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Review

Polycystic Ovary Syndrome: The influence of environmental and genetic factors

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder characterized by hyperandrogenemia, hyperinsulinemia, insulin resistance, and chronic anovulation. It is the most common endocrine disorder in women of reproductive age with an enigmatic pathophysiologic and molecular basis. The high prevalence of affected individuals and the wide range of phenotypic expression can be explained by the interaction of a number of key genes with environmental factors. Heritability of PCOS has been inferred from studies of the syndrome in various population groups (ethnic groups, twins, and PCOS families). Although evidence of familial segregation and clustering of the disease in first-degree relatives of women diagnosed with PCOS has been presented, no particular pattern of inheritance has emerged. Some of the problems in genetic studies have been the lack of uniform criteria for diagnosis, heterogeneity of phenotypic features, and the fact that the disorder is only expressed clinically in women during their reproductive years. Even within affected families and between sisters with polycystic ovaries, there is heterogeneity in presentation. However, regardless of diagnostic criteria used to identify the propositus and to determine affected status in the kindred, the genetic studies available suggest a strong familial component. Currently, PCOS is considered a polygenic trait that might result from the interaction of susceptible and protective genomic variants and environmental factors, during either prenatal or postnatal life.

Key-words: AGEs, Candidate genes, Endocrine disruptors, Environmental, Metabolic abnormalities, Molecular factors, PCOS

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POLYCYSTIC OVARIAN SYNDROME

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting up to 6.8% of women of reproductive age based on the first study conducted in Greece in 1999 on the island of Lesbos,[1] as

well as subsequent epidemiological studies which confirmed these findings.[2,3] The definition of PCOS has been an issue of great and continuous debate among experts in the field since 1990 when the National Institutes of Health sponsored a conference (NIH) on PCOS and put forward as diagnostic criteria of chronic anovulation and hyperandrogenemia.

Recently, a meeting of experts in Rotterdam (2003), sponsored by the European Society of Human Reproduction and Embryology (ESHRE)/ American Society for Reproductive Medicine,[4] suggested that the definition of PCOS should include two of the following three criteria: (i) oligo- and/or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism, (iii) polycystic ovaries on ultrasonography, and exclusion of related disorders. The syndrome is widely accepted as the commonest cause of anovulatory infertility with clinical and/or biochemical signs of excess androgen secretion, associated with hyperinsulinemia and high prevalence of significant metabolic abnormalities, implicating long-term sequellae which may affect women's long-term health.[5-10]

The signs and symptoms of PCOS usually appear during or close to the onset of puberty.[11,12] Signs of precocious pubarche as well as adolescent hyperandrogenemia with or without insulin resistance may constitute early stages of the syndrome.

Furthermore, the phenotype may change through the life cycle of the woman, making the clinical picture very unpredictable and raising the potential for inaccurate diagnosis at any point during the woman's life. Interestingly, the nature of the male phenotype remains controversial, with premature balding being considered as the more prevalent phenotype, but this has not been accepted by all investigators.[13,14]

The pathophysiology of the syndrome of polycystic ovaries is polyprismatic and analysis of the spectrum of phenotypes from a single angle of pathogenetic view cannot adequately interpret it. Environmental and genetic factors are interconnected. Risk factors causing multiple aberrations in steroidogenesis, folliculogenesis, and metabolic pathways are continuously recognised but the key abnormality escapes detection.

ENVIRONMENTAL RISK FACTORS

The rise in PCOS prevalence 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. However, even this view is too simplistic and it is likely that other factors in the environment, perhaps unrecognized or poorly understood (e.g. exposure to environmental toxins), may also play a role.

A retrospective study on PCOS patients[15] has suggested the existence of specific prenatal risk factors for the post-pubertal expression of the PCOS phenotype. The authors have found two distinct groups of patients with polycystic ovaries: (i) those who had above average birthweight and (ii) those born to overweight mothers. The second group comprised women of normal weight who had high plasma LH but normal testosterone concentrations. These women were born after term. On the basis of these findings, the authors suggest that the two forms of PCOS have different origins in intrauterine life. Obese, hirsute women with polycystic ovaries have higher than normal ovarian secretion of androgens, associated with high birthweight and maternal obesity. Lean women with polycystic ovaries have altered hypothalamic control of LH release resulting from prolonged gestation.

Obesity & diet

Undoubtedly, environmental factors, interacting with a PCOS genetic background, could affect the two major components of the syndrome, hyperandrogenemic anovulation and insulin resistance. Since androgenized animals present increased adiposity, obesity is an independent risk factor of anovulation.[16] Oligoovulation is associated with increased adiposity and concomitant hyperinsulinemia. Subsequently, hyperinsulinemia with increased

levels of LH has an additive negative effect on preovulatory follicles, terminating the differentiation of granulosa cells and causing premature cessation of the ovulatory process.[17,18] There are interesting findings among PCOS sisters revealing the role of obesity. Body weight might differ in affected PCOS sisters.[19] 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.[20] The study by Taylor et al showed that normal weight women with PCOS eat less than normal women of similar body weight.[21]

Interestingly, not only the quantity of food but also the quality and the type of nutrition may alter PCOS phenotype, possibly interacting with different genetic background. Thus, Carmina et al[22] demonstrated that women with PCOS from Sicily are less obese than women from Pennsylvania and that body mass was significantly higher in USA women with PCOS compared with Italian women. However, total caloric intake and dietary constituents were similar, except for the higher saturated fat content in the diet of USA women. Therefore, it was hypothesized that diet alone does not explain differences in body mass, since 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 in PCOS women.

Poor dietary choices, such as 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.

Advanced glycation end products

A recent study from our group showed that non-obese, normoglycemic PCOS women compared to matched controls had higher levels of glyco-toxins, advanced glycation end products (AGEs), which were positively correlated with insulin resistance indices, and hyperandrogenemia.[23]

AGEs are the end products of a chemical procedure called Maillard reaction in which the carbonyl group of carbohydrates reacts non-enzymatically with primary amino groups of proteins such as lysine or arginine.[24-26]

AGEs can be formed both endogenously and exogenously. The endogenous AGEs are produced in the body by chemical reactions which often relate to elevated blood glucose levels. Exogenous sources are tobacco and food. In food AGEs are generated in the process of industrial packaging or home cooking (during extensive cooking, baking, grilling and frying as well as during long-term storage).[27,28]

Human and animal studies have shown that serum AGEs levels can be influenced by a diet containing AGEs. It has been speculated that dietary AGEs or "glycotoxins" may represent a risk factor in diabetic and uremic patients.[29] In a recent study, a decrease in body weight, higher plasma concentration of the AGEs protein, N-e-(carboxymethyl) lysine, and a decreased insulin response was observed in rats fed a diet high in AGEs for 20 weeks.[30] In a crossover study of diabetic patients, it was found that a high-AGEs diet resulted in a slight increase in inflammatory markers such as Tumor Necrosis Factor-a and C-reactive protein.[31] A restriction of dietary AGEs in the diet for 3 days resulted in a lower AGEs level in the plasma and dialysate of renal failure patients.[32] Urinary excretion of reactive intermediate Amadori products as well as of pyrraline is significantly affected by the type of food consumption and can be decreased by diets free of Maillard compounds.[33]

AGEs crosslinking and collagen

A common consequence of AGEs in tissue is the formation of covalent crosslinks. Long-lived structural proteins such as collagen are particularly vulnerable to AGEs crosslinks by nature of their slow turnover rate.[34] AGEs crosslinking alters protein function by reducing enzymatic activity, altering biophysical properties and changing protein interactions with other enzymes.[35,36] In the case of collagen, AGEs links form throughout the molecule, contrasting with the more limited terminal positions for normal crosslinking, and this increases its tensile stiffness.

The chemistry behind crosslink formation is complex and not fully understood but is thought to involve lysine residues.[37] This is supported by *in vitro* work using the agent phenacyl thiozolium bromide which can cleave chemical crosslinks between two lysine residues. Furthermore, *in vivo* studies with a synthetic thiazolidine derivative, OPB-9195 in the kidneys of Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats, a type 2 (non-insulin-dependent) diabetes mellitus model, have shown prevention of the progression of diabetic nephropathy by blocking type IV collagen production and suppressing overproduction of the two growth factors, TGF-beta and VEGF.[38]

Physiological crosslinking which also tends to involve collagen requires the enzyme lysyl oxidase (LOX). LOX catalyzes the final enzymatic reaction required for crosslinking of collagen and elastin fibers and therefore has a crucial role in regulating the formation and maintenance of the extracellular matrix in the ovary. *In vivo* findings indicate control of LOX at endocrine, paracrine, and autocrine levels within the ovary and suggest coordinated regulation of the ovarian extracellular matrix during follicular development, with FSH determining whether local factors act as stimulators or inhibitors of LOX.[39]

The pathological crosslink formation induced by AGEs leads to increased stiffness of the protein matrix, hence impeding function as well as increasing resistance to removal by proteolytic means, which in turn affects the process of tissue remodelling. These changes occur with advancing age and are accelerated in diabetes.[40,41] Histological studies support these findings, using human aortas obtained from post-mortem examinations and showed a correlation between AGEs tissue accumulation and aortic stiffness.[42] In addition, immunostaining methods using specific antibodies have shown increased accumulation of AGEs pyrraline, crossline, and pentosidine (considered to be a good biomarker of AGEs crosslinking) in the kidneys of diabetic subjects.[43,44]

Concerning the signalling mechanisms behind crosslinks, there is some involvement of the AGEs (RAGE) receptor in the upregulation of type IV collagen by AGEs, a mechanism possibly involved in diabetic nephropathy.[45]

Among other actions, AGEs were found to upregulate growth factors, such as the IGFs and transforming growth factor-beta (TGF-beta) in human and rat mesangial cells through an AGE-receptor-mediated mechanism. The parallelism with increased extracellular matrix (ECM) deposition and altered cell growth and turnover, leading to mesangial expansion raises the speculation that the enhanced synthesis of these growth factors, resulting from advanced nonenzymatic glycation, participates in the pathogenesis of hyperglycemia-induced mesangial expansion.[46]

Additionally, AGEs upregulate alpha1 type IV collagen (Col4), one of the major components of ECM, through activation of the activin receptor-like kinase 1 (ALK1) in mesangial cells. This kinase is highly expressed in human diabetic nephropathy and modulation of its expression can be responsible for the initiation and progression of diabetic nephropathy.[47]

Also, diabetic hearts had significant increases in AGEs and elevated expression of the AGEs receptors, RAGE and AGE-R3, in association with enhancement in gene and protein expression of connective tissue growth factor (CTGF).[48]

AGEs crosslinking, collagen, and PCOS

Increased formation and deposition of collagen characterise the stroma area in polycystic ovaries, which also contribute to dysregulation of the ovarian hormonal milieu and reproductive function in these young women. A possible negative role of AGEs in the ovary as in other tissues, direct or indirect, cannot be excluded and has never been investigated in ovarian tissue from PCOS. It could also be speculated that since women with this syndrome have elevated endogenous AGEs, the environmental fortification, either by food intake or smoking would have additional detrimental effects on fertility as well as on general health issues in women with this multifaceted disorder. Furthermore, it could be an aggravating factor contributing to increased menstrual irregularity and unexplained infertility.

Additionally, the exogenous AGEs received

through food may have deleterious effects on the endothelium, as is the case with endogenous AGEs in diabetes, and via compromised circulation, may endanger not only the reproductive function of these women but also their general health and especially the cardiovascular system.

AGEs and endothelium

The progressive accumulation of AGEs, the related overexpression of RAGE and nuclear factor (NF)-kB activation have been linked to endothelial dysfunction. AGE-RAGE interactions and NF-kB activation lead to oxidative stress, vasoconstriction, and a procoagulant state.[49-52] Generation of AGEs and accumulation in the vessel wall can be due to indirect consequences of elevated blood glucose (hyperglycemia), although enhanced AGEs accumulation also occurs in euglycemia and aging, albeit to lower degrees, driven by oxidative stress and inflammation. In hyperglycemia, production of 3-deoxyglucosone, at least in part via the polyol pathway, provides an amplification loop to sustain AGEs generation, oxidative stress, and vascular activation. Furthermore, recruitment of inflammatory cells bearing S100/calgranulins, as well as ligands for RAGE, augments vascular dysfunction. It is believed that activation of RAGE is a final common pathway that transduces signals from these diverse biochemical and molecular species, leading to cardiovascular perturbation.[53,54]

Interaction of RAGE with its ligands enhances receptor expression and initiates a positive feedback loop whereby receptor occupancy triggers increased RAGE expression, thereby perpetuating another wave of cellular activation. Sustained expression of RAGE by critical target cells, including endothelium, smooth muscle cells, mononuclear phagocytes, and neurons in proximity to these ligands, sets the stage for chronic cellular activation and tissue damage.[55] However, sustained receptor expression in a microenvironment with a plethora of ligands makes prolonged receptor stimulation possible, suggesting that interaction of cellular RAGE with its ligands could be a factor contributing to the pathology in a range of important chronic disorders.[56]

Therefore, a conserved AGE-receptor complex must be present in vascular endothelium which demonstrates subtle differences to other cell-types. In response to AGE-modified molecules, this complex is subject to upregulation, while the AGE-R2 component also displays increased phosphorylation possibly leading to enhanced signal transduction.[57,58,60-62]

The general increase in reactive oxygen species generated from glucose-derived AGEs is among the key mechanisms implicated in tissue injury due to diabetes. AGE-rich foods could exacerbate diabetic injury, at least by raising the endogenous AGEs.[63,64]

AGEs linked to lipids have been shown to initiate oxidative modification with the formation of oxidised LDL and VLDL.[65,66] In diabetes a greater portion of LDL is glycated and oxidized.[67,68] The LDL receptor does not recognise modified LDL, which is taken up by macrophage scavenger receptor or AGEs receptors, resulting in lipid-laden foam cells in the arterial intima and the promotion of atherosclerosis.[69] Under conditions of hyperglycemia, glycation of HDL function can reduce paraoxonase activity, which is an HDL-associated ester hydrolase important for the prevention of LDL oxidation.[70,71] Recent research has shown that advanced glycation of ApoB contributes to the development of hyperlipidemia.[52] Furthermore, it has been shown that lipoprotein (a), Lp(a) - an independent risk factor for cardiovascular disease undergoes glycation in diabetic subjects. From *in vitro* studies, it has been established that glycation of Lp(a) attenuates fibrinolysis by inducing expression of plasminogen activator inhibitor-1 (PAI-1) and reducing expression of tissue-type-plasminogen activator.

AGEs have therefore the ability to cause platelet aggregation and fibrin stabilization, resulting in a predisposition to thrombogenesis and thereby contributing to the development and progression of diabetic vascular complications.[72-74]

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 genera-

lized mood disorders. There are studies to suggest that women with these disorders treated with valproic acid may develop stigmata of PCOS, including polycystic ovaries, hyperandrogenism, obesity, and anovulation,[75] and that these stigmata may reverse with discontinuation of the medication.[76] Although this is a highly contentious area and there may be clear ethnic differences in susceptibility, recent studies suggest that weight gain on this medication is essential for the development of the full PCOS phenotype.

GENETIC BASIS OF PCOS

Evidence for the genetic basis of PCOS is very broad but by no means adequate to interpret the pathogenesis of the syndrome. Molecular defects in gonadotrophins 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 thus far to firmly establish the role of any particular gene on PCOS pathogenesis. Furthermore, since the list of candidate genes is steadily increasing, major problems arise, while the findings are not uniform among the various studies. In fact, there is difficulty even in replicating results by the same investigator. In addition, the constantly expanding spectrum of new candidate genes makes the focus less clear. Current data favour the view that PCOS is likely to represent a complex disorder caused by multiple genetic defects.[77-79] Before we enter the analysis of the current literature on each one of the *contributory or suspicious* genes, other pertinent data will be briefly reviewed.

PCOS phenotypes in various populations

Although cases of PCOS cluster within families, genetic studies have not been conclusive. Genetic studies have been hampered by several factors such as small sample sizes, errors in statistical analysis, differences in diagnostic criteria, and prevalence of PCOS in different ethnic populations. Reports on PCOS, independent of race or ethnicity, suggested a simple Mendelian pattern of PCOS inheritance consistent with an autosomal dominant or X-linked pattern when ultrasound scan for the diagnosis of PCOS (a method not universally accepted[80,77]) was used.

The first genetic study was by Cooper et al[81] who studied 18 patients with Stein–Leventhal syndrome. Oligomenorrhea, hirsutism, and enlarged ovaries were much more common in sisters of affected subjects than in sisters of controls. In the 1970s, Givens et al[82] using as diagnostic criteria hirsutism and either polycystic or bilaterally enlarged ovaries, published reports indicating that PCOS could be inherited in an X-linked dominant fashion. In the first report,[81] two families were described in which multiple individuals in more than two generations were affected. In one of these kindreds, affected females also experienced myocardial infarction in their fifth decade; and acanthosis nigricans, insulin resistance, and hypertension were present in many family members.

In kindreds reported by Cohen et al[83] several males showed maturational arrest of spermatogenesis. Excluding index cases, Wilroy et al[84] showed that 47% of female offspring of affected females were affected. Among the offspring of males with an elevated LH/follicle stimulating hormone (FSH) ratio, 89% of daughters were affected. The fact that almost all daughters of affected males were affected is consistent with X-linked dominant inheritance.

In the UK, Ferriman and Purdie studied 381 patients with hirsutism and/or oligomenorrhea and a control group of 179 women.[85] The interesting finding was that first-degree relatives with hirsutism and enlarged ovaries had a greater incidence of oligomenorrhea and infertility compared to women who were first-degree relatives of women with normal sized ovaries and the control group.

Later British studies provided additional data in support of heritability of PCOS, specifically autosomal dominant inheritance. The significant role of diagnostic criteria in genetic studies was clearly shown by the study of Hague et al[86] who used ultrasound criteria and either hyperandrogenemia or LH hypersecretion to determine the frequency of PCOS in relatives of affected cases. PCOS was found in 45 of 52 (87%) sisters of probands and in 24 of 36 (67%) mothers. In this study the frequency of affected rel-

atives was dramatically higher than the 50% predicted for either autosomal dominant or X-linked dominant inheritance. Non-Mendelian mechanisms would need to be invoked in order to account for such a distorted segregation ratio. More likely, the criteria were overly sensitive, leading to false diagnosis.

Lunde et al[80] also conducted family studies in Norway (1989) using hirsutism and oligomenorrhea as inclusion criteria. They found only 6-15% of first-degree relatives affected. Norman et al[87] found that, in 15 probands, far more relatives were affected. Among sisters, 11 of 15 (73%) had polycystic ovaries by ultrasound, 13 of 15 (87%) had elevated testosterone, and 10 of 15 (66%) hyperinsulinemia.

In the USA, Legro et al[19] studied 80 probands diagnosed on the basis of elevated testosterone associated with oligomenorrhea (<6 menses/year); non-classical 21-hydroxylase deficiency was excluded. They found 36 of 80 (45%) sisters to be affected on the basis of hyperandrogenemia.

A recent study by our group on phenotypically healthy sisters of women with PCOS showed evidence of insulin resistance. These women had neither clinical nor laboratory evidence of hyperandrogenism, suggesting that insulin resistance is a dominant trait among PCOS families.[88]

Govind et al[89] studied 29 probands and 10 control women. Diagnostic criteria consisted of polycystic ovaries on ultrasound with or without clinical or biochemical features of PCOS; 61% of female first-degree relatives were affected and 22% of male first-degree relatives had early onset (before age 30) male-pattern baldness. The prevalence was much higher than in the control families. Of a total of 71 sibs of PCOS probands, 39 (55%) were affected, which is consistent with autosomal dominant inheritance. Kahsar-Miller et al[90] studied the frequencies of oligomenorrhea and either hirsutism or elevated testosterone among first-degree female relatives within families of 93 probands with PCOS. A significantly higher rate of PCOS was observed among first-degree relatives than in the general population, suggesting a genetic component in this disorder. Ward et al[91] used a large genealogy database to search for a founder effect and to evaluate the degree of heritability in PCOS, and showed that the degree of relatedness among a PCOS population was four-fold greater than the average degree of relatedness among a large random sample of the same database.[91] This result confirms the observation of the very first study showing that there is a familial trait in PCOS. Furthermore, studies including the PCOS morphology in the diagnostic criteria favour an autosomal dominant pattern of inheritance.[89]

Candidate genes

For a number of genes altered patterns of expression have been detected, suggesting that the genetic abnormality in PCOS affects signal transduction pathways controlling the expression of multiple genes rather than abnormal expression of a single gene. Cytogenetic studies have failed to identify common karyotypic abnormalities.

Clinical features of metabolic and steroidogenic abnormalities are a cardinal characteristic in PCOS and investigators have long sought linkage or associations between PCOS and the various genes involved in the androgen biosynthetic pathway. On the other hand, over the last ten years numerous studies have confirmed the central role of insulin action in the pathogenesis of the syndrome. Subsequent genetic studies on insulin resistance with the associated metabolic aberrations included genes also involved in chronic inflammation. A number of linkage and association studies of candidate genes in PCOS have yielded positive and/or mixed results, which are discussed below.

A. Candidate genes implicated in insulin signal transduction

Most women with PCOS, both obese and lean in comparison with normal women, have a degree of insulin resistance and compensatory hyperinsulinemia.[92] Thus genes involved in the secretion and action of insulin may play a role and are under investigation.

The insulin gene variable number tandem repeat (INS-VNTR)

A variable number of tandem repeats (VNTR) polymorphism in the promoter region of the insulin gene (INS) regulate its expression.[93] In Caucasians,

the repeats of the insulin gene VNTR are distributed in a bimodal pattern, class I alleles having an average of 40 repeats and class III alleles an average of 157 repeats.

Waterworth et al[94] found strong linkage and association between the class III allele of the insulin gene VNTR (variable number tandem repeats) in the 5' region of the insulin gene and PCOS. This allele was preferentially transmitted from heterozygous fathers but not from mothers to affected individuals.

However, in a larger study, Urbanek et al[79] found no evidence for linkage of the insulin gene and PCOS and no association between the class III allele of the insulin VNTR and hyperandrogenemia. In this study the NIHCD criteria were used to choose the population as opposed to the ultrasonographic findings used by the previous one. Other studies[96,97] have failed to show any association between the INS VNTR alleles and hyperandrogenism in PCOS. The different criteria and ethnic and geographical backgrounds might explain the conflicting results.

The insulin receptor gene

The impaired sensitivity to insulin action, both *in vivo* and *in vitro,* led to the hypothesis that genetic lesion in the insulin receptor gene or the post-receptor signalling may contribute to the pathogenesis of PCOS. Molecular studies of the coding region of the insulin receptor gene in women with PCOS have shown a large number of silent polymorphisms. The majority of these polymorphisms has also been identified in normal subjects and are considered to be common polymorphisms which do not lead to remarkable alteration in the function of the insulin receptor.

Recently, Siegel et al[98] observed a C/T single nucleotide polymorphism (SNP) in the tyrosine kinase domain of INSR, which was associated with PCOS. This SNP could be a susceptibility variant for PCOS or be in linkage disequilibrium with another INSR polymorphism. The association is pending confirmation by others.

A number of studies have examined the insulin receptor gene sequence for major mutations, since an increased insulin-dependent serine phosphorylation of the insulin receptor, causing abnormalities in post-receptor activation of the pathway and therefore reducing responsiveness, has been described.[99,100] This suggests that if perturbed insulin action is integral to PCOS, the mechanism likely involves a target downstream of the insulin receptor. After the elegant studies by Dunaif et al[99] showing that in ~50% of women with PCOS increased insulin receptor serine phosphorylation in skeletal muscle cells and fibroblasts is associated with insulin resistance, the area of the insulin receptor gene has been a major target of research. However, there are not as yet any concluding results.

Intriguing data come from three separate studies focusing on the area of the insulin receptor gene at chromosome 19p13.3. First, the National Cooperative Program in Infertility Research conducted a study linkage and an association between a marker, (D19S884) located near the insulin receptor gene and PCOS in a cohort of Caucasian families.[101] Following this, the Heritage Family Study showed evidence for statistically significant linkage between a region at chromosome 19p13.3 and androgen levels in Caucasians, providing further evidence for an important role of the region 19p13.3 in PCOS.[102] A study by Urbanek et al[95] provided strong, reproducible evidence for a PCOS susceptibility locus mapping to chromosome 19p13.2 at or near the dinucleotide repeat marker D19S884. The genes that code for three proteins known to map within 100 kb of D19S884 are: ELAVL1, a ubiquitously expressed mRNA-binding protein, CCL25, a thymus-expressed chemokine, and FBN3, the third member of the fibrillin family of extracellular matrix proteins. Although none of these genes is an obvious candidate for PCOS, their function needs further investigation.

Insulin receptor substrate proteins

Insulin signal travels downstream intracellularly towards the second main step of insulin receptor substrate proteins. A major gene defect responsible for impaired insulin action has not so far been detected.

Several polymorphisms in IRS1 and IRS2 have been implicated in PCOS pathogenesis and there is evidence of a gene dosage effect of the Gly972Arg IRS1 variant on fasting insulin and homeostasis

model assessment (HOMA) values among PCOS women. A study by Ehrmann et al[103] failed to provide any support for the IRS1 variable. Finally, the Gly972Arg variant of the IRS1 gene has also been associated with lower SHBG levels in adolescent girls with a history of precocious pubarche.[104]

Insulin-like growth factors

Although no evidence of a linkage with any of the IGF genes was initially found,[78] San Millan et al[105] tested the possibility of an association of PCOS with genomic variants related to insulin resistance in subjects from Spain. A statistically significant association between homozygosity for G alleles of the Apal variant in IGF2 and PCOS was found. Alleles of the Apal polymorphism in IGF2 induce an increase in IGF2 mRNA in leukocytes, and possibly result in increased liver IGF2 expression and secretion. IGF2 stimulates adrenal and ovarian androgen secretion, and, together with IGF1 and IGF-binding proteins, may play a role in the pathogenesis of PCOS.[106-108] The association between the Apal variant in IGF2 and PCOS needs to be confirmed by future studies.

Calpain 10

Calpain-10 is a cysteine protease that plays a role in insulin secretion and action and has been associated with susceptibility to type 2 diabetes. In a recent study, the 112/121-haplotype was associated with higher insulin levels in Afro–American women, and an increased risk of PCOS in both African–American and white women.[109]

Gonzalez et al[110,111] showed an association of the CAPN10 UCSNP-44 allele with PCOS in a Spanish population. Hadad et al[112] however, found no association between CAPN10 gene variation and PCOS.

B. Candidate Genes involved in obesity and insulin resistance

The common occurrence of insulin resistance and pancreatic β-cell dysfunction in association with PCOS and the increased risk for development of type 2 diabetes is now well recognised. Moreover, insulin acting through its own receptor and at high concentrations through the insulin-like growth factor I receptor stimulates steroidogenesis. This has led investigators to focus on insulin resistance as a potential central abnormality in PCOS and to consider the proteins promoting insulin resistance as candidate genes for PCOS.

Peroxisome proliferator-activated receptor-γ gene (PPAR-γ)

Activation of peroxisome proliferator-activated receptor (PPAR)-γ promotes differentiation of adipocytes, increasing insulin sensitivity. The PPAR-γ gene contains a common SNP, Pro12 Ala. It has been shown that Ala 12 alleles of PPAR-γ favour weight gain in obese adults and in obese hyperandrogenic girls and adolescents. They also induce insulin sensitivity in Caucausian men and women presenting with PCOS.[113] Recently, a marginally significant decrease in the frequency of the Ala12 allele in Finnish PCOS patients has been reported. However, this polymorphism has shown no association either with PCOS or insulin resistance in hyperandrogenic adolescents from the USA[114] or in women from Spain.[105]

Human sorbin and SH3 domain-containing 1 gene (SORBS1)

Human sorbin and the SH3 domain-containing 1 (SORBS1) protein is involved in insulin-mediated glucose uptake. The Thr228Ala variant of this gene (SORBS1) may play a role in the genetic predisposition to obesity and type 2 diabetes. The SORBS1 Ala228 allele was found to play a protective role against the development of these disorders in Chinese subjects;[115] however, conflicting results have been reported in a large European study.[116] Moreover, no association of this allele with either adolescent hyperandrogenism or PCOS has been found.[117]

Paraoxonase (PON1)

Paraoxonase-1 (PON1) is a serum high-density lipoportein (HDL)-associated enzyme with antioxidant properties. A polymorphism in PON1 (-108T/C) is more frequent in nondiabetic subjects showing abnormal fasting glucose concentrations (therefore suspected to have insulin resistance) compared with subjects with normal serum glucose concentrations.[118] Since the -108T alleles may reduce paraoxonase expression and secretion, increasing oxidative stress, it is possible that homozygosity for -108T al-

leles and PCOS might lead to a high oxidative stress in these women, contributing to insulin resistance.[105]

Genes encoding other molecules related to insulin resistance

Among the other genes tested, no association has been reported between PCOS and genomic variants in the genes encoding glycogen synthase,[119] resistin,[79] leptin[120] or with variants in the genes of plasma cell differentiation antigen glycoprotein, protein tyrosine phosphatase 1B, and adiponectin.[105]

C. Candidate genes involved in steroidogenesis

A common biochemical abnormality in women with PCOS is hypersecretion of androgens. The increased steroidogenic activity is due to increased 3β-hydroxysteroid-dehydrogenase and 17α-hydroxylase/17, 20-lyase activities.[121] Northern blot analysis revealed that cytochrome P450 17-hydroxylase/17, 20-desmolase (CYP17) and cytochrome P450 side-chain cleavage enzyme (CYP11A) mRNAs were more abundant in PCOS theca cells than in normal ones. In addition, transient transfection experiments indicated that the CYP17 promoter is enhanced in PCOS theca cells compared to theca cells from normal women.[122] The upregulation of steroidogenesis in PCOS theca cells suggests the presence of an intrinsic defect in the metabolic pathways of the cells responsible for androgen production independently of environmental and neuroregulatory factors. However, theca cell studies have been performed only on classical PCOS phenotype with hyperandrogenemia and there is no information if all subtypes of theca cells present a steroidogenic defect. Additional studies on theca cells isolated from different PCOS phenotypes will most likely provide valuable information.

Luteinising hormone (LH) and its receptor

A multicentre study investigating polymorphism in the LH b gene showed some interesting variations between populations but failed to find a clear causal link with PCOS.[123] Another study tested the hypothesis that an activating mutation in the LH receptor gene may be a cause of hyperandrogenism in PCOS, particularly in those subjects with normal serum LH concentrations and raised androgen levels. Five families were identified in whom polymorphic markers close to the LH gene appeared to segregate with affected subjects. However, there was no evidence of linkage in 18 other families, and in the total number of families (n=23) the non-parametric LOD score did not reach significant levels. Furthermore, no mutations were found in the relevant coding region of the LH receptor gene in the 5 affected families.

These negative data are in agreement with those from the Urbanek et al[77] study in which a total of 37 potential candidate genes were examined in 150 families.

CYP11A, coding for P450 cholesterol side chain cleavage

Investigation of the CYP11A gene, which encodes the cholesterol side-chain cleavage enzyme P450scc, showed a weak linkage between the CYP11A gene and hyperandrogenemia in PCOS women.[124] An association study of 97 women with PCOS also demonstrated a strong association between the CYP11A 5' UTR pentanucleotide repeat polymorphism with total serum testosterone levels. However, other studies have failed to find a significant association between CYP11A and PCOS.[78,125] Although perturbations in the CYP11A gene cannot easily account for altered expression of other steroidogenic enzymes, CYP11A remains a potential candidate gene.

CYP17, coding for 17-a hydroxylase, 17/20-lyase

Although initial studies suggested an association between cytochrome CYP17, which encodes 17-hydroxylase/17, 20-lyase, and PCOS, subsequent studies have failed to show any association or linkage of this gene to PCOS.[126-128] However, PCOS patients show an exaggerated serum 17-α hydroxylase response to GnRH agonists. It has recently been shown that serine phosphorylation is also involved in the post-translational regulation of 17, 20-lyase activity and therefore in androgen secretion. The serine residues that are phosphorylated and the kinase that mediates the phosphorylation remain to be identified.

CYP21 (cytochrome P450 21-hydroxylase)

CYP21 encodes 21-hydroxylase, the enzyme responsible for most cases of congenital adrenal hy-

perplasia (CAH). Recent studies have found a significant prevalence of CYP21 mutations in PCOS women with a normal 17-hydroxyprogesterone response to adrenocorticotropic hormone (ACTH) stimulation, questioning the diagnostic distinction between PCOS and CAH[127,129] by ACTH stimulation test.

Androgen receptor

Urbanek et al[78] studied 150 families and failed to find evidence for an association of the trinucleotide (CAG) repeat polymorphism in the X-linked androgen receptor gene and PCOS. However, this short CAG repeat length has been shown to be inversely associated with androgen levels.[130]

Sex hormone binding globulin (SHBG)

Hogenveen et al[131] identified a polymorphism in the coding region of SHBG that encodes a missense mutation, P156L, in 4 of 482 women with PCOS, hirsutism or ovarian dysfunction.

A recent study by Xita et al[132] shows evidence of genetic contribution to the decreased SHBG levels frequently seen in PCOS women. They investigated the possible association of the functional (TAAAA)n polymorphism in the promoter of the gene with PCOS and lower SHBG levels. It was confirmed that women with PCOS had higher frequency of longer VNTR (more than 8 repeats-range 6-11), whereas control women had higher frequency of the shorter version (less than 8). They also reported that carriers of the longer allele had lower SHBG levels within the PCOS group.

Other steroidogenic genes

Association and linkage studies have been performed by two groups with regard to variations at the CYP19 locus (coding for P450 aromatase) and significance in the etiology of PCOS. Furthermore, Urbanek and colleagues examined a series of other genes in the pathways involved in ovarian steroidogenesis, but none was identified as being a major factor in the etiology of PCOS.[77]

D. Genes involved in gonadotrophin secretion and action

Abnormalities in gonadotrophin secretion, particularly LH, are characteristic of PCOS. Because LH plays a permissive role in driving thecal androgen production, there has been interest in exploring genes related to the regulation of LH secretion and action. Although dopamine receptor genes as well as the follistatin gene seemed originally as promising fields of research regarding gonadotrophin action and regulation, subsequent studies did not meet with any success.

Dopamine receptor genes

Dopamine inhibits GnRH and prolactin secretion. Polymorphisms have been identified in the dopamine D2 and D3 receptor genes. Homozygoisity for the rare allele (allele 2) of the D3 receptor has been associated with PCOS and clomiphene resistance in Hispanic women. However, a subsequent case-control study carried out in non-Hispanic white women failed to show a significant association with alleles of the dopamine D3 receptor gene and PCOS.[133]

The follistatin gene

Follistatin is a major inhibitor of activin both *in vitro* and *in vivo*. An increase in the level or the functional activity of this gene is therefore expected to arrest follicular development, increase ovarian androgen production and reduce levels of circulating FSH. These changes are all characteristic features of PCOS. An initial study of 39 affected sibling pairs demonstrated statistically significant linkage to follistatin locus on chromosome 5. Furthermore 72% of sisters were concordant for this genotype. In their affected sibling-pair analysis, the strongest evidence for linkage with PCOS of any of 37 candidate genes studied was found.[78] However, subsequent larger studies with more families by the same authors and more detailed sequence analysis of the follistatin gene have not revealed significant linkage.[79]

E. Candidate genes of indices of chronic inflammation

Abnormalities in endothelial function and elevated cardiovascular risk factors have also been observed in PCOS women, making the relevant proteins and the corresponding gene possible candidate genes for PCOS. Additionally, the secretory products of adipose tissue, including TNF-α and IL-6 which promote insulin resistance and hyperandro-

genism, have been implicated to PCOS pathophysiology.

Plasminogen activator inhibitor-1 (PAI-1)

Elevated PAI-1 levels have been associated with increased cardiovascular risk and increased thrombogenic tendency. Recently, a 4G5G polymorphism in the promoter region of the PAI-1 gene has been associated with increased plasma PAI-1 levels in Greek women with PCOS compared with controls.[134]

Tumor necrosis factor (TNF)-a gene

TNF-α is a cytokine secreted by adipose tissue that plays a key role in mediating insulin resistance.[135,136] Serine phosphorylation of IRS1 appears to be the mechanism for TNFα-mediated insulin resistance. Serum TNFα levels are elevated in patients with hyperandrogenism and PCOS, but studies have shown no association between polymorphism in its gene (TNF) and hyperandrogenism or PCOS.[137,138] However, when considering hyperandrogenic patients and healthy controls as a whole, carriers of TNF-308A alleles presented with increased basal and leuprolide-stimulated serum androgens and 17-hydroxyprogesterone levels, suggesting that this variant contributes to hyperandrogenism.[138]

Type 2 TNF receptor gene (TNFR2)

The type 2 TNF receptor (TNFR2) mediates most of the metabolic effects of TNFα. Serum levels of TNFR2 are increased in obese individuals, correlating with indices of insulin resistance.[139,140] In an initial study of 103 hyperandrogenic patients (42 with PCOS) and 36 controls from Spain, it was observed that the Arg196 allele of the Met196Arg variant in exon 6 of the TNFR2 gene tended to be more frequent in hyperandrogenic patients than in controls,[139] suggesting a possible role of the TNFα system in the pathogenesis of hyperandrogenic disorders.

Interleukin 6 gene (IL-6)

IL-6 is secreted from the adipose tissue. It promotes liver secretion of C-reactive protein, which was found increased both in obese women and in PCOS.[141] It was recently found in a study involving 85 patients and 25 healthy women from Spain[142] that common G alleles of the -597A and -74G/C IL6 gene polymorphisms, which are in linkage disequilibrium, are associated with hyperandrogenism. Moreover, when studying controls alone, carriers of G alleles presented higher serum IL-6, 17-hydroxyprogesterone and 11-deoxycortisol levels compared with subjects homozygous for the uncommon -597A or -174C alleles, suggesting a protective role for the uncommon alleles against adrenal hyperactivity and hyperandrogenism.

IL-6 signal transducer gp 130 (IL6ST)

IL-6 actions are mediated by a heterodimeric receptor consisting of two membrane-bound glycoproteins: an 80 kDa IL-6 binding unit (IL6Ra) and a 130 kDa IL-6 signal transducer (gp130). It was recently found that the uncommon Arg148 allele of the Gly148Arg polymorphism in the gp130 gene (IL6ST) was more frequent in controls compared with hyperandrogenic patients. Controls carrying Arg148 alleles had lower 11-deoxycortisol and 17-hydroxyprogesterone concentrations, a lower response of androstenedione to 1-24 adrenocorticotropin, and a barely significant decrease in free testosterone levels.[143] As occurred with the IL-6 variants, the wild type allele was associated with hyperandrogenism, whereas the uncommon Arg148 allele in gp130 had a protective effect against androgen excess and adrenal hyperactivity. Therefore, the influence of proinflammatory genotypes on hyperandrogenism and PCOS might result from the interaction between predisposing and protective variants in several different genes.

CONCLUSION

PCOS can be considered a complex, heterogeneous metabolic syndrome triggered or maintained by the combined effect of inheritable genetic susceptibilities and environmental risk factors. Current genetic studies have failed to identify a specific gene or genes with clear clinical significance. However, it can be concluded that women with PCOS have two major genetic alterations in androgen synthesis and in insulin action and a higher incidence of different genes polymorphisms. Furthermore, studies with lifestyle modifications have shown that in women with PCOS the hormonal, metabolic, and reproductive abnormalities can improve by means of lifestyle

modification, suggesting that environmental factors such as food toxins, smoking, pollution, etc. play a fundamental role in unmasking genetic predisposition.

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