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Review

Potential Genetic Polymorphisms Predicting Polycystic Ovary Syndrome (PCOS) in Sri Lankan Women: Comparison with Different Ethnicity

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Abstract

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder of young women with long-term metabolic risk and prevalence among pre-marital Sri Lankan women is 6.3%. Inheritance of PCOS is likely to be oilgogenic; the genetic basis remaining largely unknown in view of the complex pathophysiology. The genetics of expression of PCOS requires an in-depth study, particularly among Sri Lankan women who have a greater metabolic risk from an early age. The emergence of an unanimously accepted genetic marker for susceptible PCOS was affected based on inconsistent findings. In this review, we summarize the common genetic polymorphisms of PCOS from different countries and outline some genetic polymorphisms that are potentially associated with the risk of PCOS in Sri Lankan women. This information could uncover candidate genes associating with PCOS, which will be valuable for the development of novel diagnostic and treatment method.

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Keywords: Hypothalamic pituitary gonadal axis, polycystic ovary syndrome, single nucleotide polymorphism

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Introduction

Polycystic ovary syndrome is the commonest hormonal derangement among young women from early reproductive years [1]. The syndrome is commonly associated with polycystic ovaries (PCO) with amenorrhea, hirsutism and obesity and leads to infertility in women [2]. The current clinical definition recognizes PCOS as a wide spectrum of presentations consisting of a combination of any two of the three key features: oligo-amenorrohea, hyperandrogensim and polycystic ovaries, with the proviso secondary causes are excluded. Hyperandrogensim and ovarian dysfunction are the salient features of PCOS. In addition, metabolic abnormalities such as insulin resistance and hyperinsulinemia, abdominal obesity, hypertension and dyslipidemia, type 2 diabetes mellitus (T2DM), cardiovascular disease and endometrial hyperplasia are commonly associated with the disease [3-6]. Hence, PCOS can be defined as a life-long condition which manifests from puberty and leads to severe adverse reproductive and metabolic implications [7]. It is noteworthy, that the pathogenesis of PCOS involves multiple biological pathways and hence not clearly understood [8, 9].

Albeit, due to the broad spectrum of symptoms with overlapping biochemical parameters of other disorders, the diagnosis of PCOS, often goes undetected until early adulthood due to the lack of uniform guidelines for its diagnosis and management among the adolescent population. Abnormal secretion and regulation of gonadotropins, namely the luteinizing hormone (LH), follicle stimulating hormone (FSH) and associated excess secretion and action of ovarian steroid hormones leads to subfertility in women with PCOS. Furthermore, they often have an elevated LH/FSH ratio, caused by an increased frequency and amplitude of LH pulsations due to heightened hypothalamic gonadotropin releasing hormone (GnRH) secretion that is due to a functional defect at the level of the hypothalamus. The elevated LH/FSH ratio is proposed as a useful biochemical diagnostic tool for PCOS [10].

Ethnic Variation of PCOS

The exact prevalence of PCOS is not known as the syndrome lacks a unique phenotype. The prevalence of PCOS varies among different ethnic and racial groups. The community prevalence of PCOS remains the commonest among the reproductive age with reports ranging from 2-15% among differing populations. Community based assessments showed different ethnic groups had distinct results: 4.8% and 8% in white and black women in South-Eastern United States [11], 6.8% in white women in Greece [12], 6.3% among South Asia namely Sri Lanka [13], 10.3% in Northern Finland [14], 13% in Mexican–Americans [15] and 5% in Thai women [16]. The wide variation in the prevalence of PCOS among different populations is attributed mainly due to lack of consistency in clinical diagnostic criteria, ethnicity, lifestyle, geographic location and diet [8].

Genetics of PCOS in Asian Population

Genetic factors play a major role in the etiology of PCOS. Recent studies showed that PCOS is a complex endocrine disorder involving polymorphisms in several genes under the influence of environmental factors [17-19]. Investigations were performed on more than 100 candidate genes and suggests that several mutations or polymorphisms in genes of steroid hormone metabolism, gonadotropin and gonadal hormones action, obesity and energy regulation, insulin secretion and action pathways closely interact and initiate the development of PCOS [20]. Although in various studies, several candidate genes have been identified as a susceptibility genes to PCOS, no gene has been successfully replicated and identified as to be truly susceptibility gene across all studies.

In this review, we aimed to compare the different genetic polymorphism of PCOS in Sri Lankan women with rest of the world. For this we have analyzed the genes involved in ovarian and adrenal steroidogenesis, steroid hormone effects, gonadotrophin release, regulation, and action, insulin action and secretion and obesity.

Candidate Genes in PCOS

Genes Involved in Ovarian and Adrenal Steroidogenesis

Hyperandrogenemia is the commonest biochemical abnormality in women with PCOS. Therefore, researchers have investigated the relationship between PCOS and the genes involved in the androgen biosynthetic pathway. The most common genes involved in steroidogenesis are CYP11a, CYP17, CYP19 and CYP21. It is noteworthy that, no studies have been carried out to identify the association between adrenal steroidogenesis genes with PCOS in Sri Lankan women.

CYP11a

CYP11a codes for the P450 cytochrome side chain cleavage that converts cholesterol to pregnenolone, a rate limiting step of steroidogenesis. A pentanucleotide repeat (tttta)n, at position –528 from the ATG initiation codon in the 5′-region of the CYP11a gene, seems to be associated with PCOS susceptibility. Previous studies have shown a positive association between pentanucleotide repeat alleles and susceptibility of PCOS [21-23]. A study by Wang et al., 2006 [24] in Chinese women with PCOS, demonstrated different allele combinations that either increase or decrease the risk of developing PCOS. The association of increased risk of developing PCOS and repeated polymorphisms of CYP11a was also confirmed by Shen et al., 2014 [25].

CYP17

CYP17 codes for the enzyme 17α-hydroxylase, which converts C21-steroids into androgens. A study by Carey and colleagues showed that a rare single nucleotide polymorphism (T–C) at base pair –34 in the promoter region of this gene increases the susceptibility to develop PCOS [26]. However, it is noteworthy that larger, case–control studies from the same group as well as from other studies were unable to confirm this association [27, 28].

CYP19

The CYP19 gene presents on chromosome 15q21.2 encodes the aromatase p450 enzyme that plays an important role in synthesizing estrogens from androgens [29]. Aromatase activity is decreased in lean and obese women suffering from PCOS, which may be further inhibited by hyperandrogenemia [30]. A study by Jin et al., 2009 [31] showed a significant association of intronic variant rs2414096 with an increased risk of developing PCOS among the women. In addition, a study by Xita et al., 2010 indicated that a short microsatellite (TTTA)n repeat allele in the fourth intron of the CYP19 gene is associated with suboptimal aromatase activity [32]. However, linkage and mutation screening studies did not reveal any evidence that variation at the CYP19 locus participates in the etiology of PCOS [28, 33, 34].

CYP21

One of the main steps in adrenal and ovarian steroidogenesis is the conversion of 17-hydroxyprogesterone into 11-deoxycortisol, which is catalyzed by the 21-hydroxylase enzyme encoded by CYP21. Although few research was carried out to identify the relationship between CYP21 polymorphisms and PCOS, they failed to find any significant relationship [35, 36]. Hence, CYP21 and associated mutations seem not to play a key role in the development of PCOS.

Genes Responsible for Steroid Hormone Effects

Androgen Receptor Gene

The androgen receptor (AR) is coded by the AR gene that is located on chromosome X. The AR gene contains CAG repeat polymorphisms, and the inverse correlation between CAG repeat number and AR function is known. A study by Tong et al., 2010 in Southern Chinese Han women showed that the mean CAG repeat number was significantly lower in women with PCOS than in controls, which suggests that AR function is increased in patients [37]. However, other Asian studies, did not find statistically significant CAG repeat length differences between patients and controls [38-40].

Sex Hormone-Binding Globulin Gene (SHBG)

The SHBG regulates the access of androgens to target tissues. Serum SHBG levels are commonly low in patients with hyperandrogenism, especially in association with PCOS, which contributes to increased tissue androgen availability [41]. In the general population, the SHBG gene contains at least 6–10 (taaaa)n repeat, located in an alu sequence at the 5′ boundary of the serum SHBG promoter. This (taaaa)n repeat has been reported to influence the transcriptional activity of the gene in association with downstream elements [42]. Xita and collaborators evaluated a possible association between the presence of (taaaa)n polymorphism with PCOS and observed PCOS women had a significantly greater frequency of longer (taaaa)n alleles (more than 8 repeats) than normal women who had a higher frequency of alleles with fewer than 8 repeats [43].

Nevertheless, study by Liu et al., 2008 in Chinese women showed that the (taaaa)n polymorphism was neither a determinant of PCOS nor a predictor of serum SHBG levels [44]. In addition, Ferk and colleagues compared the frequency of (taaaa)n alleles in a cohort of Slovenian women and did not find that (taaaa)n repeat frequency was significantly different in PCOS women compared with normal controls [45]. Although the findings from different studies were contradictory, no studies have been carried out to identify the association between SHBG polymorphism with PCOS in Sri Lankan women.

Genes of the Hypothalamic-Pituitary-Gonadal (HPG) Axis Pathway

The hypothalamic-pituitary-gonadal (HPG) axis plays a major role in the control of puberty and menstrual cycle. The gonadotropes respond to GnRH pulses by releasing the gonadotropins, FSH and LH, which stimulates folliculogenesis and steroid and peptidergic hormone secretion from the ovaries. Disturbance of HPG axis is suspected to be the main culprit in the development of PCOS. The genes involved in HPG axis pathway mainly KISS1, GPR54 receptor gene, GnRH (Gonotropin Releasing Hormone), GnRHR (Gonotropin Releasing

Hormone Receptor), FSH (Follicle Stimulating Hormone), FSHR (Follicle Stimulating Hormone Receptor), LHβ (Luteinizing Hormone beta subunit) and LHCGR (Luteinizing Hormone/Choriogonadotropin receptor) genes may play a role in the development of PCOS [49-67].

KISS1 gene / Kisspeptin/GPR54 Gene Pathway and PCOS

Kisspeptins are proteins (such as Kp-54 and Kp-10) encoded by the KISS1 gene and bind to the G protein–coupled receptor GPR54 [46-48]. The GPR54-KISS1 pathway plays a major role in the initiation of puberty and maintenance of mammalian fertility [49].

The association between KISS1 and GPR54 polymorphisms with PCOS have been studied in few studies. A study by Branavan et al., 2019 identified 5 SNPs in GPR54 gene and 2 SNPs in KISS1 gene in Sri Lankan women. From the 5 SNPs of GPR54 gene, two were novel polymorphisms - chr19:918686, A/G and chr19:918735, A/G. The two novel polymorphisms are located in the intron 2 of the GPR54 gene and the remaining 3 SNPs are located in exon 1 (rs10407968), intron 2 (rs1250729403), and intron 4 (rs350131). Nevertheless, sequencing of KISS1 gene revealed two SNPs, located in the untranslated variant 5 prime end (rs5780218) and exon 3 (rs4889). It is noteworthy that this study concluded, polymorphisms of GPR54 and KISS1 genes have no significant association with PCOS in Sri Lankan women [50].

GnRH and GnRHR genes

The GnRH and its receptor (GnRHR) genes play a major role in the pubertal development and sexual maturation by acting through the HPG axis [51]. The GnRH1 gene is located on chromosome 8p21.2 and synthesize GnRH1 decapaptide. The GnRHR gene is located on chromosome 4q13.2 and encodes the receptor for GnRH1 hormone. A study by Valkenburg, et al. identified a polymorphism in the first exon of GnRH1 that results in an amino acid variation at codon 16 (Trp16Ser). However, the study failed to find any association with PCOS [52]. In addition, a Sri Lankan study observed [53] that GnRH1 (rs6185) gene mutant allele (CC) was not presented in their study population and

concluded that GnRH1 (rs6185) polymorphism is uncommon in Sri Lankan women.

FSHR and FSHβ genes

The FSHR gene is located on chromosome 2 p21-p16 and various SNPs were identified from FSHR gene. It is noteworthy that, the results of association between FSHR gene polymorphisms and PCOS were contradictory. The most commonly studied polymorphisms of FSHR gene is rs6165 (Ala307Thr) and rs6166 (Ser680Asn). A Korean study by Gu et al., showed Ser680Asn of FSHR gene polymorphism was associated with PCOS and concluded Ala307Thr was not associated with PCOS [54]. Finding in Italian women by Dolfin et al., explained that the Ala307Thr polymorphism was related to PCOS [55]. However, a study by Unsal et al., in Turkish adolescent girls failed to find any associations between FSHR polymorphisms (Ala307Thr and Ser680Asn) and PCOS [56]. Whereas, Sudo et al., found significant increase in the Ala307Thr frequency among Japanese women with PCOS when compared to normal subjects [57].

Furthermore, an association study between FSHβ gene polymorphism and PCOS by Tong et al., concluded that FSHβ (rs6169) gene polymorphism is uncommon in patients with PCOS and was found to be associated with the syndrome in some women with obesity and hyperandrogenism [58]. Furthermore, study by Branavan et al., 2018 reported polymorphisms of FSHR (rs6165/rs6166) and FSHβ (rs6169) had no significant association with PCOS in Sri Lankan women [53].

LHβ and LHCGR genes

The LHβ gene that synthesize LH hormone were found to have 2 common point mutations that were associated with PCOS (Trp to Arg [codon 8] and Ile to Thr [codon 15]) [59]. A study by Kurioka et al., [60] found that these mutations were markedly associated with PCOS and Tapanainen et al., concluded that these mutations may help to identify the risk for developing PCOS among obese women [61]. The same mutations were also identified in Finland [62] and Japan [59], which

suggests that this variant LHβ gene represents a universal polymorphism [62].

In addition, 300 known polymorphisms were found in LHCGR gene [63, 64]. Many studies identified S312N polymorphism (G935A) in exon 10 of the LHCGR gene which converts Asparagine to Serine. A study by Capalbo et al., showed that S312N polymorphism is markedly associated with PCOS in the Sardinian population [65]; the findings were also supported by two other studies by Ha et al., 2015 [66] and Bassiouny et al., 2014 [67]. Whereas, a Sri Lankan study [53] in PCOS women concluded that LHCGR and LHB gene polymorphisms were not associated with PCOS in Sri Lankan women.

Insulin Receptor (INSR) Gene

Insulin resistance, defined as reduced glucose response to a given amount of insulin, is commonly associated with PCOS. Researchers revealed that 50% to 70% of women with PCOS have impaired glucose tolerance and as many as 10% develop type 2 diabetes mellitus by the age of 40 [9, 68].

Insulin acts by binding to INSR receptor which is encoded by the INSR gene located at the chromosome 19p13.2 and consists of 22 exons [69]. The tyrosine kinase domain of the receptor is encoded by the region of exons 17-21, which is necessary for insulin signal transduction. Mutations in exons 17-21 can leads to severe insulin resistance and hyperinsulinemia [70].

The INSR gene is one of the strong candidate gene for PCOS and the tyrosine kinase domain of INSR gene (exons 17–21) plays a major role in the development of PCOS [71, 72]. It was stated that the number and affinity of INSR to insulin is not altered in PCOS but its tyrosine phosphorylation status and subsequent signaling is affected, suggesting the defect may lie in the β-chain [73]. Among the SNPs in exon 17 of INSR detected to date the silent C/T SNP at His1058 (designated His1085 in dbSNP) in the tyrosine kinase domain containing the ATP binding site

of INSR has been shown to be associated with the development of PCOS [69, 74, 75].

A Chinese study revealed that the T to C variation in exon 17 led to an increased risk of insulin resistance in women with PCOS [76]; and another study from China in a cohort of lean patients confirmed that a C to T variation in exon 17 was associated with PCOS [77] and this finding was supported by a US study [74]. Moreover, an Indian study revealed that the genetic variation in exon 17 of INSR is associated with insulin resistance and hyperandrogenaemia among lean women with PCOS but not obese women [78]. Another study on Indian women with PCOS reported that the SNP rs1799817 of INSR gene is associated with an increased insulin resistance [79]. The GWAS studies by Chen et al., 2011 [80] and Shi et al., 2012 [81] confirmed an association of rs1799817 SNP of INSR gene with PCOS. Conversely, studies by Lee et al., [82], Unsal et al., [56], Ioannidis et al., [83], Xu et al., [84] and Feng et al., [85] found no significant correlation between rs1799817 polymorphism and PCOS susceptibility which is mirrored by the Branavan et al., 2018 [53] study findings in Sri Lankan women.

Fat Mass and Obesity Gene (FTO-rs9939609)

The human FTO gene is located on the chromosome 16q12.2 and expressed in a wide range of tissues, including the adipose tissue and specific areas of the brain and muscles, suggesting its potential role in body weight regulation [86].

Several studies have proven that the FTO gene variants have influences on obesity, glucose intolerance and insulin resistance among PCOS women [87, 88].

Although, several SNPs were found from FTO gene, the variant FTO rs9939609 is the most widely studied. The rs9939609 SNP is located within the first FTO intron and has two alleles - A (mutant allele) and T (normal allele). The A allele has been linked with an increased risk for developing obesity and type 2 diabetes mellitus [89]. Approximately 40

– 80% of PCOS women are overweight or obese [3, 86, 90], which indicate that the genes associated with obesity might play a role in the pathogenesis of PCOS.

Several studies have been conducted to identify the relationship between FTO rs9939609 polymorphism and PCOS susceptibility, but the results were conflicting. Genome-wide association studies revealed FTO gene is markedly associated with PCOS in Asian women [91]. The mechanism of the association of FTO gene variant with PCOS is unclear. However, a few studies have proved that the FTO gene impact PCOS mainly by the obesity or obesity-related parameters such as BMI [86, 87, 88, 92] and other metabolism-related traits such as insulin resistance, impaired fasting glucose, glucose intolerance. It has also been showed that the influence of the FTO gene polymorphism on the metabolic parameters could be due to its effect on BMI [93]. A study by Kowalska et al., revealed that the FTO gene interacts with other susceptibility genes and leads to the polygenic disorder of PCOS [88].

A study on UK population by Barber et al., is the first to demonstrate that the SNPs of FTO gene is significantly associated with PCOS [94]. This was also confirmed by the studies by Yan et al., 2009 [95], Sokkary et al., 2014 [96] and Farhan et al., 2015 [97] in PCOS women. In addition, a Sri Lankan study [53] confirms that the rs9939609 SNP of FTO gene is clearly associated with PCOS among a cohort of women. However, studies by Tan et al., 2010 [92], Wehr et al., 2010 [86], Ewens et al., 2011 [98] and Ramos et al., 2015 [99] failed to find such association between FTO gene SNP and PCOS.

Epigenetics of PCOS

Recently, epigenetic factors have gained considerable attention in the pathogenesis of PCOS, which involves the changes in the content of DNA methylation, histone acetylation and noncoding RNAs. However, no studies have been carried out in epigenetics of PCOS in Sri Lankan women.

DNA Methylation and PCOS

DNA methylation is a biological process consisting of enzymatic reaction involving the addition of a methyl group onto the carbon in the 5′ position of the pyrimidine ring of a cytosine followed by a guanine (CpG dinucleotides) and results in the formation of 5-methylcytosine [100]. Studies have shown alterations in DNA methylation in the peripheral and umbilical cord blood, suggesting a correlation between the PCOS phenotype and epigenetic changes in cells from systemic and fetal circulation [101-105]. In addition, variation in DNA methylation have also been seen in tissues affected in the disease, including the ovary [106], adipose tissue [107] and skeletal muscle [108].

Next generation sequencing method revealed numerous differentially methylated genes in peripheral blood of women with PCOS when compared with women without the disease. Most detected genes correlated with cancer, immune response, transcription regulation and metabolism [101].

Studies focusing on the analysis of the DNA methylation in the specific genes revealed, an elevation of DNA methylation in the promoter of FST (which encodes follistatin), LMNA (encodes Lamin A/C) and PPARGC1A (encodes the peroxisome proliferator-activated receptor gamma coactivator 1-α), while reduced levels of this epigenetic mark have been reported in the promoter of the LHCGR gene (encodes the LH receptor) and EPHX1 gene (encodes epoxide hydrolase 1) in women with PCOS compared with controls [102 -110]. These alterations in DNA methylation correlated with various molecular pathways and physiological processes that are dysregulated in PCOS such as follicular development [111], infertility [112], steroidogenesis [113], glucose metabolism and insulin signaling [114]. In addition, DNA methylation defects in PPARGC1A have been related to insulin resistance and high serum androgen levels in women with PCOS, as well as with a decline in the mitochondrial DNA content, a well-known marker of metabolic disease when reduced in the peripheral blood [109].

In addition, alterations in DNA methylation have been reported in several genes associated with the ovary function and morphology in granulosa cells of women with PCOS, which, in turn, correlates with the response to gonadotropins, insulin signaling and steroidogenesis [109, 115-117].

MicroRNAs and PCOS

It is noteworthy, recently many studies have been carried out to identify the relationship between MicroRNAs (miRNAs) and PCOS. MicroRNAs are small noncoding single-stranded RNA molecules 18-24 nucleotides in length that directly regulate gene expression post-transcriptionally. Studies have shown that miRNAs regulate 60% of human protein-coding genes and are involved in various physiological processes, including development, metabolism, apoptosis, differentiation, and cell cycle [118, 119]. Evidence suggests that miRNAs are differentially expressed between women with PCOS and women without the disease [120, 121]. Moreover, miRNAs are stable in serum and easy to detect due to their resistance of nuclease activity. Therefore, miRNAs may be candidate diagnostic markers for patients with PCOS [122]. Few studies have shown the expression of small non-coding miRNA changes in PCOS and their expression in follicular fluid. However, the relationship between miRNAs and PCOS is at a preliminary stage, and the possible roles of miRNAs in the pathogenesis of PCOS is limited. Hence, further study is recommended to identify the association between miRNA and PCOS.

Conclusion

PCOS is a common endocrine disorder among women with complex trait. Many risk factors affect the occurrence and development of PCOS, including ovary abnormalities, obesity, and environmental factors. Multiple studies have suggested that genetic factors are essential in the etiology of PCOS. Although a number of candidate genes associated with PCOS have been identified in case–control studies around the world, only a few studies have been carried out in Sri Lankan women. It

is noteworthy, only FTO (rs9939609) polymorphism seems to be associated with PCOS in different ethnicity including Sri Lankan population. Hence, we can conclude FTO (rs9939609) polymorphism as a risk factor associated with the manifestation of PCOS. However, the genetic significance and the exact mechanism of action of most of these genes with PCOS, are still unclear and need to be confirmed. There is a huge gap in genetic studies of PCOS in Sri Lanka, hence future studies to identify the genetic polymorphisms of different biochemical and metabolic pathways and their association with PCOS is recommended. In addition, for the putative candidate genes identified so far, more studies are needed for a better understanding of their molecular and physiological roles in PCOS. These data will clarify the risk factors of PCOS and contribute to the prediction of the disorder, which could improve diagnosis and treatment of PCOS patients.

Moreover, several candidate genes have been associated with the PCOS susceptibility in different ethnicity. The controversial findings in PCOS are mainly attributed to the diverse populations and genotyping methods. There is a long way to go before we entirely understand the genetic effects on this complex and heterogeneous disorder. In addition, epigenetics of PCOS should be studied in different population. Therefore, further studies should consider address genetic and environment factors, including obesity and nutrition, and epigenetics genes associated with PCOS which will provide a better understanding of the etiology of PCOS.

Conflicts of Interest

The authors declare no conflict of interest.

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