Original Article

An Investigation of Steroid Biosynthesis Pathway Genes in Women with Polycystic Ovary Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a common endocrinopathy whose heterogeneous genetic basis results in a variable clinical presentation. One of the main clinical features of PCOS is hyperandrogenism which occurs due to dysregulation of ovarian and adrenal steroidogenesis. **Aims:** This study aimed to investigate potentially pathogenic variants in steroidogenic genes associated with PCOS. **Settings and Design:** This was a hospital-based observational study. **Materials and Methods:** We recruited 51 women who presented with PCOS. Fasting blood samples were drawn from the participants and their whole-exome sequencing analysis was carried out to look for pathogenic variants involved in steroidogenic pathways. The variants were predicted for their probable deleterious effects on proteins through *in silico* prediction tools. We evaluated the variants with respect to the hormonal characteristics and clinical outcomes of the patients. **Statistical Analysis Used:** All variables were analysed using GraphPad Prism 8. Kruskal–Wallis *t*-test and Fisher's exact test were used to compare clinical parameters and frequency differences among PCOS patients with and without variants. **Results:** The data presented here reveal eight heterozygous exonic variants, namely CYP21A2 (p.Ala392Thr, p.Gln319Ter and p.I143N), steroidogenic acute regulatory (p.Arg53 Leu), AKR1C3 (p.Phe205Val), P450 oxidoreductase (p.Val334Ile and p.Val251Met) and HSD17B6 (p.Gly40Ser), of which three were pathogenic, and four variants of uncertain significance in 8 out of 51 patients (15.68%). The identified variants were predicted to cause protein destabilisation, thus likely contributing to the pathogenesis of PCOS. Some of the variants showed significant differences between PCOS patients and population database ($P < 0.05$). **Conclusion:** The results of this study add to the mutational spectrum of steroidogenic genes and their association with PCOS.

Keywords: *Polycystic ovary syndrome, rare variants, steroidogenesis, whole-exome sequencing*

Introduction

Polycystic ovary syndrome (PCOS) is a common and complex endocrinopathy which is the major cause of anovulatory infertility, affecting 5%–10% of women of reproductive age.[1,2] It presents with multiple combinations of signs and symptoms, resulting in a wide spectrum of phenotypes including reproductive, endocrine as well as metabolic disruptions. Like the other ovulatory disorders, PCOS is characterised by hypothalamic–pituitary–gonadal dysfunction along with anovulation, but unlike other ovulatory disorders causing ovulatory dysfunctions, PCOS is often associated with androgen excess. Apart from being an

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apparent reproductive disorder, PCOS is also frequently associated with extra-reproductive manifestations such as metabolic syndrome, low-grade chronic inflammation and insulin resistance.[3-5] PCOS is one of the major causes of female infertility, comprising approximately 40% of the cases, and a leading cause of endometrial carcinoma.[6]

PCOS is often considered a multifactorial disorder in which the women with a pre-disposition to PCOS development are affected by individual gene, gene-gene or gene-environment interactions. Multiple studies have opened new avenues for the exploration of genetic basis of PCOS by identifying PCOS as a familial condition.[7,8] A number of studies have previously reported a genetic pre-disposition to PCOS; however, a consensus has not yet been reached about the genetic markers. While some studies are in favour of an autosomal dominant inheritance,[9,10] others point at a polygenic basis of the syndrome.[11,12] In addition, PCOS has an estimated heritability of 70%, as demonstrated by mono- and dizygotic twin studies.[13]

One of the cardinal features of PCOS (phenotypes A, B and C) is androgen excess. The sisters of women with PCOS are at an increased risk of androgen excess, which implies that hyperandrogenism is a genetically inherited abnormality. The androgen levels in PCOS women are increased as a result of alterations observed in steroid biosynthesis pathway.[14] The Δ5 and Δ4 pathways are the major pathways through which large amounts of dehydroepiandrosterone (DHEA), androstenedione (Δ4A) and testosterone are synthesised in the ovary in PCOS women.[15-18] Because the levels of dehydroepiandrosterone-sulphate (DHEA-S), a marker of adrenal androgen precursors, are elevated in PCOS women, it seems likely that adrenal glands are also the sites of these pathways. Adrenal and gonadal steroid hormone biosynthesis is dependent on enzymes of which majorly fall into two major classes: the cytochrome P450 heme-containing enzymes and hydroxysteroid dehydrogenases. The P450 enzymes are membrane-bound proteins linked with the mitochondrial membrane, which involve CYP11A, CYP11B1 and CYP11B2, or the endoplasmic reticulum which involves CYP17, CYP19 and CYP21. Ovaries are the prominent sites of steroidogenesis, any aberration in which can lead to hyperandrogenism in PCOS women.[19] Androgen excess, in turn, promotes follicular arrest, altered oocyte development and maturation and anovulation.[20] This is suggestive of the fact that ovarian function is affected by androgen excess. The potential role of abnormal adrenal steroidogenesis in the development of PCOS has also long time attracted the attention of researchers. This could possibly be because of the enzymatic defects in 21-OH deficiency which redirects steroid precursors to the synthesis of adrenal androgens or activation of enzymes like CYP17 which are directly involved in androgen synthesis. To date, many genes have been identified as causal factors in the pathogenesis of PCOS, as clear from the mutations and/or polymorphisms which have been discovered.[21] However, the exact role of these genes is still ambiguous. In the present study, we hypothesise that the inherited factors associated with PCOS may involve genes which regulate steroidogenesis and androgen metabolism.

Methodology

Subjects and ethics statement

The current study was a retrospective, cohort study conducted over a period of 3 years. For this study, 51 PCOS patients were recruited. The patients were recruited based on the National Institutes of Health criteria, 2012, according to which two of the three characteristics are required for PCOS: hyperandrogenism, ovulatory dysfunction and/or polycystic ovarian morphology (PCOM). The presence or absence of multiple follicles over the surface of ovaries was detected by ultrasonography. PCOM, examined through ultrasonography, is defined as the presence of antral follicles (≥12) measuring 2–9 mm in diameter and/or ovarian volume (>10 cm^3). Clinical hyperandrogenism was diagnosed on the basis of the modified Ferriman–Gallwey score (≥8).[22] Biochemically, hyperandrogenism is determined through elevated total testosterone (TT) levels according to the laboratory criteria. The inclusion criteria were aged between 18 and 35 years and hormonal analysis to study the profile of thyroid (T4 and thyroid-stimulating hormone), serum prolactin, luteinising hormone (LH), follicle-stimulating hormone (FSH), oestradiol (E2), TT and fasting insulin (FI) from morning fasting samples on D2-5 of menstrual cycle. The exclusion criteria were based on the clinical diagnosis of congenital adrenal hyperplasia (CAH), hypothyroidism, pre-mature ovarian failure and ovarian neoplasm. Women under medication affecting the hypothalamic–pituitary–gonadal axis were also excluded.

The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2013. Written informed consent was obtained from the subjects before sample collection. Our research was approved by the Institute Ethics Committee.

Ethics Committee approval number: IEC-730/29.12.2017.

The sample size calculation was not performed for the present study.

Hormonal analysis

The serum levels of TT, DHEA-S, LH, FSH, anti-Mullerian hormone and 17-hydroxyprogesterone (17-OHP) were measured through ELISA. Blood samples of the patients were collected after overnight fasting on days 2–5 of menstrual cycle.

DNA extraction and whole-exome sequencing analysis

Genomic DNA was extracted from fresh peripheral blood samples of the patients using the salting-out method. The DNA samples of 51 PCOS women were subjected to whole-exome sequencing (WES). Targeted gene capture was performed using Agilent Sure Select V5 exome capture kit. The libraries were sequenced to mean >80–100× coverage on Illumina sequencing platform.

Bioinformatics

The sequences obtained were aligned to human reference genome (GRCH37/hg19) using Sentieon aligner and analysed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon haplotypecaller was used to identify variants which are relevant to the clinical indication. Gene annotation of the variants was performed using Variant effect predictor (VEP) programme against the Ensembl release 91 human gene model. In addition to single-nucleotide variants and small indels, copy number variants were detected from targeted sequence data using the ExomeDepth (version 1.1.10) method. Clinically relevant mutations were annotated using published variants in literature and a set of disease databases – ClinVar, OMIM, GWAS, HGMD (version 2018.3) and SwissVar. Common variants are filtered based on allele frequency in 1000 Genome Phase 3, ExAC (version 1.0), gnomAD (version 2.1), EVS, dbSNP (version 151), 1000 Japanese Genome and an internal Indian population database. Non-synonymous variant effect was calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region were not reported.

Variant filtering strategy and effect prediction

Rare variants with minor allele frequency (MAF) more than 0.05% in ExAC were retained while those with higher MAF (>0.05%) were excluded from the data, unless reported pathogenic or likely pathogenic in ClinVar. Variants with expected pathogenicity were selected based on their variant class followed by their functional impact prediction based on scores obtained from *in silico* tools CADDphred score, SIFT score, PolyPhen-2 and LRT. Variants with a low CADDphred score were excluded unless reported as pathogenic or likely pathogenic in ClinVar. In addition, I-Mutant 2.0 and MUpro Tool were used to assess the effect of single amino acid substitution on protein stability.

Pathogenicity interpretation of the variants

The American College of Medical Genetics and Genomics (ACMG) guidelines, 2013, were used to interpret the pathogenicity of the identified variants.

Statistical analysis

Continuous quantitative data are expressed as the mean ± standard deviation. Kruskal–Wallis *t*-test ($P < 0.05$) was applied to compare the differences in clinical parameters among PCOS subjects carrying variants and those without variants. In addition, the frequency differences of the identified variants were analysed by applying two-tailed Fisher's exact test ($P < 0.05$).

Results

Whole-exome sequencing

Whole-exome sequencing data from 51 PCOS patients were analysed for the presence of potentially pathogenic exonic variants in genes associated with ovarian and adrenal steroidogenesis. The detailed information of the variants is given in Table 1. We identified eight heterozygous pathogenic variants in six different genes, namely CYP21A2 (p.Ala392Thr; p.Gln319Ter; p.I143N), HSD17B6 (c.118G>A), HSD3B1 (p.Thr220Ser), P450 oxidoreductase (POR) (p.Val334Ile and p.Val251Met), steroidogenic acute regulatory (StAR) (p.Arg53 Leu) and AKR1C3 (p.Phe205Val). The most common of these genes was CYP21A2 which was found in 50% ($n = 4$) of the patients. Rest all other variants were found in single cases. Importantly, all the variants exhibited heterozygosity. All except one of the CYP21A2 variants belonged to missense class of mutations. Only one case of compound heterozygosity was found in a patient (Case 3) carrying CYP21A2 missense variant (p.Ala392Thr) and a non-sense variant (p.Gln319Ter) which has been predicted to cause pre-mature termination of protein synthesis. In addition, the variants CYP21A2 (c.1174G>A, c.955C>T and c.428T>A) exhibited medium to high Combined annotation dependent depletion (CADD) score and are pathogenic/likely pathogenic according to ClinVar database. These variants were also interpreted as pathogenic/likely pathogenic according to the ACMG guidelines. Furthermore, each of the eight variants was found to have a decreasing effect on their respective protein stability [Table 2]. The remaining variants in other genes were interpreted as variants of uncertain

Table 1: Details of the pathogenic variants identified through whole-exome sequencing in polycystic ovary syndrome patients

Gene	CDS	Case number	Variant class	Zygosity	RefSeq ID	Amino acid change	CADDphred score	ClinVar significance	ACMG interpretation	ExAC AF
CYP21A2	c. 1174G>A	2, 3, 4	Missense	Heterozygous	NM_000500.7	p.Ala392Thr	Missense	Likely pathogenic	Pathogenic (PS3 + PM3 + PP5 + PS4 + BS1)	0.01357
	c. 955C>T	3	Non-sense	Heterozygous	NM_000500.7	p.Gln319*	Heterozygous	Pathogenic	Pathogenic (PS3 + PM3 + PP5 + PS4 + BS1)	0.0025
	c. 428T>A	5	Missense	Heterozygous	NM_001128590	p.I143N	Missense	Pathogenic	VOUS (PM1 + PP3)	0.0004
AKR1C3	c. 613T>G	5	Missense	Heterozygous	NM_000941	p.Phe205Val	Missense	NA	VOUS (PM1 + PP2)	0.00000239
StAR	c. 158G>T	6	Missense	Heterozygous	NM_000349.2	p.Arg53Leu	Missense	Uncertain significance	VOUS (PM2 + PP3)	0.0008
POR	c. 1000G>A	7	Missense	Heterozygous	NM_000941.2	p.Val334Ile	Missense	NA	VOUS (PM1 + PM2 + PP3)	0.001
POR	c. 751G>A	8	Missense	Heterozygous	NM_000941	p.Val251Met	Missense	NA	VOUS (PM2 + PM2)	0.0000122
HSD17B6	c. 118G>A	1	Missense	Heterozygous	NA	p.Gly40Ser	Heterozygous	NA	VOUS (PM1 + PM2 + PP3)	0.00014

NA=Not available, StAR=Steroidogenic acute regulatory, CDS=Coding sequence, ACMG=American College of Medical Genetics and Genomics, ExAC=Exome aggregation consortium, POR=P450 oxidoreductase, AF=Allele frequency, VOUS=Variants of uncertain significance, CADD=Combined annotation dependent depletion

significance. Moreover, the results of the various *in silico* prediction tools showed that majority of the identified variants were deleterious. All the variants exhibited low allele frequencies. Importantly, significant differences in the frequency of the variants CYP21A2 (c.1174G>A and c.428T>A), AKR1C3 (c.613T>G), HSD17B6 (c.118G>A) and POR (c.751G>A) were observed between PCOS patients and healthy population database ($P < 0.05$) [Table 2]. The variants were mapped onto their respective genes and visualised on the ProteinPaint platform (https://proteinpaint.stjude.org). The results revealed that of the nine identified variants, eight were located in important functional domains of their respective proteins [Figure 1].

Clinical and hormonal findings

The clinical and hormonal characteristics of the patients are given in Table 3. Interestingly, all except one patient (Case 3) exhibited all three cardinal features of PCOS, thus belonging to phenotype A of PCOS. The incidence of oligomenorrhoea was 100%, while hirsutism was found among 75% (n = 7) and biochemical hyperandrogenism among 37.5% (n = 3) of patients with variants. However, none of these patients showed signs of hyperinsulinaemia as clear from the normal FI values. In addition, the basal metabolic rate (body mass index [BMI]) was also lower among these women (11.1%, n = 1). The hormonal profile revealed altered steroidogenesis in the patients with pathogenic variants. In particular, testosterone (0.57 ± 0.30 ng/mL) and oestrogen (59 ± 27.47 pg/mL) levels were higher in these patients as compared to those without variants [Table 4]. However, this difference was not statistically significant. In total, 44.4% (n = 4) of patients with pathogenic variants had abnormally high levels of testosterone. The levels of 17-OHP (2.74 ± 2.72 ng/mL) and DHEA-S (206.82 ± 68.36), on the other hand, were lower in these patients, but this difference too was not statistically significant.

ProteinPaint platform (https://proteinpaint.stjude.org) was used to map and visualise the variants onto their respective genes. The results revealed that each of the eight variants was present in important catalytic domains of the protein [Figure 1].

Discussion

Abnormal androgen synthesis in PCOS women is a consequence of aberration in genes involved in steroid hormone biosynthesis.[23] A number of such genes have been studied through GWAS for their possible association with PCOS; however, the results have been contradictory. Hence, identifying pathogenic mutations

Table 2: Evaluation of the variant effect and assessment of protein stability done through *in silico* prediction tools

Gene	Variant effect				Protein stability		PCOS subjects with variant	AF (ExAC)	*P*
	Variant	LRT prediction	SIFT prediction	PolyPhen-2 prediction	I-Mutant 2.0	MUpro tool			
CYP21A2	p.Ala392Thr	Deleterious	Deleterious	Probably damaging	Decreased	Decreased	3/51	0.0137	0.0378*
	p.Gln319*	-	-	-	-	-	1/51	0.0025	0.216
	p.I143N	Deleterious	Deleterious	Probably damaging	Decreased	Decreased	1/51	0.0004	0.0403*
AKR1C3	p.Phe205Val	Deleterious	Deleterious	benign	Decreased	Decreased	1/51	0.0000239	0.0012*
StAR	p.Arg53Leu	Deleterious	Tolerated	Benign	Decreased	Decreased	1/51	0.0008	0.078
POR	p.Val334Ile	Deleterious	Deleterious	Probably damaging	Decreased	Decreased	1/51	0.001	0.0964
POR	p.Val251Met	Neutral	Tolerated	Benign	Decreased	Decreased	1/51	0.0000122	0*
HSD17B6	p.Gly40Ser	Deleterious	Deleterious	Probably damaging	Decreased	Decreased	1/51	0.00014	0.0083*

The Fisher's exact test ($P<0.05$) showed significant differences in variant distribution between the PCOS cohort and that of ExAC database. PCOS=Polycystic ovary syndrome, StAR=Steroidogenic acute regulatory, POR=P450 oxidoreductase, ExAC=Exome aggregation consortium, AF=Allele frequency, *Statistically significant

in genes associated with abnormal steroidogenesis in PCOS women can be an important attribute in carrying out the molecular diagnosis and specific treatment of the syndrome. In the present study, we tried to look at the involvement of these genes by finding pathogenic variants through WES.

The present study describes whole-exome sequencing in 51 women who were affected by classical clinical signs of PCOS. The initial analysis of the data was focused on the steroidogenic genes which were selected from public databases and have been found to be squarely associated with PCOS. More stringent filters such as low MAF, SIFT, LRT, PolyPhen 2 software screening and variant class (non-synonymous mutations) filters were applied to facilitate the selection of rare/pathogenic variants which may have damaging effects on proteins. Through these criteria, we identified eight rare and pathogenic variants in five genes, namely CYP21A2 (p.Ala392Thr, p.Gln319Ter and p.I143N), POR (p.Val334Ile), StAR (p.Arg53 Leu), HSD17B6 (c.118G>A) and AKR1C3 (p.Phe205Val) which have been clearly linked with steroid hormone biosynthesis.

The gene encoding 21-hydroxylase (CYP21A2) is located on the long arm of chromosome 6, within the human leucocyte antigen locus. Mutations in CYP21A2 cause complete loss of function of 21-hydroxylase, causing salt-wasting CAH. On the other hand, non-classical CAH occurs when mutations cause a partial loss of enzyme activity such that 20%–60% of the enzyme activity is preserved. The phenotypes associated with NC-CAH range from isolated hyperandrogenism to PCOM to the different combinations observed in PCOS.[24] Consequently, these overlapping phenotypes of PCOS and NC-CAH often cause difficulties in the diagnostic approach.[25,26] The prevalence of heterozygosity of mutations in CYP21A2 gene among women with PCOS has been extensively studied; however, the results have been contradictory. In a study by Witchel *et al*.,[27] the prevalence of heterozygous mutations was 35.2% among adolescents with hirsutism and/or irregular menses and 6% among healthy controls. Similarly, a study conducted by Escobar-Morreale *et al*.[28] showed that 13.3% of the women with ovarian Hyperandrogenemia were carriers of CYP21A2 mutations, while only 7.7% of the healthy women were heterozygous for the same mutations. In addition, in a later study by Witchel and Aston, 33% of the women in the hyperandrogenic group and 7% in the control group exhibited heterozygosity in mutations in CYP21A2 gene.[29] The prevalence of CYP21A2 heterozygous mutations was not significant among the PCOS and control groups in a study conducted by Glintborg *et al*.[30] However, these studies did not interpret the nature of the mutations and did not segregate the potentially deleterious mutations from benign ones. In the present study, the identified CYP21A2 variants have been interpreted for their potential pathogenicity and impact on protein stability using multiple *in silico* tools. Of the three patients carrying CYP21A2 mutations, one patient (Case 3) was a compound heterozygote for CYP21A2 mutations, carrying two mutations: a missense mutation (p.Ala392Thr) and a non-sense mutation (p.Gln319Ter). Interestingly, CYP21A2 was the most commonly mutated gene found in the cohort, observed in 5.8% of patients. Of all the mutations studied, CYP21A2 p.Ala392Thr was the most common one, found in three patients (Case 2, Case 3 and Case 4). The mutations were located in the catalytic cytochrome P450 domain of the enzyme, which suggests that the mutations have an adverse effect on protein activity. The variants were predicted to be deleterious, impacting the functional activity of the enzyme. In addition, the frequency of the mutations CYP21A2 p.I143N and p.Ala392Thr in the cohort differed greatly from the frequency in healthy population ($P < 0.05$). The levels of testosterone and

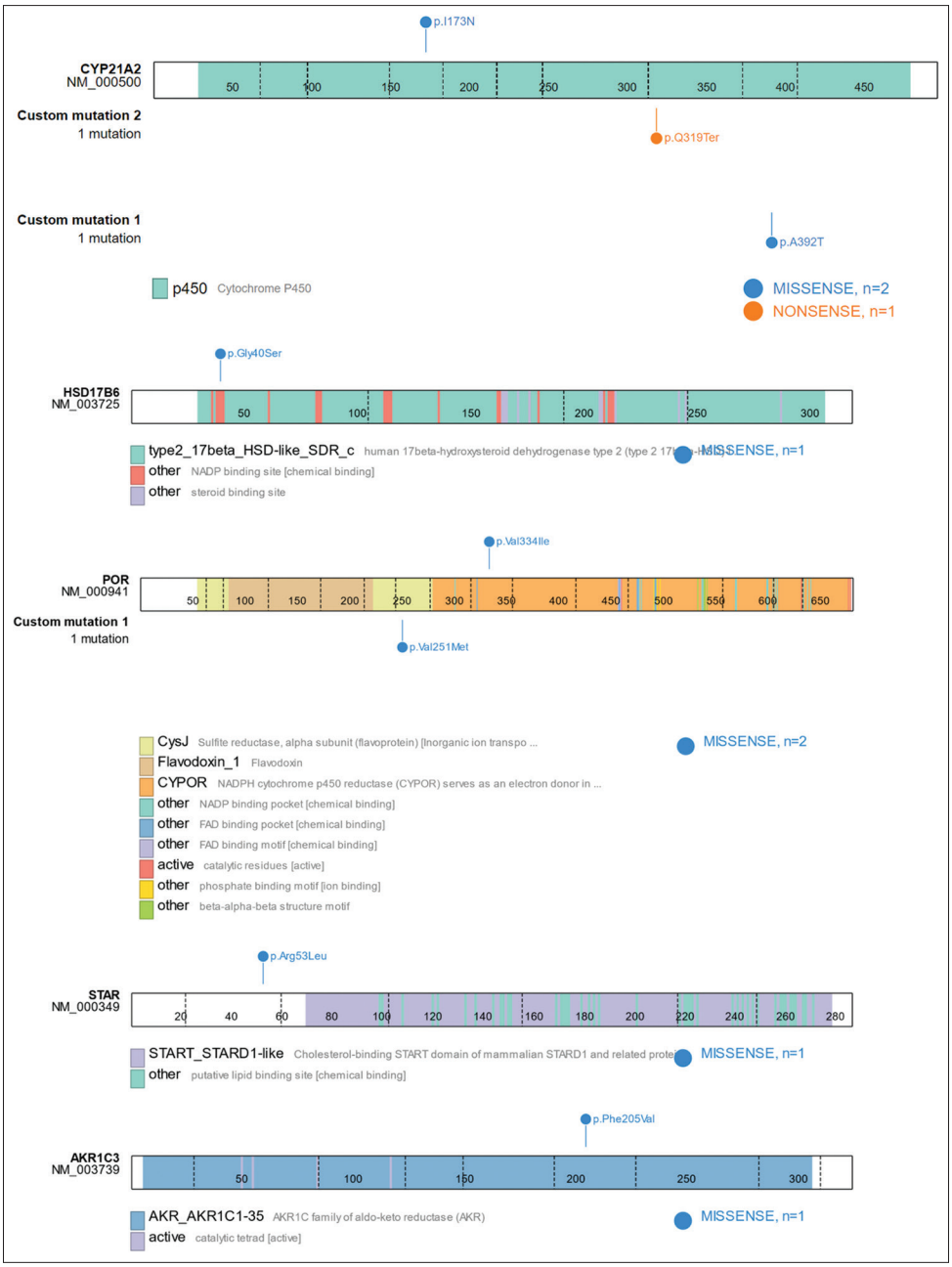

Figure 1: The precise location of the pathogenic variants on their respective genes visualised by ProteinPaint

17-OHP in the patients carrying these mutations were comparable [Table 3].

Cytochrome POR, encoded by POR gene, participates in electron transfer from NADPH to cytochrome P450 enzymes and thus plays an essential role in toxin and drug metabolism and steroid metabolism.[31] POR knockout mice have been shown to cause embryonic lethality.[32] People with deficiency of POR, on the other hand, show multiple clinical manifestations, including adrenal insufficiency, disordered steroidogenesis and disordered sex development.[31,33-35] It has also been seen that the hormonal profiles of patients with POR deficiency are similar to those with partial deficiencies of 21-hydroxylase.[36] For this reason, POR deficiency is sometimes considered a rare form of CAH disease. Insufficient glucocorticoid production with modestly elevated serum 17-OHP has been observed in patients of both sexes, along with polycystic ovary.[36] These clinical features are largely explained by compromised activities of CYP51A1 (lanosterol 14α-demethylase) involved in cholesterologenesis and of CYP21A2 (21-hydroxylase), CYP17A1 (17-α hydroxylase and 17, 20-lyase) and CYP19A1 (aromatase) involved in steroidogenesis. In addition, a number of studies have also reported infertility in phenotypically normal adults with POR

Table 3: Clinical parameters of the patients with identified variants

Clinical and hormonal parameters	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Identified variant (s)	HSD17B6 (p.Gly40Ser)	CYP21A2(p.Ala392Thr)	CYP21A2 (p.Ala392Thr; p.Gln319Ter)	CYP21A2 (p.Ala392Thr)	CYP21A2 (p.I143N); AKR1C3 (p.Phe205Val)	StAR (p.Arg53Leu)	POR (p.Val334Ile)	POR (p.Val251Met)
PCOS phenotype	A	A	D	A	A	A	A	A
Compound heterozygosity	No	No	Yes (CYP21A2)	No	No	No	No	No
Age (years)	18	28	23	25	26	19	27	21
BMI (kg/m^2)	21	24.5	23.8	24.5	19.2	27.5	24.9	18.3
FG score	14	13	4	8	13	11	9	26
Basal FSH	4.48	5.39	4.73	6	6.57	2.47	4.31	4.95
Basal LH	5.78	10.81	9.78	6.48	11.57	1.4	9.8	4.97
LH/FSH	1.2	2	2	1.1	1.76	<1	2.2	1.004
Basal T (ng/mL)	0.8	0.43	0.42	0.4	0.50	0.28	1.24	0.45
Basal E2	43.52	23.48	80	52	63	46	114	50
Basal 17-OHP (ng/mL)	1.18	1.47	1.30	1.04	1.78	2.45	9.11	1.37
AMH (ng/mL) above	19.41	18	9.49	13.05	17.3	5.6	<0.16	9.75
FI	9.1	4.55	8.2	8.8	8.5	12.3	9.8	4.6

BMI=Body mass index, FSH=Follicle-stimulating hormone, FG=Ferriman-Gallwey, LH=Luteinising hormone, T=Testosterone, E2=Oestradiol, 17-OHP=17-hydroxyprogesterone, AMH=Anti-Mullerian hormone, PCOS=Polycystic ovary syndrome, FI=Fasting insulin, StAR=Steroidogenic acute regulatory

Table 4: Basal levels of testosterone (ng/mL), dehydroepiandrosterone-sulphate (µg/dL), 17-hydroxyprogesterone (ng/mL) and oestrogen (pg/mL) in patients with and without pathogenic variants

Hormones	With variants	Without variants	*P*
T	0.57±0.30	0.53±0.32	0.703
DHEA-S	206.82±68.36	225.92±148.49	0.84
17-OHP	2.74±2.72	1.62±1.39	0.361
Oestrogen	59±27.47	64.90±69.97	0.726

17-OHP=17-hydroxyprogesterone, T=Testosterone, DHEA-S=dehydroepiandrosterone sulphate

deficiency.[33,34,35,37,38] The pathogenic mechanisms of this manifestation involve disrupted biosynthesis of androgens and conversion of the androgens to oestrogen.[39,40] Although patients with POR deficiency present with low levels of testosterone and other androgens, normal levels of testosterone in Cytochrome P450 oxireductase deficiency (PORD) cases have also been reported.[41] This is mainly due to the fact that PORD disrupts the classical pathway of steroidogenesis while the alternate pathway remains intact. In the present work, we describe the cases of two PCOS patients (Case 7 and Case 8) heterozygous for pathogenic mutations located in important catalytic domains in POR. Both the patients showed elevated levels of testosterone, while one of the patients (Case 7) exhibited abnormally high levels of 17-OHP. These results strongly suggest a relevant role of these variants in PCOS pathogenesis.

StAR protein is abundantly expressed in the cells of steroidogenic organs including adrenal glands, ovaries and testis and is crucial for adrenal and gonadal steroidogenesis. It carries out the rate-limiting step of steroidogenesis wherein cellular cholesterol is transported from the outer mitochondrial membrane to inner membrane before getting converted to pregnenolone, the precursor for all steroids.[41] Since StAR participates in steroidogenesis, it can be viewed as a suitable candidate for hormonal abnormalities associated with PCOS. In a study by Jakubowski in 2005, StAR was included in the list of candidate genes of PCOS.[42] In addition to this, it was later suggested that any variant in StAR gene, especially in the cholesterol-binding domain, can lead to hormonal abnormalities such as lipoid CAH, PCOS[43] and endometriosis.[44] In the present study, the StAR p.Arg53 Leu variant identified in a patient (Case 6) was located outside the functional cholesterol-binding domain of the protein. The patient carrying this variant had normal levels of testosterone, which could possibly be due to the location of the variant outside the functional domain. However, the mutation has been predicted to be mildly deleterious and is present

at an extremely rare frequency in the population which makes it important to be studied with regard to abnormal steroidogenesis.

HSD17B6 is expressed in multiple tissues including ovary, and the encoded protein has an elevated affinity for the androgenic substrate than the retinoic substrates.[45] The product of this gene possesses both epimerase and oxidative activities and is found to be associated with androgen metabolism and increased BMI.[46] Increased levels of type 6 17β-deoxyhydrogenase (HSD17B6) mRNA has been found in ovarian theca-cell expression data in PCOS.[47] In addition, significant differences in the allele distribution for SNP rs898611 of HSD17B6 have been observed between PCOS cases and controls in a Caucasian population, suggesting that HSD17B6 might be responsible for altered androgen metabolism in PCOS women. An association of SNP rs898611 with increased risk of developing PCOS has also been reported in a large Caucasian cohort.[48] In our data, we report a variant in HSD17B6 in a woman (Case 1) with abnormally high levels of testosterone, suggesting that this variant might have a role to play in hyperandrogenism seen in PCOS women.

AKR1C3 (type 5 17β-hydroxysteroid dehydrogenase) belongs to the Aldo-keto reductase superfamily which comprises a number of multifunctional enzymes that have different tissue-specific expression profiles and substrate specificity.[49] The enzyme encoded by the gene carries out the reduction of androstenedione (A4) to testosterone (T) in the ovaries, adrenal glands and adipose tissues, which are the main sources of testosterone production in women.[50,51] Ju *et al*. have reported an association of polymorphisms in AKR1C3 with PCOS in Chinese population.[51] Increased expression of AKR1C3 has been observed in the adipose tissues of PCOS women.[52] In the present study, a missense variant in AKR1C3 was reported in a PCOS woman with hyperandrogenism. The protein instability caused due to this variant would affect the activity of the resultant protein. However, considering that increased expression of AKR1C3 is considered to be a contributing factor to hyperandrogenism, it seems likely that there are other pathway(s) leading to the synthesis of testosterone responsible for hyperandrogenism seen in the patient.

Taken together, our study adds information to the genetic aetiology of PCOS. We consider that additional rare and potentially pathogenic variants in genes participating in ovarian and adrenal steroidogenesis could contribute to the variable phenotypes of PCOS. Further NGS studies, performed in large panels of PCOS patients, can reveal more about the involvement of causative pathogenic variants. Hence, an early diagnosis of pathogenic variants in genes associated with steroidogenesis in PCOS patients is imperative as it can render the disorder treatable and possibly reduce the severity of the consequent symptoms. The limitations of the present study were lack of the control group and the sample size.

Conclusion

We identified eight rare variants in five genes associated with abnormal steroidogenesis and hyperandrogenism in PCOS women. The patients with these variants presented with abnormal levels of androgens suggestive of impaired steroidogenesis. Our study supports the hypothesis that exonic rare variants in genes related to steroidogenesis drive the progression of PCOS.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

All data obtained and analysed in this study are included in this article. Details are available from the corresponding author on reasonable request.

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