POLYCYSTIC OVARY SYNDROME – CANDIDATE GENES INVOLVED IN INSULIN RESISTANCE

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Abstract: Polycystic ovary syndrome (PCOS) is a complex, heterogeneous endocrinopathy, being considered the most common endocrine disorder in women reproductive period. It reflects multiple potential aetiologies and variable clinical presentations. The diagnostic criteria refer to the most pointed features: clinical hyperandrogenism and/or biochemical hyperandrogenemia, oligo/anovulation and polycystic ovarian morphology; insulin resistance is present in the majority of cases. Despite the progress in the definition of the clinical aspects of the syndrome, only very few certain data are available about the ethiopathogenetic mechanisms in PCOS. It seems that the PCOS phenotype derives from the interaction between the environmental and genetic factors. Many studies suggest that genetic factors play a major part in the etiology of PCOS. This article presents a brief overview of the most investigated candidate genes involved in PCOS’s insulin resistance.

Cuvinte cheie: sindromul ovarelor polichistice, insulin rezistență, candidate gene

Rezumat: Sindromul ovarelor polichistice (SOPC) este o endocrinopatie heterogenă și complexă, fiind considerată cea mai frecventă afeție endocrină din perioada fericită feminină. Reflectă multiple potențiale etiologice și manifestă clinice variabile. Crietiile de diagnostic includ cele mai pregnante trăsături: hiperandrogenismul clinic și sau hiperandrogenemia biochimică, oligo/anovulația și morfologia ovariană polichistică; rezistența la insulină este prezentă în majoritatea cazurilor. În ciuda progreselor realizate în definirea aspectelor clinice ale sindromului, doar puține date certe există în ceea ce privește mecanismele etiopathogene din SOPC. Se pare că fenotipul SOPC derivă din interrelația factorilor genetici și de mediu. Multiple studii sugerează că factorii genetici joacă un rol major în etiologia SOPC. Cu toate acestea, modul de transmitere rămâne neelucidat. Acest articol prezintă o sinteză a celor mai investigate gene candidate posibil implicate în insulin rezistență din SOPC.

Definition, pathogenesis, clinical picture and epidemiology of PCOS

The definition of polycystic ovary syndrome (PCOS) has been an issue of great and continuous debate among the experts in the field. PCOS is a highly prevalent endocrine disorder affecting approximately 7% of reproductive-aged women. There is no consensus on the diagnostic criteria and definition of PCOS. The most widely used 1990 National Institute of Child Health and Human Development (NICHD) conference diagnostic criteria include (a) clinical and/or biochemical signs of hyperandrogenism, (b) oligo-ovulation, and (c) exclusion of other known disorders such as Cushing’s syndrome, hyperprolactinemia and nonclassic adrenal hyperplasia. In 2003, a consensus workshop sponsored by ESHRE/ASRM in Rotterdam, indicated PCOS to be present if any 2 out of 3 criteria are met: (3)

1. oligoovulation and/or anovulation,
2. excess androgen activity,
3. polycystic ovaries (by gynecologic ultrasound).

Other entities with the same signs should be excluded. (4) The Rotterdam definition is wider, including many more patients, most notably patients without androgen excess. (5)

Ovarian dysfunction usually manifests as oligo/amenorrhoea resulting from chronic oligo-ovulation/anovulation. However, prolonged anovulation can lead to dysfunctional uterine bleeding which may mimic more regular menstrual cycles. The majority of PCOS patients, 70% to 80%, have oligo- or amenorrhoea. Among those with oligomenorrhoea, 80% to 90% will be diagnosed with PCOS. (6) Among those with amenorrhoea, only 40% will be diagnosed with PCOS as the hypothalamic dysfunction is a more common cause. (7) PCOS is the most common cause of anovulatory infertility. (6)

Hyperandrogenism. The clinical and/or biochemical signs of androgen excess in PCOS result from increased synthesis and release of ovarian androgens. Elevated luteinizing hormone and insulin synergistically increase androgen production. Insulin resistance leads to hyperinsulinaemia, reduces SHBG (sex hormone binding globulin) and raises free circulating testosterone, impairing ovarian follicle development. Clinical hyperandrogenism primarily includes hirsutism (60%), acne (30%) and male pattern alopecia. (8, 9)

Metabolic features - Mechanisms involved in:

- **insulin resistance** are likely to be complex with genetic and environmental contributors. Specific abnormalities of insulin metabolism identified in PCOS include reductions in secretion, reduced hepatic extraction (10, 11), impaired suppression of hepatic gluconeogenesis and abnormalities
in insulin receptor signalling.(9,12) Insulin resistance in PCOS associates diverse and complex effects on regulating lipid metabolism, protein synthesis and modulation of androgen production.(13)

- **dyslipidaemia** - is common in PCOS compared with BMI (body mass index) matched controls (14,15), with higher triglycerides and lower high density lipoprotein cholesterol. Dyslipidaemia occurs independently of body mass index (BMI) and the causes seem to be multifactorial. Insulin resistance appears to have a central role, modulating lipolysis and altering the expression of lipoprotein lipase and hepatic lipase.(14,15)

### Methods used in genetic studies of PCOS

Different approaches have been applied to elucidate the complex polygenic origin of PCOS: (a) association studies where a predisposing allele is expected to be encountered more frequently in the effected population than the normal individuals and (b) linkage studies where the subjects and their families are investigated to determine if particular genomic landmarks are distributed independently or in linkage (together) with the phenotype. While the mode of inheritance is not required for the association studies, it requires that a relatively large set of individuals are needed for a clear conclusion. The canonical linkage studies require that the mode of inheritance should be known for the analysis procedure. These studies are quite robust to identify single genes causing Mendelian disorders but are poorly suited to the genetic architecture of complex traits such as PCOS.(16) Linkage studies define the resolution and the mapping power of the study. The whole genome scan approach using the SNP (single nucleotide polymorphism) microarray to identify the punctiform mutations in candidate genes in PCOS.(17)

### Candidate genes involved in insulin resistance

It has been demonstrated that most women with PCOS either obese or nonobese, compared with normal women, exhibited variable degrees of insulin resistance and compensatory hyperinsulinemia, at present, being well known that hyperinsulinemia and insulin resistance are common features of PCOS patients.(18) Therefore, numerous genes involved in insulin action and secretion have been explored as candidate genes in PCOS pathogenesis.

#### 1. The insulin gene

The INS includes variable tandem repeats (VNTR) embedded at the 5′ regulatory region. The VNTR polymorphism regulates the transcriptional rate of the INS and probably that of the gene encoding IGF-II (insulin growth factor).(19,20) The number of the repeats of the INS VNTR ranges from 26 to 200, and due to this feature, INS VNTR polymorphism has three size classes. In Caucasians, the repeats of the insulin gene VNTR are distributed in a bimodal feature, class I alleles having an average of 40 repeats and class III alleles an average of 157 repeats, with class II alleles being rare. Transcriptional activity of the longer polymorphic region is greater than that of the shorter one.(19)

It is not known whether hyperinsulinemia detected in PCOS is an outcome of primary insulin resistance or the direct effect of pancreatic β-cell disorder. Therefore, in order to clarify the genetic basis of PCOS and to determine its association with defects in insulin secretion and action, firstly, Waterworth et al. evaluated the linkage and association of the INS VNTR polymorphisms in families with and without PCOS.(21) They have found an association between PCOS and allelic variation at the INS VNTR locus in three separate populations. Furthermore, they found that class III alleles were associated with anovulatory PCOS, and were more frequent among the women with polycystic ovaries with symptoms than among those without symptoms.(21) These data support the idea that the VNTR polymorphisms have a functional role on the establishment of hyperinsulinemia and/or insulin resistance in PCOS. The same group also reported that class III alleles were transmitted significantly more commonly from fathers than from mothers to affected daughter, suggesting a “parent of origin” effect in the transmission of alleles.(21) The latter finding was confirmed by Eaves et al.(22)

However, in a larger study, Urbanek et al. (1999) found no evidence for the linkage of the insulin gene and PCOS and no association between the class III allele and hyperandrogenemia.(23) Other studies (Calvo et al., 2002; Vankova et al., 2002) failed to show any association between the INS VNTR alleles and hyperandrogenism or PCOS.(24,25) A recent study on PCOS in a Han Chinese population came out with similar conclusion.(26) Using different selection criteria, studying patients with variable ethnic and geographical backgrounds, and most importantly working with small to, at best, modest sample sizes might explain the presence of these conflicting results; associations with a small sample size is known to be a major risk factor for the generation of the results that cannot be replicated on consecutive examinations.(27)

#### 2. The insulin receptor gene INSR

The impaired sensitivity to insulin action led to the hypothesis that genetic lesion of the insulin receptor gene or the postreceptor 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 those has also been identified in normal subjects.(28)

First direct sequencing of INSR did not reveal any mutations in PCOS women.(29) Consequently, Conway et al. (30) analyzed the sequence of the tyrosine kinase domain of INSR in 22 hyperinsulinemic patients with PCOS, and Tabibzadeh et al. (31) investigated the mutations by molecular scanning of the entire coding region of INS in 24 hyperinsulinemic women with PCOS, and none of these groups detected any significant mutations related to insulin resistance in PCOS.(31) Siegel et al. (2002) observed a C/T single nucleotide polymorphism (SNP) in the tyrosine kinase domain of INSR, which was associated with PCOS.(32) Recently, a comprehensive study published by Urbanek et al. demonstrated a linkage with PCOS. A broad region of the chromosome 19p13.2 was investigated and the strongest evidence for association was found with INSR polymorphism.(33) In a large case-control association study Godarzi et al. (2011) found that several novel INSR SNPs were associated with PCOS.(34)

#### 3. Insulin receptor substrate proteins IRS

Insulin binds to the α subunits of the insulin receptor, which increases glucose transport and causes autophosphorylation of the β subunit of the receptor, which induces tyrosine kinase activity. Tyrosine phosphorylation, in turn, activates via insulin receptor substrates (IRS), such as IRS-1 and IRS-2, a cascade of intracellular signaling proteins to promote the metabolic and mitogenic actions of insulin. When IRS-1 is dysfunctional, IRS-2 is the main messenger for the intracellular transmission, but it requires a higher insulin concentration for activation.(35)

Several polymorphisms of IRS1 and IRS2 genes have been involved in insulin resistance. The Gly972Arg polymorphism for IRS-1 and Gly1057Asp for IRS-2 have been shown to increase susceptibility to type-2 diabetes mellitus.(36,37) Although initially no evidence for linkage or association with PCOS was found with IRS-1 by Urbanek et al. (23), the potential roles of these SNPs of IRS genes in insulin resistance have further been investigated in PCOS. Sir-
Petermann et al. reported a higher frequency of the Arg972 allele in PCOS patients in Chilean population. Ehrmann et al. investigated the influences of Gly972Arg and of Gly1057Asp polymorphisms in nondiabetic women with PCOS. Although the IRS-1 genotype was not found to be associated with any clinical or hormonal measures in nondiabetic PCOS subjects, carrying IRS-2 Gly/Gly genotype was associated with significantly higher glucose levels in 2-h OGTT compared with Gly/Asp and Asp/Asp genotypes. Contrary to this report, El Mkadem et al. could not find any differences in the distribution of IRS-1 Gly972Arg and IRS-2 Gly1057Asp alleles in PCOS patients and controls; however, they demonstrated that the Gly972Arg IRS-1 was more prevalent in insulin-resistant patients compared with the noninsulin resistant patients or control subjects. Confirming the report of El Mkadem et al., Villuendas et al. showed that these polymorphisms had an equal distribution pattern among PCOS patients and controls from Spain. Dilek et al. reported a higher frequency of the Gly972Arg polymorphism for IRS-1 in Turkish women with PCOS in accordance with the data of Sir-Petermann et al. Moreover, similar to the data obtained by El Mkadem et al. and Villuendas et al., they found that the Gly972Arg carriers were more obese, more insulin-resistant and had higher fasting insulin levels when compared with the other PCOS patients and controls.

Overall, the association studies of IRSs with PCOS and insulin resistance phenotype of this syndrome exhibit many conflicting properties as seen with the other candidate genes for PCOS. We should emphasize however that these IRS polymorphisms seem to be associated separately with insulin resistance rather than PCOS in these studies.

4. Calpain10 gene

Calpain-10 is a cysteine protease that plays a role in insulin secretion and action, and genetic studies have shown that variation in the gene (CAPN10) encoding calpain-10 is associated with type-2 diabetes. Due to the fact that PCOS and type-2 diabetes share a number of etiologic factors, Ehrmann et al. sought to determine whether variation in the CAPN10 is associated with quantitative traits related to the pathogenesis of PCOS and type-2 diabetes. They found an association between the 112121 haplotype of this gene and higher insulin levels in African-American women and an increased risk of PCOS in both African-American and white women. Gonzales et al. investigated whether four SNPs (SNP-19, SNP-43, SNP-44, and SNP-63) of the CAPN10 were associated with PCOS. In support of the latter, they reported in their consecutive studies that SNP-44 of the gene is associated with PCOS in Spanish women. Nevertheless, using the same SNPs, Haddad et al. and Escobar-Morreale et al. could not confirm any association with PCOS in a more comprehensive study.

5. Peroxisome proliferator-activated receptor-γ gene

Activation of peroxisome proliferator-activated receptor (PPAR-γ) promotes differentiation of adipocytes, increasing insulin sensitivity. The PPAR-γ gene (PPARG) 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.

There are two PPAR-γ gene polymorphisms that have been thoroughly investigated in various populations. The first is the silent CAC478-CAT substitution which resides in the exon 6 and the second is a proline to alanine missense variant at the codon 12 of the exon 2.

Most studies proved no statistical significance link between PCOS and PPAR-γ polymorphisms; although functional investigations still point out the suspicious role of PPAR-γ on PCOS.

In a recent study, there was no statistically significant difference in the genotype and allele distribution of the Pro12Ala polymorphism between PCOS and control women.

Other candidate genes involved in insulin resistance

Among the other genes tested (Insulin-like growth factors, human sorbin and SH3 domain-containing 1 gene, paraoxonase, genes encoding glycogen synthase, as resistin, leptin, adiponectin) no significant association has been reported between PCOS and genomic variants in these genes.

Conclusions

PCOS is a complex genetic disease reflecting the interplay of susceptibility genes and environmental factors. Almost all recent studies indicate that this disorder could be a complex trait, meaning that several genes are interacting with environmental factors to induce the phenotype.

Despite the efforts to dissect the variants of genes from multiple logical pathways which are involved in the pathophysiology of the syndrome, today no gene has been emerged as universally accepted susceptibility gene for PCOS.

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