REVIEW ARTICLE

Metabolomics approach for the etiopathogenesis of polycystic ovarian syndrome

Sukanti Bhattacharyya¹, Sukumar Barik², Siddhartha Gupta³, Sudarsan Saha², Madhuri Mandal Goswami¹, Utpal Goswami⁵, Samashaptak⁶

¹Department of Physiology, ICARE Institute of Medical Sciences and Research and Dr. Bidhan Chandra Roy Hospital, Haldia, West Bengal, India, ²Department of Obstetrics and Gynaecology, ICARE Institute of Medical Sciences and Research and Dr. Bidhan Chandra Roy Hospital, Haldia, West Bengal, India, ³Department of Biochemistry, ICARE Institute of Medical Sciences and Research and Dr. Bidhan Chandra Roy Hospital, Haldia, West Bengal, India, ⁴School of Materials Sciences, Indian Association for the Cultivation of Science, Kolkata, West Bengal, India, ⁵Department of Pathology, ICARE Institute of Medical Sciences and Research and Dr. Bidhan Chandra Roy Hospital, Haldia, West Bengal, India, ⁶ICARE Institute of Medical Sciences and Research and Dr. Bidhan Chandra Roy Hospital, Haldia, West Bengal, India

Correspondence to: Sukanti Bhattacharyya, E-mail: sukantib514@gmail.com

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex and multifaceted disorder that has been extensively studied from various angles, including genomics, metabolomics, and other multiomics approaches. This narrative review seeks to provide an updated understanding of PCOS, with a particular focus on metabolomics. PCOS encompasses four primary phenotypes: Classic PCOS with or without polycystic ovary morphology, ovulatory PCOS, and non-hyperandrogenic PCOS. Each phenotype exhibits distinct clinical and biochemical characteristics. Classic PCOS, characterized by hyperinsulinemia, insulin resistance (IR), and metabolic complications, has garnered significant attention in recent studies. In the pursuit of unraveling PCOS’s pathophysiology, researchers have embraced a multiomics approach, exploring the genome, epigenome, transcriptome, exosomes, proteome, and metabolome. The genomic landscape of PCOS includes genes related to androgen synthesis, regulation, insulin receptors, and growth factors, among others. Epigenetic mechanisms have also been investigated, revealing the roles of non-coding RNA, histone acetylation, and deacetylation. Transcriptomic biomarkers and mitochondrial RNAs targets, such as miR-9 and miR-32, have been explored in granulosa cells of PCOS patients. Metabolomics studies have identified various biomarkers and metabolic pathways that differentiate PCOS from healthy individuals. A diverse range of alterations in carbohydrate and lipid metabolism has been observed, shedding light on the underpinnings of PCOS-related metabolic dysfunction. These studies have uncovered numerous biomolecules with potential relevance to PCOS, including kininogen 1, cytoketatin 9, and many others. In addition, the intricate relationships between IR, inflammation, and metabolic factors have been elucidated. Recent research extends beyond high-end research centers, even using urine samples to identify metabolomic markers for PCOS. This approach has led to the discovery of distinct biomarker panels related to metabolic pathways that differentiate PCOS from healthy subjects. In summary, PCOS is a complex and heterogeneous condition, with multiomics studies offering promising avenues for a deeper understanding of its pathophysiology. These studies may ultimately provide valuable insights that can revolutionize the diagnosis and treatment of PCOS, ending a century-long scientific expedition with a crescendo of discoveries.

KEY WORDS: Metabolomics; Multiomics; Polycystic Ovary Syndrome
INTRODUCTION

In a recently published article, Bhattacharyya et al. mentioned that not only genomics but also many other factors should be studied through different tools of multomics to explore etiopathogenesis of multifactorial and multiphenotypic syndromic disorder polycystic ovary syndrome (PCOS). In that article, a comprehensive account of genetic basis of PCOS was elaborated. In this brief narrative review, updated knowledge on different studies on metabolomics related to PCOS will be elucidated.[1]

CLINICAL MANIFESTATIONS

Different phenotypic attributes of PCOS have been systematically classified by different professional bodies. To cut short the detailed accounts of all those recommendations, here we reproduce a table from the article by Bhattacharyya et al. for easy representation of the phenotypes [Table 1].[2]

To summarize, there are four different phenotypic expressions of PCOS: A and B fall under classic PCOS category, C is categorized as ovulatory variety, D as non-hyperandrogenic type. Classic PCOS, represented by phenotypes A and B, has hyperinsulinemia, insulin resistance (IR), obesity, metabolic syndrome, atherogenic dyslipidaemia, hepatic steatosis, and significantly high titer of anti-müllerian hormone (AMH).

Ovulatory PCOS (phenotype C) is characterized by normal menstrual cycles but still exhibits high hirsutism scores and mild IR, atherogenic dyslipidaemia, and metabolic syndrome, possibly due to excessive diet and lack of physical activity among individuals in higher socioeconomic groups where these phenotypes are more common. Non-hyperandrogenic PCOS (phenotype D) is marked by regular menstrual cycles with occasional irregularities, elevated sex hormone-binding globulin (SHBG) levels, normal androgen levels, and lower levels of thyroid hormones (T3 and T4), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). This phenotype also demonstrates the least metabolic dysfunction among the four.[3]

MULTIOMICS APPROACH

Over the last two decades, multi-omics approaches have elucidated many alterations in the genome, epigenome, transcriptome, exosomes, proteome, and metabolome. Pinpointing a single gene has become a misadventure in this polygenic, multifactorial condition. Many alterations of biomolecules, such as amino acids, nucleic acids, fatty acids, carbohydrates, lipids, glycosphospholipids, sphingolipids, and hormones have also been elucidated. A decrease in FSH, increase in LH, androgen, and insulin among extraovarian factors; alteration in growth factors, cytokines, leptin, and homocysteine (Hcy) among intraovarian factors; and decrease in FSH, poor follicular growth, increase androgen, LH and AMH among genetic variant factors have been found to be of prime importance in pathophysiology of PCOS.[4]

Genes encrypting for androgen synthesis, transportation, regulation, insulin receptor synthesis, secretion and action, signaling cascade protein, and growth factors are the main candidates in the genomic study.[5] Several epigenetic mechanisms, involving post-transcriptional alteration of expression in non-coding RNA,[6] histone acetylation by histone acetyltransferases,[7] and elimination of acetylation by histone deacetylases[8] have been extensively studied to find out specific biomarkers. For transcriptomic biomarker mitochondrial RNAs (miRNA) target-based 284 enriched pathway comprising Hippo signaling pathway, MAPK signaling, and insulin signaling pathway were analyzed.[9]

Different circulating miRNAs, such as upregulation of miR-9, miR-18b, miR-32, miR-135a, miR-146a, miR-486, and downregulation of miR-132, miR-320, miR-370, have been found in human follicular fluids and granulation cells of patients with PCOS.[10] Similarly, several alterations in long non-coding RNAs were also reported by many.

As of now, many studies were conducted using ovarian follicular fluid, omental fatty tissues, plasma/serum, T-cells, urine, granulosa cells, and other body fluids through non-targeted proteomic techniques.[11] Results of these studies identified many biomolecules having significant differences in “area under the curve” of “receiver operating characteristics” with respect to age-matched controls in different chromatographies with mass spectrometry (MS).[12]

Kininogen 1, cytoketatin 9, antithrombin, fibrinogen γ-chain, apolipoprotein A-IV (apoA-IV)-precursor, and α-1-β-glycoprotein (A1BG) were found to be amplified in follicular fluid of patients with PCOS by two-dimensional-gel-electrophoresis coupled to matrix-assisted laser desorption

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<tr>
<th>Phenotype</th>
<th>Clinical criteria</th>
<th>Prevalence (%)</th>
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<tr>
<td>Polycystic ovarian morphology in USG</td>
<td>Oligo/anovulation</td>
<td>Hyperandrogenism</td>
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<tr>
<td>A</td>
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PCOS: Polycystic ovary syndrome
ionization – time-of-flight mass spectrometry (2-DE MALDI-TOF MS) and nano liquid chromatography with tandem mass spectrometry (LC-MS/MS),[10] of which A1BG and apoA-IV precursors were also found to be exacerbated in follicular fluid of patients with PCOS in comparison to controls by western blot analysis.[12] A1BG was reported to be elevated in Type 1 diabetes mellitus and decreased with control of hyperglycemia in them.[13]

Serum in patients with PCOS-IR (PCOS with IR) as compared with patients with PCOS-NIR (PCOS with no IR) showed differentiation in four proteins: Afamin, serotransferrin, complement C3, apolipoprotein C3; apoc3 in serum was further analyzed by WB and ELISA showing higher expression in PCOS-IR cohort.[14]

Pro-inflammatory factors, such as calgranulin (S100A8) and CD14, were found to be raised, while IR factors, for example, IGF-1, IGF-2, IGFBP-2, and IGFBP-4 were less expressed by 3-D quantitative proteomic analysis of follicular fluid of PCOS patients, came for in-vitro-fertilization.[15] By 2-DE-MS of CD4-T cells, phosphatidyl ethanolamine-binding protein 1, proteasome activator complex subunit 1 (PSME1), and triosephosphate isomerase 1 (TP11) were more expressed in PCOS cases than controls.[16] TP11 was found to be directly related to TH2 and autoantibody production-cum-humoral response in PCOS. PSME1 expression in CD4 cells was also seen in obesity, Type 2 diabetes mellitus.[16,17]

Although various biomolecules had already been identified by non-targeted proteomic studies, those were found to have a variable relationship with the candidate disease entity. Moreover, these studies were mostly undertaken at high-end research centers with follicular fluids. Such fluids could be obtained from cases under intervention for some other purposes.

Zhou et al.[18] applying OPLA-DA and GC-MS on urine samples identified 9-biomarker panel (butanitrol, trans-aconitic acid, isotiric acid, 3-deoxypentitol, xylose, phosphoric acid, D-allole, D-threitol, psicose) involving metabolic pathways of propanoate metabolism, cysteine metabolism, urea cycle, malate-aspartate shuttle, glyceral phosphate shuttle, etc. differentiating PCOS from healthy subjects. 8-biomarker panel (ribitol, trans-aconitic acid, 4-hydroxyxyclohexanecarboxylic acid, tartronic acid, glycolic acid, myo-inositol, oxalic acid, ribonic acid) involving pathways of glyoxylate and dicarboxylate metabolism, inositol phosphate metabolism, riboflavin metabolism, etc., along with homeostatic model assessment -IR and free androgen index (FAI) were used to distinguish PCOS with hypothalamic amenorrhea (HA) from that with IR.[18]

More extensive metabolomics studies were conducted over the last two decades identifying alterations in carbohydrate and lipid metabolisms.[19] Significantly increased lactate and gluconeogenic amino acids, and decreased plasma glucose in non-obese PCOS patients were explained by increased muscle glycolysis and decreased hepatic gluconeogenesis in them. Increased lactate in non-obese PCOS-IR following insulin-stimulated uptake and breakdown of glucose in muscle could indicate a central (not peripheral) type of IR in them.[20,21]

Besides surge in low-density lipoprotein-cholesterol (LDL-C), very LDL-C, and fall in high-density lipoprotein-cholesterol (HDL-C), increased hormone-sensitive lipase activity in white adipose tissue (as described in adipose tissue expandability hypothesis) was found to yield raised titer of free fatty acids, like palmitic, stearic, linoleic acids in patients with PCOS.[22,23] Linoleic acid being proinflammatory[24] could halt the maturation of developing oocytes.[25]

Similarly, alterations in a number of biomolecules, such as decrease in citrate, 2-ketoisocaprate, and many amino acids, for example, arginine, citrulline, histidine, methionine, proline, glycone, leucine, and glutamine; on the other hand, increase in n-acetyl glycoprotein, phenylalanine, threonine, tyrosine, valine, etc., in PCOS patients could be explained by metabolic variations in them.[26] Leucine might mitigate IR by the mTOR pathway.[27]

Linoleic acid was found to be increased in serum;[28] possibly due to increased hsLipase activity in IR and obesity.[23] Linoleic acid having pro-inflammatory activities[24] could inhibit the maturation of developing oocytes.[25] In other studies, low levels of linoleic acid were also noted.[21]

Lower level of palmitoleic acid and other long-chain fatty acids (LCFAs), glycerol, and high level of lactate were found among PCOS cases than control – both being non-obese. This could be metabolically explained by insulin-mediated suppression of lipolysis, and increased glucose uptake and conversion in muscles. This also could happen in a situation of increased insulin activity following central IR, with no peripheral IR effect on adipose and muscle tissues. In addition, catecholamines-induced lipolysis in subcutaneous fatty tissue was seen to be suppressed in non-obese female with PCOS.[29-31] On the contrary, high levels of glycerol and palmitoleic acid, and low lactate level were found among obese PCOS patients, indicating peripheral IR.[32] This could also be explained by the adipose tissue expandability hypothesis.[33] A study[34] reported an inverse relationship between LCFA levels and androgen levels in PCOS patients; contradicting the findings of some other similar studies.[34-38]

Less activation of the mTOR pathway and thereby less restoration of insulin signaling due to decreased leucine level could lead to IR.[27] However, Sun et al.[28] and Zhao et al.[21] reported conflicting results of decreased and increased level of leucine, respectively, in PCOS.

Hyperandrogenic state might alter pentose phosphate pathway by down-regulating glucose-6-phosphate dehydrogenase,
and also tricarboxylic acid (TCA) cycle by decreasing citrate level in oocytes to affect oocyte maturation; even without inducing IR.

Decreased levels of citrate indicating impaired TCA cycle were observed in PCOS patients. Furthermore, levels of many amino acids involved in the TCA cycle were found to be altered in PCOS. Low levels of arginine, citrulline, glutamine, histidine, methionine, and proline; and high levels of phenylalanine, threonine, tyrosine, and valine were reported, as well.\textsuperscript{[20,21,28]} Low levels of citrate were seen in PCOS, irrespective of IR\textsuperscript{[25]} or HA.\textsuperscript{[37]}

IR-induced increased levels of branched-chain amino acid (BCAA) in PCOS were found to be catabolized to Phe, which in turn competed with BCAA for transportation in mammalian cells yielding a high levels of Val, and low levels of Gly in IR-independent ways.\textsuperscript{[21]}

Phe and other aromatic AAs were seen to be related to IR and DM. Gly, on the other hand, could mitigate proinflammatory state, also upregulate adiponectin gene expression in vitro study.\textsuperscript{[39]} Another study also found Lys, Phe, and 2-aminoisadipic acid to be associated with IR.\textsuperscript{[40]}

Many adipokines could act differently in metabolism; and get altered differently in PCOS. Adiponectin, though helpful against IR, was found to decrease in PCOS.\textsuperscript{[41]} Proinflammatory resistin increased in PCOS, irrespective of obesity. Levels of leptin, visfatin, and apelin also increased in PCOS. Leptin was found to regulate food intake on long term, also energy expenditure; and visfatin to promote IR. Apelin was seen to be associated with androgenic obesity, increased H-W ratio, polycystic ovary morphology, impairment between LH/FSH interactions,\textsuperscript{[42]} and also high triglycerides (Tg), dyslipidemia and high free testosterone level.\textsuperscript{[43]}

Cholecalciferol (Vitamin D3) being more of endocrinal category was found to regulate bone metabolism and Ca/P balance. It also had beneficial effects in proinflammatory and IR conditions. It was found to be elevated in obese than non-obese PCOS patients.\textsuperscript{[44]}

High ferritin level was found in obese PCOS patients. Ferritin was seen to be linked with obesity, IR, and also mediating inflammation.\textsuperscript{[45]}

Hey level was often found very high in PCOS patients with obesity and high waist measurement in comparison to non-obese PCOS patients and normal controls. Hey could result in many cardiometabolic disorders in obesity and PCOS. Hyperinsulinemia could inhibit hepatic cystathione $\beta$ synthase to raise Hey level in serum.\textsuperscript{[46]}

Plasminogen activators inhibitor Type 1, a SERPIN group of protein with proinflammatory property, was found in a number of studies to be raised in PCOS with obesity, in particular. It basically blocks plasminogen to retard fibrin degradation and is related to IR, RAAS, and tissue remodeling. It is thus considered to be associated in the pathogenesis of PCOS.\textsuperscript{[47]}

**RECENT STUDIES**

Diet with excess fat and sugar could alter the transcriptome and metabolome of the ovary to impair female reproduction; justifying similarity in PCOS.\textsuperscript{[48]} Untargeted metabolomic approach using serum samples of patients with PCOS indicated increased steroid hormone biosynthesis, altered sphingolipid, and phospholipid metabolism, and also fatty acid metabolism, as well as citric acid cycle, gamma-glutamyl cycle, vit B metabolism, and altered amino acid-like Trp, Phe, His, Ala.\textsuperscript{[49]} High plasma levels of bisphenol a among women with PCOS in comparison to healthy women with low bisphenol A level were found to have disturbed various lipid metabolisms and steriodogenesis.\textsuperscript{[50]} Perfluorooctanoic acid accumulation in the follicular fluid of PCOS women was seen to diminish the ovarian reserve function.\textsuperscript{[51]} Increased pyroglutamic acid in cumulus cells in women with PCOS decreases glutathione synthesis and oxidative stress-related deterioration of the quality of oocyte in them.\textsuperscript{[52]} Hyperandrogenaemia was found to override the effect of body mass index in causing gut dysbiosis and altered metabolism in PCOS.\textsuperscript{[53]} A pilot study by Brinca et al. showed the prospect of getting potential biomarkers through volatilomics using the follicular fluid of women with PCOS and other causes of infertility.\textsuperscript{[54]}

There is also a recent trend in combining metabolomics with machine learning models to study endocrine and metabolic changes in PCOS.\textsuperscript{[55]}

**PHARMACOTHERAPEUTIC TRIALS**

A network meta-analysis by Zhao et al. concluded that myo-inositol with D-chiro-inositol and metformin with thiazolidinedione improved IR and decreased total testosterone more than metformin alone. The first combination was effective in menstrual recovery and the second improved lipid metabolism better.\textsuperscript{[56]} Zhang et al. conducted a prospective randomized control trial and showed that canagliflozin with metformin combination more effectively reduced total testosterone level than metformin alone, but either of these two therapies improved menstrual frequency, controlled body weight, relieved IR, and hyperandrogenemia to a similar extent.\textsuperscript{[57]} Karamali et al., through a randomized controlled trial (RCT), conducted that supplementing coenzyme Q10 to PCOS women over 3 months yielded a beneficial effect on the levels of high-sensitivity C-reactive protein (hs-CRP), total testosterone, dehydroepiandrosterone sulfate, SHBG, total antioxidant capacity (TAC), and malondialdehyde.\textsuperscript{[58]} An RCT by Shahmoradi et al. showed beneficial effect of
magnesium supplementation on IR and lipid profile in women with PCOS. A randomized triple-blinded placebo-controlled clinical trial by Abbasi showed that propolis supplementation resulted in control in fasting insulin, ameliorating IR, and reduce in testosterone level, LDL/HDL ratio in PCOS women. Beneficial effects on body weight, control of blood sugar, reduction in LDL and Tg and gene expression of peroxisome proliferator-activated receptor-γ and LDL receptor following 12-week supplementation of chromium and carnitine together to overweight PCOS patients were noted in a randomized double-blind placebo-controlled trial conducted by Jamilian et al. 4000 IU of Vit D supplementation on daily basis over 12 weeks to PCOS patients had beneficial impacts on total testosterone, SHBG, hs-CRP, FAI, and plasma TAC level, compared to low dose (1000 IU/day) Vit D and placebo groups, as shown in an RCT by Jamilian et al. An RCT by Aubuchon et al. showed that metformin had no beneficial effect on reproductive or metabolic profile in women with PCOS.

Various herbal preparations, like quercitrin, Chinese Bu-Shen-Tian-Jing formula, Berberine, Mogroside, and Oligopin, were claimed to have beneficial effects on several phenotypes of PCOS in a number of clinical trials. Circadian disruption-associated dyslipidemia in PCOS could be ameliorated by manipulating the L-reuteri-capric acid-hepatic galanine receptor 1 axis. This could be a therapeutic strategy.

CONCLUSION

Over the last nine decades, scientists across the world are unveiling layers after layers to understand the basic pathophysiology of polycystic ovarian syndrome. Right from the gross macroscopic observations, through histopathologic evaluation, transabdominal, and transvaginal sonographic screening to biochemical and molecular biological exploration, using classical to modern state-of-the-art facilities, we are in a state of beating around the bush. Recent multiomics studies are basically non-targeted type of study and such studies could help us to find hidden diamonds deep from coal mines. Further intensive exploration over the extensive arena of multiomics studies could make us to reach the treasure trove culminating our scientific expeditions near for a century in a cascade of crescendo.

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