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The Role of Genes and Environment in the Etiology of PCOS

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Both genes and the environment contribute to PCOS. Obesity, exacerbated by poor dietary choices and physical inactivity, worsens PCOS in susceptible individuals. The role of other environmental modifiers such as infectious agents or toxins are speculative. Phenotype confusion has characterized genetic studies of PCOS. Although several loci have been proposed as PCOS genes including *CYP11A*, the insulin gene, the follistatin gene, and a region near the insulin receptor, the evidence supporting linkage is not overwhelming. The strongest case can be made for the region near the insulin receptor gene (but not involving this gene), as it has been identified in two separate studies, and perhaps most importantly has not yet been refuted by larger studies. However, the responsible gene at chromosome 19p13.3 remains to be identified. To date, no gene has been identified that causes or contributes substantially to the development of a PCOS phenotype.

Key Words: Insulin resistance; familial studies; association studies; linkage; obesity.

Introduction

Polycystic ovary syndrome (PCOS) is the most common, but least understood, endocrinopathy. Although a major genetic contribution is suspected, there have been no clear genes or family of genes identified that cause or contribute to PCOS. There are a number of environmental factors that clearly also contribute to PCOS, most notably obesity. The rapid emergence of PCOS as a major endocrinopathy has gone hand in hand with the epidemics of obesity and type 2 diabetes sweeping developed countries. While these disorders also have a genetic component, clearly the environment has changed radically in the last century to further these epidemics and it is reasonable to assume that similar changes have also factored in the PCOS epidemic. This article will explore the contributions of genes and environment in PCOS, and unfortunately there has been little work in gene–environment interactions in this area. *The overall hypothesis is that a genetic predisposition combined with a favorable environment leads to PCOS.*

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The Diagnostic Dilemna of PCOS

The diagnosis of PCOS has traditionally been based on a history of *oligomenorrhea* and/or *hyperandrogenism*, either clinical, i.e., most commonly hirsutism, or biochemical, i.e., elevated circulating total or bioavailable androgens; and/or *polycystic ovaries*. The criteria that emerged from the 1990 NIH–NICHD conference identified PCOS as unexplained hyperandrogenic chronic anovulation, making it in essence a diagnosis of exclusion *(1)*. The "consensus" definition did not include the polycystic ovary morphology, most commonly found today on ultrasound consisting of multiple 2–8 mm subcapsular preantral follicles and increased ovarian volume *(2)*; however, these ultrasound criteria for polycystic ovaries are also a shifting target *(3)*. Ultrasound criteria were recently incorporated in the revised 2003 Rotterdam diagnostic criteria, which requires two out of the three outlined cardinal stigmata for PCOS: oligomenorrhea, hyperandrogenism, and/or polycystic ovaries *(4)*.

The Difficult Genetics of Complex Diseases

None of these expert generated definitions include insulin resistance, a common but not inevitable finding in PCOS. This diagnostic dilemma has hampered clinical and genetic studies of PCOS. The larger the number of distinct phenotypes within the affected category, the more complex the genetic analysis and the greater the likelihood that investigators using different diagnostic criteria will arrive at different conclusions. Other factors that contribute to difficulty in performing genetic studies in PCOS include the associated infertility and low fecundity (selection bias against the transmission of PCOS genes). Thus, it is rare to find large pedigrees with multiple affected women with which to perform linkage analysis.

Another difficulty is assigning phenotypes to premenarchal girls and postmenopausal women (who lack menses, and have indeterminate ovarian morphology, and no clear cutoffs for hyperandrogenemia), a problem that also limits the utilization of pedigrees. Although a male phenotype has

been postulated, there are no rigorously established clinical or biochemical features that can be used to identify "PCOS males." This makes formal segregation analysis as well as genetic linkage studies more difficult. Finally, the lack of animals that spontaneously develop a PCOS-like phenotype, especially mice, precludes the use of usually powerful tools of genetic mapping. The animal model (rhesus monkey) that best reflects the complex metabolic and reproductive abnormalities found in women with PCOS has resulted from prenatal iatrogenic androgen exposure *in utero*, at least providing support for the idea of some potential endocrine disruptor contributing to PCOS in humans, but most importantly the role of the environment in shaping PCOS *(5)*.

The idea that PCOS is a heritable disease is supported by studies documenting ethnic predisposition, familial aggregation, concordant twin studies, and association with other Mendelian disorders. This chapter will review the evidence that PCOS is a genetic disease and will briefly overview the largely *negative* genetic marker data that have emerged in the last decade.

Familial Aggregation

The foundation of genetic studies is the evidence that disease clusters in families. Although familial clustering may also occasionally have environmental causes (selective exposure to the putative agent differentially affects family members), but there has been no environmental endocrine disruptor or infectious agent identified to date that causes PCOS. None of the existing family studies of PCOS convincingly establishes a mode of inheritance either because the number of families studied was too small; the parental phenotypes could not be firmly established; and/or the male phenotype is uncertain. Despite the heterogeneity in study design and the inability to obtain comprehensive phenotype information to permit a formal segregation analysis, collectively the existing literature strongly suggests the clustering of PCOS in families with a mode of inheritance suggestive of an autosomal dominant pattern (Table 1). This may equally suggest a publication bias as subsequent analyses of genetic markers utilizing larger sample size often utilize a non-Mendelian model mode of genetic analysis (see below). This may also reflect that fact that retrospective series tend to identify large families through selection bias, whereas prospective series tend to identify smaller families, perhaps better indicative of low fecundity in these families.

These studies need to be critically judged based on their strengths and weakness. Diagnostic criteria used to assign affected status differed among the studies as did the methods with which the status of first- and second-degree relatives were ascertained (Table 1). By and large, ovarian morphology determined from tissue biopsy, direct visualization, or diagnostic imaging, in association with menstrual disturbances and evidence for hyperandrogenism, have been used in most studies as the criteria for diagnosing PCOS in probands. Earlier studies tended to characterize relatives on the basis of questionnaires, whereas later studies focused on more intensive phenotyping. More recent studies have focused on hyperandrogenemia, which appears to be one of the strongest genetically inherited characteristics in familial cases *(6,7)*.

These studies strongly support the clustering of reproductive abnormalities such as clinical and biochemical hyperandrogenism, polycystic ovaries, and, to a lesser degree, oligomenorrhea in first degree relatives. Similarly, there appears to be an associated increased prevalence of biochemical insulin resistance, hyperinsulinemia in first degree relatives. In one study 50% of sisters of women diagnosed with PCOS had elevated total or bioavailable testosterone levels, suggesting that hyperandrogenemia is a common trait, and has a bimodal distribution suggesting a dominant form of inheritance *(6)*.

Twin Studies

There have been a paucity of studies examining twins affected with PCOS. Twin studies offer a method for examining the relative contributions of genes and environment by comparing the prevalence and penetrance of traits in monozygotic (MZ) to that in dizygotic (DZ) twins. A higher prevalence of the trait and more complete penetrance in MZ compared to DZ twins is strongly supportive of a trait under genetic influence. A twin study from Australia *(7)* reported on both MZ and DZ twins, who were studied with ultrasound as well as clinical and biochemical parameters. This study noted a high degree of discordance among the twins for polycystic ovaries on ultrasound; however, there was a significant genetic component to androgen and insulin levels *(7,8)*. A recent large twin series applied Rotterdam PCOS diagnostic criteria to a large existing sample (with the exception of no ultrasound data). Clinical signs and symptoms from 1332 monozygotic twins (genetically identical) and 1873 dizygotic twins/singleton sisters of twins registered with The Netherlands Twin Register pointed to a strong contribution of genetic factors to PCOS. The resemblance in MZ twin sisters (tetrachoric correlation = 0.71, these correlations are an index of twin similarity with higher rates associated with higher heritability) for PCOS was about twice as large as in DZ twin and other sisters (tetrachoric correlation = 0.38) *(9)*.

Modes of Genetic Analysis Utilized in PCOS Studies

A variety of approaches have been used to find genes that contribute to PCOS including both genetic association studies in unrelated probands and familial linkage studies. Both approaches to date have been characterized by relying on the candidate gene approach, which is again ham-

Table 1
Summary of Diagnostic Criteria for the Proband in Familial Studies of PCOS and Proposed Mode on Inheritance

Author	Diagnostic criteria for PCOS	Number studied	Mode of inheritance/familial cluster
Cooper et al. *(41)*	Oligomenorrhea, hirsutism, polycystic ovaries (by culdoscopy, gynecography, or wedge resection)	18 PCOS women and their first degree relatives and a control group	Autosomal dominant with reduced penetrance
Givens et al. *(42)*	Oligomenorrhea, hirsutism, and polycystic ovaries (exam and surgery)	3 multigeneration kindreds	(?X-linked) dominant
Ferriman and Purdie *(43)*	Hirsutism and/or oligomenorrhea, 60% with polycystic ovaries (by air contrast gynecography)	381 PCOS women and relatives and a control group	Modified dominant
Hague et al. *(44)*	Clinical symptoms (menstrual dysfunction, hyperandrogenism, obesity, and infertility) and polycystic ovaries by transabdominal ultrasound	50 PCOS women and 17 women with CAH and a control group	Segregation ratios exceeded autosomal dominant pattern
Lunde et al. *(45)*	Clinical symptoms (menstrual irregularities, hirsutism, infertility, and obesity) and multicystic ovaries on wedge resection	132 PCOS women and first and second degree relatives and a control group	Unclear, most consistent with autosomal dominant
Carey et al. *(15)*	Polycystic ovaries (by transabdominal ultrasound)	10 kindreds and 62 relatives	Autosomal dominant with 90% penetrance
Norman et al. *(46)*	Elevated androgens, decreased SHBG, and polycystic ovaries on ultrasound	5 families with 24 females and 8 males	Not stated, metabolic abnormalities associated with insulin resistance in families
Legro et al. *(6)*	Elevated testosterone levels combined with oligomenorrhea (≤ 6 menses/yr)	80 PCOS probands and 115 sisters	Hyperandrogenemia consistent with an autosomal dominant trait
Govind et al. *(47)*	Polycystic ovary morphology on ultrasonograpy	29 families with 53 sisters and 18 brothers	Autosomal dominant
Kahsar-Millar et al. *(48)*	Oligomenorrhea and either hirsutism or elevated testosterone levels	90 PCOS probands, 50 sisters, 78 mothers	Increased prevalence of symptoms in in first degree relatives suggesting genetic trait
Legro et al. *(25)*	Elevated testosterone levels combined with oligomenorrhea (≤ 6 menses/yr)	346 PCOS probands, 307 sisters	Increased prevalence of hyperinsulinemia in sisters segregating with hyperandrogenemia
Legro et al. *(49)*	Elevated testosterone levels combined with oligomenorrhea (≤ 6 menses/yr)	87 PCOS probands, 119 brothers	Increased DHEAS levels in brothers
Sir-Petermann et al. *(50)*	Hyperandrogenism (elevated free androgen index or hirsutism) AND oligomenorrhea	106 PCOS Probands, 200 parents	Increased diabetes (approx twofold) and insulin resistance among PCOS parents
Yildiz et al. *(51)*	Elevated testosterone levels AND oligomenorrhea	52 PCOS Probands, 102 first degree relatives	Elevated testosterone levels in sisters and mothers, increased rates of glucose intolerance in first degree family members
Kaushal et al. *(52)*	Polycystic ovary morphology on ultrasonograpy	20 PCO probands and 20 brothers	Increased prevalence of insulin resistance and endothelial dysfunction in brothers.

pered by the same drawbacks as our diagnostic criteria, inadequate understanding of the fundamental pathophysiology of PCOS. Molecular defects in such candidate genes as gonadotropins and their receptors, in enzymes involved in steroidogenesis, as well as those underlying insulin action and secretion pathways, have been under continuous and intense investigation with variable results.

Although this scientific race is quite stimulatory, it has not been possible to establish firmly the role of any particular gene or region, and it has not so far helped us to get in any way closer to the exit of this genomic labyrinth. There has been no genome-wide scan performed in either association or linkage studies, and one problem has been the failure to accumulate a sufficient number of individuals or families to perform adequately powered studies using these broader tools. Thus, findings of positive and negative genes in PCOS must be corrected for the relatively small sample size and the limitations of our vision.

Table 2
Common Flaws in Genetic Association Studies

Small sample size
Subgroup analysis and multiple comparisons
Poorly matched control group
Failure to attempt study replication
Failure to detect linkage disequilibrium with adjacent loci

Adapted from ref. *11*.

Association Studies

Association studies are case control studies that test whether a particular allele occurs at a higher frequency among affected other than unaffected individuals. They involve correlation within a population, and not the inheritance of alleles within a family *(10)*, and therefore family members are not required to participate. Association studies are most commonly performed with alleles of a gene thought to have biological significance to the etiology of the complex trait or where familial-based linkage studies have shown linkage disequilibrium. This does not preclude their use in studying random alleles of seemingly unrelated genes.

Association studies have been criticized by a number of researchers for their tendency to highlight positive results leading to a publication bias, and the tendency to overinterpret these results as identifying etiologic genes *(10, 11)*. This has certainly been the case with PCOS where innumerable negative and positive associations have been reported. Association studies have provided a number of potential loci with genetic variants that may create or add to a PCOS phenotype (many of these borrowed from similar studies in families with type 2 diabetes), including, to mention a few such genes, *Calpain 10 (12)*, *IRS-1* and *IRS-2 (13)*, and *SHBG (14)*. Positive associations between a candidate allele and a complex disease are subject to many potential errors (Table 2). One of the most common is that smaller sample sizes are more likely to yield positive relationships that do not hold up when the sample size increases, as we have seen in PCOS with a *CYP17* (which encodes a key enzyme involved in androgen biosynthesis) polymorphism *(15)*. These were not replicated even by the same group in independent data sets *(16)*.

Population stratification between cases and controls is another problem. Failure to match cases with controls on the basis of racial background can lead to spurious results based on racial differences in allele frequencies unrelated to disease status. Results should be adjusted for multiple comparisons, as frequently both multiple genes and multiple alleles of these genes (at different sites) are tested. Furthermore, *a priori* phenotypes should be used to avoid *post hoc* phenotype drift, which can occur if the pirmary analyses do not reach significance with the initial proposed phenotypes. Finally, an association may exist, but not because the disease allele is the chosen candidate gene, but because the allele is in linkage disequilibrium with a nearby gene. Thus, the association is real, only the gene of interest is wrong.

Family-Based Association Studies

To control for genetic mismatching in case-control studies, Spielman and Ewens have suggested further assessment of positive associations with a transmission disequilibrium test (TDT) *(17)*. This requires the genotyping of both parents for, preferably, heterozygous alleles as well as having affected and unaffected offspring. The parent who is heterozygous for this allele should more often transmit the disease allele to the affected offspring than the other allele(s) *(17)*. Chi square testing can document increased transmission of putative disease markers. Family-based tests, such as this one, provide for an appropriate "internal control" with the use of unaffected siblings, whose environment and overall genetic background (i.e., same ethnic and family group) are as closely similar to the proband as possible *(18)*.

Linkage Analysis

Finally, there are modes of linkage analysis, which use DNA sequence polymorphisms (normal variants) that are near or within a gene of interest to track within a family the inheritance of a disease-causing mutation in that gene. These have classically been considered the most powerful means of identifying disease genes, and have the most complex forms of analyses. Parametric methods assume a specific model of inheritance (i.e., autosomal recessive, etc.), whereas nonparametric methods do not. The nonmodel based Affected Relative Pair analyses (sib pairs, cousin pairs, grandparent/child, etc.), have the advantage of not specifying a genetic model and of eliminating the problem of misclassifying young or "nonpenetrant" relatives, who are clinically unaffected but may carry the trait allele and have been used for PCOS studies. Linkage with PCOS was observed over a broad region of chromosome 19p13.2 in a recent large study of 367 PCOS families. The strongest evi-dence for association was observed with D19S884 (chi square = 11.85; nominal $p < 0.0006$; permutation $p = 0.034$) (Fig. 1) *(18)*. The present analysis suggests that a PCOS susceptibility locus maps very close to D19S884.

Pitfalls of PCOS Genetic Studies: Examples

Some of the pitfalls with specific genes will be illustrated by the following examples of two candidate genes follistatin and side chain cleavage that have been implicated in the etiology of PCOS.

Follistatin

Follistatin, an activin binding protein, is widely expressed, as is activin and could play an important role in PCOS. Activin stimulates pituitary FSH secretion and acts directly

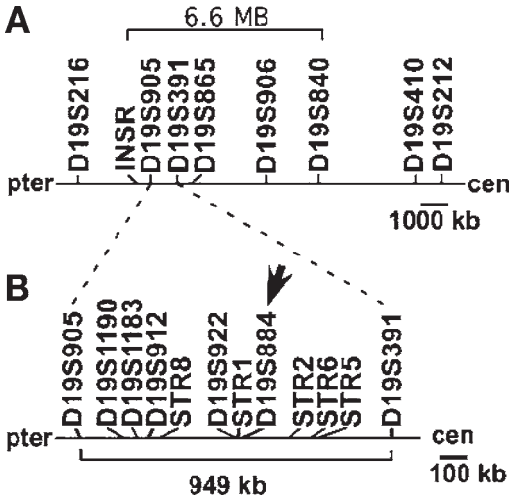

Fig. 1. Map of D19S884 region of chromosome 19p13.2. Locations of the short tandem repeat polymorphisms used in the analysis of 367 PCOS families, including those for (**A**) the 6.6 MB region of strongest evidence for linkage with PCOS in the *INSR* region and (**B**) the narrow region around D19S884. D19S884 is indicated by an arrow. Adapted from ref. *18*.

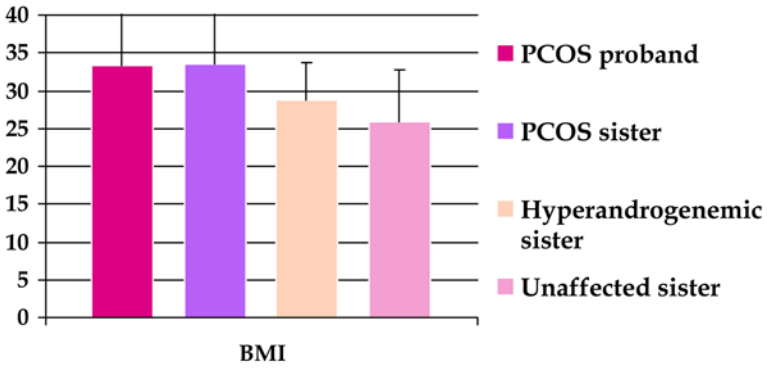

Fig. 2. Influence of body weight in women with PCOS (proband) and their sisters who have varying phenotypes, those with fully penetrant PCOS, those with regular periods and hyperandrogenemia alone, and those who are completely unaffected with regular menses and normal serum androgens. Mean BMI steps down as the phenotype improves. Adapted from ref. *6*.

on the ovary to promote follicular maturation. Neutralization of activin would be expected to cause reductions in FSH levels and arrested follicular maturation as occurs in transgenic mice overexpressing follistatin. In addition, activin inhibits thecal androgen biosynthesis and its removal by binding to follistatin might lead to unrestrained theca androgen synthesis driven by LH. The follistatin locus emerged as a promising candidate from the study of 39 affected sib-pairs *(19)*, although the strength of the link faded in a larger follow-up series that included detailed sequence analysis of the follistatin gene (Fig. 2) *(20)*. The follistatin gene has also not been found to be linked to PCOS in studies by other investigators.

CYP11A

The rate-limiting step in sex steroid biosynthesis is determined by the activity of side-chain cleavage encoded by $P450_{scc}$ encoded by the *CYP11A* gene. Gharani et al. examined 20 families with multiple affected women and found evidence for weak linkage to the *CYP11A* locus *(21)*. An association study conducted on 97 PCOS women and matched controls revealed significant association of a pentanucleotide repeat (tttta)n polymorphism at position –528 from the ATG initiation codon in the 5'-region of the *CYP11A* gene, which encodes the cholesterol side-chain cleavage enzyme and total serum testosterone levels *(22)*. Although no regulatory role has been assigned to this polymorphism in terms of *CYP11A* gene transcription, the investigators suggested that allelic variants of the *CYP11A* gene have a role in the hyperandrogenemia of PCOS. This conclusion was supported by another study that found an association between at least four repeats and PCOS, and within the PCOS group an association of this allele group with a lower testosterone level *(23)*. In a study with a larger sample size, Urbanek et al. found modestly increased sharing of alleles at the *CYP11A* locus among affected sib-pairs *(19)*, but the sharing was not statistically significant. Most recently, the original group in a larger replication study, using both case-control and family-based association methods, report no association with the *CYP11A* gene *(24)*.

The Environment and PCOS

An overemphasis on genes is a simplistic explanation of the PCOS epidemic. The rise in PCOS in populations where the gene pool has been relatively constant confirms that environmental factors are assuming an ever more important role. The development of obesity is linked to the development of PCOS in susceptible individuals. The modern living environment in developed countries is characterized by low daily energy expenditure and an abundant and inexpensive food supply, making positive energy balance common, but we propose this too is simplistic and it is likely that other factors in the environment perhaps unrecognized or little recognized may also play a role, for instance exposure to infectious agents, or environmental toxins.

Obesity and Diet

Obesity is critical in the development of the PCOS phenotype and weight exacerbates many (most notably insulin resistance), if not all, of the PCOS symptoms. Family studies provide an interesting perspective on the role of weight in the PCOS phenotype. There are interesting findings among PCOS sisters revealing the role of obesity. Body weight differs between the phenotypes in affected PCOS sisters *(6)*. Sisters with irregular cycles and hyperandrogenemia are heavier than sisters with regular cycles and hyperandrogenemia, while unaffected sisters have lower body weights than the affected ones (Fig. 2) *(25)*. Retrospective studies suggest that a different intrauterine environment is linked to different PCOS phenotypes like PCOS women with increased birth weight born from overweight mothers *(26)*, or girls with premature pubarche and lower birth weights who develop a more severe PCOS phenotype at menarche *(27)*.

For obese women with PCOS, dietary therapy is often the first line recommended therapy and marked improvement including restoration of ovulation has been noted with minimal weight loss approaching 5% of the baseline weight *(28,29)*. The mechanisms for this are complex for weight loss is associated with simultaneous improvements in circulating androgens, gonadotropins, and insulin levels. It is difficult to tease out the benefits of exercise per se on improving PCOS, but intervention programs that have combined both dietary and exercise interventions have had excellent outcomes in terms of improved ovulation and/or pregnancy rates.

Poor dietary choices including relying on energy dense foods instead of unprocessed grains, fruits, and vegetables, and larger portion sizes have been implicated as contributors to the obesity epidemic. Interestingly not only the quantity of food but the quality and the type of nutrition as well may alter PCOS phenotype, possibly interacting with different genetic patterns. One study demonstrated that women with PCOS from Sicily are less obese than women from Pennsylvania *(30)*. However, total calorie intake and dietary constituents were similar between countries, except there was higher saturated fat content in diet of U.S. women. Therefore, it was hypothesized that diet alone does not explain differences in body mass, because their food differed only in the quality of consumed fats and not in quantity. From these data it was concluded that genetic and lifestyle factors contribute to body weight differences, and there may be substantial geographic differences in the activities of daily life including exercise- and nonexercise-related activities.

Randomized trials of varying hypocaloric diets in women with PCOS have resulted in appropriate short-term weight loss after 4 wk (with a 1000 kcal deficit/d designed to lose 1 kg/wk, mean weight loss at 4 wk was 4 kg) *(31)* and after 12 wk (on an approx 1400 kcal/d diet designed to lose about 0.45 kg/wk, subjects lost approx 8 kg) *(32)*. There was also an improvement in body composition, insulin levels, androgen levels, and menstrual frequency with dietary-induced weight loss *(31,32)*. Neither of these studies found a benefit to a particular dietary composition (high protein versus low protein) although their sample size was small and both had high dropout rates *(31,32)*.

Environmental Toxins

Organochlorine pesticides were extensively used throughout the world until they were banned in the 1970s *(33)*. Owing to their resistance to enzymatic degradation and to their concentration in fat, these products are particularly persistent in living organisms and accumulate in their fat over years *(33)*. Since then, several organochlorine compounds have been characterized as estrogenic and as hormone-disrupters *(34)*. Furthermore, organochlorines were found in greater concentrations in the adipose tissue of women affected by breast cancer than in healthy controls *(35)*. However, their role in the development of a PCOS phenotype are unknown.

Medications

A better example of an "environmental" substance implicated in the development of a PCOS phenotype is valproic acid, which is a short-chained fatty acid that is widely used to treat epilepsy and bipolar disorders as well as migraines and generalized mood disorders. There are studies to suggest that women with these disorders and treated with valproic acid may develop stigmata of PCOS, including polycystic ovaries, hyperandrogenism, obesity, and anovulation *(36)*, and that these stigmata may be reversible with discontinuation of the medication *(37)*. Although this is a highly contentious area and there may be clear ethnic differences in susceptibility, recent studies suggest that weight gain on medication is essential to developing the full PCOS phenotype.

Infectious Causes

The possibility that one or more viruses could have contributed to the epidemic of PCOS has received little consideration. Most people view the likelihood that an infection

causes PCOS or obesity with the same skepticism that the scientific community once viewed the causes of peptic ulcer disease (interestingly also as a fault of the individual—too much stress in their life!). There is a significant literature that demonstrates that viruses may cause obesity in animals *(38)*. A human virus, adenovirus-36 (Ad-36), has produced obesity in chickens, mice, and monkeys. About 70–100% of animals inoculated with Ad-36 became obese (100% of monkeys) *(39)*. A study in humans demonstrated that both obese and nonobese subjects positive for Ad-36 antibodies had significantly higher BMIs compared to AB– subjects, thus demonstrating a syndrome similar to animals infected with Ad-36 in the laboratory *(40)*. These data show an association, but causality for human obesity has not been proven. There are no comparable studies of infectious agents in PCOS, and these data are cited for edification.

Summary

There is evidence that both genes and the environment contribute to PCOS. Recognizing environmental modifiers of PCOS in addition to poor diet and physical inactivity should be a priority for future researchers. There are likely man-made common substances that affect predisposition to PCOS, and one may also even widely speculate that infectious agents contribute to the risk.

Thus, there have been many tantalizing starts, but no finishes identifying genes associated with PCOS. This may be partially due to the fact that the majority of genes studied have been candidate genes based on current understanding of the pathophysiology of PCOS. Given that the pathophysiology is poorly understood, it is not surprising that these candidates have yielded little. No one to date has performed a genome-wide scan on an adequate sample size to discover new genes and regions unrelated to the expert's best guesses, although these studies are now in progress. The PCOS gene or genes are awaiting clearer unfolding

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