J Assist Reprod Genet (2013) 30:883–895
DOI 10.1007/s10815-013-0030-1

REVIEW

Genetic variants associated with insulin signaling and glucose homeostasis in the pathogenesis of insulin resistance in polycystic ovary syndrome: a systematic review

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Received: 10 March 2013 / Accepted: 31 May 2013 / Published online: 22 June 2013

Abstract
Background Polycystic ovary syndrome must be recognized as a serious issue due to its implication on long term health regardless of an individual's age. PCOS and insulin resistance are interlinked, as approximately 40 % of women with PCOS are insulin resistant. However, the detailed molecular basis for insulin resistance that is coupled with PCOS remains poorly understood.
Objective To review the published evidence that polymorphisms in genes that are involved in insulin secretion and action are associated with an increased risk of PCOS.
Methods We reviewed articles published through November 2012 which concerned polymorphisms of genes related to insulin signaling and glucose homeostasis as well as their associations with PCOS. The articles were identified via Medline searches.
Conclusions No consistent evidence emerged of a strong association between the risk of PCOS and any known gene that is related to insulin signaling and glucose homeostasis. Moreover, recent genome-wide association studies are inconsistent in identifying the associations between PCOS and insulin metabolism genes. Many of the studies reviewed were limited by heterogeneity in the PCOS diagnosis and by not have having a sufficient number of study participants. Further studies are warranted to determine predisposing risk factors which could modify environmental factors and thus reduce the risk of PCOS. Large genome-wide association studies devoted solely to PCOS will be necessary to identify new candidate genes and proteins that are involved in PCOS risk.

Keywords Insulin resistance · PCOS · SNP · Genetics

Capsule Majority of women with polycystic ovary syndrome are insulin resistant. Polymorphisms of genes related to insulin signaling and glucose homeostasis as well as their associations with PCOS were reviewed. The studies are limited by heterogeneity in the PCOS diagnosis and no strong evidence for association between the risk of PCOS and these gene polymorphisms was found.

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Introduction

Polycystic ovary syndrome (PCOS) must be considered a serious issue because of its implication on long term health regardless of a woman's age. It needs to be seen as a lifelong condition, not one tied only to pregnancy. Polycystic ovary syndrome (PCOS) is a very common and complex female endocrine disorder. It affects women in their reproductive years with an estimated prevalence of 4–8 % [13]. The ESHRE/ASRM consensus conference held in Rotterdam in 2003 defined the syndrome as having two of the following three conditions diagnosed as PCOS: oligo-ovulation; clinical or biochemical evidence of androgen excess; and multicystic ovaries. The diagnostic criteria for defining the PCOS are as heterogeneous as the disease itself and have been amended in recent years. During the first international conference on PCOS held at the National Institutes of Health (NIH) in the US in 1990, three key features of PCOS were generally agreed on, including chronic anovulation; hyperandrogenism (clinical or laboratory evidence); and the absence of other endocrine disorders (e.g., congenital adrenal hyperplasia, hyperprolactinaemia or thyroid abnormalities) [46]. Polycystic ovary syndrome affects women of all races and nationalities. Indeed, this heterogeneous condition affects 7–10 % of women worldwide [10, 12] irrespective of their ethnic background [64]. An estimated prevalence of 20 % of the normal female population has polycystic ovaries [104]. PCOS is the most common cause of oligoanovulatory infertility which is characterized by insulin resistance (IR), while hyperinsulinemia is found in 50–70 % of women diagnosed with PCOS. Women with PCOS are at increased risk for diabetes, dyslipidemia, atherosclerosis [22, 59, 117] as well as endometrial carcinoma

[36, 111]. Furthermore it is suggested that women with PCOS are at an increased risk for miscarriages, gestational diabetes, pre-eclampsia and preterm labour [19]. Due to the clinical and biochemical heterogeneity of PCOS, several studies have focused on the aspects of hormonal, genetic and environmental factors involved in the development of the syndrome. A study of PCOS subjects representing three different ethnic groups revealed that obesity and hirsutism varied with genetic and environmental factors. At the same time, the prevalence of adrenal androgen excess and insulin resistance among these subjects appeared fairly uniform [106]. More recently, DeUgarte [39] observed that ethnicity and PCOS were associated with independent and additive defects of insulin action in Caribbean-Hispanic PCOS women. However, women with PCOS undergo several interrelated features including ovarian hyperandrogenism, chronic anovulation, polycystic ovaries; these are coupled with anomalous androgen and insulin-related parameters irrespective of other standard reproductive factors [4]. The genetic basis of the disease is not clearly known, which is largely due to the difficulties in determining the inheritability of PCOS. The genes that regulate insulin secretion and action, ovarian and adrenal steroidogenesis and energy regulation act as candidate genes which determine the expression of several integral phenotypes of PCOS. The present review concentrates on the polymorphisms in the genes that are involved in insulin secretion and action (Table 1).

Insulin resistance and PCOS

PCOS and insulin resistance are interlinked, as approximately 40 % of women with PCOS have been found to be insulin resistant [135, 162, 169]. Insulin resistance is a common feature in both polycystic ovary syndrome (PCOS) and non-insulin-dependent diabetes mellitus (NIDDM); however, persistent reproductive disturbances were limited to the PCOS, suggesting that insulin resistance in the ovary itself may be responsible for this susceptibility [173]. Insulin resistance refers to a state in which circulating insulin does not bind to the insulin receptors on the cell, or it does bind but its effects are deficient, thus giving a less than normal reduction of glucose to a given amount of insulin [30]. The pancreas then continues to secrete more insulin, leading to higher levels in the blood and ensuring normal glucose tolerance [58]. The association between insulin resistance and PCOS has provided significant insight into the pathogenesis of PCOS [141]. Several studies indicated altered insulin levels which can directly stimulate ovarian androgen production in PCOS [130, 137]. Hyperinsulinemia leads to hyperandrogenemia by stimulating ovarian androgen production [38, 63]. Insulin can also stimulate adrenal steroidogenesis by enhancing sensitivity to adrenocorticotrophic hormone (ACTH) and can increase pituitary LH release [45, 154]. Increased androgen levels lead to menstrual disturbances, development of ovarian cysts, hirsutism and other related disorders [22, 59, 117]. Important physiological processes including cellular glucose uptake [25, 133], metabolism [124, 133] and gene expression [109] are regulated by insulin. Specific abnormalities of insulin metabolism have been identified in PCOS. These include reduction in secretion, reduced hepatic extraction [116], impaired suppression of hepatic gluconeogenesis [44] and abnormalities in insulin receptor signaling [43].

Insulin signaling pathway

Insulin regulates both metabolism and gene expression. The insulin signal passes from the plasma membrane receptor to insulin-sensitive metabolic enzymes and then reaches the nucleus where it stimulates the transcription of certain genes. The insulin receptor is a heterodimeric complex consisting of 2 extracellular α-subunits and 2 transmembrane β-subunits. The α-subunit contains the insulin binding domain**.** The binding of insulin to the α subunit activates the tyrosine kinase activity of β subunit to transphosphorylate one another. This allows association of insulin receptor substrates, such as IRS-1 and IRS-2 a cascade of intracellular signaling proteins to the regulatory subunit of P13k kinase. The activated P13k further phosphorylates the membrane phospholipids and produces the phosphatidylinositol-3, 4, 5 triphosphate (PIP3). This PIP3 activates the enzyme protein kinase B (PKB: also known as Akt) which helps in the translocation of GLUT4 to the cell surface and results in the increased glucose uptake of the cells [2]. Defects in the insulin signaling system may result in insulin resistance, obesity, and type II diabetes [24, 95, 100]. Nevertheless, the detailed molecular basis for insulin resistance that is coupled with PCOS remains poorly understood.

Genes involved in insulin resistance

The majority of the evidence supports the finding that most women with PCOS have both insulin resistance and compensatory hyperinsulinemia. Insulin resistance in PCOS predisposes the individual to type 2 diabetes**.** The heritability of beta-cell dysfunction observed in families of women with PCOS demonstrated beta-cell dysfunction, a significant factor that predisposes to type 2 diabetes [31]. Therefore, several candidate genes involving signaling pathways (insulin secretion and action) are examined for PCOS.

Insulin gene (*INS*)

Insulin is composed of 2 dissimilar polypeptide chains, A and B, which are linked by 2 disulfide bonds. The gene coding for

Table 1 List of polymorphisms that studied in different regions of the world

Variants studied	Population	Study design	Samples	Association	Reference
INS					
INS-VNTR	United Kingdom	Family based	17 Families	Yes	[168]
INS-VNTR	European	family based	150 Families	No	[157]
INS-VNTR	UK	family based	74 families	Yes	[105]
INS-VNTR	Czechoslovakia	case-control	38 cases, 22 controls	No	[159]
INS-VNTR	Spanish	case-control	96 cases, 38 controls	No	[20]
INS-VNTR	Irish	case-control	185 cases, 1,062 controls	No	[125]
INS-VNTR	United Kingdom	family based association trios	255 parent-offspring trios	No	[125]
INS-VNTR	Finnish	Cohort	1599	No	[125]
INS-VNTR	Estonian women	case-control	30 cases, 75 controls	No	[72]
INS-VNTR	Slovene	case-control	117 cases, 108 controls	Yes	[56]
INS-VNTR	Han chinese	case-control	216 cases, 192 controls	No	[175]
INS-VNTR	Korean	case-control	218 cases,141 controls	No	[178]
INSR					
His1058C/T	United Kingdom	case-control	22 cases, 8 controls	No	[32]
Mutation scanning	United Kingdom	case-control	108cases, 5 control	No	[148]
D19S884 & other loci	European	family based	150	Yes	[157]
D19S884 & other loci	Caucasian	case-control	85 cases, 87 controls	Yes	[155]
C/T -C10923T	US	case-control	99 cases, 136 controls	Yes	[138]
His1058 C/T	Chinese			No	[164]
His1058 C/T	Korean	case-control	9 cases, 9 controls	No	[55]
His1058C/T	Chinese	case-control	120 cases, 40 controls	Yes	[26]
Cys1008	Chinese	case-control	109 cases, 107 controls	Yes	[83]
176447C > T	Korean	case-control	134cases, 100 controls	Yes	[94]
His1058C/T	Indian	case-control	180 cases, 144 controls	Yes	[110]
INSR exon17 C/T	Turkish	case-control	44 cases, 50 controls	No	[156]
IRS					
IRS1-Gly972Arg, IRS2-Gly1057Asp	France	case-control	53 cases, 102 controls	Yes	[50]
IRS1-Gly972Arg	Caucasians	case-control	69 cases, 15 controls	Yes	[172]
IRS1-Gly972Arg	Chile	case-control	82 cases, 70 controls	Yes	[140]
IRS1-Gly972Arg, IRS2-Gly1057Asp	African-American	case-control	227 cases, 175 controls	No	[49]
IRS1-Gly972Arg	Chile	case-control	143 cases, 97 controls	Yes	[139]
IRS1-Gly972Arg	Turkish	case-control	60 cases, 60 controls	Yes	[40]
IRS1-Gly972Arg	USA	case-control	114 cases, 95 controls	No	[171]
IRS1-Gly972Arg, IRS2-Gly1057Asp	Spanish	case-control	103 cases, 48 controls	No	[163]
IRS1-Gly972Arg, IRS2-Gly1057Asp	Germany	case-control	57 cases, 567 controls	No	[69]
IRS1-Gly972Arg	Taiwanese	case-control	47 cases, 45 controls	No	[97]
IRS1-Gly972Arg	Japanese	case-control	123 cases, 380 controls	Yes	[14]
IRS1-Gly972Arg	Chile	case-control	50 cases, 75 controls	No	[158]
IRS1-Gly972Arg, IRS2-Gly1057Asp	Greece	case-control	183 cases, 88 controls	Yes, No	[29]
IRS1-Gly972Arg	Italian	case-control	65 cases, 27 controls	Yes	[120]
IRS1-Gly972Arg	Slovak	case-control	53 cases, 21 controls	No	[42]
IRS1-Gly972Arg	Greece	case-control	162 cases, 122 controls	No	[101]
IRS2-rs7997595, rs7987237, rs1865434	Caucasians	discovery cohort replication cohort	273 cases, 173 controls 526 cases, 3,585 controls	Yes	[65]

Table 1 (continued)

Variants studied	Population	Study design	Samples	Association	Reference
RS1-Gly972Arg, IRS2-Gly1057Asp	Iranian	case-control	48 cases, 52 controls	No	[127]
IRS1-Gly972Arg and G2323A	Indian	case-control	250 cases, 299 controls	yes	[35]
IGFs					
IGF1,IGF2 Apa1,IGF1 RECEPTOR,IGF2 RECEPTOR	Spanish	case-control	72 cases, 42 controls	Yes	[134]
IGF2 Apa1		case-control	153 cases, 178 controls	No	[89]
IGF2-3′UTR GA rs680		case-control	117 cases, 105 controls	No	[126]
PPARG					
Pro12Ala	USA	case-control	124 cases, N/A	Yes	[73]
Pro12Ala	Finnish	case-control	135 cases, 115 controls	Yes	[92]
CAC ^{478} CAT, Pro12Ala	Italy	case-control	120 cases, 120 control	Yes	[119]
Pro12Ala	Spanish	case-control	72 cases, 42 controls	No	[134]
Pro12Ala	Turkey	case-control	60 cases, 60 controls	No	[153]
Pro12Ala	Turkish	case-control	100 cases, 100 controls	No	[176]
Pro12Ala, Gly482Ser	Han Chinese	case-control	201 cases, 147 controls	No	[166]
His 447 His in exon6, Pro12Ala	Los angeles	case-control	285 cases, 187 controls	No	[7]
Pro12Ala	Greek	case-control	156 cases, 56 controls	No	[90]
Pro12Ala, 1431C/T	Korean	meta-analysis	238 cases, 125 controls	Yes	[67]
Pro12Ala	Greek	case-control	180 cases, 140 controls	No	[174]
D3S1263	European	family based	150 Families	No	[157]
Pro12Ala and His447His	Indian	Case-control	250 cases, 299 controls	yes	[35]
CAPN10					
UCSNP 44,43,19,63	Spanish	case-control	55 cases, 93 controls	Yes	[61]
UCSNP 43,19,63	White of European ancestry	only Cases	124	No	[48]
	African-American		57		
	Hispanic		13		
	Asian-American		13		
	Middle Eastern		5		
UCSNP 44,43,19,63	Europid	case-control	185 cases, 525 controls	Yes	[70]
UCSNP 43,44,45	Caucasians	case-control	81 cases, 37 controls	Yes	[53]
UCSNP 44,43,19,63	Spanish	case-control	146 cases, 93 controls	No	[62]
UCSNP 44,43,45	Caucasians	case-control	57 cases, 567 controls	No	[69]
UCSNP 43,19,63	Brazil	case-control	59 cases, 29 controls	Yes	[170]
UCSNP 43,44,58, 19,56,63,22	Europid	case-control	146 cases, 606 controls	No	[165]
UCSNP 43,19,63	chile	case-control	50 cases, 70 controls	Yes	[102]
UCSNP 43,19,63	Spanish	population based	899	Yes	[132]
UCSNP 43,19,63	Korean	case-control	188 cases, 439 controls	Yes	[93]
UCSNP 44,43,19,63	Turkish	case-control	107 cases, 114 controls	Yes	[177]
UCSNP 44,43,19,63	Turkish	case-control	44 cases, 50 controls	Yes	[156]
UCSNP 44,43,56,19,63	Indian	case-control	250 cases, 299 controls	Yes	[34]

insulin is localized to 11p15.5 [74] and is located between the genes for tyrosine hydroxylase and the insulin-like growth factor-II (IGF-II) [84]. The human insulin gene contains three exons: exon 2 encodes the signal peptide, the B chain, and a fraction of the C peptide, while exon 3 encodes the balance of the C peptide and the A chain [144]. Insulin hormone is not synthesized as an active protein; insulin mRNA is initially translated into a single chain precursor called preproinsulin. The preproinsulin is 110 amino acids long and made up of a signal peptide, the A, B and C chains. The preproinsulin enters

the endoplasmic reticulum and loses its signal peptide and converts into proinsulin which is 86 amino acids long. Later, the proinsulin is exposed to several specific endopeptidases and further loses the C chain; it is thus left with only the A and B chains, which is considered as insulin hormone.

The transcription factor Pur1 initiates transcription after binding to the promoter element that is located 596 bp upstream of the insulin gene translation initiation site. This promoter element is known to have a variable number of tandem repeat (VNTR) regions with varying repeats: 26–63 repeats (Class I); 80 repeats (Class II); and 140-200repeats (Class III) [16]. Class I and class III alleles are common in Caucasians. While the class II alleles are very rare in Caucasians, they are common in Africans [143] as the HphI T/A SNP at the locus −23 (rs689) polymorphism of the insulin promoter region which is in strong linkage disequilibrium and acts as a surrogate marker to *INS*-VNTR [99]. Hence, the Class I and III alleles of *INS*-VNTR were determined by −23 HphI A and T alleles, respectively. Class III alleles are associated with reduced expression of *INS* and *IGF2* in the pancreas and placenta [121].

The first evidence for linkage and an association between VNTR and PCOS subjects revealed that the class III alleles were only associated with women who were anovulatory and hyperinsulinaemic [168]. Another study on 74 UK women with PCOS reported an association between the class III allele and lower insulin sensitivity [105]. In Slovene PCOS subjects, class III *INS* VNTR alleles were found to be more frequent and their interaction with body mass index was a significant predictor of serum insulin level [56]. On the other hand, no association between *INS* VNTR polymorphism and PCOS was reported in Czech [159] or Spanish women [20]. Subsequently, in a large-scale study using 255 nuclear families and 3,000 subjects from Irish and Finnish populations, *INS*-VNTR was not found to be a key factor in the pathogenesis and progress of PCOS [125]. A comparative study of *INS* VNTR between PCOS and tubal infertility groups found that INS VNTR genotypes are not associated with PCOS. However, they could have a certain influence on the phenotypic spectrum of the syndrome [72]. No association between PCOS and *INS*-VNTR polymorphism was observed in either the Han Chinese [175] or Korean populations [178].

Insulin receptor gene (*INSR*)

The insulin receptor is a heterotetrameric glycoprotein with two alpha and beta units. Its gene is located at chromosome 19p13.2, spanning 120Kb with 22 exons [136]. The tyrosine kinase domain of the receptor, which is necessary for insulin signal transduction, is encoded by exon 17–21. Alpha and beta subunits of the insulin receptor were derived by the proteolytic processing of a common 1,382 amino acid preproreceptor [47]. Two compound heterozygote mutations which behave in a cis-dominant fashion to decrease mRNA transcription levels have been identified in the insulin-receptor gene of a patient with leprechaunism. Within this single allele there is a nonsense mutation at codon 897, while the other alleles map outside the coding sequence of the gene [167]. More recently, the direct sequencing of all 22 exons of the *INSR* gene in three women with PCOS did not reveal any mutations [142]. The screening of 22 hyperinsulinemic patients for mutations of the insulin receptor gene revealed that these mutations are not involved in causing insulin resistance in UK PCOS subjects [32]. Furthermore, the screening of 24 severe insulin resistance patients revealed several mutations, but none of their missense or nonsense mutations contributed to the insulin resistance found in UK subjects with PCOS [148]. A His1058 C/T SNP at exon 17 of INSR is not associated with decreased insulin resistance in Chinese [26, 164]; Korean [55]; or Turkish women with PCOS [156]. However, this polymorphism did show a significant association with the lean rather than the obese US [138] and Indian PCOS women [60]. A novel SNP in intron 21 (176477 C > T) of INSR showed strong association with the pathogenesis of PCOS in the Korean population [142]. A meta-analysis of eight studies comprising 795 cases and 576 controls found no significant evidence for an association between PCOS and *INSR* His1058 C/T polymorphism [107]. By contrast, linkage analysis using STRs encompassing the *INSR* region of chromosome 19 did find evidence for an association between the D19S884 locus on *INSR* [85, 150, 155]. Furthermore, the DNA sequence surrounding D19S884 conferred in vitro promotes activity in lymphoblastoid cell lines [11]. A recent study using pathway-based tagging SNP identifies new *INSR* SNPs associated with PCOS; moreover, a large replication cohort confirmed association of PCOS with rs2252673 [152]. A family-based association study using 260 trios of Han Chinese origin did not reveal significant evidence of association or linkage of the *INSR* gene to PCOS [78]. According to a Chinese study, a novel T/C SNP at codon Cys1008 of the *INSR* gene is associated with decreased insulin sensitivity in Chinese PCOS women. The study found that the association is not caused by the change of synthesis or secretion of the INSR beta-subunit, but most probably by the effects of this novel SNP on the function of the *INSR* beta-subunit [83]. A novel SNP in the *INSR* gene, +176477 C > T, was associated with the pathogenesis of PCOS in a Korean population [94]. The study found a significant association of C/T polymorphism at His1058 of *INSR* with PCOS in lean rather than obese Indian women [110].

Insulin receptor substrates (*IRS*)

Tyrosine phosphorylation is the result of insulin binding to its receptor; this in turn leads to the phosphorylation of several

protein and insulin receptor substrates (*IRS*), including primarily *IRS-1* and *IRS-2* for initiating and coordinating multiple downstream pathways [145, 146]. A series of gene "knockout" experiments demonstrated the critical role of both *IRS1* and *IRS2* where both aid in activating multiple signaling pathways for the regulation of glucose homeostasis by insulin [8, 149]. The human *IRS1* gene contains the entire 5′-untranslated region and the protein coding region in a single exon and is localized on chromosome 2q36-37 by in situ hybridization [9]. The *IRS2* gene is mapped on chromosome 13q34 [86]. The open reading frames of *IRS1* and *IRS2* predict a molecular weight of 131 and 136 kD. Arg972Gly, a common variant of *IRS1*, lies between two potential sites of tyrosine phosphorylation involved in binding the p85 subunit of the PI-3 kinase. Although the G972R variant is not associated with abnormal expression of the *IRS-1* protein [147], it does impair signaling [6]. Asp1057Gly, a common *IRS2* variant, has not been associated with changes in insulin sensitivity in lean or obese adults [5].

Although the initial study did not reveal any association of PCOS with the *IRS1* gene [157], many subsequent studies have been concentrated on Arg972Gly and Asp1057Gly polymorphisms in PCOS; this continuing interest is due to the complementary role of *IRS1* and *IRS2* in insulin signaling. The higher frequency of the *IRS1* variant was observed in adolescent girls with hyperandrogenism [172], but the G972R variant acted as a modifier locus among women who are heterozygous carriers of CYP21, which indicates its limited role in the development of PCOS [171]. Recently, attention has also been focused on insulin receptor substrates and the association with PCOS of SNPs at the *IRS1* and *IRS2* loci. The results, however, are contradictory. A slightly higher frequency of Arg972 was observed in PCOS women of Chilean [139, 140] and Turkish populations [40]. The *IRS1* Gly972Arg polymorphism is significantly associated with PCOS in the Japanese [14] and Greek populations [29]. The *IRS1* Gly972Arg has the highest frequency reported worldwide and is associated with insulin resistance and higher fasting insulin in Southern Italian women [120]. Furthermore, the *IRS1* genotype also influenced the fasting insulin levels and HOMA indices in PCOS women on metformin therapy [52]. No significant association between insulin receptor substrate genes and PCOS was reported in the French [50], Spanish [163], German [69], Taiwanese [97], Chilean [158], Slovak [42], Greek [101], Indian [35] or Iranian populations [127]. Very few studies reported an association between *IRS2* Gly1057Asp and PCOS. The Gly1057Asp polymorphism influenced blood glucose levels in nondiabetic Caucasian and African-American women with PCOS [49]. An analysis of US Caucasian women revealed three additional *IRS2* SNPs that are associated with PCOS (rs7997595, rs7987237, rs1865434) [65]. A recent genome-wide association study (GWAS) of PCOS in Han Chinese women failed to detect associations between the polymorphism of the *IRS* gene and PCOS [27]. However, two independent meta-analyses suggest that *IRS1* Gly972Arg polymorphism causes significant risk for PCOS, but that *IRS2* Gly1057Asp polymorphism has not shown such risk [80, 131].

Insulin-like growth factors *(IGFs)*

The *IGF*s are peptide hormones secreted from many different cells and exhibiting a high sequence of similarity to insulin. There are two principal *IGF*s, known as *IGF-1* and *IGF-2*. Their functions include: mediation of growth hormone action; stimulation of growth of cultured cells; stimulation of the action of insulin; and involvement in development and growth. Each of these has a number of variant forms, a result of the use of alternative gene promoters and alternative splicing. The gene *IGF2* is located on chromosome 11p15.5 [115]. A single nucleotide polymorphism (SNP) in the 3′ untranslated region of the *IGF2* gene (*ApaI*; rs680) is known to increase *IGF2* mRNA in leukocytes due to increased liver *IGF2* expression and secretion. Together with *IGF1* and *IGF*-binding proteins, *IGF2* stimulates adrenal and ovarian androgen secretion. The association between PCOS and G alleles of the ApaI polymorphism (*IGF2* 3′UTR GA; rs680) was first established in Spanish women [134]. A subsequent study found that the ApaI polymorphism in the *IGF2* cluster in combination with the −108 polymorphism (rs705379) in *PON1* increased the risk of PCOS in German women [89]. A recent study showed a predominance of ApaI GA + AA genotypes in younger Brazilian women with PCOS [126].

Peroxisome proliferator-activated receptor γ (*PPARG*)

Peroxisome proliferator-activated receptors are members of the nuclear receptor super family of ligand-activated transcription factors [81]. The *PPAR-γ2* is formed by an alternative mRNA splicing pathway and regulates the transcription and expression of numerous target genes. These genes have been shown to be involved in adipocyte differentiation, lipid and glucose metabolism, and atherosclerosis [96]. The gene coding for *PPAR-γ* has been mapped to chromosome 3q25 [66]. The human *PPAR-γ* gene is composed of 9 exons; it spans more than 100 kb of genomic DNA [54]. A common *C* to *G* base exchange leads to the substitution of proline with alanine at codon 12, which has been associated with reductions in both DNA binding and transcriptional activity in vitro. Recent studies have indicated that the Ala12 allele is involved in increased insulin sensitivity by enhanced suppression of lipid oxidation, thereby permitting more efficient glucose disposal.

Several studies have found similar genotype and allele frequencies of the PPAR-γPro12Ala polymorphism in PCOS

women and healthy controls in Italy [118, 119], Spain [134], China [166], Turkey [153], Chile [68], Korea [23], Greece [28, 90, 174], Los Angeles [7], Germany [69, 88], Poland [18] and Slovenia [41]. Although *PPAR-γ*Pro12Ala polymorphism is equally distributed in PCOS women and healthy controls, it showed a modifier effect on insulin resistance in both German [71] and multi-ethnic populations [73]. Contrary to these findings, however, some studies have shown that the Pro12Ala polymorphism is significantly more frequent in control subjects when compared with PCOS women, indicating a protective effect by the Ala allele against the development of PCOS in Finland [92], Turkey [176], India [35], and Korea [67]. PCOS subjects carrying Pro12Ala showed higher leptin levels than the Pro12Pro and Ala12Ala genotypes, indicating that the single Ala12 allele may play a protective role in respect to hyperleptinemia [17]. Although the protective trend of the G allele existed, a recent meta-analysis did not show a significant association between Pro12Ala and PCOS [151]. A meta-analysis using 17 case control studies from Europe and Asia supports the finding that the *PPAR-γ*Pro12Ala polymorphism is capable of reducing the PCOS in European but not in Asian women [179]. Yet another meta-analysis using 17 studies reported that the Ala12 variant would decrease the risk of PCOS and result in lower BMI and fast insulin levels in Europeans, but would have no impact on HOMA-IR in PCOS patients [75].

Calpain-10 (CAPN10)

Calpains are calcium-dependent intracellular nonlysosomal proteases that are capable of hydrolyzing specific substrates involved in calcium-regulated signaling pathways [51]. Calpain-10 is an atypical member of the calpain family and is expressed at the mRNA and protein levels by several tissue types including pancreatic β islet cells; liver; skeletal muscle; and adipocytes [21, 123]. The gene encoding calpain-10 (*CAPN10*) consists of 15 exons and is located on chromosome 2q37.3. It was shown to be related to proinsulin processing, insulin secretion and insulin resistance [15, 181]. *CAPN10* variants are known to influence cholesterol levels, blood pressure values, and insulin resistance phenotypes in the Spanish population [132]. Several SNPs in *CAPN 10* (UCSNP-63, −44, −43, −19) have been the focus of PCOS researches; however, the results are contradictory.

CAPN10 UCSNPs associated with PCOS varies in different populations. The *CAPN10* UCSNP-44 allele showed significant association in the populations of Spanish [61, 62], Turkish [177], and Indian women [34]. A significant association between the UCSNP-43 polymorphism and the PCOS metabolic phenotype was found in hirsute southern Brazilian patients [170] as well as Chilean PCOS women [102]. The UCSNP-45 C allele is associated with idiopathic hirsutism in Spanish PCOS women [53]. The UCSNP-56 and ins/del-19 are found to be in strong linkage disequilibrium and showed significant association with PCOS in German women. The TGG3AGCA and TGA2AGCA haplotypes showed both decreased and increased risk for PCOS [165]. The more common allele of UCSNP-63 showed evidence for excess transmission in a single-locus transmission disequilibrium analysis of European trios. However, this association was not replicated in the case-control study from that region [70]. In contrast to these associations, none of the *CAPN10* polymorphisms were associated with PCOS in German Caucasians [69] or Turkish adolescent girls [156]. Although there is no significant association between individual polymorphisms of *CAPN10* and PCOS, neither the haplotype nor the diplotypes of this gene showed significant associations with PCOS in African-American [48], Korean [93] or Indian populations [34]. A recent meta-analysis using 11 case control studies demonstrated that the *CAPN10* UCSNP-63 homozygous allele and the UCSNP-19 insert allele are protective factors for PCOS [77].

Metformin and PCOS

Several studies have postulated that the use of metformin in women with PCOS may reduce the endocrine and metabolic features of PCOS. The first and foremost study of metformin in obese women with PCOS demonstrated a restoration of normal menses and reduced hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure [161]. Ensuing studies have reported that metformin can alter sex hormone binding globulin *(*SHBG*)* and free T levels [112]; increase the rate of ovulation [114, 160]; and improve the efficacy of ovulation induction medications including clomiphene citrate [113] and exogenous gonadotropins [37]. A meta-analysis using 13 randomized trials involving 543 women with PCOS reported that the use of metformin significantly increased ovulation frequency compared to placebo. Furthermore, this meta-analysis confirmed that the metformin in combination with clomiphene citrate showed superior ovulation when compared to the clomiphene alone [98]. Although metformin's actions are mediated by activation of AMP-activated protein kinase (*AMPK*) [180], its exact molecular mode of action remains unclear.

Several lines of evidence indicate that metformin treatment in women with PCOS results in a decline of insulin as well as total bioavailable T [91], which leads to a significant reduction in hyperinsulinemia and hyperandrogenism [128]. It has been shown that luteinizing hormone (LH) and insulin reduction with metformin increases progesterone [103]; serum glycodelin; and insulin-like growth factor-binding protein 1 concentrations [82] during luteal phases. Importantly, this indicates an improved endometrial milieu for the establishment and maintenance of pregnancy in PCOS women. A meta-

analysis of 8 randomized controlled studies investigating metformin in PCOS women found a significant reduction in the risk of ovarian hyperstimulation syndrome, while at the same time showing no improvement in pregnancy rates after metformin treatment [33]. Moreover, significant teratogenicity is also not evident. Despite its advantages, metformin is listed as a "Category B" drug because its safety in pregnancy has not yet been established.

Environmental factors and PCOS

Many studies have concentrated on possible environmental factors that contribute to the development or progression of PCOS. Several environmental factors are known to unveil genetically programmed susceptibility to PCOS and contribute to its phenotypic expression. These factors interact chiefly with early stages of human development and convert a predisposed genotype to the phenotypic expression of PCOS. Low-birth-weight infants show an increased incidence of precocious puberty, hyperinsulinemia, and hyperandrogenism compared to normal-weight infants [79]. The foetus or infant with retarded growth will develop PCOS when exposed to nutritional surplus later in life [3]. A nutritional surplus with the consumption of high-calorie diets leads to obesity and induces the development or progression of the clinical spectrum of PCOS [108, 122]. Furthermore, environmental determinants may influence the clinical severity of PCOS, ranging from a less-severe phenotype to the mature phenotype of classic PCOS. The exposure of pregnant non-human primates and sheep to excess androgens can cause the development of a syndrome similar to PCOS, indicating that the exposure to androgen-like chemicals absorbed by the human body can lead to PCOS [1, 129]. A retrospective study demonstrated that disposable plastic drinking cups, cooking oil fumes and indoor decorations made of plastic increased the PCOS risk, indicating that environmental endocrine-disrupting chemicals are associated with the risk of PCOS [76]. Bisphenol A (BPA), a known hormone disrupter which is present in our environment, food, and consumer products, is elevated and associated with higher levels of male hormones in the blood of women and results in a deviation from normal homeostasis or reproduction. Studies using experimental animals have demonstrated that neonatal exposure to BPA leads to PCOS development [57]. Moreover, serum BPA levels were positively associated with serum androgen levels and insulin resistance indices in both lean and obese PCOS women [87].

Conclusions

The cited genetic studies which focused on PCOS using several different approaches in different populations are limited by heterogeneity in the PCOS diagnosis as well as the relatively small number of participants in the researches. No consistent evidence emerged of a strong association between the risk of PCOS and any of the known genes related to insulin signaling and glucose homeostasis. Although an individual's geographic location, ethnic origin, and cultural or social practices are known to alter manifestations of PCOS, earlier studies did not consider these factors. Moreover, the failure of researchers to replicate the results of more recent genome-wide association and linkage studies leaves this field with the both phenotypic variability and lack of a male phenotype as well as the associated comorbidities of PCOS.

Future directions

Future research should focus on early detection of the predisposing risk factors in PCOS development, including long-term studies with the goal of modifying environmental factors so that risk may be significantly reduced. Large genome-wide association studies devoted solely to PCOS will be necessary to identify new candidate genes and proteins that are involved in PCOS risk. Experiments related to pathophysiological perturbances and interventions which will normalize signal transduction of these pathways should be conducted in a number of cell culture and animal models to shed more light on our understanding of the pathophysiology of PCOS. The use of Systems Biology approaches in analyzing biochemical networks will enable us to better comprehend the multisystem cross-talk underlying the etiology of PCOS.

Acknowledgments Dr. LVKSB and Dr. GUR acknowledge funding from the Department of Biotechnology (DBT), Government of India (Project Ref. No. BT/PR14090/GBD/27/275/2010).

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