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GENETICS OF POLYCYSTIC OVARY SYNDROME **GENETIKA SINDROMA POLICISTIČNIH JAJNIKA**

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Review

Key words: polycystic ovary syndrome (PCOS), multifactorial pathogenesis, PCOS candidate genes

SUMMARY. Polycystic ovary syndrome (PCOS), a heterogeneous complex disease, is presented in the light of its multifactorial pathogenesis. In particular, the genetic background of the syndrome and the most promising PCOS candidate genes are discussed in more detail.

Pregled

Ključne riječi: policistični ovarijski sindrom (PCOS), multifaktorska patogeneza, PCOS geni kandidati

SAŽETAK. Prikazan je sindrom policističnih jajnika (PCOS) kao heterogena kompleksna bolest, uglavnom iz vidika njegove multifaktorske patogeneze. Genetska pozadina ovog sindroma i najvjerojatniji PCOS geni kandidati detaljno su obrazloženi.

Clinical presentation of polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is probably the most common endocrinopathy in women of reproductive age. As a co-occurrence of polycystic ovary morphology (PCO) and amenorrhoea, it was first described by Stein and Leventhal in 1935. Nowadays, PCOS is considered not only a syndrome of ovarian dysfunction but a *complex disorder* with an extremely *heterogenous* systemic clinical presentation. The prevalence of the syndrome is estimated to be 5–10% in different ethnic populations and as much as 22% of women in general population have PCO on ultrasound.¹ PCOS accounts for about three-quarters of all cases of anovulatory infertility and for about 90% of the causes of hirsutism.² According to the 2003 Rotterdam Consensus criteria,³ PCOS is diagnosed when, after the exclusion of other possible etiologies (hyperprolactinemia, non-classical congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's disease, acromegalia), two out of three of the following criteria are fulfilled: (i) chronic oligo- or an-ovulation resulting in irregular menstrual cycles (oligo- or amenorrhoea) with sub-/ infertility; (ii) clinical hyperandrogenism (hirsutism, acne, androgenic alopecia) and/or biochemical hyperandrogenism (hyperandrogenemia); (iii) PCO on ultrasound (asymptomatic PCO): the presence of 12 or more follicles measuring 2–9 mm in diameter, in both ovaries, and/or increased ovarian volume (> 10 ml).⁴ Regarding the above criteria, three different PCOS phenotype subgroups should be considered.⁵

Other clinical and laboratory manifestations of PCOS are: LH hypersecretion; metabolic disturbances: insulin resistance and compensatory hyperinsulinemia, glucose

intolerance, dyslipidemia;⁶ (abdominal) obesity.⁷ Premature pubarche might be the earliest recognizable phenotype of PCOS.⁸ As well as the short-term health implications for PCOS women, significant consequences for long-term quality of life are evident: increased risk for type 2 diabetes mellitus,^{9,10} for cardiovascular events,¹¹ for endometrial cancer and for some other disorders.¹

Pathogenesis of PCOS

From the molecular point of view, the following pathological changes have been observed in PCOS patients:¹²

- abnormalities in biosynthesis, metabolism and/or androgen action,
- disturbances in insulin secretion and/or action,
- disturbances in hypothalamic-pituitary pathway,
- changes in cortisol metabolism,
- lipid metabolism disturbances,
- chronic subclinic inflammatory processes.

However, the three major pathophysiologic hypotheses for PCOS are the following:¹³

1. Ovarian androgen hypothesis. Hyperandrogenism is probably mainly the result of excessive biosynthesis (predominantly in ovaries) and/or action of androgens. It could affect hypothalamic control and pituitary gonadotropin secretion. In addition, increased local ovarian androgen levels may impair follicular maturation through direct effects on the follicles or by promoting stromal hypertrophy/hyperplasia with stromal products retarding follicular maturation.

A stable biochemical and molecular phenotype in PCOS theca cells is evident. Enhanced production of

dehydroepiandrosterone, progesterone, 17-hydroxyprogesterone and androstenedione was observed. Increased steroidogenic activity is probably due to increased 3 β -hydroxysteroid-dehydrogenase and 17 α -hydroxylase/17,20-lyase activities. 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 as compared to normal theca cells. In addition, transient transfection experiments indicated that in PCOS theca cells, the *CYP17* promoter activity is enhanced.¹⁴

2. LH hypothesis. Exaggerated LH pulse frequency and amplitude could also reflect primary disturbances in gonadotropin secretion, resulting in the stimulation of ovarian androgen production as well as in anovulation.

3. Insulin hypothesis. Since insulin is postulated to act as a cagonadotropin, increased serum insulin levels could augment the stimulatory effect of LH on ovarian androgen biosynthesis. Hyperinsulinemia could also directly stimulate ovarian androgen secretion or contribute to hyperandrogenism by suppressing hepatic sex-hormone binding globulin (SHBG) production. Low serum SHBG levels result in higher serum free (i.e. bioavailable) androgen levels. Similar effects on upregulation of androgen biosynthesis have been described for insulin-like growth factors.

Although most previous data suggest that ovarian androgen hypersecretion (with subsequent abnormal LH secretion, insulin resistance and anovulation) is the primary abnormality in the etiology of PCOS, the origin of the whole spectrum of disturbances in PCOS is not yet precisely determined and different pathways seem to be in complex interactions.¹⁵

Regarding extreme phenotypic heterogeneity of the syndrome, *multifactorial pathogenesis* has been proposed. Accordingly, the influence of a combination (interaction) of several genetic (specific genotype combinations) and/or environmental (nutrition, exercise) susceptibility factors have been considered.¹

Genetic basis of PCOS

A genetic contribution to the etiology of PCOS is based on familial clustering of cases. There is no general agreement on its mode of inheritance. However, due to its extreme heterogeneity, PCOS is usually considered to have an *oligogenic* (polygenic) rather than a monogenic basis: a minor contribution of several genes to the final complexity of PCOS phenotype has been suggested. Genetic susceptibility for PCOS differs between individuals, even within affected families.¹⁵ Recently, a hypothesis on as early as prenatal genetic programming of PCOS phenotype has been introduced.¹⁶

Since the genome screen approach is impractical in analyzing genetic background of complex diseases (such as PCOS), the *candidate gene approach* is much more widely used. *Linkage analyses* have been performed in order to demonstrate the co-segregation of particular ge-

netic variants with disease loci. *Association studies* are the most common approach and are used to investigate whether a genetic variant of a candidate gene or a nearby genetic marker is linked to a disease locus on a population scale. Moreover, *transmission/disequilibrium tests* have been performed to test whether parents who are heterozygous for an allele hypothesized to be associated with PCOS, transmit that allele more often to their affected children than the non-disease transmitting allele.¹⁷

Difficulties in analyzing the genetic background in PCOS are mainly associated with the fact that PCOS is a complex heterogeneous genetic disease with multifactorial pathogenesis: the results from different studies cannot be readily compared, since different diagnostic criteria are used. Therefore, large numbers of patients with clear inclusion criteria should be tested to obtain more reliable results. Moreover, PCOS is associated with infertility, thus the availability of large pedigrees for linkage analyses is limited. Also, a male phenotype for PCOS has not yet been clearly defined, although premature male pattern baldness has been proposed.¹⁸

PCOS candidate genes

On the basis of their known or expected role in the pathogenesis of PCOS, numerous PCOS candidate genes have been proposed and studied till now.^{1,2,15,17} Attention has been focused on defects in: (i) ovarian androgen biosynthesis and action; (ii) insulin secretion and action; (iii) hypothalamic-pituitary axis; (iv) adipose tissue biochemical disturbances.

Till now, several nucleotide sequence changes, mutations as well as genetic polymorphisms, have been shown to influence transcriptional activity of target genes and consequently, to be involved in the pathogenesis of several human genetic diseases.¹⁹ The most promising PCOS candidate genes are introduced below.

Genes involved in ovarian androgen production and action

Genes involved in ovarian androgen biosynthesis. Increased transcriptional activity of genes encoding steroidogenic enzymes could lead to upregulation of androgen biosynthesis. The most widely studied PCOS candidate genes from this group are *CYP17 gene* (cytochrome P450 17-hydroxylase/17, 20-desmolase gene), *CYP21 gene* (cytochrome P450 21-hydroxylase gene) and *CYP11A gene* (cytochrome P450 side-chain cleavage enzyme gene) (15q23-q24).

The *CYP11A* gene product, cholesterol desmolase, catalyzes a rate-limiting step in steroidogenesis. The (TTTTA)_n microsatellite polymorphism in the *CYP11A* promoter was proposed as influencing *CYP11A* gene transcriptional activity. In the initial study, evidence of a weak linkage between the *CYP11A* gene and hyperandrogenemia in PCOS women and a strong association of the *CYP11A* microsatellite polymorphism with total serum testosterone levels in PCOS patients were

found.²⁰ Furthermore, an association between the polymorphism and the presence of PCOS in Greek patients was confirmed.²¹ However, a large multicentric study as well as certain other studies, failed to find a significant association between *CYP11A* gene and PCOS.¹

AR gene (*androgen receptor gene*) (Xq11-q12). Genetically determined androgen receptor hypersensitivity could result in clinical hyperandrogenism even in normoandrogenemic PCOS patients. Microsatellite polymorphism (CAG)_n in exon 1 of the *AR* gene has been suggested as influencing *AR* transcriptional activity. Initially, no evidence for association of the polymorphism (CAG)_n with PCOS was provided.²² However, the polymorphism was shown to be inversely correlated with serum androgen levels.²³ In Australian patients, a significantly higher frequency of longer CAG alleles in PCOS compared to control women was found.²⁴ In contrast, an association between shorter *AR* alleles and precocious pubarche was found in Spanish girls.²⁵ However, results of a recent study on Finnish PCOS patients led to the conclusion that *AR* (CAG)_n polymorphism is not a major determinant of PCOS.²⁶

SHBG gene (*sex hormone-binding globulin gene*) (17p13-p12). The (TAAAA)_n microsatellite polymorphism in the promoter of the *SHBG* gene might influence transcriptional activity of the *SHBG* gene. Consequently, disturbed SHBG production and secretion with further changes in serum bioavailable androgen levels might occur. An association between *SHBG* alleles with more than eight TAAAA repeats and PCOS as well as a highly significant influence of the polymorphism on serum SHBG levels were observed in different ethnic populations, with longer alleles predisposing to lower serum SHBG levels.^{27,28}

Genes involved in insulin secretion and action

INSR gene (*insulin receptor gene*) (19p13.2). Several sequence analyses in the *INSR* gene have been performed, but negative results were obtained. However, increased insulin receptor serine phosphorylation in skeletal muscle cells and fibroblasts has been described in approximately 50% of PCOS women. Moreover, tyrosine autophosphorylation of the insulin receptor has been found to be decreased in PCOS ovaries. An association between PCOS and a C/T single nucleotide polymorphism in the *INSR* gene segment encoding tyrosine kinase has been shown.¹ In addition, three separate studies have found linkage and an association between D19S884 marker, located near the *INSR* gene at position 19p13.3, and PCOS.²⁹ Further evidence for probably important role of this region in the pathogenesis of PCOS was provided by statistically significant linkage between the region and serum androgen levels in Caucasians. However, the putative PCOS gene at this chromosomal location remains to be identified.¹

INS gene (*insulin gene*) (11p15.5). Minisatellite polymorphism (VNTR) in the promoter of the *INS* gene might influence transcriptional activity of the gene. Ini-

tially, strong linkage and association between class III *INS* VNTR alleles and PCOS were found.⁹ Afterwards, no association between *INS* VNTR polymorphism and the presence of PCOS was obtained in two separate studies. In addition, no evidence of linkage of the *INS* gene and PCOS as well as no association between class III *INS* alleles and hyperandrogenemia was found in a larger study.³⁰

IRS-1 gene (2q36), ***IRS-2 gene*** (13q34). The *IRS* genes encode for insulin receptor substrates (IRSs) that are critical proteins for signal transduction in insulin target tissues. Despite some promising results, no convincing evidence for involvement of IRSs in the pathogenesis of PCOS has been reported.¹

CAPN10 gene (2q37.3). The *CAPN10* gene encodes for calpain 10, an ubiquitously expressed member of a calpain-like cysteine protease family. It affects insulin action and has been described as being associated with an increased likelihood of developing type II diabetes mellitus. However, no obvious involvement of *CAPN10* polymorphic variants in the development of PCOS has been reported.¹

IGF2 gene (11p15.5). The *IGF2* gene encodes for insulin-like growth factor II, which stimulates insulin action as well as adrenal and ovarian androgen secretion. A G/A single nucleotide polymorphism on position +582 relative to termination codon has been described as influencing *IGF2* gene expression. The G alleles are reported to increase *IGF2* mRNA levels in leukocytes and probably result in increased IGF2 expression and secretion in liver. An association study on Spanish patients showed a significantly higher frequency of *IGF2* G/G homozygotes in PCOS compared to control women.³¹

Genes involved in gonadotropin release, regulation and action

LH gene (19q13.32). Since increased serum LH (luteinizing hormone) levels are frequently observed in PCOS patients, possible *LH* gene abnormalities have been sought. Indeed, a variant with two point mutations, functionally different from the wild type, has been found in PCOS women, but its role in the pathogenesis of PCOS is considered not to be pivotal.¹

LH receptor gene (2p21). The presence of LH receptor gene mutations has been examined in PCOS patients with normal serum LH levels and hyperandrogenemia. No mutations were found in two different studies. However, a linkage analysis in five affected families showed segregation of polymorphic markers, close to the LH receptor gene, with PCOS.¹

FST gene (5q11.2). Since activin plays an important role in follicular maturation and regulation of ovarian androgen production, excessive neutralization of activin by follistatin, its binding protein, may result in the PCOS phenotype. Although a strong linkage between the gene encoding follistatin (*FST* gene) and PCOS has

been reported,¹⁷ no PCOS susceptibility genetic variants have been found.¹

Genes involved in adipose tissue metabolism

Several other genes, including those involved in adipose tissue metabolism, have been proposed as PCOS candidate genes. Genetic variants in the *leptin gene* (7q31.3), *leptin receptor gene* (1p31), in the gene encoding peroxisome proliferator-activated receptor γ -*PPAR γ gene* (3p25), in the *adiponectin gene* (3q27), in the gene encoding plasminogen activator inhibitor-1-*PAI-1 gene* (7q21.3-q22) and in many other genes have been investigated; however, no convincing results were obtained.¹

Microarray analyses

Recent cDNA microarray analyses have shown consistent differences in gene expression profiles between human PCOS and normal ovarian theca cells. Accordingly, several *new* PCOS candidate genes, involved in different biological pathways and being either over-expressed or down-regulated in PCOS patients, might be proposed.^{32–36} Examples are genes encoding proteins involved in Wnt signalling, proteins involved in apoptosis and stress response, components and remodelling factors of extracellular matrix, components of the immune system, heat shock proteins, proteins with a role in proliferation and differentiation. Data from microarray analyses may contribute to a better understanding of abnormal biochemical pathways in PCOS and to novel clues for ovarian dysfunction in PCOS. Consequently, novel leads for therapeutic interventions might be applied in future.

Conclusion

Despite extensive investigations, the pathogenesis of PCOS remains only partly elucidated. Results from numerous studies on PCOS candidate genes are contradictory, suggesting that specific PCOS susceptibility genes or regions have yet to be identified. To overcome some of the limitations in studying the genetic background of PCOS, large multicentric studies should be performed and statistical methods that were developed especially for complex diseases should be applied.³⁷

Regarding extreme heterogeneity of the syndrome, a combination of specific genetic polymorphisms might be indirectly involved in the PCOS clinical status. New molecular genetic approaches, such as microarray technology, hold promise for elucidating pathophysiological mechanisms of several human diseases, including PCOS. Successful identification of PCOS candidate genes would have enormous potential for both the diagnosis and management of PCOS. The prospect arises of being able to identify genetic susceptibility loci in individual patient profiles to aid diagnosis, highlight long-term risks, and allow tailoring of therapy for a patient's particular needs.³⁸

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