In addition to effects on hyperandrogenism and reproductive function, weight gain induced insulin resistance in PCOS, as in metabolic syndrome [97], is also thought to be a major contributor to its associated cardiometabolic dysfunction. This includes the effects of insulin resistance on development of dyslipidemia [98], nonalcoholic fatty liver disease [4], and T2D [3]. Given the known association of PCOS with insulin resistance, obese women with PCOS often manifest heightened insulin resistance from the combined effects of PCOS and obesity: a metabolic "double-whammy" [2,6]. Such a scenario is likely to explain heightened risk of development of T2D (around 10%) and increased risk of early-onset impaired glucose tolerance (around 30%–40%) in women with PCOS [5]. Heightened insulin resistance in obese women with PCOS is also likely to underlie the increased risk of OSA in this condition: risk of OSA is 5- to 10-fold greater in women with PCOS compared with BMI-matched control women [5].

Our current understanding of the central role played by insulin resistance (and its worsening through weight gain) in the etiology of PCOS has an important implication for its effective management. Weight loss, through its beneficial effects on insulin resistance and therefore

hyperinsulinemia, remains the most effective management strategy for obese women with PCOS. Benefits conferred from improved insulin sensitivity in PCOS include metabolic and ovulatory function, androgenicity, and fertility [2]. Lifestyle advice for obese women with PCOS has focused primarily on dietary modification [99]. Maintenance of weight loss remains a challenge for the longer-term management of PCOS [100]. This can be compounded in women with PCOS due to increased levels of low self-esteem, psychological distress, and depression that can interfere with the longer-term effective implementation of healthy lifestyle strategies [101]. Although bariatric surgery represents a promising solution, it is not a solution that is easily scalable to the population level. It is therefore important that research focus is maintained on development of novel, effective and durable weight-loss strategies in PCOS, including perhaps exploration of the effects of administration of novel weight-loss therapies. Our current therapeutic armamentarium for PCOS provides little more than symptomatic relief (with the exception of fertility treatments). We need to develop novel therapies for PCOS that address underlying etiological factors. Weight-related insulin resistance provides such a target.

GUT MICROBIOTA

In recent years, gut microbiota have assumed increasing prominence with regard to their effects on general health, but particularly their relationship to chronic dysmetabolic conditions such as T2D. The human gut contains >100 trillion microbes, vastly outnumbering human cells [102]. Although we lack studies in women with PCOS, there are recent reports from rodent studies alluding to a potential role of gut microbiota in the development of PCOS. Many potential mutidirectional effects of gut microbiota may pertain (including involvement of the immune system, hormonal signaling [both levels of hormones and their receptor sensitivities], and nervous system [including effects on appetite and behavior]), but the "leaky gut hypothesis" has perhaps received most attention in recent years regarding effects on metabolic health. The leaky gut hypothesis purports that certain gut microbiota profiles (related to dietary and genetic factors) can predispose the colon to leakage of endotoxins (lipopolysaccharides produced by Gram-negative bacteria) from the gut into the serum [103]. Gut-derived endotoxinemia can then stimulate a chronic inflammatory response in the adipose and other tissues, which in turn can influence adipokine release and heighten insulin resistance and contribute to other metabolic disturbance [103]. Given the important role of insulin resistance in the etiology of PCOS [2,94], the potential role of gut microbiota in the development of this condition is an important consideration worthy of focused research.

Guo and colleagues reported on the effects of gut microbiota in a rat model of PCOS [102]. Female Sprague-Dawley rats ($n=32$) were studied, with random assignment into four groups of eight rats each. These consisted of a control group and three PCOS groups (induced through letrozole treatment, an aromatase inhibitor that results in elevated levels of testosterone and suppressed estradiol levels). Fecal samples were collected, including at 21 days following letrozole treatment. One of the PCOS groups then underwent fecal microbiota transplantation (FMT) and another PCOS group underwent *Lactobacillus* transplantation. On day 36, fecal samples were collected and examined using real-time polymerase chain reaction (PCR). Real-time PCR and sequence analysis were used to analyze fecal microbiota. Hormonal levels of ostradiol and testosterone were also analyzed from blood samples and estrous cycle determination from vaginal smears. There were some important data generated. First, letrozole-induced PCOS was shown to influence gut microbiota: compared with fecal samples from control rats, those from letrozole-induced PCOS rats contained lower levels of *Lactobacillus*, *Ruminococcus*, and *Clostridium* and higher levels of *Prevotella*. In the PCOS rat groups administered FMT and *Lactobacillus*, the increased fecal *Lactobacilli* was associated with increases in estradiol. The associated reduction in fecal *Prevotella* in those fecal-transplanted PCOS rats was also associated with a reduction in serum testosterone and androstenedione. The fecal-transplanted rats also had improved estrous cycles and ovarian functions [102].

A further letrozole-induced PCOS rodent-based study on 4-week-old female mice reported by Kelley and colleagues also showed association of letrozole treatment with reduced microbiome diversity [104]. In this study, testosterone levels were inversely associated with diversity and abundance of large intestinal bacterial species. Letrozole treatment was associated with reduced fecal *Bacteroidales* and increased fecal *Clostridiales* and majority of *Firmicutes* [104]. As with the study reported by Guo and colleagues [102], the study outlined here also demonstrated an effect of letrozole-induced PCOS on reduced diversity of gut microbiota, presumed to be secondary to the hormonal changes (including elevated serum testosterone) induced by letrozole administration [104].

The studies outlined earlier show proof of concept that at least in a rodent model gut microbiota are implicated in reproductive function, and therefore may also play a role in the development of PCOS [102]. The interplay between gut microbiota and levels of sex hormones appears to be multidirectional. *Lactobacillus* is known to have health-promoting qualities and hypothesized to produce short-chain fatty acids within the colon that strengthen the gut barrier and reduce bacterial endotoxin

translocation across the gut wall [105]. It has been shown that administration of *Lactobacillus* through probiotic supplements could reduce insulin resistance in humans in response to high-fat and calorie-laden diets [106]. More human-based studies on the role of fecal microbiota in the etiology of PCOS are required. Future study of fecal transplant or probiotic administration (including *Lactobacillus*) as a preventive and treatment strategy for women with PCOS should be prioritized, including effects of gut microbiota on insulin sensitivity and metabolic, biochemical, and clinical features of PCOS.

LACK OF EXERCISE AND PHYSICAL ACTIVITY

Over recent decades, there has been mounting evidence reported in the literature to promote the health benefits of exercise and physical activity. Avoidance of adopting a sedentary lifestyle is topical and forms a cornerstone of general healthy living advice. It has been proposed that physical inactivity is an important cause of most chronic diseases [107]. Booth and colleagues argue, based on existing evidence from the literature, that physical activity prevents and delays onset of chronic disease affecting most systems that include metabolic, cardiovascular, musculoskeletal, gastrointestinal, psychological, and bone health [107]. Promotion of physical activity forms an important part of healthy lifestyle advice for women with PCOS. As such, exercise and physical activity are important environmental considerations when considering the pathogenesis of PCOS. Unfortunately, however, there is a lack of well-controlled studies reported in the literature that evaluate the benefits of exercise and physical activity both as a preventive and management strategy for PCOS [108]. The relevant literature is outlined here.

Physical Fitness

A useful measure of physical fitness is the "VO_2 max." This is a measure of maximal aerobic capacity. There is controversy in the literature regarding VO_2 max in women with PCOS. In one study, this measure of physical fitness is equivalent between women with PCOS and age- and BMI-matched controls [109], while another study showed an impairment of VO_2 max in young overweight women with PCOS [110]. It has been proposed that such discrepancies of VO_2 max in PCOS between studies could be influenced by differences in insulin sensitivity [108]. With regard to muscle strength [109] and levels of free-living physical activity [111], there appears to be no difference between women with PCOS and healthy controls.

Reproductive Function

There is some evidence that exercise can improve reproductive function in women with PCOS. In one study, restoration of normal menstrual cyclicity was shown in 60% of anovulatory women with PCOS who underwent a 3-month aerobic training program [112]. Menstrual cyclicity and ovulation rates also improved in overweight women with PCOS following 24 weeks of aerobic exercise [113]. In a further study that compared energy restricted diet alone or in combination with exercise, the combined group experienced more ovulatory cycles than the diet-alone group [114]. Overall, the data in the literature suggest that through exercise interventions, around 50% of women with PCOS will experience improvements in menstrual cyclicity and/or ovulation [108]. Although the mechanisms implicated are incompletely understood, it has been hypothesized that the reproductive benefits of exercise in PCOS are mediated, at least in part, through improvements in insulin resistance [108].

Although exercise appears to promote ovulation in women with PCOS, it should be noted that excessive exercise may have the opposite effect. In a systematic review of the literature on exercise and the effects on ovulation, Hakimi and colleague describe an *increased* risk of anovulation in extremely heavy exercisers (>60 min per day), but exercise duration 30–60 min per day appeared to *reduce* the risk of anovulatory infertility [115]. Excessive and heavy exercise (>60 min per day) may interfere with levels of leptin and opioids that in turn may affect both adrenal and gonadal function [115]. Promotion of *moderate* exercise and avoidance of *excessive* exercise may represent important health messages for women with PCOS.

Cardiovascular Health

In one study that included 90 overweight women with PCOS, a 3-month aerobic exercise program improved fasting insulin levels, BMI, and waist circumference compared with nonexercising controls [112]. In a comparison between 24 weeks of aerobic exercise vs a hypocaloric diet in 40 obese women with PCOS, the exercise group had greater improvements in waist circumference, insulin levels, and free androgen index [113]. In a more detailed and complex study that included comparisons of diet-alone, with diet combined with different forms of exercise (aerobic and aerobic-resistance) over a 20-week period in obese and overweight women with PCOS, there were improvements in body weight, blood pressure, lipid profile, glucose, and insulin levels in all groups [114]. However, the combined exercise groups experienced more favorable changes in body composition than the diet-alone

group, with relative reductions in fat mass and preservation of fat-free mass [114].

Psychological Well-Being

There are few studies that report psychological effects of physical activity in PCOS. Body image distress score was improved in women with PCOS following a brisk-walking program that was self-directed for a duration of 6 months [116]. In a broader cohort of obese infertile women who followed lifestyle modification programs (that included increased exercise), there were improvements in self-esteem, depression, and anxiety [117]. More recently, Banting and colleagues published self-reported measures of depression and anxiety in women with and without PCOS. Regardless of PCOS status, physically active women had less severe depression than their inactive counterparts [118]. Such associative data are difficult to interpret given that causality cannot be inferred, and multidirectional effects could pertain. Although women with PCOS identified more sources of support than the control women, there were more barriers to exercise for women with PCOS compared with controls (including lack of confidence, fear of injury, and physical limitations) [118].

To summarize this section, although the literature is limited in this field, there does appear to be good evidence to support the reproductive (including menstrual cyclicity and ovulatory rate) and cardiovascular (including insulin resistance and body composition) health benefits conferred by moderate exercise and physical activity in overweight and obese women with PCOS. There are likely to be many other benefits of exercise in PCOS (as in the general population) that include psychological well-being, although direct evidence for this is limited due to paucity of published relevant studies. Physical fitness and muscle strength per se do not appear to be impaired in PCOS, although in some women with this condition, other impediments to exercise may pertain (such as reluctance to attend a gym due to impaired self-esteem, for example). An important learning point from the available literature is that exercise and physical activity in PCOS (as in the general population) appear to confer health benefits that are independent of dietary change and that these benefits occur even without weight loss. This important public health message needs to be promulgated widely: although weight loss is often an important target in obese women with PCOS, significant improvements in reproductive and cardiovascular health can also be achieved simply through increased exercise and physical activity, even *without* attendant weight loss. Optimal lifestyle change of course combines dietary change with increased exercise and physical activity, which in most cases would also be expected to result in some weight loss.

Finally, much of the discussion in this section has been focused on studies on exercise and physical activity in women with an established diagnosis of PCOS: severity of reproductive and cardiovascular manifestations of PCOS appears to be influenced by physical activity. The role of physical activity (or lack thereof) as an "environmental" (i.e., nongenetic) contributor to *future* development of PCOS has not been demonstrated conclusively in any longer-term prospective study. However, given the well-established contribution of weight gain in the development of PCOS in women who are genetically predisposed [2], and the known contribution of lack of exercise on weight gain and insulin resistance [119], it seems reasonable to hypothesize that female sedentariness, especially when associated with weight gain, would likely be an important environmental contributor to future development of PCOS.

SOCIOECONOMIC STATUS

It is well-established that lower socioeconomic status (SES) associates with engagement in unhealthy lifestyles that include poor nutrition, lack of physical activity, and smoking [120]. There is also an association between low SES and obesity in women [121]. Given the link between obesity and PCOS [2], it is important to consider the role of SES as an environmental contributor to the etiology of PCOS.

Merkin and colleagues studied women ($n = 1163$) from the Coronary Artery Risk Development in Young Adults (CARDIA) Women's Study, a prospective study of risk factors for coronary artery disease in young adults [122]. Self-reported data on menstrual cyclicity and hirsutism were ascertained, and testosterone and SHBG measured. Parental education was used as a marker of childhood SES, and personal education of each respondent as a measure of adult SES. SES trajectories were constructed for each subject, based on childhood and adult SES scores (4 trajectories with SES for each marked as "high" or "low"). Within the cohort, the overall prevalence of PCOS was 10.7%. Those women with lower childhood SES scores tended to have a higher prevalence of PCOS development. Interestingly, the subgroup with both low childhood SES and high adult SES appeared to be most at risk of developing PCOS [122]. It was hypothesized by the authors that compared with those with a low adult SES, women with high adult SES may have better recall of features relating to menstruation and hirsutism, and therefore may be more likely to have a diagnosis of PCOS. Consistent with this hypothesis is the observation that serum testosterone had the strongest correlation with PCOS in the subgroup of women with both low childhood and adult SES [122]. Another proposed possible explanation for the data is that upwardly

mobile women may experience increased peripubertal stress that in turn could contribute toward weight gain and menstrual irregularity [122].

Although self-reporting was a limitation of the study reported by Merkin and colleagues [122], a clear strength was the size of the cohort and the availability of biochemical data. The effects of SES mobility and transition in the peripubertal period on metabolic health and future risk of PCOS development should be a focus for future research. It is fairly clear from the study reported [122], however, that low childhood SES is associated with increased risk for future development of PCOS. It has been suggested that poor intrauterine or childhood nutrition may drive association between low childhood SES and risk of developing PCOS [123]. Other possible explanations for this association include childhood adoption of poor health-related behaviors that persist into adulthood [122] and associated excessive weight gain in infancy and early childhood [124].

Intriguingly, the effects of SES on risk for development of PCOS may be dependent upon the population studied and its prevailing cultural and economic milieu. While studies in western countries reveal an association between low SES in childhood and future development of PCOS, as exemplified by Merkin and colleagues [122], the situation in those countries that over recent decades have undergone, and are undergoing rapid westernization may be different. In such regions of the world such as much of the Indian subcontinent, *affluence* rather than poverty is likely to be an important driver of weight gain and associated metabolic dysfunction. In one study on women from Mumbai in India, PCOS developed in predominantly middle-class populations: presumably those who could afford to adopt a "western-type" lifestyle with the unfortunate associated weight gain [125]. The observation by Pathak and colleagues [125], and the juxtaposition with PCOS prevalence and SES status in western countries, reveals a profound insight: ultimately, caloric intake is likely to mediate association between SES and PCOS development. In poorer populations, affluence may provide a key to excessive caloric ingestion which the poorer members of those societies can simply not afford. In more affluent populations, relative poverty may drive excessive caloric ingestion through relative affordability of cheaper and unhealthy "convenience" foods. Although there are likely many other lifestyle factors at play, the effects of SES on caloric ingestion and PCOS development in different populations are important considerations.

In addition to risk for development of PCOS, SES may also influence the phenotypic expression of PCOS. In one study of 244 women with PCOS who completed a questionnaire regarding family income and school education, Di Fede and colleagues reported an association between income and education with ovulation (assessed through measurement of day 22 serum progesterone following a spontaneous or induced menstrual cycle) [126]. The proportion of women with PCOS who manifested ovulation was lower in those with low-medium income (21%) vs those with high income (43%). Similarly, those women with PCOS who had low education also had lower rates of ovulation (12%) compared with the subgroup with high education level (47%) [126]. The authors hypothesized that the differences in ovulation between the subgroups are likely related to differences in BMI and insulin levels (with an inverse correlation between family income and BMI and insulin levels and an inverse relationship between insulin and progesterone levels) [126].

SES as an entity is difficult to study based on a number of factors. These include differing definitions between studies on different populations in different cultures, and also the transitory nature of SES, particularly in our modern-day environment, with upward trajectories fairly commonplace particularly in rapidly westernized populations. A further difficulty with SES however, other than its definition and transitory nature, is that SES is often intertwined with, and has complex interactions with a person's lifestyle and behavior, for a multitude of reasons. Any association with SES is therefore likely to be complex and manifest multidirectionality. Therefore, any discussion of epidemiological data regarding SES and PCOS is necessarily associative by nature, and firm conclusions regarding SES as a *causal* environmental factor in the etiology of PCOS are inevitably limited. Notwithstanding these limitations, however, the available data reveal population-specific associations of SES with development of PCOS. While low SES in childhood appears to associate with later development of PCOS, in poorer populations, the reverse may pertain, with higher SES and associated affluence associated with weight gain and increased risk for development of PCOS. As outlined, phenotypic subgroup of PCOS may also be influenced by SES. It is important to focus on the actual mechanisms that mediate effects of SES on PCOS development in each specific population and translate these into meaningful and targeted public health messages. Instead of focusing primarily on SES per se, focus should instead rest on targeting those SES groups most at risk in any given population, and facilitating change toward a more salutary lifestyle for all. Focused and SES-relevant dietary advice should form an important part of this initiative.

ADVANCED GLYCATION END PRODUCTS

In recent years, there has been much interest in the deleterious metabolic effects of a diverse group of reactive molecules called advanced glycation end products (AGEs), through a plethora of possible mechanisms that

includes activation of a chronic inflammatory response and cellular damage [127]. AGEs are formed endogenously through nonenzymatic advanced glycation and oxidation (glycoxidation) reactions of lipids, nucleic acids, and free amino groups of proteins with carbohydrates [128]. AGEs also occur in our food, particularly cooked "fast-food" diets, uncooked animal-derived foods, high protein and fat content, and thermal processing of foods [129]. Dietary intake, together with tobacco represents an important source of exogenous AGEs. Evidence has emerged in recent years to implicate a potential role for dietary AGEs as an environmental contributor to the etiology of PCOS, including its dysmetabolic and reproductive features [128,130].

It is known that the harmful proinflammatory effects of AGE are mediated via their interaction with the receptor for advanced glycation end products (RAGE), which are transmembrane receptors. AGE-RAGE interaction results in a proinflammatory response, oxidative stress (including activation of nuclear kappa B [NF-κB]), and ultimately tissue damage [128]. AGE-RAGE interaction also upregulates RAGE expression acting like a positive feedback loop [128,131]. Conversely, presence of soluble receptor for advanced glycation end products (sRAGE) acts like a brake on this mechanism and confers protection from the harmful effects of AGE through antiinflammatory effects [128]. Unlike RAGE, sRAGE circulates throughout the body and forms a decoy receptor that can bind to circulating AGEs, thereby preventing the harmful effects of AGE-RAGE interaction [128]. It has been reported that circulating levels of AGE and ovarian RAGE are elevated in women with PCOS [128,132]. Furthermore, ingestion of a diet that is low in AGE-content in women with PCOS has been shown to be associated with improved hormonal and metabolic profiles [128].

It has been demonstrated that serum levels of AGE (from dietary intake) actually correlate with inflammatory markers that include C-reactive protein and HOMA IR (a marker of insulin resistance) [128]. Production of reactive oxygen species within tissues following accumulation of dietary AGEs can enhance cellular damage [133]. PCOS is a condition characterized by chronic low-grade inflammation, which likely underlies some of the metabolic and reproductive features of this condition [134]. It has been hypothesized that dietary AGEs may contribute to the chronic inflammatory state of PCOS, and may therefore contribute toward its pathogenesis [128]. However, further studies are required to validate this hypothesis.

One type of AGE is termed methylglyoxal (MG). MG is metabolized by an enzyme called Glyoxalase 1 (GLO-1), thereby providing some protection against the deleterious effects of AGEs [135]. It has been demonstrated in Wistar rats that ovarian accumulation of AGE results from reduced ovarian activity of GLO-1, which in turn can result from increased dietary intake of AGEs combined with excessive exposure to androgens within the ovary [136]. It has been hypothesized that such a mechanism may result in elevated inflammatory markers within the ovary, which in turn may contribute toward ovarian dysfunction that typifies PCOS [136]. There is accumulating evidence from the literature to support deleterious effects of AGE-RAGE on folliculogenesis, oocyte maturation [137], mitochondrial function, apoptosis, and DNA damage within the oocyte [137].

There is evidence to suggest that a diet low in AGE content in women with PCOS (compared with high AGE content) is associated with beneficial effects on metabolic factors and oxidative stresses [138]. In one study, women with PCOS were fed a high-AGE vs a low-AGE diet for 2 months, with marked improvement in insulin sensitivity (HOMA IR marker) following the low-AGE diet [138].

It has been demonstrated in vitamin D-deficient women with PCOS that dietary vitamin D supplementation results in increased serum sRAGE [139]. Furthermore, metformin intake for 6 months in women with PCOS has been shown to result in a reduction in serum AGE levels [140]. It is believed that metformin reduces expression of RAGE, thereby preventing AGE-induced cell damage [141]. Metformin may also convert MG to dihydroimidazole, thereby reducing oxidative stress [140].

To summarize, there is evidence in the literature from a variety of sources that include animal models of PCOS, in vitro studies and studies on women with PCOS, to support association between dietary AGE intake and deleterious inflammatory and metabolic effects (including cellular damage from oxidative stress) and ovulatory dysfunction. However, much of the literature in this field is based on associative data, from which we should be cautious about inference of causality. Although a plausible causal mechanism between AGE-RAGE and inflammatory, metabolic, and reproductive features of PCOS has been hypothesized, further focused studies are required to confirm or refute such a causal mechanism, to elucidate which, if any pathways link precisely AGE-RAGE with PCOS pathogenesis, and how such insight can be used to develop potential novel therapies. Given the association of high AGE content in food with unhealthy "fast food," it remains difficult to disentangle the harmful metabolic effects of fat and carbohydrate in food highly laden with these macronutrients, from those of AGE content per se. However, despite our incomplete understanding of causality, on the basis of current evidence, it would seem a reasonable approach for general healthy lifestyle advice to include limitation of our intake of dietary AGEs and advice on healthier cooking methods: AGE content of food is limited through preparation of food at low temperature, with high moisture, brief heating time, and use of acidic marinades (including lemon juice and vinegar) [128].

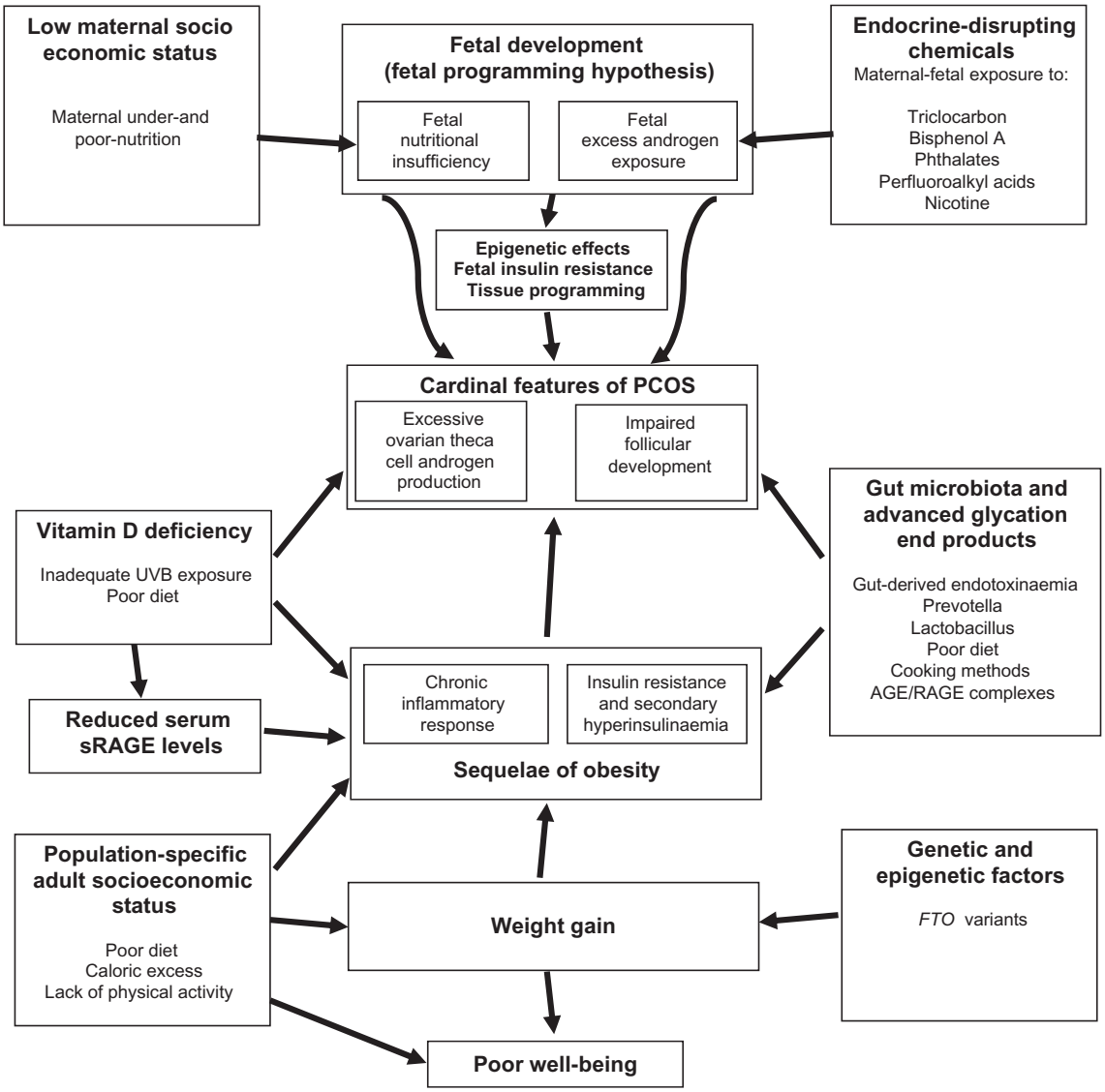

FIG. 2 Summary of the mechanisms by which environmental factors are implicated in the etiology of PCOS (where relevant, genetic, and epigenetic factors are also shown).

A summary of mechanisms by which environmental factors are implicated in the etiology of PCOS is shown in Fig. 2.

CONCLUSIONS

As the burgeoning obesity epidemic ensues, the prevalence of PCOS around the world is likely to increase. PCOS is a condition associated with significant physical and mental morbidity, and one which, potentially, confers a substantial cardiovascular burden. A fundamental premise of any human condition is to understand its etiology. Unfortunately, despite decades of focused research across many fields, our understanding of the etiology of PCOS remains incomplete. There are many possible reasons for this, which include the complex and heterogeneous nature of PCOS and the inherent difficulties associated with its effective study as outlined earlier. However, despite these barriers, we must not let these detract us from striving in the future to gain a clear understanding of PCOS etiology.

In this chapter, we have explored the three main components of the etiology of PCOS: genetic, epigenetic, and environmental. Within each section, the relevant published data have been discussed, including implications for effective management, and future directions for further research. It is of course possible that other hitherto unknown components of PCOS etiology play contributory roles and these will become evident as data from

further studies emerge. Although we understand heritability as a statistical likelihood of developing PCOS, and the roles of weight gain and insulin resistance in the expression and manifestation of PCOS, we still lack a clear and detailed understanding of the underlying mechanisms at play. A general observation from the PCOS literature is that there seems to be a predominance of cross-sectional studies reported. Unfortunately, such studies are inherently limited in their capacity to shed insight into underlying mechanisms, providing only a "snap-shot" vista. To truly understand mechanisms, we need more long-term prospective studies that explore the factors (genetic, epigenetic, and environmental) that may influence the clinical course of PCOS in women with this condition over time. Such studies will provide important insights into all the etiological pathways discussed in this chapter.

We may never fully understand the true complexity of PCOS and its intricate etiology. However, improved understanding of the etiology of PCOS will enable us to manage this condition more effectively. Our ability to accurately diagnose, predict, and prevent the onset of PCOS is also likely to improve. Such measures will hopefully reduce both the prevalence and the burden and morbidity on the many women and girls, globally, who suffer from PCOS.

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Conflicts of Interest

None.

Financial Disclosure

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