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Research Paper

Comprehensive assessment of expression of insulin signaling pathway components in subcutaneous adipose tissue of women with and without polycystic ovary syndrome

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ABSTRACT

Objective: Insulin resistance is a common feature of polycystic ovary syndrome (PCOS). The insulin signaling pathway consists of two major pathways, the metabolic and the mitogenic cascades. The many components of these pathways have not been comprehensively analyzed for differential expression in insulin-responsive tissues in PCOS. The goal of this study was to determine whether the core elements of the insulin signal transduction cascade were differentially expressed in subcutaneous adipose tissue (SAT) between PCOS and controls.

Materials/methods: Quantitative real-time PCR for 36 insulin signaling pathway genes was performed in subcutaneous adipose tissue from 22 white PCOS and 13 healthy controls.

Results: Genes in the insulin signaling pathway were not differentially expressed in subcutaneous adipose tissue between PCOS and controls ($P > 0.05$ for all). Components mainly of the mitogenic pathway were correlated with both androgens and metabolic phenotypes. Expression levels of five genes (*MKNK1*, *HRAS*, *NRAS*, *KRAS*, and *GSK3A*) were positively correlated with total testosterone level ($\rho > 0$, $P < 0.05$). Inverse correlation was found between expression of six genes (*HRAS*, *MAP2K2*, *NRAS*, *MAPK3*, *GRB2*, and *SHC1*) and metabolic traits (body mass index, fasting glucose, fasting insulin, and HOMA-IR) ($\rho < 0$, $P < 0.05$).

Conclusions: Differential expression of core insulin signaling pathway components in subcutaneous adipose tissue is not a major contributor to the pathogenesis of PCOS. Correlation between clinical phenotypes and expression of several genes in the mitogenic limb of the insulin signaling pathway suggests mitogenic signaling by insulin may regulate steroidogenesis and glucose homeostasis.

Introduction

Polycystic ovary syndrome (PCOS), characterized by hyperandrogenism, polycystic ovaries and oligo-ovulation, affects 7–10% of reproductive age women [1]. Women with PCOS disproportionately manifest insulin resistance, obesity, diabetes mellitus and cardiovascular disease. Approximately 50%–70% of all PCOS women demonstrate some degree of insulin resistance, as determined by a variety of methods [1]. As a consequence of insulin resistance, PCOS patients often present with compensatory hyperinsulinemia, which exacerbates hyperandrogenemia by promoting ovarian androgen

Abbreviations: polycystic ovary syndrome, PCOS; subcutaneous adipose tissue, SAT; single nucleotide polymorphism, SNP; quantitative real-time PCR, qRT-PCR; MAP kinase, MAPK; phosphatidylinositol-3-kinase, PI3K; homeostasis model assessment, HOMA; body mass index, BMI.

Author contribution: M. Goodarzi and D. Geller conceived and designed the study. N. Xu and D. Geller wrote the manuscript. R. Azziz and M. Goodarzi critically revised the manuscript. M. Jones, V. Funari, and R. Azziz acquired the data. N. Xu and M. Jones analyzed the data. All authors approved the final manuscript.

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production and suppressing sex-hormone binding globulin levels [1,2]. That many features of PCOS improve on treatment with insulin sensitizing medications suggests that insulin resistance may be integral to the pathogenesis of PCOS [3].

Given that both PCOS and insulin resistance are heritable [4], the insulin signaling pathway provides a number of candidate genes that might illuminate the pathogenesis of PCOS. Only a handful of the many possible genes coding for components of the insulin signaling pathway were initially investigated in single nucleotide polymorphism (SNP) association studies [5]. We extensively interrogated the entire regions of ~30 genes comprising the core components of the classic metabolic limb of the insulin signaling pathway as well as the novel CAP/Cbl pathway, identifying several SNPs associated with PCOS in a discovery cohort; association with PCOS of one SNP, rs2252673, in the insulin receptor (*INSR*) gene was confirmed in a replication cohort [6]. Until the latter study, the majority of association studies of *INSR* in PCOS had focused only on a single variant [7]. Several studies describing association with PCOS of the D19S884 microsatellite provide further support of a potential role of *INSR* in the inheritance of PCOS [8,9]. Most convincingly, a recent genome-wide association study identified eight new risk loci for PCOS, including rs2059807, a SNP within an intron of the *INSR* gene [10]. This confirms the significance of *INSR*, and suggests a possible effect on the downstream insulin signaling events that follow binding of insulin in PCOS pathogenesis.

The insulin signaling cascade is initiated when insulin binds to the α-subunit of the insulin receptor, activating the tyrosine kinase activity in the β-subunit, which results in phosphorylation of the intermediates phosphatidylinositol-3-kinase (PI3K) and MAP kinase (MAPK) (Fig. 1). Activation of PI3K leads to a cascade of downstream signals (termed the PI3K pathway or metabolic pathway) that promote metabolic effects such as glucose uptake and glycogen synthesis. The activation of the MAPK cascade promotes gene expression and cell growth, comprising the mitogenic pathway [11] (Fig. 1).

Table 1
Clinical characteristics of PCOS and control subjects

	Control ($n = 13$)	PCOS ($n = 22$)	*P* value
Age (yr)	33.0 (13.0)	28.0 (3.3)	0.042
BMI (kg/m^2)	25.5 (8.7)	31.0 (8.0)	0.23
Insulin (pmol/l)	57.0 (48.0)	54.0 (69.0)	0.46
Glucose (mmol/l)	4.14 (1.28)	4.97 (0.55)	0.030
HOMA-IR	1.09 (0.46)	1.01 (1.08)	0.99
HOMA-%B	122.4 (75.3)	89.0 (44.6)	0.17
Total testosterone (nmol/l)	0.97 (0.29)	1.11 (0.99)	0.079
Free T (nmol/l)	0.080 (0.074)	0.18 (0.14)	0.0013
DHEAS (μmol/l)	5.41 (4.32)	6.60 (3.68)	0.038
mFG	0 (1)	7 (5)	<0.0001

BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-%B, homeostasis model assessment of beta-cell function (insulin secretion); mFG, modified Ferriman-Gallwey score to evaluate hirsutism in women.
Data are median (interquartile range). *P* values are derived from the Mann–Whitney test.

Women with PCOS often have abdominal obesity, and gene expression profiling experiments suggest dysfunction of visceral fat plays an important role in PCOS [12]. However, visceral fat is not the only determinant of insulin resistance, and a substantial proportion of lean PCOS women are insulin resistant as well [13]. In addition to visceral adipose tissue, dysfunction of subcutaneous adipose tissue (SAT) also contributes to insulin resistance and obesity; SAT mass and capacity for fat storage influence insulin sensitivity, independent of visceral fat [14,15]. Investigators have successfully utilized SAT to demonstrate adipose tissue dysfunction in PCOS. A

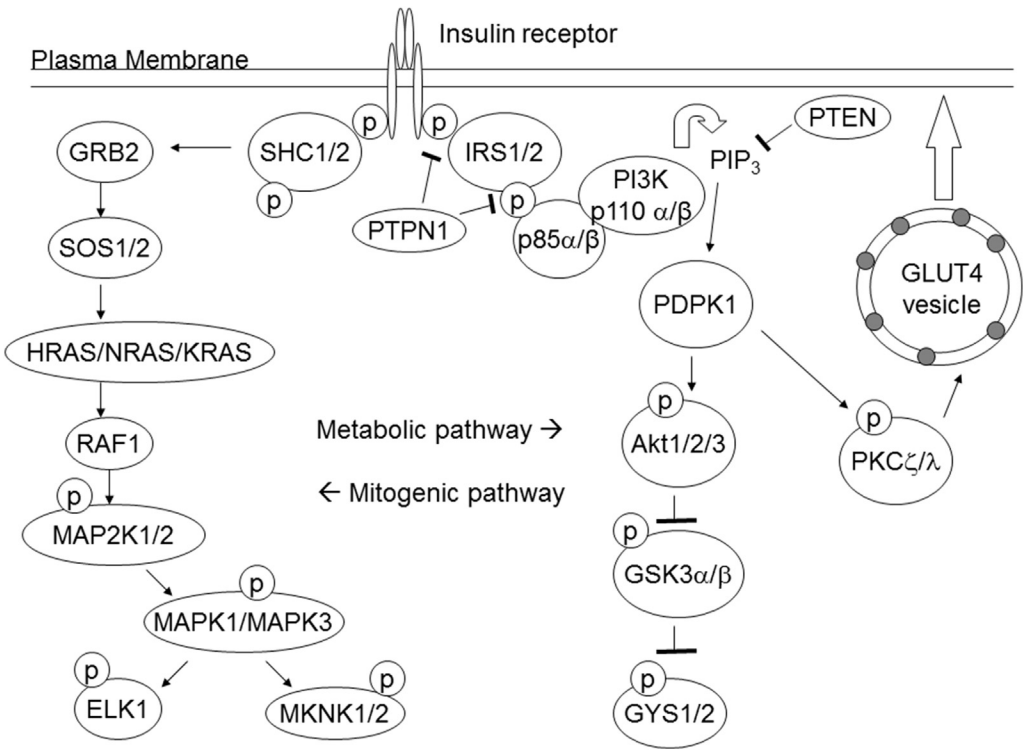

Fig. 1. Core components of the insulin signaling pathway. The metabolic (PI3K) and mitogenic (MAPK) limbs of the insulin signaling pathway were studied. The products of the 36 genes whose expression was analyzed are indicated. Names containing a slash indicate isoforms encoded by different genes. Flat arrows indicate inhibition. p = phosphate group; PIP_3 = phosphatidylinositol (3,4,5)-trisphosphate. Full gene names are listed in Supplemental Table 1.