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Polycystic Ovarian Syndrome Genetics and Epigenetics

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Abstract: Polycystic ovarian syndrome and its associated endocrine abnormalities comprise one of the most common metabolic spectrum disorders within the human race. Because of the variance in phenotypic expression among individuals and within family lineages, attention has been turned to genetic and epigenetic changes in which the root cause of the disorder may lie. Further understanding of DNA/histone methylation and microRNA patterns may help to improve the accuracy of diagnosis and lead to future treatment options.
Key words: PCOS, genetics, epigenetics, DNA, methylation, MicroRNA

Introduction

Polycystic ovarian syndrome (PCOS) and its associated endocrine abnormalities comprise one of the most common metabolic spectrum disorders within the human race.[1] On the basis of the frequency in which PCOS is seen clinically and the ramifications the disease process can have on reproductive and metabolic health, research is underway with the hopes of finding not only the underlying etiology of the disorder but also better tools for diagnosis and treatment. Because of the variance in phenotypic expression among individuals and within family lineages, attention has been turned to genetic and epigenetic changes in which the root cause of the disorder may lie.

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Genetics

PCOS is known to be a complex disorder in which heritable genetics has long been suspected because of familial clustering of symptoms. Sisters often share hyperandrogenic and menstrual similarities with their mother, whereas their male siblings may demonstrate hyperandrogenism through symptoms such as early balding.[2] Initial studies into a truly genetic etiology revealed a possible autosomal dominant mode of inheritance, however, further investigation has driven the revelation that PCOS is multigenic in origin. Work continues within this

investigative arena, however, challenges exist because of a lack of consistent diagnostic criteria and similarities in phenotypic expression across different ethnic groups.[3]

Multiple population-wide genome-wide association studies (GWAS) have been leveraged to investigate PCOS with results revealing numerous pathogenic variants. Conservation has been noted across many promoter genes, *including the following major contributors: luteinizing hormone/chorionic gonadotropin receptors (LHCGR), thyroid adenoma-associated gene (THADA), and DENN domain-containing protein 1 (DENND1A)*.[3–6] *LHCGR* are found in theca and mature granulosa cells of the adult ovary with associated polymorphisms driving excess androgen production.[7] DENND1A encodes for DENN proteins with overexpression also driving excess ovarian steroidogenesis.[8] Changes in expression of the THADA alter insulin secretion and subsequent insulin resistance by modifying pancreatic β-cell function. Further associated genes and their respective association with PCOS pathophysiology can be seen in Figure 1.

The large volume of data generated by GWAS and conclusions drawn from its analysis were not without controversy. Ultimately, no single-gene loci were identified as the common etiology for PCOS. The discovery of these loci did, however, open the door for further study into their DNA foundations and the changes that cause associated differences in expression.[3,4]

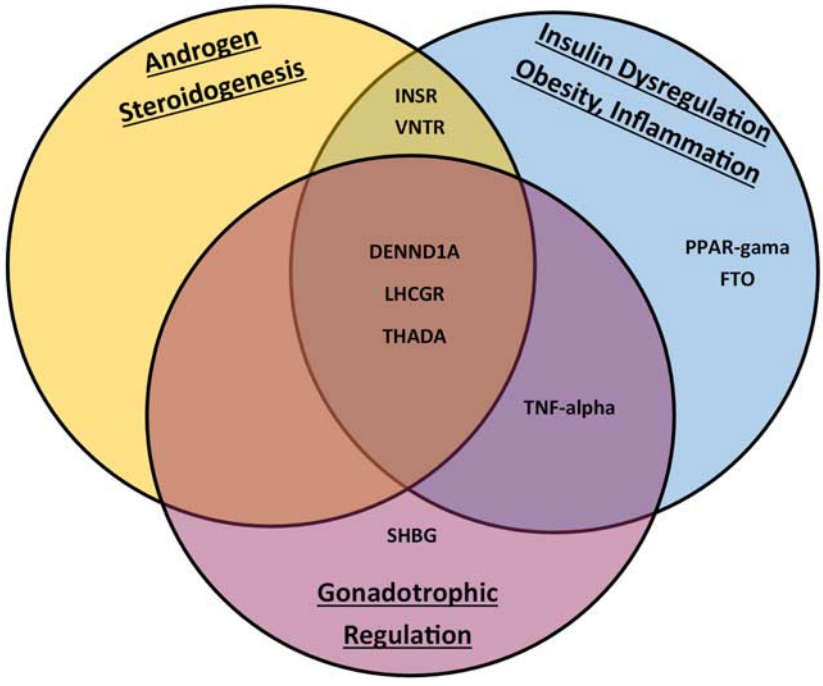

FIGURE 1. Promoter genes identified by genome-wide association studies. *VNTR, variable number tandem repeats insulin gene; INSR, insulin receptor gene; PPAR-γ, peroxisome proliferator-activated receptor-γ; FTO, fat mass/obesity gene*; DENND1A, DENN domain-containing protein 1; LHCGR, luteinizing hormone/chorionic gonadotropin receptor; THADA, thyroid adenoma-associated protein; *TNF-α, tumor necrosis factor-α; SHBG, sex hormone-binding globulin)*. full color online

Mitochondrial DNA (mtDNA) heritability may be another contributing factor to the genetic lineage of PCOS.[9] This topic will be covered in further detail at the conclusion of this chapter.

Epigenetics

Given the lack of specific, heritable findings as a single etiology for PCOS, investigators began looking at other factors, which could change gene expression characteristics. Environmental exposures, specifically increased exposure to in utero androgens, has become a leading theory in fetal programming for PCOS. Dumesic and colleagues proposed maternal insulin resistance leads to fetal exposure to hyperinsulinemia, which in turn drives fetal ovarian steroidogenesis with excess androgen production. In a normal pregnancy, this excess androgen should be resolved by placental aromatase activity, however, placental dysfunction in PCOS pregnancies has been previously reported. This 2-hit mechanism of hyperinsulinemia driving excess androgen production, with decreased placental aromatase activity to counterbalance the system, likely results in a hyperandrogenic fetal environment.[5,10] Proven by elevated testosterone levels in second-trimester amniocentesis and umbilical vein sampling of female infants born to mothers with PCOS, the Barker hypothesis correlates this exposure with reprogramming of offending genes, such as those identified through GWAS.[4,5,11] Animal models using sheep, mice, and monkeys have confirmed this hypothesis with the reported development of PCOS phenotypes and associated DNA methylation changes following exposure to increased androgen levels in utero.[3,10]

This epigenetic reprogramming is facilitated by modifications to chromatin without additions nor deletions to the existing DNA and can be accomplished through 1 of 2 mechanisms, or both in combination. The first includes methylation, hydroxymethylation, formylation, or carboxylation modifications of cytosine at its fifth carbon in the pyrimidine ring followed by a guanine, also known as CpG islands.[3,10] Methylation of DNA is generally thought to inhibit gene expression, whereas hydroxymethylation increases expression.[12] The second mechanism is histone modification by acetylation, methylation, phosphorylation, ubiquitylation, or sumoylation. Epigenetic reprograming can occur in both somatic and germ cells, however, only germ cells have the potential to pass these changes to offspring.[3]

Once epigenetic changes occur, DNA transcription is either downregulated or upregulated. This is followed by a correlated change in translation and protein production, which ultimately affects gene expression. Confirmation of these methylated/hypomethylated changes to genome-wide DNA have been studied through peripheral blood sampling through next-generation sequencing in women with PCOS, revealing numerous genes that differ in methylation status between women with and without the disease. Similar to previous GWAS studies, the LHCGR gene encoding for luteinizing hormone receptor presence was again identified as having modified expression levels in PCOS. Others include *FST—encodes for follistatin, LMNA—encodes for Lamin A/C, PPARGC1A—encodes for peroxisome proliferation,* and *EPHX1—encodes for epoxide hydrolase*. As demonstrated when searching for a single, global genetic cause for PCOS, none of these individual gene methylation alterations are responsible for the disease process. They are instead building blocks that encode for the physiological processes of follicular development, steroidogenesis, glucose metabolism, insulin regulation, and inflammatory mediation.[10] Changes in their methylation status results in the derangement of multiple systems leading to syndromic outcomes.

Although initially promising, concerns for lack of statistical significance in these serum genome-wide methylation findings were presented due to the inability to specify which

cell line or tissue type produced detectable levels of epigenetic changes. This led to further study of tissue-specific cell lines, such as those within granulosa cells of PCOS women.[10,12,13] Again found with decreased levels of methylation and subsequent overexpression were LHCGR and PPAR-γ genes.[14]

Xu and colleagues took this analysis perspective further by examining the differences between obese versus non-obese PCOS patients in regard to DNA methylation levels. Their findings indicate that obese PCOS patients have higher levels of serum global methylation present than non-obese PCOS patients. This indicates obesity may play a role within the disease process, not only as a phenotypic expression but also as an epigenetic modifier itself.[12] Further evaluation of adipose tissue by this same group in 2011 demonstrated the presence of elevated DNA methylation levels, contributing to the above findings. Interestingly, different adipose-specific gene sets were methylated in infant versus adult rhesus monkey models who were exposed to androgens in utero.[15] This alludes to the constant change present within epigenetic modifications and furthers the notion that, whereas PCOS may originate from preprogramming in utero, the disease process continues to be driven by epigenetic changes resulting from internal environmental changes such as body habitus.

Histone epigenetic modifications, most commonly through acetylation or methylation, complements the direct methylation changes in DNA. As mentioned previously, these modifications may be paired and potentiate one another or seem in opposition. Proper gene expression requires the presence of the correct pattern of both DNA and histone epigenetic modifiers.[10,16] Hosseini et al[16] confirmed the pathophysiological link between histone acetylation and PCOS while studying *CYP19A1* in ovarian cumulus cells when compared with controls. Increased serum levels of acetylation in histone H3 and methylation of H3K9 were found in PCOS patients, reducing the expression of *CYP19A1*, ultimately reducing cytochrome P450 aromatase activity. This loss in aromatase function is likely a contributing factor to the hyperandrogenic phenotype and subsequent pathology in PCOS.

Another facet of epigenetic modifications influencing gene expression involves the presence of microRNA (miRNA). Previously studied in other adult disease states, changes in miRNA expression are known to be associated with diabetes, insulin resistance, inflammation, and cancer.[6] These noncoding single-stranded RNA molecules regulate gene expression following translation and are capable of modulating DNA methyltransferases and histone deacetylases.[17] Although present in large, stable quantities within the serum, tissue origins are difficult to trace, as is the case with the previously discussed correlation of serum DNA methylation levels. In regards to PCOS, a multitude of miRNAs have been associated with disease pathogenesis to include metabolism/insulin and androgen regulation, as well as adipose generation and inflammation.[18] These are listed in Figure 2 with their pathogenesis correlate.

Perhaps one of the most well-studied of these miRNAs is miR-222. Previously linked to type 2 and gestational diabetes, miR-222 has consistently been found to be elevated in serum quantities across multiple studies.[6] MiR-93 is also known to correlate with cellular lipid and glucose metabolism and is directly linked to *GLUT4* expression. *GLUT4* acts at the major insulin-mediated transporter of glucose into adipocytes and decreased expression has been demonstrated in PCOS patients with inversely proportional MiR-93 levels in serum.[19] These miRNA correlations may contribute to insulin resistance through overexpression, thus establishing one of the major pathophysiological pillars of PCOS.

Although much is still be learned regarding miRNA and its role within the foundation for PCOS, its stable presence within the serum may be useful for future diagnostic testing as opposed subjective choice

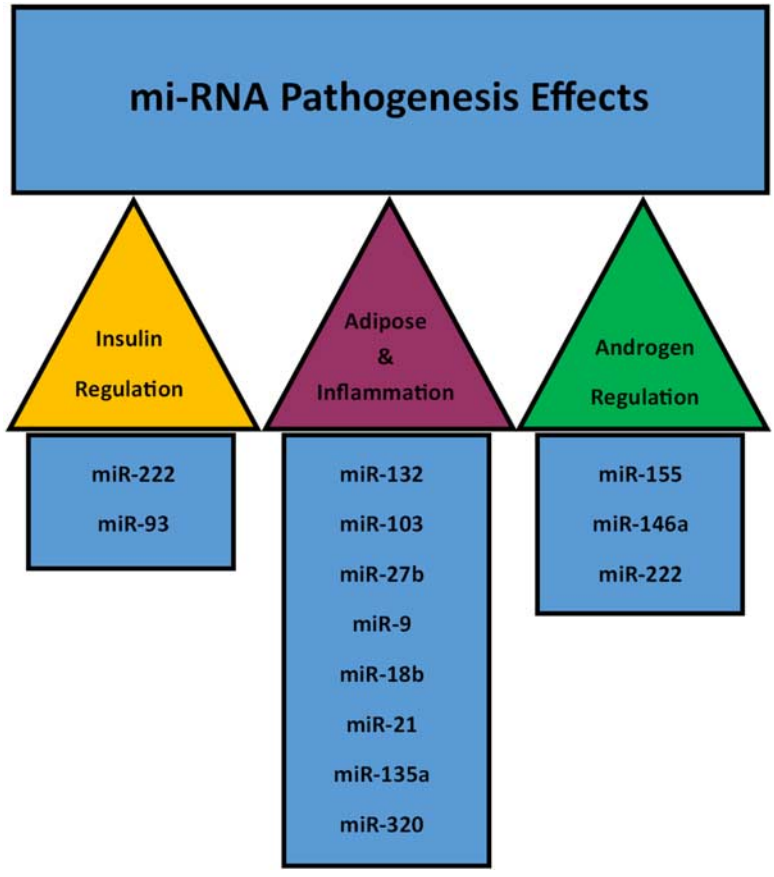

FIGURE 2. microRNAs (MiRNAs) and their associated pathophysiological effects. full color online

between multiple organization-defined diagnostic criteria.[6] This standardization would allow for more defined research into the etiology of PCOS when studying a cohort meeting the same inclusion threshold, research-wide.

Finally, mtDNA may be another frontier in the research of PCOS and its heritability through genetic and epigenetic mechanisms. Studies have identified 16 variants in the mitochondrial transfer RNA of PCOS women, contributing to inflammation through reactive oxygen species formation and insulin resistance. As mtDNA is maternally inherited, linkages could exist between family members with similar PCOS phenotypes.[9] Epigenetic control is also suspected through the methylated regulation of genes such as *PARK2, ESR1*, and *INS*, all of which have demonstrated control of mitochondrial activity.[20] Known controversy exists regarding the above mitochondrial associations with PCOS and more studies are need for further validation.

Conclusions

Much work has been done in an attempt to identify the cause of PCOS and its associated comorbidities. Although genetic linkages have been found, these seem to be just as complex as the disease process itself. The continuing growth of knowledge regarding the multitude of genes associated with the PCOS, and their epigenetic regulators, provides promise for improvement in future

understanding of the disease, its diagnosis, and treatment modalities. Continued research into the epigenetic root cause of PCOS needs to not only validate what has been recently discovered but also to continue to grow the common knowledge base.

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