Review Article

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Molecular & genetic factors contributing to insulin resistance in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of unknown etiology. Insulin resistance is very common and plays a central pathogenic role in PCOS. During last decade several studies have been conducted to understand the mechanisms contributing to the state of insulin resistance and insulin-induced hyperandrogenemia in PCOS. Insulin signaling pathways have been dissected in different insulin responsive tissues such as skeletal muscles, adipose tissues, fibroblasts as well as ovaries to elucidate the mechanism. These studies suggest a post receptor signaling defect where metabolic action of insulin is affected but not the steroidogenic and mitogenic actions. Despite advancement in these studies gaps exist in our understanding of the mechanism of insulin resistance as well as insulin-induced steroidogenesis in PCOS. The syndrome is now considered as a complex multigenic disorder. Efforts are ongoing to dissect the variants of genes from multiple logical pathways which are involved in pathophysiology of the syndrome. But still today no gene has been emerged as universally accepted susceptibility gene for PCOS. This review briefly describes the lacunae along with the current status of molecular events underlying insulin resistance and the contribution of insulin signaling pathway genes in pathogenesis of PCOS along with future researchable areas.

Key words Hyperandrogenemia - insulin signaling - PCOS - steroidogenesis - phosphorylation

Introduction

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder, affecting approximately 7 per cent of women in reproductive age[1]. It is characterized by chronic anovulation, hyperandrogenemia, altered LH: FSH ratio (>2/3:1) and polycystic ovaries. The syndrome is a major cause of anovulatory infertility[2]. The aetiopathogenesis of this syndrome still remains elusive but likely to be mutifactorial consisting of genetic and environmental components.

Insulin resistance (IR) is now known to be intrinsic to this disorder, present in approximately 50-70 per cent of these women independent of obesity, and contributing in a major way to its pathogenesis[2,3]. Women with PCOS are frequently obese which contributes an extrinsic component of IR. It is known that IR progresses towards the development of compensatory hyperinsulinemia, which drives hyperandrogenemia in these women[4]. Excess androgen levels lead to menstrual disturbances, development of ovarian cysts, hirsutism and other related disorders. IR also increases the risk for development of glucose intolerance, type 2 diabetes mellitus (T2DM), hypertension, dyslipidaemia and cardiovascular abnormalities in these women[3,5] (Fig. 1).

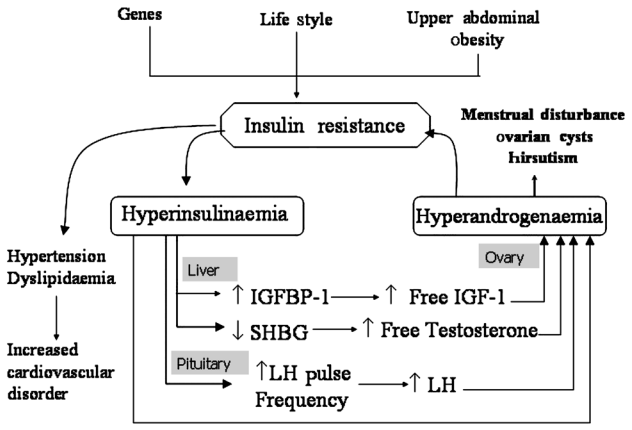

Fig.1. Pathways linking hyperinsulinemia and hyperandrogenemia and related disorders: Insulin directly acting on ovary alone or/ and along with LH can enhance ovarian androgen production. It indirectly also can increase androgen levels by reducing hepatic production of SHBG (sex hormone binding globulin) and IGFBP-1 (insulin like growth factor binding protein -1) and thus elevates free testosterone and free IGF-I, IGF-II (insulin like growth factor) levels. Excess androgen impairs folliculogenesis resulting in menstrual disturbance, development of multiple cysts in ovary. Insulin resistance increases the risk of development of metabolic disorders including diabetes, hypertension, dyslipidemia and cardiovascular events in these women.

Hyperinsulinemia and hyperandrogenemia are thus two principal features of PCOS and their cause and effect relationship is still debated[2,6]. However, several evidences suggest hyperinsulinemia to be the primary factor contributing to the ovarian hyperandrogenemia. Pharmacological reduction of insulin levels has been found to improve hyperinsulinemia as well as hyperandrogenemia and restore ovulation in the women with PCOS[2,4,6]. However, reduction of androgen levels by bilateral oophorectomy or administration of GnRH agonist[6] or antiandrogenic compounds[2] were seen to have no effect on IR or hyperinsulinemia in the PCOS women which would have been expected, if hyperandrogenemia was the cause of hyperinsulinemia.

Insulin can augment ovarian steroidogenesis directly, alone and/or augment LH-mediated androgen production[4,6,7]. It may act indirectly; to reduce hepatic biosynthesis of sex hormone–binding globulin (SHBG)[4,6], the key circulating protein which controls the bioavailability of testosterone; and/or to reduce production of IGF binding proteins in liver and ovary and therefore elevate levels of free IGF-1[4,6]; upregulate ovarian IGF type-I receptor[4] and thus amplify IGF-I, IGF-II action in the ovary and enhance amplitude of GnRH-stimulated LH pulses[6] (Fig. 1). However, recent evidence suggests that abnormality in GnRH pulse generator in PCOS are independent of hyperinsulinemia or insulin resistance[8].

In PCOS the "central paradox" is that ovary remains sensitive to insulin action to produce androgens in spite of systemic insulin resistant state; whereas classical target organs of insulin as well as ovary remain resistant to its metabolic activity. The number and affinity of insulin receptors have been found to be optimal in different insulin target tissues and ovary, and also no structural and mutational abnormalities could be detected in the PCOS women[2,4,9,10]. Thus a post-receptor binding defect in the insulin signaling pathway appears to play an important role in the etiology of selective IR. Although several *in vitro* and *in vivo* studies have been carried out in various tissues (adipocytes, fibroblasts, myocytes and ovarian cells)[2,4,11-28] to elucidate the potential mechanism of IR and insulin derived hyperandrogenemia (ovarian cells)[1,24-28] in PCOS women, the data is not yet conclusive. This review briefly describes the current understanding of the mechanism of IR and insulin induced hyperandrogenemia in PCOS, emphasizing the need for future research.

Signaling pathways of insulin action: A brief overview

Insulin exerts a wide range of pleiotropic actions at the target tissue such as cellular metabolism, growth and differentiation, via different signaling pathways. Binding of insulin to extracellular α subunits of insulin receptor (INSR) leads to activation of intrinsic tyrosine kinase and autophosphorylation of its β subunits. Activated INSR phosphorylates a number of substrates like the insulin receptor substrate family (IRS 1-4), Gab-1, Cbl, APS and Shc isoforms, and signal regulatory protein (SIRP) family members which bind to INSR[29]. Phosphorylated IRS proteins act as docking sites for several intracellular proteins such as Grb2, NcK and the regulatory subunit p85 of phosphatidylinositol 3-Kinase (PI3K), which mediate different actions of insulin. PI3K activation is crucial for metabolic actions, such as GLUT4 translocation, glucose transport, glycogen synthesis and protein synthesis, however the downstream signaling proteins of PI3K pathway is still not clear, probably it activates Akt and atypical PKC isoforms λ and ζ. A substrate of Akt, AS160 is involved in GLUT4 translocation from intracellular vesicles to the plasma membrane, which results in rapid entry of glucose into the cell[22]. Another pathway leading to GLUT4 translocation involves

the insulin receptor-mediated phosphorylation of the scaffolding protein CAP (c-Cbl Associated Protein) and formation of the CAP:Cbl:CrkII complex. This complex, through its interaction with flotillin, localizes to lipid rafts facilitating GLUT4 translocation[30]. The mitogenic action is mediated through binding of phosphorylated IRS1/2 or Shc with Grb-2/SOS complex leading to p21Ras and Raf-1 activation of mitogen-activated protein kinase pathway (MAPK)[21]. PI3K probably facilitates the mitogenic response as well (Fig. 2).

Studies with ovarian tissues

Increased androgen production by ovarian cells is the classical endocrine phenotype of PCOS[11,31]. The genomic and molecular studies demonstrated multiple alterations in the steroidogenic machinery of theca cells from PCOS women *viz*., overexpression of various proteins including luteinizing hormone (LH) receptor, INSR, lipoprotein receptor [high-density lipoprotein (HDL) and low- density lipoprotein (LDL)], steroidogenic acute regulatory protein (StAR), P450 side-chain cleavage (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD) and cytochrome P450c17 (CYP-17)[1,11]. All these contribute to excess production of progesterone (P_4), 17α-hydroxyprogesterone and testosterone as compared to normal theca cells[1,11]. The role of insulin in ovarian function becomes evident from the observations of severe ovarian hyperandrogenemia in women with syndromes of extreme insulin resistance[4]. Further, identification of INSR throughout the ovary and the ability of insulin to stimulate biosynthesis of androgens, estrogen and P_4 in ovarian cell culture suggest that ovary is another target organ of insulin action[4,24]. These findings clearly indicate that insulin may play a role in normal follicular development and hence in a variety of insulin resistant states ovarian dysfunctions are manifested[4].

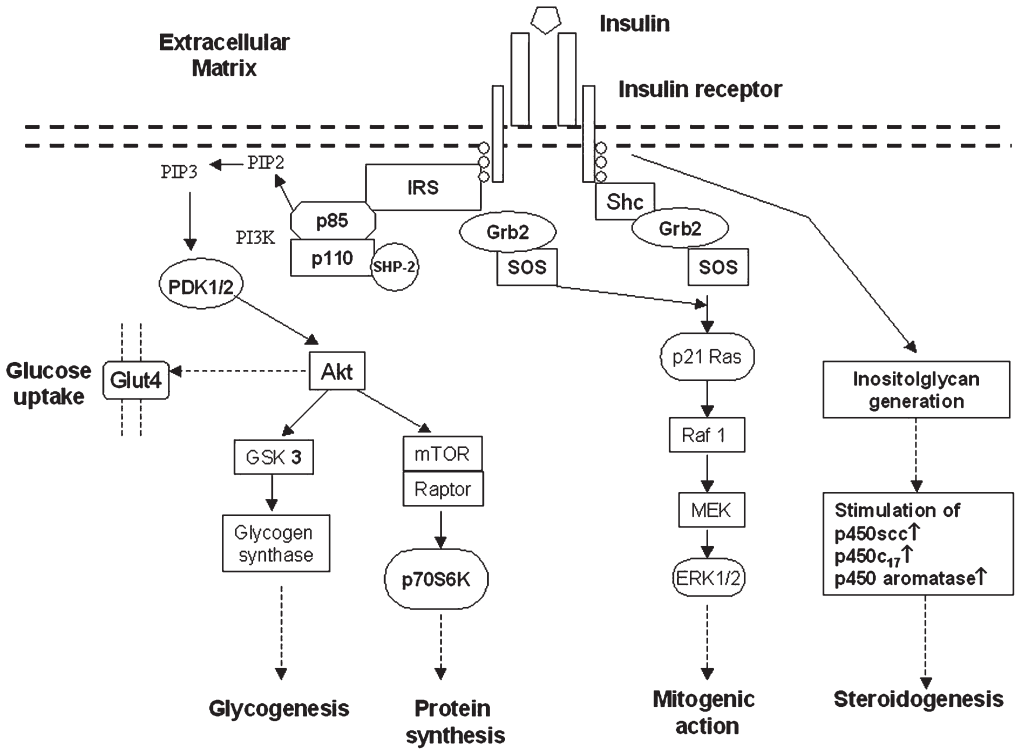

Fig. 2. Schematic illustration of major signaling pathways of insulin action: Binding of insulin to its receptor results in autophosphorylation and tyrosine kinase activation of the receptor which furthers phosphorylates other downstream mediators [insulin receptor substrate (IRS) and Src homology domain containing transforming protein 2 (Shc)]. These mediators then differentially activate various downstream signaling proteins. Phosphatidylinositol 3-Kinase (PI3K) plays a major role in glucose transport, glycogenesis and protein synthesis. On the other hand, Grb2/SOS (growth factor receptor-bound receptor 2/ Son of sevenless) complex activates mitogen-activated protein kinase pathway (MAPK) playing a crucial role in mitogenic response. Another pathway via inositolglycan generation has been suggested which may play a vital role in steroidogenesis. Abbreviations: PIP2-phosphatidylinositol 4,5-bisphosphate, PIP3- phosphatidylinositol 3,4,5-triphosphate, mTOR- mammalian target of rapamycin, Raptor-regulatory associated protein of mTOR, Glut4- glucose transporter type 4, GSK3-glycogen synthase kinase 3, PDK1/2-phosphoinositide dependent kinase1/2, p70S6K- ribosomal protein S6 kinase, MEK- mitogen-activated protein kinase kinase, ERK1/2- extracellular signal regulated kinase ½, SHP-2- SH_2 domain containing protein tyrosine phosphatase.

The molecular mechanism by which insulin augments androgen synthesis in ovary of PCOS women, in the systemic insulin resistant state, is still elusive. However, to explain this 'paradox' several hypotheses have been proposed[4]. The first one suggests that the steriodogenic action of insulin might be mediated via IGF-1 receptor or hybrid insulin/IGF-1 receptor. However, insulin can act through IGF-1 receptor only when its circulating levels are extraordinarily high but in PCOS women it is only moderately high. Evidence indicates that insulin stimulates steriodogenesis in granulosa cells (GCs) and theca cells from both normal and PCOS ovaries via its cognate receptor[4]. The second hypothesis proposes that the signaling cascade for metabolic action of insulin in ovary becomes divergent from the one, which mediates steroidogenic action after binding of insulin to INSR. Insulin may activate steroidogenesis via several pathways, either via cross talk with LH-induced cAMP accumulation, which in turn might activate PI3K activity or MAPK pathway or via alternate pathways of insulin signaling. Primarily, steroidogenesis in theca cells is regulated by LH, which acts via cAMP dependent protein kinase pathway (PKA)[11]. Insulin alone was not found to increase cAMP but it enhanced LH-induced cAMP accumulation in porcine theca cells[25]. LH and insulin independently and also synergistically could increase expression of genes in androgen biosynthesis pathway *viz*., StAR and CYP17, and also augment androstenedione and P_4 biosynthesis in primary culture of porcine theca cells[25]. A stable cAMP analog (8-Br-cAMP) could mimic the same effect on steroidogenesis, StAR and CPY17 mRNA expression but when it was co-administered with insulin it could enhance only P_4 synthesis and StAR gene expression but not CPY17 expression and androstenedione biosynthesis[25]. This indicated the mechanism involved in the expression of CPY17 is not perhaps cAMP dependent or may involve steps downstream of cAMP accumulation. On the other hand, study with cultured human theca cells showed insulin alone had no effect on 17α-hydroxylase activity or CPY17 expression but requires concomitant activation of cAMP signaling pathway by forskolin[27]. This different effect of exogenous or endogenous cAMP along with insulin on CPY17 gene expression indicates the possibility of different cellular response mediated by exogenous or endogenous cAMP. The stimulation of 17α-hydroxylase activity was blocked by PI3K inhibitor but not by MAPK kinase (MEK) inhibitor, indicating insulin action on 17α-hydroxylase activity is mediated via PI3K pathway not via MAPK pathway[27]. However, this response of insulin is different from its effect on glucose metabolism which do not require co-activation of cAMP pathway, again emphasizing the divergence of signaling pathways distal to PI3K for metabolic and steroidogenic action[28]. However, both MEK1/2 and ERK1/2 phosphorylation were markedly reduced and were associated with increased CYP17 mRNA abundance and androgen production in theca cells from PCOS women compared to cells from normal women[28]. Besides, MEK1/2 activity was reduced and CYP17 mRNA abundance and dehydroepiandrosterone (DHEA) activity was increased even in absence of insulin treatment in PCOS cells suggesting that alteration in MAPK pathway may plays a role in pathogenesis of excess androgen biosynthesis via an insulin independent mechanism[28].

Insulin alone or with FSH can augment P450 aromatase activity in human GCs[32] and can stimulate oestradiol (E_2) production by GCs isolated from polycystic ovary (PCO)[33]. Aromatase activity has been reported to be low in GC from PCOS ovary[33] . On the contrary a significant increase in basal aromatase activity had been reported in GCs isolated from PCO ovary and these GCs were more sensitive and hyperresponsive not only to FSH but also to insulin and IGF-1[34] . In these studies the stimulatory effect of insulin on aromatase activity was observed only at supraphysiological dose. Insulin even at physiological concentration could enhance E_2 and P_4 production by GCs from both PCOS and normal ovaries[24]. Interestingly, insulin along with FSH augmented P_4 production by GCs from normal and PCOS ovary but enhanced E_2 secretion only by GCs from anovulatory PCOS women and not from normoovulatory PCOS and normal women[24]. As anovulatory PCOS women are known to be more insulin resistant than normoovulatory PCOS, these suggest that the steroidogenic machinery may be more responsive to insulin in a systemic insulin resistant state. Recent data also showed that insulin can regulate the expression as well as activity of aromatase and also the expression of 3β-HSD in human luteinized GCs[35]. Experimental data from cultured human GCs from both control and PCOS women suggest that neither PI3K nor MAPK pathway are involved in insulin induced steriodogenesis[36] which is in contrast to theca cells where PI3K has been shown to be involved [27]. Cholesterol which is the substrate for steroid biosynthesis is transported into ovarian cell via LDL receptor. Sekar and Veldhuis[26] demonstrated insulin and LH synergistically upregulated transcription of LDL receptor in porcine GCs via mechanism that

involves PKA, PI3K and MAPK pathways. Insulin treatment elevates the P450ssc mRNA levels in bovine luteal cell[37]. Insulin when co-administered with LH the expression of P450ssc mRNA was much higher that that occurred only with LH[26]. All these data support that elevated insulin levels can affect steroid production in human GCs and alter the menstrual cycle and fertility. Though steriodogenic action of insulin is maintained, the metabolic action is affected in PCOS women. A decrease in lactate production but not P_4 secretion by granulosa-lutein cells, isolated from PCOS women have been reported [38] (Table I).

Another hypothesis proposed to explain the 'paradox' is serine phoshorylation theory, based on the observation that serine phosphorylation of the main regulatory enzyme of androgen biosynthesis *i.e.*, P450c17 appears to modulate its 17, 20 lyase activity and subsequent androgen production[6]. In a subgroup of PCOS women, IR appears to be related to excess serine phosphorylation of the β subunit of INSR[2]. The mechanism of serine phosphorylation is still unknown but evidence suggests it may be due to serine/threonione kinase extrinsic to INSR or due to an inhibitor of a serine/threonine phosphatase[19]. This leads to the postulation that a single hypothetical kinase might phosphorylate both INSR and P450c17 and thus account for both hyperandogenemia and hyperinsulinemia in a subgroup of PCOS women[6]. Identification of the kinase or the regulatory factors responsible for the serine phosphorylation is required to prove this hypothesis.

Recently it has been debated whether PCOS is a state of ovarian hypersensitivity to insulin or a state of preserved ovarian sensitivity inspite of systemic IR. Baillargeon and Nestler[39] postulated the former view as the cultured theca cells from PCOS women secrete more androgen upon insulin stimulation compared to normal cells. Furthermore, normoinsulinemic PCOS women treated with insulin sensitizing drugs exhibit substantial reduction in ovarian androgen production though the reduction in insulin level is modest, suggesting increased insulin sensitivity of the androgenic pathway. Conversely, Poretsky[40] believes ovarian sensitivity is maintained in PCOS women inspite of systemic IR, as ovarian cells from PCOS women secrete higher amount of androgens not only in response to insulin but regardless of the stimulus used. He emphasized that the signaling pathways mediating ovarian effects of insulin other than glucose transport are different from classical insulin signaling pathways. Further studies are required to delineate the alternate signaling pathway mediating ovarian effect of insulin, their interaction with IGF-1 pathway or any other pathways that are hypersensitive to physiological concentrations of insulin in PCOS.

Table I. Insulin effect on steroidogenic machinery in ovary

Targets	Cell	Effect	Stimulant	Exerted via	References
LDL Receptor	GC	↑	Insulin +LH	PKA, PI3K, MAPK	Sekar *et al*[26]
StAR mRNA	Theca	↑	Insulin, LH , Insulin +LH	via cAMP	Zhang *et al*[25]
CYP11A mRNA	LGC	↑	Insulin	N.d	Mamluk *et al*[37]
		↑	Insulin	N.d	Sekar *et al*[26]
CYP17 mRNA	Theca	↑	Insulin, LH, Insulin + LH	Not completely cAMP dependent	Zhang *et al*[25]
		↑	Insulin + Forskolin	cAMP	Munir *et al*[27]
17α-hydroxylase activity	Theca	↑	Insulin + Forskolin	PI3K	Munir *et al*[27]
P_4 secretion	GC	↑	Insulin	Not via PI3K and MAPK	Poretsky *et al*[36]
		↑	Insulin	N.d	Willis *et al*[24]
					Rice *et al*[38]
P450 aromatase	GC	↑	Insulin	N.d	Pierro *et al*[34]
	LGC	↑	Insulin	N.d	Fedorcsak *et al*[35]
3β-HSD mRNA	LGC	↑	Insulin	N.d	Fedorcsak *et al*[35]

LDL-R, low density lipoprotein receptor; StAR, steroidogenic acute regulatory protein; 3β-HSD, 3β-hydroxysteroid dehydrogenase; P_4, progesterone; PKA, protein kinase A; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; LGC, luteal granulosa cell

In recent years, PCOS research has focused on comparative gene expression profiles of different components of ovarian tissue such as theca cells, ovary and oocytes of PCOS and normal women[41-44]. These studies have provided some leads about the expression profile of insulin signaling and functional pathway related genes and their possible transcriptional regulation. The first study by Wood *et al*[41] on theca cells from PCOS and normal ovary showed differential expression of 346 genes. To understand the transcriptional regulation of these genes, the promoter regions were analyzed by *in silico* approach in our lab[45]. The analysis identified four putative transcription factor binding sites (TFBS) *i.e.*, Staf, E47, CCAAT Box and CRE-BP in the promoter regions of co-expressed genes which were overexpressed in the PCOS theca cells. Members of CCAAT box family are known to be associated with CYP11A1, CYP17 and 3βHSD as well as genes related to their action such as GLUT4 and peroxisome proliferator activated receptor-gamma (PPAR-γ). Multiple transcriptionally active CRE-BP sites have been found in human insulin gene promoters. E47 plays an important role in the regulation of insulin transcription in the pancreatic β-cells and thus in maintenance of insulin sensitivity[45]. Another *in vitro* gene expression study with normal and PCOS ovaries revealed altered expression of several genes involved in insulin function like PDK4 (pyruvate dehydrogenase kinase 4), PIGH (phosphatidylinositolglycan class H) and UDP-GalNAcPP (UDP-GalNAc pyrophosphorylase) and some genes involved in insulin mitogenic pathway, mainly those in MAPK pathway, such as RPS6KA2[44]. PDK4 has recently been shown to play a role in the pathogenesis of insulin resistance in T2DM[44]. Similar studies with oocyte revealed the presence of putative binding sites for androgen receptor (AR), PPAR-γ, PPAR-γ/Retinoic X Receptor (RXR) in the promoter regions of many genes differentially expressed in PCOS. PPAR-γ is a ligand-activated transcription factor involved in glucose and lipid metabolism are known to modulate insulin signaling pathway. Over expression of PPAR-γ gene in PCOS ovary[43] and the presence of PPAR-γ binding sites in differentially expressed genes suggest its role in pathogenesis of the syndrome but the mechanism needs to be elucidated. It has been demonstrated that insulin can phosphorylate and activate PPAR-γ in absence of endogenous PPAR-γ ligand[42]. Thiazolidinidiones (TZDs), the synthetic PPAR-γ agonist can restore insulin sensitivity and ameliorate hyperandrogenemia in PCOS women[4]. TZDs have been shown to exert direct effect on ovarian androgen production through both insulin independent and insulin sensitizing effect. They directly enhance P_4 and IGFBP-1 synthesis and inhibit E_2 and testosterone production and also reduce insulin induced testosterone production[46]. The indirect effect of TZDs is mediated by its systemic insulin-sensitizing action through reduction in insulin level. Insulin and TZDs independently have been shown to increase expression of INSR, IRS-1, PPARγ and StAR protein in human ovarian cells[46]. This indeed indicates a crosstalk between insulin signaling, PPARγ and steriodogenic pathways which may constitute a novel regulatory mechanism in ovarian function.The effect of insulin on steroidogenesis machinery in ovarian cell is summarized in Table 1. Further molecular studies are needed to dissect the role of these genes and their downstream pathways in the pathophysiology of this syndrome.

Studies with adipocytes

Both lean and obese women with PCOS exhibit significant decrease in maximal insulin-stimulated glucose utilization *in vivo* and in isolated adipocytes, suggesting an intrinsic abnormality, independent of obesity[2]. Ciaraldi *et al*[12] observed reduced insulin sensitivity but normal insulin responsiveness in adipocytes isolated from PCOS women with IR. The receptor kinase activity was optimal inspite of decrease in insulin induced INSR autophosphorylation suggesting a defect in signaling cascade between INSR and glucose transport[12]. On the contrary, Lystedt *et al*[13] reported no differences in the insulin sensitivity to glucose uptake in cultured adipocytes of PCOS women compared to controls. This discrepancy might be result of the fact that former group[12] studied fat cells immediately after surgery, when the cells were in the state of IR due to surgical stress whereas, the later group[13] allowed adipocytes to recover before initiating the study. The expression of IRS-1, Akt1/2, PKCζ as well as their phosphorylation status and PI3K activity were similar in adipocytes isolated from both control and PCOS women[12]. On the contrary a reduction in IRS-1 expression as well as its tyrosine phosphorylation has been reported[14,15]. The expression of IRS-2 was normal but phosphorylation was decreased in adipocytes from insulin resistant PCOS group compared to noninsulin resistant PCOS women and controls[16]. Rosenbaum's group[20] observed GLUT4 expression, was significantly reduced in adipocytes from PCOS women, independent of obesity and correlated well with diminished insulin responsiveness, but Ciraldi *et al*[12] found no alteration in

expression. The expression of CAP or cbl protein was not different in adipocytes from control and PCOS[12]. A recent study with subcutaneous preadipocytes of PCOS women concluded that there is no intrinsic defect of insulin signaling in adipose cell lineage, the IR is due to the factors present in the *in vivo* environment[18].

Visceral adiposity is the commonest phenotype seen in both lean and obese PCOS, which releases increased amounts of free fatty acids (FFA), known to plays a pathophysiological role in IR directly by affecting insulin signaling. Catecholamine-activated lipolysis was shown to be reduced in subcutaneous (SC) adipocytes[47], but enhanced in visceral fat[48] of PCOS women which may be due to altered expression of lipolysis regulatory proteins in these two fat depots of controls and PCOS women. This partly explains the metabolic defect observed in PCOS subjects, where lipolysis resistant SC fat cells promotes obesity and enhanced lipolysis in visceral fat increases FFA levels.

Adipose tissue factors in pathogenesis of PCOS

It is now well recognized that adipose tissue functions as a highly specialized endocrine and paracrine organ producing an array of adipokines like adiponectin, leptin, resistin, TNF-α etc. which affect insulin sensitivity. The relationship of these adipokines with IR and obesity in PCOS women are conflicting.

Adiponectin, a key adipokine in mediating relationship between body weight and insulin sensitivity, protects against IR and T2DM[49]. Several studies reported lower serum adiponectin levels in PCOS women compared to controls[49-52] and some of them have observed no significant difference[53]. Recent findings indicate that the effect of adiponectin on insulin sensitivity is mediated primarily by high molecular weight (HMW) form of adiponectin, but studies showed no difference in HMW adiponectin in PCOS and controls[53]. A recent metanalysis revealed lower adiponectin levels in PCOS women which showed association with IR but not with total testosterone levels[53].

Resistin, another adipokine, which is significantly increased in insulin-resistant mice and genetic or diet-induced obese mice has been shown to be down regulated by insulin sensitizing agents[54], suggest to mediate a link between obesity and IR in mice, but this relationship remains controversial in human. Circulating resistin level is proportional to degree of adiposity and decreased adiponectin and increased resistin have been linked to the development of IR[50]. Most of the studies have not found any difference in resistin levels between PCOS and controls[50,52] however, increased expression of resistin mRNA[55] in adipocytes as well as elevated serum resistin levels was observed in PCOS women[55,56].

Leptin, is a key hormone in energy homeostasis and neuroendocrine function, may involve in pathogenesis of hyperandrogenism and infertility in women with PCOS[57]. High leptin levels have been deemed as a component of the metabolic syndrome, obesity, IR, hypertension and dyslipidemia[58], and hyperleptinemia may lead to leptin resistance. Some studies showed similar leptin levels in both PCOS women and weight matched controls but showed significant association between leptin levels and BMI[50,59], and others reported high circulating levels of leptin in the PCOS women[49,59].

Retinol-binding protein 4 (RBP4) has been implicated in IR and reported to be high in insulin resistant humans with obesity and T2DM[51]. Elevated levels of serum RBP4 as well as increased mRNA and protein expression in adipose tissue were reported in overweight PCOS women[60] . On the other hand, similar serum levels of RBP4 were observed in PCOS and controls but its relationship with IR is contradictory[51,61]. Barber *et al*[51] clearly demonstrated that RBP4 levels correlate with visceral fat depot. Recently, Diamanti-Kandarakis *et al*[62] observed lower levels of both RBP4 and free plasma RBP4 (a more sensitive marker of circulating RBP4) in insulin-resistant PCOS subjects than controls, suggesting lack of relationship between IR and RBP4. Aigner *et al*[63] reported a positive correlation between RBP4 and androgen levels and also clinical hirsutism scores in women with PCOS, suggesting a role in steroid metabolism, which needs further investigation. The serum levels of visfatin, another adipokine and its mRNA and protein expression in adipocytes were reported to be increased in PCOS women[64,65].

A gene expression study carried out by Corton *et al* [66] with omental adipose tissue from PCOS women showed overexpression of ectoenzyme nucleotide pyrophosphate phosphodiesterase 1 (ENPPI), which is a negative regulator of INSR tyrosine kinase activity and the regulatory p85α subunit of PI3K which resulted in decreased PI3K activity. Besides, several actin and myosin isoforms were downregulated which may result in reduced glucose transport. The upregulation of a serine/threonine kinase PKN2 was observed which may be involved in excess serine phosphorylation of

different proteins in insulin signaling pathway as well as steroidogenic enzymes leading to impaired insulin action and enhanced steriodogenesis in PCOS.

Studies with fibroblasts

Increased basal autophosphorylation of serine residues of INSR and subsequent decrease in insulin stimulated tyrosine autophosphorylation of INSR were reported in 50 per cent of cultured skin fibroblasts from women with PCOS[19]. Similar defect was also observed *in vivo*, indicating that the defect might be intrinsic. Excess serine phosphorylated INSR showed reduction in tyrosine phosphorylation of an artificial substrate, which again provides evidence of association between increased serine phosphorylation and decreased tyrosine kinase activity [67]. On the other hand, the remaining 50 per cent of PCOS women in whom INSR autophosphorylation was normal, had similar degree of IR, which clearly indicate a signaling defect downstream of INSR. Fibroblast from PCOS women showed similar mitogenic action like other tissues but glycogen synthesis was affected[10]. Optimal expression of IRS-1 and its associated PI3K activity in cultured fibroblasts of these women suggests that metabolic signaling defect lay either on a different signaling pathway or at steps that are downstream of IRS-1 mediated PI3K activation[10]. On the contrary, Ciaraldi *et al*[20] found no difference in both metabolic and mitogenic actions of insulin in fibroblast culture from obese PCOS women, though adipocytes of same women demonstrated impaired glucose transport suggesting that the insulin signaling defect is possibly tissue specific[20]. In cultured fibroblast, isolated from PCOS women, though Akt phosphorylation was normal, a decreased phosphorylation of GSK-3 was observed which led to decreased activation of glycogen synthase and diminished glycogen synthesis [21].

Studies with skeletal muscle

Skeletal muscle is the major site for insulin mediated glucose uptake. *In vivo* studies, where muscle biopsies were serially taken at different time points during euglycemic hyperinsulinemic glucose clamp, revealed normal steady state insulin levels but reduced insulin mediated glucose uptake (IMGU) in PCOS women along with decrease in insulin mediated IRS-1 associated PI3K activity[19]. The expression of signaling proteins upstream of PI3K like INSR, IRS-1 and also p85 subunit of PI3K were normal but that of IRS-2 was markedly increased[19,12]. However, as IMGU was still decreased in PCOS which indicate that increased IRS-2 associated PI3K activity could not completely compensate for the defect. In muscle biopsies from PCOS women, impaired IMGU were paralleled by reduced insulin induced phosphorylation of Akt at Ser^{473}, Thr^{308} and AS160, despite normal basal and insulin stimulated P13K activity, which improved by treatment with TZDs[22]. This suggests that impaired glucose metabolism in skeletal muscle of PCOS women is mediated via signaling defect at Akt and AS160 level[22]. Further research on other insulin sensitive tissues is warranted to determine whether similar defects exist or not.

Like fibroblasts, skeletal muscle from women with PCOS also showed impaired insulin action along with constitutive serine phosphorylation and decreased tyrosine kinase activity of INSR *in vitro*[2]. In contrast to *in vivo* studies, basal and IMGU was significantly high in the cultured myotubes from obese PCOS women[68]. Expression of INSR β subunit, IRS-2, p85 of PI3K as well as basal and insulin induced tyrosine phosphorylation of INSR were similar to controls. The IRS-1 expression and its phosphorylation at Ser^{312} were significantly increased in PCOS women which might have inhibited its tyrosine phosphorylation and downstream signaling[68]. Though IRS-1 related PI3K activity was normal, when normalized for increased IRS-1 expression, it was significantly decreased. IRS-2 mediated insulin signaling was also impaired as evident by decreased basal IRS-2 associated PI3K activity and decreased binding of p85 to IRS-2 upon insulin stimulation[68]. In skeletal muscle, inspite of intrinsic defects in insulin signaling increased IMGU was observed *in vitro*, which indicates that interaction with *in vivo* environmental factors are required to manifest IR[69]. Expression of GLUT4 was normal but GLUT 1 was significantly increased in PCOS which positively correlated with increased basal glucose uptake in skeletal muscle[69]. On the other hand, Ciraldi *et al*[12] observed that myotubes from PCOS women displayed reduced IMGU and normal insulin sensitivity. The expression of GLUT4, insulin signaling proteins and insulin induced phosphorylation of Akt in skeletal muscle and myotubes did not differ between PCOS and controls[12]. *In vivo* and *in vitro* studies with skeletal muscle showed that the mitogenic signaling via MEK1/2 and ERK 1/2 is constitutively enhanced in PCOS women and possibly at the level of Raf-1[68]. Inhibition of MEK activity by specific inhibitor resulted in decreased phosphorylation of IRS-1 at Ser^{312} and increased association of IRS-1 with p85 of PI3K in

both control and PCOS. This suggests that constitutive activation of ERK1/2 or ERK1/2 regulated kinase may inhibit the association of IRS-1 with p85 via IRS-1 Ser^{312} phosphorylation and thus lead to impaired metabolic action of insulin in skeletal muscle of PCOS women[68].

In the light of these studies it becomes evident that mechanism underlying insulin resistance in PCOS women is tissue specific and pathway selective involving various molecules of insulin signaling and related pathways and the results are still inconclusive. The molecular mechanisms of insulin resistance and insulin mediated steroidogenesis are still elusive. The studies conducted so far have been mainly focused on PI3K, MAPK pathways and data suggest that these pathways may not be the primary ones involved in steroidogenesis therefore, alternate pathways of insulin signaling also need to be explored in PCOS. As PCOS is a heterogenous disorder it is also possible that the different subgroups of PCOS women may exist, having defects at different levels of insulin signaling. Recent reports on altered expression profile of several steroidogenesis and insulin signaling pathways genes in PCOS creates a need of integrated approach to study their molecular interactions to unravel the underlying mechanism involved in PCOS pathogenesis.

Genetic variants involved in insulin resistance in PCOS

Evidence from twin and family based studies have demonstrated an increased prevalence of PCOS and its phenotypic features in the relatives of women with PCOS, suggesting genetic factors underlying the syndrome[70,71]. However, the mode of inheritance is still not clear and recent studies indicate that the disorder could be a complex trait where several gene variants interact with each other and along with the environmental factors in the manifestation of the syndrome. Ethnic variations in the prevalence of IR, obesity and PCOS and also their association with different gene variants have also been observed. A series of linkage association studies carried out in women with PCOS from USA showed a strong linkage between susceptibility to the disease itself and a dinucleotide marker in chromosome 19p13.2[72-74]. Most of the genetic studies have focused on candidate gene approach, selecting genes from multiple logical signaling pathways, implicated in the pathogenesis of PCOS, such as genes involved in steroid hormone biosynthesis and metabolism, insulin signaling, gonadotropin action and its regulation, and proinflammatory genes[70-72, 75,122]. We briefly discuss here the most important findings published to date regarding the molecular genetic mechanisms underlying the association of PCOS with IR (Table II).

Insulin: The pancreatic β-cell dysfunction in PCOS women appears to have a genetic predisposition. The minisatellite variations (variable number of tandem repeats, VNTR) upstream of the insulin gene locus (*INS*) regulates insulin expression. Watherworth *et al*[77] reported strong linkage and association between *INS* VNTR and PCOS but only in the form of preferential transmission of class III allele from heterozygous father but not from mother to daughter with PCOS. However later studies failed to confirm any linkage of *INS* with PCOS and also any association between class III *INS* VNTR alleles with hyperandrogenemia[71,72]. Recently Ferk *et al*[78] reported a significant association of class III *INS* VNTR alleles with PCOS suggesting that an interaction of obesity and III/III *INS* VNTR genotype increases risk for development of PCOS.

Insulin receptor: Available evidence suggests IR in PCOS could be due to post-binding defects in insulin signaling[2,11]. Hence INSR, being an integral part of insulin signaling, has been explored as a potential candidate gene. Linkage analysis studies have found an association of PCOS with the microsatellite marker D19S884, located on chromosome 19p13.2 and relatively close (1cM) to *INSR*[72-74]. However, this association has not been supported by a case control study carried out in Spanish and Italian women with PCOS[79]. As the number and affinity of INSR is not altered in PCOS but its tyrosine phosphorylation status and subsequent signaling is affected it suggests that the defect may lie in the β chain[2,12]. Several polymorphisms have been identified in women with PCOS of which more frequent were at exon 17 which encodes the partial tyrosine kinase domain of INSR[71,76,80-84]. Among these polymorphisms, a C/T SNP at His1058 in exon 17 have been reported in studies with Caucasian, Chinese and Korean women[80,81,84]. In the first two studies the frequency of T allele was found to be significantly different between obese and lean PCOS women, which suggest an association of this SNP with lean PCOS women[80,81]. Our study in Indian women also confirmed this association with PCOS in the lean rather than obese women along with significant association with indices of IR and hyperandrogenemia in the same subgroup[83]. Our findings suggest that the genetic pathogenesis of IR in PCOS could be different in lean and obese women. A Korean study however failed to confirm this

Table II. Gene variants related to insulin resistance in PCOS

Gene/References	Variant/Locus	Subject	Phenotypic traits	Association
INS				
Urbanek *et al*[72]	VNTR	PCOS	PCOS, hyperandrogenemia	No
Watherworth *et al*[77]	VNTR	PCOS	PCOS	Yes
Ferk *et al*[78]	VNTR	PCOS	PCOS	Yes
INSR				
Urbanek *et al*[72]	D19S884	PCOS	PCOS	Yes
Tucci *et al*[74]	D19S884	PCOS	PCOS	Yes
Villuendas *et al*[79]	D19S884	PCOS	PCOS	No
Siegel *et al*[80]	His1058	PCOS	PCOS in lean Caucasian	Yes
Chen *et al*[81]	His1058	PCOS	PCOS	Yes
Jin *et al*[82]	Cys1008	PCOS	PCOS	Yes
			Insulin sensitivity	Yes
Mukherjee *et al*[83]	His1058	PCOS	PCOS in lean Indians, IR, hyperandrogenemia	Yes
Lee *et al*[84]	+176447	PCOS	PCOS	Yes
IRS1/2				
Urbanek *et al*[72]	IRS	PCOS	PCOS	No
Ehrmann *et al*[86]	Gly972Arg, IRS-1	PCOS	PCOS	No
			Insulin and glucose	No
	Gly1057Asp, IRS-2	PCOS	PCOS	No
			↓2h- glucose	Yes
EL Mkadem *et al*[87]	Gly972Arg, IRS-1	PCOS	PCOS	No
			↑ Fasting insulin	Yes
	Gly1057Asp, IRS-2	PCOS	PCOS	No
			↑ 2h- glucose	Yes
Villeundas *et al*[88]	Gly972Arg, IRS-1	PCOS	PCOS	No
			↑ Fasting glucose	Yes
	Gly1057Asp, IRS-2		PCOS	No
			↓2h- glucose	Yes
Dilek *et al*[89]	Gly972Arg, IRS-1	PCOS	PCOS, obese, fasting insulin, IR	Yes
Lin *et al*[90]	Gly972Arg, IRS-1, Ala513Pro, IRS-1	PCOS	PCOS, Metabolic parameters	No
Sir-Petermann *et al*[91]	Gly972Arg, IRS-1	PCOS	PCOS	Yes
			Obesity	Yes
Baba *et al*[92]	Gly972Arg, IRS-1	PCOS	PCOS	Yes
Valdés *et al*[93]	Gly972Arg, IRS-1	PCOS	PCOS	No
Haap *et al*[94]	Gly972Arg, IRS-1	PCOS	PCOS	No
	Gly1057Asp, IRS-2	PCOS	PCOS	No
ENPP1				
Baba *et al*[92]	K121Q	PCOS	PCOS	No
Heinonen *et al*[97]	K121Q	PCOS	PCOS	Yes
San Millan *et al*[98]	K121Q	PCOS	PCOS	No
Shi *et al*[99]	K121Q	PCOS	PCOS	No
PPARγ				
San Millian *et al*[98]	Pro12Ala	PCOS	PCOS	No
Korhonen *et al*[100]	Pro12Ala	PCOS	PCOS	Yes

Gene/References	Variant/Locus	Subject	Phenotypic traits	Association
Gu *et al*[101]	Pro12Ala	PCOS	PCOS	Yes
	1431 C/T	PCOS	PCOS	Yes
Orio *et al*[102]	Pro12Ala	PCOS	PCOS	No
			BMI, glucose, lipid	No
			IR	No
	1431 C/T	PCOS	PCOS, ↑ BMI, ↑serum leptin	Yes
Wang *et al*[103]	Pro12Ala	PCOS	PCOS	No
			BMI, Reproductive hormones	No
Hara *et al*[104]	Pro12Ala	PCOS	PCOS	No
			Insulin sensitivity in Caucasian	Yes
Xita *et al*[105]	Pro12Ala	PCOS	PCOS	No
Tok *et al*[106]	Pro12Ala	PCOS	PCOS	No
			↑Obesity,↑ Fasting insulin	Yes
			↓IR	Yes
			Reproductive hormones	No
Yilmaz *et al*[107]	Pro12Ala	PCOS	PCOS	No
			↓ Androgens	Yes
			↓ Insulin and IR	Yes
Antoine *et al*[108]	Pro12Ala	PCOS	PCOS	No
	His447His in exon 6	Control	↓ Testosterone	Yes
			↓ Insulin and IR	Yes
Koika *et al*[109]	Pro12Ala	PCOS	PCOS	No
			↓BMR, hyperandrogenemia	Yes
CAPN 10				
Ehrmann *et al*[110]	Genotype 112/121	PCOS	PCOS, ↑ fasting insulin	Yes
Lee *et al*[111]	Haplotype 111, diplotype 111/121 and 111/111	PCOS	PCOS	Yes
	Haplotype 112, diplotype 112/121		↓PCOS risk	Yes
Gonzalez *et al*[112]	UC SNP-44	PCOS	PCOS	Yes
	Haplotype 1121		hypercholesterolemia	Yes
Escobar-Morreale *et al*[113]	UC SNP-45	PCOS	PCOS	Yes
	Haplotype 2111 and 1221		Hirsutism	Yes
Haddad *et al*[114]	UCSNP -44	PCOS	PCOS	No
Vollmert *et al*[115]	UC SNP-19 ins/del	PCOS	PCOS	Yes
	UC SNP-56			Yes
	UC SNP-44			No
PON1				
San Millan *et al*[98]	-108C/T	PCOS	PCOS	Yes
	L55M		PCOS	No
	Q192R		PCOS	No
ADIPOQ				
Escobar-Morreale *et al*[52]	T45G	PCOS	PCOS	No
	G276T		PCOS	No
San Millan *et al*[98]	T45G	PCOS	PCOS	No
	G276T			No
Xita *et al*[116]	T45G	PCOS	PCOS	No
			Hyperinsulinemia	Yes
	G276T		PCOS	No
			↑Serum adiponectin	Yes
			↓Insulin	Yes

Gene/References	Variant/Locus	Subject	Phenotypic Traits	Association
Zhang *et al*[117]	T45G	PCOS	PCOS	Yes
	G276T		PCOS	Yes
			↓Insulin, and IR	Yes
			↑Serum adiponectin	Yes
Haap *et al*[94]	T45G	PCOS	IR	No
Baba *et al*[118]	-11377	PCOS	PCOS	No
RETN				
Escobar-Morreale *et al*[52]	-420C/G	PCOS	PCOS	
Baba *et al*[118]	-420C/G	PCOS	PCOS	Yes
Urbanek *et al*[119]	-420C/G	PCOS	PCOS	No
			Obesity	No
			IR	No
Xita *et al*[120]	-179C/G	PCOS	PCOS	No
			BMI	Yes
LEP				
Oksanen *et al*[121]	Coding region	PCOS	PCOS	No
LEPR				
Oksanen *et al*[121]	K109R	PCOS	PCOS	No
	Q223R		PCOS	No
	K656N		Low insulin	Yes
	3´ UTR		Low insulin	Yes
Erel *et al*[122]	Q223R	PCOS	PCOS	No

association, rather reported an association of a novel C/T SNP at +176477 with PCOS[84]. Another novel T/C polymorphism at Cys1008 in exon 17 of *INSR* has been reported to be associated with PCOS and decreased insulin sensitivity[82]. A recent meta-analysis has reported no significant association of His 1058 C/T polymorphism with PCOS[85].

Insulin receptor substrates: IRS proteins are critical for insulin mediated signal transduction in insulin target tissues. Two common polymorphisms in *IRS* particularly Gly972Arg in *IRS*-1 and Gly1057Asp in *IRS*-2 have been shown to influence the susceptibility to T2DM and are associated with phenotypic features of PCOS[71,76,86-90]. Several studies have reported a higher frequency of Gly972Arg allele of *IRS*-1 in PCOS women[89,91,92] but other studies failed to confirm this association[86,88,93]. In two of these studies, this variant showed association with increased fasting insulin[87,93] whereas the study by Ehrmann *et al*[86] showed no association with glucose, insulin and androgen levels. A recent study in Taiwanese women reported absence of Gly972Arg and another Ala513Pro polymorphism in *IRS-1* though majority of women with PCOS were either insulin resistant or glucose intolerant, thus suggesting *IRS-1* variants may not play a role in glucose dysmetabolism in their population[90]. Gly1057Asp variant of *IRS*-2 was shown not to associated with PCOS[86,87,94] However, a gene dosage effect of Arg972, *IRS-1* on fasting insulin and Asp1057 variant of *IRS-2* on 2 h glucose and insulin levels were observed among PCOS women[87]. Contradicting reports are also available linking wild type genotype with increased 2 h glucose levels[86,88]. Interestingly, PCOS women with both *IRS* variants were found to have a higher degree of IR compared to those with only one *IRS*-2 variant and wild type allele, indicating functional impact of these polymorphisms on IR component of PCOS[87]. A recent Mendelian meta analysis confirmed association of *IRS-1* Gly972Arg with PCOS, which support the overall data and indicates a role of IRS in IR component of PCOS[85].

Ectoenzyme nucleotide pyrophosphate phosphodiesterase (ENPP1): ENPP1 (also known as PC-1, plasma cell differentiation antigen 1) has been identified as a factor potentially contributes to IR by binding to INSR and affecting its signaling. It has been reported to be overexpressed in different insulin target tissues of insulin resistant subjects[95] and also in adipose tissue of PCOS women[96]. Recent linkage and association studies suggest *ENPP1* to be a susceptibility gene for IR and related abnormalities[95]. A functional missense

polymorphism K121Q in exon 4 has been reported to be associated with PCOS in a study from Finland[97] but other three studies showed negative results[92,98,99].

Peroxisome proliferator activated receptor gamma (PPAR-γ): Genetic association of *PPAR-γ* with IR and PCOS has evoked considerable interest in recent years, following an understanding of its role in diabetes. A common Pro12Ala polymorphism in *PPAR-γ* has shown to be associated with PCOS in Finish and Korean women[100, 101]. But some other studies have failed to confirm this association[98,102-105]. However reports are available on association of this polymorphism with insulin sensitivity[104,106-108] with decreased basal metabolic rate and hyperandrogenemia[109]. Another *PPAR-γ* polymorphism 1431C/T (His447His) in exon 6 showed association with PCOS[101] and also with increased BMI and higher serum leptin levels[102]. On the contrary, Antoine *et al*[108] reported no association with PCOS but T allele showed association with decreased free and total testosterone levels and as well as decreased IR in control women. A variant in PPAR- coactivator-1 (Gly482Ser) showed no association with PCOS[103].

Calpain-10: Calpain-10, a cysteine protease, has been shown to be associated with IR and susceptibility to T2DM[71]. The potential association between several nucleotide polymorphisms (SNPs) in the *CAPN10* (UCSNPs -45, -44, -43, -19, and -63) and PCOS susceptibility have been investigated with contradictory results. Ehrmann *et al*[110] found a haplotype combination (genotype 112/121) associated with higher insulin levels in African-Americans and which increases 2-fold risk of PCOS susceptibility in both African-Americans and Caucasian women. In Korean population specific haplotype and diplotypes of *CAPN10* significantly increases the risk of development of PCOS, and few others decrease the risk[111]. Studies in Spanish women reported association of UCSNP-44[112] or UCSNP-45[113] with PCOS susceptibility and showed further association of specific haplotypes with phenotypes, but no association observed between UCSNP-44 and PCOS in women from UK[114]. A recent, study with German women genotyped eight variants of *CAPN10* and a significant association of UCSNP-19 ins/del and UCSNP-56 with PCOS[115] were observed. Their meta-analysis showed a significant association only with ins/del-19 with PCOS and they emphasized that *CAPN10* may be an interesting candidate gene for PCOS[115].

Paraoxonase 1: Paraoxonase 1 encoded by the *PON1* gene, is a serum high-density lipoprotein (HDL) associated enzyme with antioxidant property. Oxidative stress plays a central role in the pathogenesis of IR and cardiovascular disease. Three genomic variants of *PON*1 have been studied in a Spanish population, which showed increased frequency of -108 C/T polymorphism in promoter region of *PON1* in PCOS women, whereas other two Leu55Met, and Gln192Arg polymorphisms in coding region showed no association[98]. However, subjects homozygous for Met55 alleles presented with a higher BMI and increased indices of IR[98].

Adiponectin: Most of the studies investigating the association of genetic variations in the adiponectin gene (*ADIPOQ*, T45G in exon 2 and G276T in intron 2) with pathogenesis of PCOS have provided negative results[52,98,116], but some of them showed relation with serum adiponectin levels[116,117]. A study with German PCOS women showed a higher prevalence of homozygous G allele of T45G polymorphism in PCOS women than in controls, but this was not associated with insulin resistant phenotype[94]. Another study in Chinese women showed association of both T45G and G276T polymorphisms with PCOS and carriers of polymorphic genotype at G276T had decreased levels of serum adiponectin[117]. Recently a Japanese study failed to show any association with -11377 polymorphism in promoter region of *ADIPOQ* with PCOS[118].

Resistin: The resistin gene (*RETN*) maps to the region on chromosome 19 which harbors possible candidate gene for PCOS[72,73]. Three groups investigated the possible association of promoter region polymorphism (-179C/G or -420C/G) in *RETN* with PCOS, its phenotypes and also with serum resistin levels with none reporting any association with development of PCOS[52,119,120]. However Xita *et al*[120] observed that PCOS women with CC genotype of -179C/G SNP had increased BMI as compared to women with polymorphic genotype, indicating an association of this polymorphism with adiposity in PCOS. The other SNP -420C/G showed no association with subphenotypes of PCOS (IR and obesity) or with serum levels of resistin[52, 119] but another study with Japanese women showed association with PCOS[118].

Leptin and leptin receptor: Leptin (*LEP*) and leptin receptor (*LEPR*) are attractive candidate genes for PCOS. A study in Finish population has not found any variation in coding region of *LEP* in PCOS women[121]. Regarding *LEPR* no association between its polymorphisms (exon 2, 4, 12 and 3' UTR pentanucleotide insertion) and PCOS susceptibility were observed[121,122]. However in this Finnish study, carriers of polymorphic genotype

of exon 12 (Lys656Asn) and *LEPR* 3'UTR (insertion) showed low serum insulin levels compared to wild type, suggesting a possible role of *LEPR* in regulation of insulin levels[121].

From the above review it is clear that although a genetic basis of PCOS is univocally accepted inspite of having several positive results, till today no gene has emerged as universally accepted susceptibitily gene for PCOS. The conflicting results might be attributed in part due to lack of universally accepted diagnostic criteria, relatively small sample size, failure to replicate results in independent studies, inadequate number of gene and their variants analyzed, presence of clinical heterogeneity among PCOS and ethnic variation between populations. To overcome this, uniformity in diagnosis of PCOS and sub-classification of cases according to sub-phenotypes, improved application of the candidate gene approach by selecting genes from expression studies, using haplotype-based analysis, replicating results in large cohorts and genome-wide approaches to maximize the chance of identifying an association is needed.

Conclusions

Although a large body of research has been devoted to understand the mechanisms underlying IR and insulin induced hyperandrogenaemia in PCOS, the data are still inconclusive. Evidence suggests that there is a post-receptor divergence in the insulin signaling pathway where metabolic activity is affected but not its steroidogenic and mitogenic activity. Use of high throughput techniques like pathway specific genomic and proteomic approaches may help to identify novel factors associated with insulin action in the target tissues. Moreover, subphenotyping PCOS women according to their insulin resistant status, may provide further insights to understand the molecular mechanism of insulin resistance in PCOS and also to develop novel therapeutic approaches to overcome the defect. The upcoming metabolomic research approach needs to be adapted to elucidate the aetiological mechanism involved in PCOS. Most of the genetic studies so for have used candidate gene approach. As PCOS is a heterogeneous and complex disorder, studies to elucidate function of genes in isolation will not be of much relevance. A genome wide scan or SNP microarray with phenotypically defined subgroup of PCOS may help to elucidate the different molecular defects associated with different phenotypes. As PCOS is a multigenic disorder an integrated approach of functional genomics (gene variations, gene expressions, protein–protein interaction and phenotypic variations) is needed to understand the genotype-phenotype correlation and extend it to understand interaction between different genes in manifestation of the syndrome and also its underlying pathogenesis.

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