Article

Genetics of polycystic ovarian syndrome

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Abstract

Polycystic ovarian syndrome (PCOS) is a reproductive system disorder characterized by irregular menses, anovulation, clinical and/or biochemical signs of hyperandrogenism (hirsutism and/or acne), ovarian micropolycystic appearance and metabolic abnormalities, such as hyperinsulinaemia and obesity. The aetiopathogenesis of this syndrome is not well known. Several pathogenetic hypotheses have been proposed to explain the full array of symptoms and signs, but with elusive results. A genetic abnormality causing PCOS is supported by the observation that different members of the same family are often affected, and about half of the sisters of PCOS women have elevated serum testosterone concentrations. Therefore, the presence of gene abnormalities in women with PCOS has been widely explored in the attempt to establish whether their mutations or polymorphisms may cause PCOS. The main genes evaluated are those involved in steroidogenesis, steroid hormone effects, gonadotrophin release regulation and action, insulin secretion and action, and adipose tissue metabolism. Despite the vast body of literature produced, none of the genes evaluated seems to play a key role in PCOS pathogenesis. It is likely that PCOS may represent the final outcome of different, deeply inter-related genetic abnormalities that influence each other and perpetuate the syndrome.

Keywords: *gene abnormalities, genetic causes, polycystic ovarian syndrome*

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disease. Its prevalence has been reported to be 4% in an unselected group of 369 USA women examined in their pre-employment physical examination (Knochenauer *et al*., 1998). A cross-sectional study on 192 women aged 17–45 years living in the Greek island of Lesbos showed that the prevalence of PCOS, defined by the presence of oligo-amenorrhoea and biochemical hyperandrogenism, was 6.8% (Diamanti-Kandarakis *et al*., 1999). A similar prevalence (6.5%) of PCOS, defined by the NIH 1990 criteria, was found in 154 Caucasian women from Spain of reproductive age reporting spontaneously for blood donation (Asuncion *et al*., 2000). In all, these data suggest that PCOS is one of most common reproductive endocrinological disorders of women.

Despite the great heterogeneity of its clinical manifestations, PCOS is characterized by hyperandrogenism and chronic anovulation, which, according to the leading American opinion, are the fundamental criteria for its diagnosis after having excluded specific endocrine abnormalities (Zawadazki and Dunaif, 1992). It is noteworthy that an absolute consensus on PCOS diagnostic criteria does not exist, and that according to the British school, the typical ovarian ultrasonographic appearance, after which the syndrome was named, remains an essential diagnostic criterion (Balen, 1999). However, it should be kept in mind that ovarian morphological abnormalities are also present in a significant percentage of women without any evidence of reproductive system abnormalities. Therefore, the exact meaning of these microcysts remains controversial. Recently, it has been reported that polycystic ovarian morphology (PCOM) in non-hirsute women with a history of ovulatory cycles is associated

with normal 17β-oestradiol, progesterone and gonadotrophin dynamics, but higher androgen and insulin concentrations and lower sex-hormone-binding globulin (SHBG) serum concentration. This suggests that PCOM may represent the mildest form of ovarian hyperandrogenism (Adams *et al.*, 2004). Longitudinal studies are, however, necessary to establish whether PCOM predisposes to PCOS development. The last consensus conference, held in Rotterdam in the year 2003, included ovarian morphology among the diagnostic criteria of PCOS. Hence, the diagnosis is based on the presence of at least two of the following criteria: (i) oligo-amenorrhoea and/or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism; and (iii) ovarian micropolycystic appearance (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003).

In addition to oligo-amenorrhoea, hirsutism, acne and/or micropolycistic ovaries, PCOS is characterized by obesity and metabolic abnormalities that have been and are currently being investigated. Altogether, these data have given a new clinical and nosographic collocation to PCOS, which is no longer regarded as a syndrome affecting the reproductive system, but a syndrome with systemic implications.

The clinical heterogeneity of PCOS and the lack of consensus on the diagnostic criteria has been a major confounding factor in those studies aimed at establishing PCOS aetiopathogenesis because heterogeneous cohorts of patients with PCOS have been selected. Therefore, the aetiopathogenesis of PCOS is, at present, not completely understood. A number of hypotheses have been advanced, but none of them explains the full spectrum of PCOS symptoms and signs. It is likely that some of these mechanisms are variously inter-related, and therefore contribute to the onset of PCOS. The main theories, some of which have nowadays only a historical connotation, are reported in **Table 1**.

LH–theca interstitial cell hypothesis

This hypothesis was based on the observation that patients with PCOS have elevated serum LH concentrations (Patton *et al.*, 1975). Since LH is the main regulator of androgen production from the ovarian theca cells, elevated concentrations of this hormone would explain the hyperandrogenism found in PCOS women and the resulting hirsutism and follicular atresia (Kunle and Kenneth, 1999). In addition, it has been shown that the LH molecule of PCOS women has elevated LH bioactivity and a preponderance of basic LH isoforms (Ropelato *et al.*, 1999). It was therefore postulated that an intrinsic hypothalamic–pituitary abnormality was responsible for the PCOS features. However, this hypothesis cannot explain the wide clinical spectrum of the syndrome.

Table 1. Main pathogenetic hypotheses for PCOS.

LH–theca interstitial cell hypothesis
Oestrone hypothesis
Ovarian hyperandrogenism hypothesis
Genetic hypothesis

Oestrone hypothesis

This theory postulated that the increased aromatization of adrenal androgens into oestrone causes PCOS. Although it has only weak oestrogenic activity, oestrone is able to exert a positive feedback on LH secretion, resulting in increased serum LH concentrations and consequently increased ovarian androgen production responsible for the auto-maintenance of the process (Yen, 1991). Although this hypothesis explains some aspects of the syndrome, no convincing confirmatory experimental evidence has been produced.

Ovarian hyperandrogenism hypothesis

This theory was based on the observation that cultures of theca cells obtained from women with PCOS release, in the culture medium, greater amounts of testosterone compared with cultures of theca cell obtained from normal women (Gilling-Smith *et al.*, 1997; Strauss, 2003). These observations suggested that intrinsic ovarian factors might cause hyperandrogenism in PCOS. However, the genes responsible for ovarian hyperandrogenaemia of PCOS have not been identified. A microarray analysis, conducted to define the gene networks involved in excess androgen synthesis, showed that PCOS theca cells have an expression profile distinct from normal theca cells. This included the over-expression of aldehyde dehydrogenase 6 and retinodehydrogenase 2, which play a role in all-trans-retinoic acid biosynthesis and the transcription factor GATA 6. On this account, the authors showed that retinoic acid and GATA 6 increased the expression of 17α-hydroxylase, providing a functional link between altered gene expression and intrinsic abnormalities in PCOS theca cells (Wood *et al.*, 2003).

Genetic hypothesis

The presence of gene abnormalities in patients with PCOS has been widely explored, since a genetic abnormality causing PCOS is clearly supported by the observation that different members of the same family are often affected (Givens, 1988; Hague *et al.*, 1988). Indeed, about 50% of the sisters of PCOS women have elevated serum testosterone concentrations (Hong *et al.*, 1998). Many genes have been suggested as playing a role in PCOS pathogenesis and the presence of mutations and/or polymorphisms has been explored. Several genes have been proposed to play a pathogenetic role PCOS, but their role has not been proven (Ben Shlomo, 2003). The main genes explored, reported in **Table 2**, are reviewed in this article.

Genes involved in steroidogenesis

The main steroidogenetic genes that have been suggested as playing a role in the pathogenesis of PCOS are *CYP11a*, *CYP17* and *CYP21*.

CYP11a

CYP11a codes for the P_{450} cytochrome side chain cleavage that converts cholesterol to pregnenolone, a rate limiting step of steroidogenesis. A polymorphic sequence, a pentanucleotide repeat $(tttta)_n$, at position –528 from the ATG initiation codon in the 5′-region of the *CYP11a* gene, seems to be associated with PCOS susceptibility. The most common genotype, named 216 and comprising four repeats, is present with a frequency of

Table 2. Genetic hypothesis for PCOS: main genes examined.

Genes involved in steroidogenesis
CYP11a
CYP17
CYP21
Genes involved in steroid hormone effects
Androgen receptor gene
Sex hormone-binding globulin gene
Genes involved in gonadotrophin release regulation and action
LH gene
LH receptor gene
Follistatin
Genes involved in insulin secretion and action
Variable number tandem repeats
Insulin receptor gene
Insulin receptor substrate proteins (IRS-1 and IRS-2)
Calpain 10
Genes involved in adipose tissue metabolism
Leptin gene
Leptin receptor gene
Peroxime proliferator-activated receptor-γ
Other genes
Plasminogen activator inhibitor-1

0.59. The genotype 216–, encompassing patients with six or more repeats, has instead been reported to be associated with higher testosterone serum concentrations (Gharani *et al*., 1997). The role of $(tttta)_n$ in the pathogenesis of PCOS has also been supported by studies showing an association between this polymorphism and PCOS or hyperandrogenaemia (Franks *et al*., 1998; Diamanti-Kandarakis *et al*., 2000). Urbanek and colleagues also reported a statistically significant association between PCOS and $(tttta)_n$ polymorphism by linkage analysis in 39 affected siblings; however, the significance of this association was lost after correction for multiple analyses (Urbanek *et al*., 1999). Accordingly, a more recent study completely ruled out that $(tttta)_n$ polymorphism of the *CYP11a* gene promoter region plays a pathogenetic role at least in Spanish women with hyperandrogenism. Indeed, the absence of the four-repeat-units allele (4R– genotype) was found equally distributed among patients and controls, independently of the presence of PCOS and/or ovarian or adrenal hyperandrogenism. In addition, no difference in serum testosterone concentrations was found in 4R– patients compared with patients with at least one four-repeat-units allele (San Millian *et al*., 2001). To fully understand the role, if any, of the $(tttta)_n$ polymorphism in PCOS, structure–function studies using expression systems need to be performed, since the effects of this polymorphism of the CYP11a promoter region on gene transcription are not known.

CYP17

CYP17 codes for the enzyme 17α-hydroxylase, which converts C_{21}-steroids into androgens. Carey and colleagues reported that a rare single nucleotide polymorphism (T–C) at base pair –34 in the promoter region of this gene increases the susceptibility to develop PCOS (Carey *et al*., 1994). However, subsequent larger, case–control studies from the same group as well as from other centres were unable to confirm this association (Gharani *et al*., 1996; Urbanek *et al*., 1999). Furthermore, linkage analysis excluded *CYP17* as a major susceptibility gene for PCOS within families (Carey *et al*., 1994).

CYP21

The *CYP21* gene, which codes for $P_{450}C_{21}$, an enzyme involved in cortisol biosynthesis, has been called into play because its deficiency is associated with clinical and biochemical PCOS-like manifestations. Therefore, the existence of *CYP21* gene mutations in patients with PCOS has been explored. Escobar-Morreale and collaborators found a positive relationship between gene mutations and PCOS susceptibility. In particular, they identified the V281L polymorphism to be associated with an elevated production of 17α-hydroxyprogesterone following ACTH stimulation (Escobar-Morreale *et al*., 1999). Nevertheless, the normal 17α-hydroxyprogesterone concentrations found in subjects with the same gene mutation and the absence of signs of hyperandrogenism in heterozygote patients for *CYP21* gene mutations do not assign a definitive role to this gene in the aetiopathogenesis of PCOS. Alterations in the activity of phase I drug metabolizing enzymes and phase II detoxification enzyme (glutathione *S*-transferase, GST, genes) have been shown to cause follicular cyst formation. On this basis, Babu and colleagues found the presence of hyperinducible *CYP1A1* and its mutant with *GSTM1* and *GSTT1* null genotypes in PCOS women which may cause an imbalance between phase I and II enzymes and therefore predispose to PCOS development (Babu *et al*., 2004).

Genes involved in steroid hormone effects

Androgen receptor gene

All androgens act through the androgen receptor, whose gene is located in the short arm of the X chromosome. The N-terminal domain of this nuclear protein contains a poly-glutamine stretch codified by a CAG triplet whose length is polymorphic. An inverse relationship between the CAG repeat length and androgenicity has been shown. Since a subset of PCOS patients have normal androgen serum concentrations, it has been postulated that these patients may have an androgen receptor gene with a low number of CAG repeats. However, Urbanek and colleagues failed to find any relationship between this trinucleotide repeat and PCOS in 150 families using the transmission disequilibrium test which analyses the excessive transmission of an allele to affected individuals (Urbanek *et al*., 1999).

Likewise, Mifsud and colleagues found no differences between infertile and fertile women for CAG repeat distribution pattern in a group of predominantly Chinese women resident in Singapore (Mifsud *et al*., 2000). Indeed, a detailed analysis of these data shows that no difference in mean CAG length was found between PCOS patients and

controls. Only a downward trend was found in CAG biallelic length among patients with normal or elevated testosterone serum concentrations. The difference reached statistical significance only when the shorter allele for each individual was considered. However, the difference between the number of CAG repeats was very small (**Table 3**). Hickey and colleagues found that infertile Australian Caucasian women with PCOS have a significantly greater frequency of alleles with longer CAG repeats (>22 repeats) than fertile women of the same ethnicity (Hickey *et al*., 2002). Despite the different outcome, however, both studies (Mifsud *et al*., 2000; Hickey *et al*., 2002) highlighted a direct relationship between testosterone concentrations and the number oif CAG repeats in the androgen receptor gene.

The role of androgen receptor gene CAG repeat polymorphism has also been evaluated in girls with precocious pubarche (pubic hair <8 years) on the assumption that precocious pubarche represents a predisposing condition to PCSO (Ibanez *et al*., 2000). Girls with precocious pubarche had shorter mean CAG repeats than controls and, in addition, post-menarcheal precocious pubarche girls with shorter CAG repeats had higher 17α-hydroxyprogesterone concentrations. Therefore, the results of this study seem to suggest that shorter CAG repeats increase the risks for precocious pubarche and subsequent ovarian hyperandrogenism (Ibanez *et al*., 2003).

Sex hormone-binding globulin gene

The low serum SHBG concentrations typically found in patients with PCOS contribute to increased tissue exposure to free androgens. Recently, a $(taaaa)_n$ pentanucleotide repeat, located in an alu sequence at the 5′ boundary of the serum SHBG promoter, has been reported to influence the transcriptional activity of this gene in association with downstream elements (Hogeveen *et al*., 2001). On this ground, Xita and collaborators evaluated a possible association between the presence of $(taaaa)_n$ polymorphism with PCOS in 185 patients and 324 normal controls. PCOS women had a significantly greater frequency of longer $(taaaa)_n$ alleles (more than 8 repeats) than normal women who had a higher frequency of alleles with fewer than 8 repeats (Xita *et al*., 2003). More recently, Cousin and colleagues to a large extent confirmed these findings, and showed that a point mutation (D327N) in exon 8 of the *SHBG* gene, which increases SHBG protein half-life, has a strong disequilibrium linkage with the eight $(taaaa)_n$ polymorphism (Cousin *et al*., 2004). These results suggest that the decreased SHBG serum concentrations observed in PCOS have a genetic background. Therefore, the *SHBG* gene may be regarded as a candidate gene for PCOS development.

Table 3. Number of CAG repeats in the androgen receptor gene in 91 patients with PCOS (Misfud *et al*., 2000).

	Normal testosterone concentrations	*Elevated testosterone concentrations*
CAG biallelic length	22.5 ± 0.4	23.3 ± 0.3
Shorter CAG allele length	20.4 ± 0.5	22.0 ± 0.3[a]

[a] $P < 0.05$.

Genes involved in gonadotrophin release, regulation, and action

LH gene

The increased serum LH concentrations frequently observed in PCOS patients led to the evaluation of possible *LH* gene abnormalities (as distinct from genetic isoforms). Indeed, the presence of a genetic LH variant (v-LH) with two point mutations in the *LH β* gene, shown to be functionally different from the wild type (wt), was found to be 5- to 7-fold lower in obese PCOS subjects compared with non-obese patients from The Netherlands and Finland. A similar tendency was found in patients from the United States, but not in those from the United Kingdom. These observations suggest that v-LH somehow protects obese women from developing symptomatic PCOS. However, the national differences observed suggest that v-LH does not play a pivotal pathogenetic role in PCOS (Tapanainen *et al*., 1999).

LH receptor gene

The presence of LH receptor mutations was examined in PCOS patients with normal serum LH concentrations and hyperandrogenaemia. Using linkage analysis in families with multiple cases of PCOS, Franks and colleagues identified five families in whom polymorphic markers close to the *LH receptor* gene segregate with the syndrome (Franks *et al*., 2001). However, in a subsequent preliminary study, these authors did not find any mutations after gene sequencing in these affected families. A similar negative outcome was previously reported (Urbanek *et al*., 1999).

Follistatin gene

Activin may play an important role in PCOS because it stimulates FSH secretion and follicular maturation and inhibits LH-stimulated ovarian androgen production. Therefore, excessive activin neutralization by follistatin, its binding protein, may result in reduced FSH concentrations, follicular maturation arrest and increased androgen production. Urbanek and colleagues reported strong evidence for linkage between the follistatin gene and PCOS (Urbanek *et al*., 1999). Follistatin gene sequencing in 85 women of 19 families of PCOS patients showed sequence variants at 17 sites. Of these, 16 sites had variants too uncommon to be major contributors to PCOS susceptibility; the only frequent variant is a single base pair change in the last exon at a non-translated site. However, the linkage between PCOS and this variant is weak, suggesting only a marginal contribution of the follistatin gene to PCOS pathogenesis. Accordingly, the same authors reported similar expression of the follistatin gene mRNA in cultured fibroblasts from PCOS and control women (Urbanek *et al*., 2000). Similarly, another study conducted on 65 Chinese PCOS patients did not show any mutation of the follistatin gene that could be related to the pathogenesis of this syndrome (Liao *et al*., 2000).

Genes involved in insulin secretion and action

The central paradox is the high responsiveness of the ovary to insulin in contrast with the resistance observed in the other tissues (Ben Shlomo 2003). Insulin has been shown to stimulate ovarian androgens by multiple mechanisms. It acts directly on the ovarian function by: (i) increasing steroidogenesis; (ii) stimulating $P_{450}C_{17}$ activity; (iii) up-regulating LH and IGF-1 receptors; and (iv) inducing theca–interstitial cell proliferation. Indirect effects of insulin on ovarian function have also been reported. Indeed, insulin potentiates the effect of GnRH on pituitary LH release and acts synergistically with gonadotrophins to stimulate steroidogenesis (see Poretsky *et al*., 1999, for review). A gonadotrophin-mediated effect of insulin on ovarian function has been further demonstrated by treating PCOS women with insulin suppressor drugs, such as troglitazone and metformin (Velazquez *et al*., 1994; Dunaif *et al*., 1996), which results in lower serum LH concentrations. To further clarify the relationship between insulin and LH in PCOS women, Ciampelli and collaborators evaluated the LH response to GnRH in 110 PCOS patients subdivided into four groups according to their BMI and serum insulin concentrations. Hyperinsulinaemic patients had an increased LH response to GnRH compared with normoinsulinaemic PCOS patients, but the difference reached the statistical significance only in lean patients (Ciampelli *et al*., 1999). These findings clearly suggest that insulin plays a relevant role in PCOS pathogenesis. Indeed, hyperinsulinaemia and insulin-resistance are common features of PCOS patients (see Dunaif, 1997, for review). On these grounds, many genes involved in insulin secretion and action have been evaluated as possible candidate PCOS genes. The most relevant are: the variable number tandem repeats (VTNR), the insulin receptor gene, the insulin receptor substrate (IRS) proteins and calpain 10 (CAPN 10) (**Table 2**).

Variable number tandem repeats

Insulin–VNTR, which lies in the 5′ regulatory region of the gene, is involved in the regulation of insulin gene expression and has been implicated in the pathogenesis of type II diabetes mellitus. This locus has a bimodal distribution of repeats that have been divided into class I alleles, with a low number of repeats (mean 40), and class III, with a greater number of repeats (mean 157). Waterworth and colleagues reported that class III VNTR is associated with PCOS and that insulin serum concentrations are more elevated in families in which the linkage is present compared with families without evidence of linkage. This suggests a functional role of this VTNR variant in the expression of hyperinsulinaemia/insulin resistance in PCOS (Waterworth *et al*., 1997). However, no evidence for excess allele sharing has been reported in subsequent studies (Urbanek *et al*., 1999; Vankovà *et al*., 2002).

Insulin receptor gene

Since mutations of the insulin receptor cause insulin resistance associated with acanthosis nigricans and PCOS (Moller and Flier, 1988), a number of studies have explored whether patients with PCOS have mutations of this gene. No abnormalities have been found in the tyrosine kinase domain of the insulin receptor gene in 22 PCOS patients (Conway *et al*., 1994). Similarly, molecular analysis of the entire coding sequence of the insulin receptor gene resulted in no significant detection of mutations (Sorbara *et al*., 1994; Talbot *et al*., 1996). Therefore, mutations of the insulin receptor gene do not play a pathogenetic role in PCOS. Nevertheless, the insulin receptor appears to have a pivotal role in PCOS pathogenesis. Indeed, the insulin resistance in at least 50% of PCOS women appears to be related to excessive serine phosphorylation of the insulin receptor. A factor extrinsic to the insulin receptor, presumably a serine/threonine kinase, is responsible for this abnormality. Interestingly, serine/threonine phosphorylation appears to modulate the 17,20 lyase activity of $P_{450}C_{17}$ (Miller *et al*., 1997), the regulatory enzyme of androgen biosynthesis. Thus, it has been postulated that a single defect produces both the insulin resistance and the hyperandrogenism in some PCOS women (see Dunaif, 1997; Sam and Dunaif, 2003, for review).

Insulin receptor substrate protein genes

IRS proteins are critical to signal transduction in insulin target tissues (Sesti *et al*., 2001) and polymorphisms of IRS genes, particularly Gly972Arg of *IRS-1* and Gly1057Asp of *IRS-2*, have been shown to increase susceptibility to type 2 diabetes mellitus (Burks and White, 2001). Although no novel mutations have been found in *IRS-1* and *IRS-2* genes in PCOS women, by direct sequencing, gene-dosage effects were found on fasting insulin by the IRS-1 Gly972Arg variant and on 2-h serum glucose concentrations by the Gly1057Asp IRS-2 variant. In addition, the Gly972Arg IRS-1 variant seems to be more frequently present in insulin resistant patients compared with non-insulin resistant women or control subjects (39 versus 4 or 17%). These findings led the authors to conclude that polymorphic alleles of both IRS-1 and IRS-2, alone or in combination, may have a functional impact on the insulin-resistant component of PCOS (El Mkadem *et al*., 2001). This conclusion has, however, been disputed by a subsequent study which showed no evidence of the Gly972Arg IRS-1 polymorphism on glucose or insulin concentrations during an oral glucose tolerance test (OGTT) in either white or Afro–American women with PCOS (Ehrmann *et al*., 2002). In addition, in contrast to what was previously reported (El Mkadem *et al*., 2001), this latter study showed that Gly1057Asp polymorphism was associated with lower glucose concentration at 2-h point OGTT in both white and Afro-American PCOS women. The discrepancy between these two studies may relate, as also pointed out by the authors (Ehrmann *et al*., 2002), to the different criteria used to define PCOS as well as to the lack of stratification of PCOS patients on the basis of measures of insulin resistance or hyperinsulinaemia by Erhmann and collaborators (2002). More data are therefore needed to understand the role, if any, of IRS proteins on PCOS pathogenesis.

Calpain 10 gene

Polymorphisms of CAPN10, a ubiquitously expressed member of the calpain-like cysteine protease family, have been associated with an increased likelihood of developing type II diabetes mellitus (Horikawa *et al*., 2000). Gonzales and colleagues evaluated whether four polymorphisms (SNP-19, SNP-43, SNP-44 and SNP-63) of the *CAPN10* gene were

associated with PCOS. This study showed that the allele SNP-44 is associated with PCOS in Spanish women, supporting the role of CAPN10 in the pathogenesis of PCOS (Gonzales *et al*., 2002). In contrast, using both family-based and case–control association resources focusing on the same four single nucleotide polymorphisms of the previous study (Gonzales *et al*., 2002), Haddad and co-workers found no association between *CAPN10* gene polymorphism and the likelihood of developing PCOS (Haddad *et al*., 2002).

Genes involved in adipose tissue metabolism

Leptin gene

Obesity, a common feature of PCOS patients, led to the hypothesis that leptin may be involved in the pathogenesis of PCOS. Oksanen and collaborators sequenced the leptin gene in 38 PCOS patients, but no mutations of the coding exons were found (Oksanen *et al*., 2000).

Leptin receptor gene

Single-stranded conformational polymorphism (SSCP) analysis and subsequent sequencing of the leptin receptor gene revealed previously identified amino acid variants in exons 2, 4 and 12 as well as the pentanucleotide insertion in the 3′-untranslated region (3′-UTR) in these patients. However, the frequency of these polymorphisms did not differ from that found in the general population, suggesting that the leptin receptor gene does not play a role in PCOS (Oksanen *et al*., 2000).

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

PPAR-γ, a gene involved in adipose tissue differentiation, has recently been called into play because obesity is a frequent feature of PCOS. To shed some light on the role of this transcription factor on PCOS pathogenesis, Orio and collaborators evaluated the frequency of exon 2 and six polymorphisms of the *PPAR-γ* gene. This study showed that exon 6 T allele frequency is higher in PCOS women compared with controls. In addition, the body mass index (BMI) and leptin serum concentrations were higher in PCOS patients carrying the C–T substitution than in controls. Exon 2 Pro12Ala polymorphism was instead unrelated to BMI and/or leptin serum concentrations in PCOS women (Orio *et al*., 2003). Accordingly, Korhonern and colleagues found that the frequency of exon 2 Pro12Ala *PPAR-γ* gene polymorphism is significantly reduced in 135 PCOS patients compared with 115 controls (Korhonen *et al*., 2003). These findings support a role for *PPAR-γ* gene polymorphism in PCOS pathogenesis with the presence of the Ala isoform being protective against the development of PCOS.

Other genes

Plasminogen activator inhibitor-1 (PAI-1) gene

In addition to metabolic aberrations, PCOS women have an increased activity of PAI-1 (Dahlgren *et al*., 1994). The polymorphism 4G/5G in the promoter region of the *PAI-1* gene has been associated with increased plasma PAI-1 concentrations in patients with type II diabetes mellitus and myocardial infarction. Indeed, the concentrations of PAI-1 in homozygous 4G/4G subjects are approximately 25% higher than those carrying the 5G/5G polymorphism (Eriksson *et al*., 1995). On this ground, the poymorphisms of the *PAI-1* gene have been evaluated in PCOS patients. The results of this study showed a statistically significant difference in the distribution of *PAI-1* gene variations between patients and controls. The PCOS group had significantly higher 4G/4G and 4G/5G combinations than the control group, whereas there were significantly fewer 5G/5G. In the 4G/4G genotype subgroup, 75% were PCOS and 25% controls, in the 4G/5G were 68.42 and 31.58% and in the 5G/5G were 31.58 and 62.26% respectively. As previously shown (Dahlgren *et al*., 1994), PCOS women had significantly higher PAI-1 concentrations Diamanti-Kandarakis, 2004).

Conclusion

Although many pathogenetic hypotheses have been advanced, none of them fully explains the variegate symptoms and signs of this syndrome. A large body of research has been devoted to establishing the genetic basis of PCOS exploring, in particular, the role of genes involved in androgen biosynthesis and action, gonadotrophin release and action, insulin secretion and action and adipose tissue metabolism. A wide range of gene mutations or polymorphisms have been reported, but most of them do not seem to play a pivotal role in PCOS pathogenesis. It is likely that PCOS phenotype may represent the final outcome of different genetic abnormalities that are so deeply inter-related to influence each other and to perpetuate the syndrome. Therefore, the clinical heterogeneity of PCOS may result from its pathogenetic heterogeneity.

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