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Custom exome panel revealed new mutations in *MAPK14* and novel mutation in *RUNX2* gene in patients with PCOS

Yunus Arikan[1]✉ & Taylan Onat[2]

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy and is both phenotypically and genotypically heterogeneous. A large number of genetic variants have been found in different genes, so far. Based on the literature, we identified 7 genes and aimed to find new causative variants in these genes. We created a targeted PCOS panel including major genes in the steroidogenezis, WNT, MAPK, and TGFβ pathways and analyzed whole-exome sequencing results. We compared the minor allele frequency (MAF) values of different variants with our results and calculated deleterious scores of newly found variants using various web-based prediction tools and ACMG pathogenicity criteria. We found a novel missense mutation (p.Thr355Ile) in the *RUNX2* gene in one patient and heterozygous mutations in the *MAPK14* gene (c.306_5delT and c.*8G>T) in another patient with PCOS. Five novel pathogenic moderate (PM2) intronic variants in 4 different genes in total were introduced for the first time. We also decoded 7 genes in patients with PCOS in our cohort. Two more candidate genes (*MAPK14* and *RUNX2*) may be related to PCOS.

Keywords PCOS, Targeted exome panel, MAPK14, RUNX2

Polycystic ovary syndrome is the most common endocrine disorder in women of reproductive age[1]. Worldwide, 4-20% of women of childbearing age are affected[2]. Clinical findings of PCOS include the presence of PCO morphology (PCOM), infertility, acne, ovulatory dysfunction (OD), hirsutism, insulin resistance, obesity, hyperandrogenism (HA) and dyslipidemia[3,4]. National Institute of Health (NIH)-1990, Rotterdam-2003 and Androgen Excess (AE)-2006 criteria are used for diagnosis of PCOS[5,6]. PCOS is studied in 4 different phenotypes as phenotypes A, B, C and D. It is known that the most common (50%) phenotype among the selected clinical populations is phenotype A (HA + OD + PCOM), which together with phenotype B (HA + OD) is also referred to as classical PCOS[7,8]. These phenotypes differ based on the presence or absence of hyperandrogenism, ovulatory dysfunction, and polycystic ovary morphology.

Many genetic changes have been studied in PCOS. They include genes involved in ovarian and adrenal steroidogenesis (*AR*, *CYP11A*, *CYP17*, *CYP19*, *CYP21*, *SHBG*, *StAR*, *SRD5A2*)[9–15], in gonadotropin axis (*ESR1*, *LH*, *LHR*, *AMH*, *AMHR2*, *FSH*, *FSHR*)[16–22], in insulin signaling (*INS*, *INSR*, *IRS1*, *IRS2*)[23–25], and in obesity-related genes (*FTO*, *MC4R*)[26,27]. In recent years, especially with the development of next generation sequencing technologies, new candidate genes or their spesific variants have been found either in family-based studies or in case-control studies[28–32].

In a study investigating the molecular changes in the egg cell microenvironment at miRNA level in PCOS patients, certain differences were detected in MAPK, insulin, Wnt and TGFβ signaling pathways[33]. Today, there is no certain diagnostic PCOS genetic panel currently used to detect PCOS in patients. In our previous study we identified novel *INSR* gene variations by employing exome sequencing in PCOS patients[34]. In the current study, we investigated the variations of genes that we hypothesized, might be important for the signaling pathways that were mentioned above where differences in miRNA levels were found in PCOS patients. We also aim to highlight variants of interest for future functional studies that may contribute to understanding the complex genetic architecture of PCOS.

[1]Faculty of Medicine, Department of Medical Genetics, Yozgat Bozok University, Yozgat, Turkey. [2]Faculty of Medicine, Department of Obstetrics and Gynaecology, Inonü University, Malatya, Turkey. ✉email: yunus.arikan@yobu.edu.tr

Parameter	Min	Max	Mean	SD	Reference range	Patients*/+
BMI (kg/m^2)	18.65	36.20	26.85	5.55	–	26.67/31.41
FSH (mIU/mL)	3.21	8.72	5.59	1.84	1.5–33.4	**8.72**/4.29
LH (mIU/mL)	1.28	27.80	8.26	6.33	1.24–58.64	7.86/4.24
LH/FSH ratio	0.40	4.61	1.52	1.16	–	0.90/0.99
E2 (pg/mL)	11.00	114.00	43.27	28.21	27.00–127.00	41.70/37.75
PRL (mIU/mL)	6.34	45.61	14.34	9.87	2.74–26.72	**45.61**/11.03
TSH (mIU/mL)	0.57	2.71	1.58	0.62	0.38–5.33	**0.57**/1.42
FT4 (nmol/mL)	0.60	1.48	1.05	0.21	0.61–1.12	1.07/1.22
Total testosterone (ng/mL)	18.00	63.30	29.43	14.21	1.75 –7.81	**63.30**/29.6
DHEAS µg/dL)	59.00	425.00	259.11	110.58	18.00–391.00	328.00/355.0
HbA1C (mmol/mol)	4.80	5.80	5.28	0.25	4.00–6.00	5.20/5.40
Cholesterol (mg/dL)	109.00	259.00	196.00	40.44	0.00–199.00	136.00/167.0
HDL (mg/dL)	32.50	65.00	47.45	10.82	50.00–65.00	56.00/42.90
LDL (mg/dL)	62.00	168.00	102.22	32.48	30.00–130.00	66.60/109.30
VLDL (mg/dL)	7.20	32.00	18.86	7.99	2.00–3.00	12.80/14.80
HOMA-IR	0.38	7.37	2.61	1.92	–	2.00/**6.30**

Table 1. Biochemical outputs of descriptive parameters in PCOS patients. *SD* standart deviation, *patient with *MAPK14* mutation, +patient with *RUNX2* mutation. For example, the bolded values of 8.72 and 45.51 were observed in the patient with compound heterozygous *MAPK14* mutations (c.306-5delT/c.*8G > T).

Gene	Transcript ID	Type	cDNA	Protein	Polyphen2	SIFT	Grantham
RUNX2	NM_001024630.4	SNV	c.1064 C > T	p.Thr355Ile	0.001	0.07	89.0

Table 2. Computational prediction scores of novel mutation in *RUNX2* gene.

Results

We customised PCOS panel containing 7 genes in different pathways in 16 patients with phenotype A who were diagnosed according to the AE-PCOS diagnostic criteria. All patients were presenting not only hirsutism (Ferriman-Gallwey scores ≥ 8) but also had PCO morphology (the presence of ≥ 20 follicles in the ovary with a size of 2–9 mm or total ovarian volume > 10 ml, based on 2018 International Guidelines for the assessment of PCOS) with ovarain dysfunction profile. The menstrual cycle profile of all patients except one case was > 35 days.

Demographic profiles of PCOS patients

The mean age of patients was 22.3 ± 4.3 and BMI was 26.8 ± 5.5 kg/m2. The ratio of patients whose BMI that bigger than 25 (> 25 kg/m2) was 62.5% (n = 10). The hormon levels of patients was following; FSH (5.6 ± 1.8 mIU/mL), LH(8.3 ± 6.3 mIU/mL), E2 (43.3 ± 28.2 pg/mL), PRL (14.3 ± 9.8 mIU/mL), TSH (1.6 ± 0.6) mIU/mL), FT4 (1.1 ± 0.2 nmol/L), total testosteron (29.4 ± 14.2 ng/mL), DHEAS (259.1 ± 110.6 µg/dL), HbA1C (5.3 ± 0.3 mmol/mol), cholesterol (169.0 ± 40.4 mg/dL), HDL (47.5 ± 10.8 mg/dL), LDL (102.2 ± 32.5 mg/dL), VLDL (18.8 ± 7.9 mg/dL) (Table 1). Among the hormones evaluated in the early phase of menstruation, the average LH/FSH ratio was 1.5 ± 1.2, while two cases (12.5%) with a ratio > 2 were detected. HOMA-IR values of the PCOS patients were 2.6 ± 1.9. The proportion of patients with HOMA-IR values exceeding 2.5, the upper threshold of the normal range, was 31.3% (5 out of 16 patients).

Among all patients, the one carrying the *MAPK14* mutation exhibited the highest levels of FSH, PRL, and total testosterone, alongside the lowest TSH level, suggesting a potential genotype-phenotype correlation with this mutation. Similarly, the patient with the *RUNX2* mutation had the second highest HOMA-IR value, indicating a possible link between this genetic variant and insulin resistance, a common feature in PCOS (Table 1).

Custom exome panel results-novel candidate variants

We found a novel mutation in *RUNX2* (NM_001024630.4) gene and compound heterozygous mutation in *MAPK14* gene (NM_139012.3) in two different patients with PCOS. The novel mutation (c.1064 C > T) was found to be located in exon 8 region of *RUNX2* gene as missense mutation (p.Thr355Ile) in patient 8 (Supplemental Fig. 1). PolyPhen2, sorting intolerant from tolerant (SIFT) and Grantham scores (Table 2) of this missense mutation showed different scores but we found that phyloP value of novel mutation that causing amino acid change from threonine to isoleucine at 355th position of RUNX2 protein has a value of 0.032. Based on ACMG criteria it was classified in PM1, PM2 (pathohenic moderate) level.

On the other hand, one nucleotide deletion (c.306_5delT, a.k.a rs61763106, MAF = 0.156) in splice site region was in compound heterozygous state with c.*8G > T (a.k.a rs115711278, MAF = 0.003) which is located at 3'UTR region of *MAPK14* gene in second patient (Table 3, Supplemental Figs. 2 and 3). Rs11511278 was classsified as VUS, while rs617336106 was scored as BA1 and BS2 according to ACMG criteria and varsome prediction tools.

Gene	cDNA	Type	dbSNP	Localisation	MAF (gnomAD)
MAPK14	c.306-5delT	indel	rs61763106	Splice site	0.156
	c.*8G > T	SNV	rs115711278	3'UTR	0.0029

Table 3. Nomenclature of compound heterozygous mutations in *MAPK14* gene. *MAF* minor allele frequency, *UTR* untranslated region.

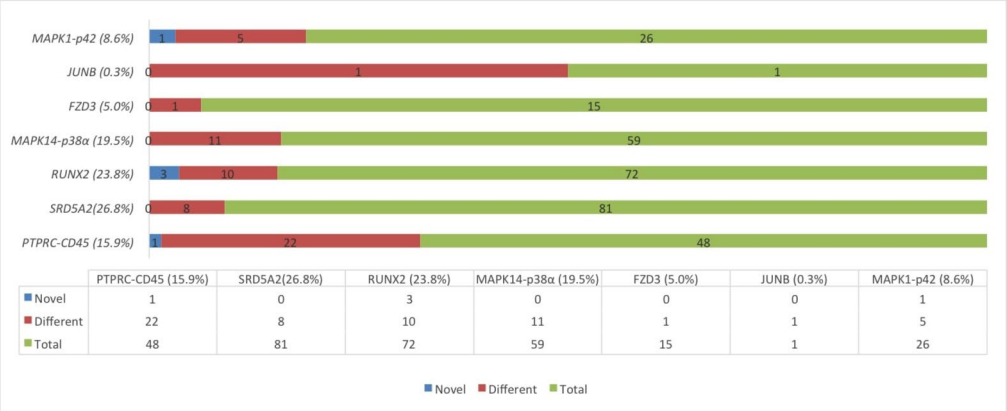

Fig. 1. Fifty-eight different genomic variations were identified in PCOS panel.

Gene	cDNA	Mutated allele frequency	ACMG classification
RUNX2	c.1021 + 62delG	0.375	PM2
	c.1021 + 51insG	0.375	PM2
PTPRC	c.1696 + 45 A > G	0.031	PM2
MAPK14	c.448-113insT	0.312	PM2
MAPK1	c.120-4delT	0.343	PM2

Table 4. Five novel intronic variants in custom PCOS panel. *ACMG* American College of Medical Genetics and Genomics Guideline, *PM* pathogenic moderate.

Custom exome panel results-other variations

After filtering out the minor allele frequency (MAF), we counted a total of 302 variations in 7 genes in the user-defined PCOS panel. Fifty-eight different variations (19.2%) together with 5 novel variants have been found in these genes (Fig. 1, supplemental Table 2).

The proportion of intronic variants amounted to 60.3% out of a total of 58 different variants. Five novel intronic variants were c.2179 + 45 A > G in *PTRPC* gene, c.1021 + 51insG and c.1021 + 65delG in *RUNX2* gene, c.448-113insT in *MAPK14* gene, c.120-4delT in *MAPK1* gene (Table 4). An intron variant (rs556118862) in the *PTPRC* gene that has no MAF value in the gnomAD database but has a value of 0.01% in the Estonian population and was found heterozygous in 10 of 16 PCOS patients (62.5%, $n = 10$) in our cohort. rs769673108 in the *MAPK1* gene with a MAF value of 0.02% was found heterozygous in a PCOS patient in our study. The allele frequency of rs41270086 in the *MAPK14* gene was higher in our PCOS cohort than in the gnomAD database (0.031 versus 0.007). Based on the mutation taster prediction, this is a polymorphism.

Six missense variants (10.3%) were uncovered in three genes (*SRD5A2*, *PTPRC* and *RUNX2*). A new variant (p.Thr355Ile) of the 6 missense variants was found heterozygous in the *RUNX2* gene in a patient. The mutation causing p.Thr355Ile was calculated as a benign polymorphism with respect to the databases of mutation taster, SIFT and polyphen-2 with a phlyloP value of 0.032. The most common benign polymorphism in the *SRD5A2* gene was rs523349, which causes p.Leu59Val and was found in 15 of 16 patients (93.75%) with PCOS. A heterozygous, potentially damaging missense variant (rs41269905, p.Asp123His) in the *PTPRC* gene was found in a patient with a much higher MAF level than the normal population (1.5%). In *SRD5A2* gene, we found a homozygous benign frameshift mutation (rs142200057, p.Ser31fs) in 14 PCOS patients. The MAF value of this SNP was 99.9% in the gnomAD database. Another heterozygous missense variant (rs150672767, p.Val1224Ile) with a MAF value of 0.1% was found in 2 patients with PCOS in the *PTPRC* gene. Six synonymous variants were found in 4 genes (*PTPRC*, *RUNX2*, *FZD3* and *MAPK1*). Among them, the heterozygous rs17612648 (p.Pro59Pro) in the *PTPRC* gene had a MAF value of less than 1% which was found in one patient (6.25%) with PCOS. The ratio of 3'UTR variants was the same as that of missense and synonymous variants and was found in

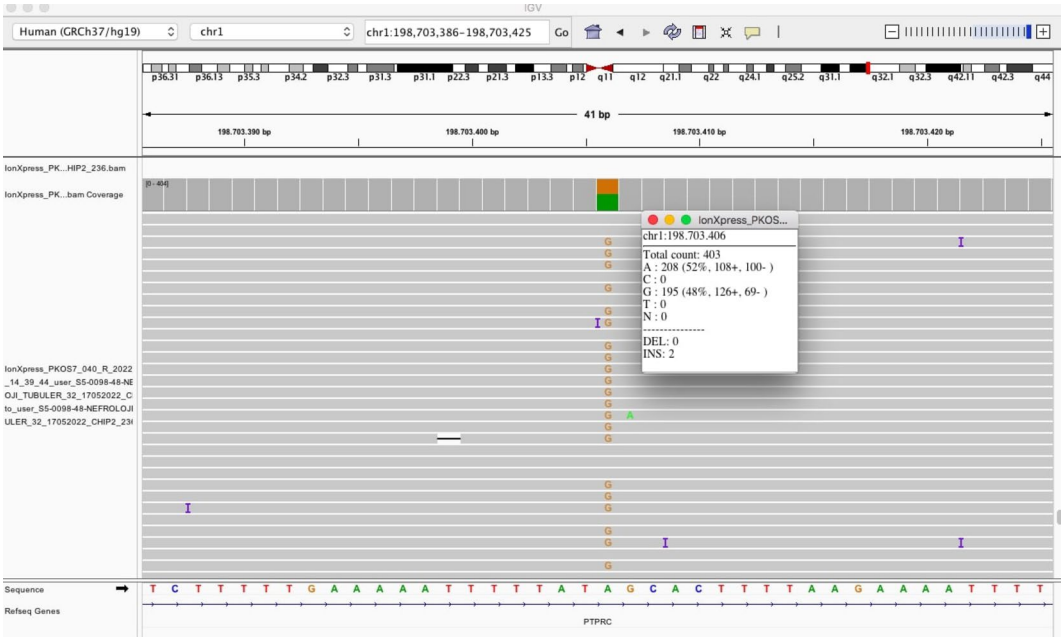

Fig. 2. A novel intron variant in *PTPRC* gene.

3 genes (*PTPRC*, *SRD5A2* and *MAPK14*). Among them, the heterozygous insertion of (AT)8 (rs1055645201) in *SRD5A2* gene has the lowest MAF value of 0.01% and was found in our 3 PCOS patients. rs115711278 had the second lowest MAF value of 0.29% and was found only in one patient as a compound heterozygous with a more common (MAF value is 15.6%) splice region variation (rs61763106, c.306-5delT) in the *MAPK14* gene. As to 5'UTR variants there were only 2 variants (MAF > 5.0%); one is rs56156688 in *MAPK14* gene and the second is rs17886698 in the *JUNB* gene.

Among the 7 genes in the custom made PCOS panel, the total number of variations in the *SRD5A2* gene ranked first in 16 patients ($n = 81$) (Fig. 1) and the lowest number of variations was detected in the *JUNB* gene (rs17886698, $n = 1$). On the other hand, rs2241802, the only genetic variation we found in the *FZD3* gene, was found in the majority of PCOS patients (93.75%, $n = 15$). The total number of *MAPK1* gene variations was 26 and the most common variation in this gene was a novel intronic variation (c.120-4delT) found in 11 of 16 PCOS patients. The total number of variations in the *PTPRC* gene was 48. Twenty-two different variations (45.8%) together with a novel intronic variation were detected in the *PTPRC* gene. The position of the new variation is c.2179 + 45 A > G (Fig. 2). The pyhloP value for this new variation was calculated to be 0.42 using the mutation taster prediction tool.

Discussion

This study was conducted to identify potential genetic variations contributing to the pathogenesis of polycystic ovary syndrome (PCOS), with a focus on key signaling pathways (steroidogenic, Wnt, TGFβ, MAPK) previously linked to PCOS pathophysiology. Given the multifactorial and complex nature of PCOS, the aim was to explore new candidate genes that could provide further insights into the genetic underpinnings of the disorder. Through a customized exome panel, we sought to investigate potential causative variations in PCOS patients and evaluate their potential clinical relevance.

The most striking result of our study was the identification of a novel missense mutation (p.Thr355Ile) in the *RUNX2* gene, which could be a candidate for autosomal dominant PCOS. This mutation, found in one patient, has not previously been associated with PCOS. Given that RUNX2 plays a role in the Wnt signaling pathway, our finding suggests that this pathway may be implicated in PCOS pathogenesis, specifically in this phenotype. However, further investigation, including segregation analysis in family members, is required to determine its pathogenicity.

Additionally, the identification of a compound heterozygous mutation in the *MAPK14* gene in another patient supports the hypothesis that mutations in MAPK pathway genes may contribute to PCOS. Although the clinical significance of the identified variants (c.306_5delT and c.*8G > T) remains uncertain, these mutations may play a role in *MAPK14* gene expression regulation, which warrants further functional studies. This finding highlights the potential for the MAPK signaling pathway to be a target for future diagnostic or therapeutic approaches. Previous studies have implicated RUNX2 and MAPK14 in ovarian function and androgen synthesis, with RUNX2 linked to estrogen deficiency and follicular development in PCOS, while MAPK14 enhances androgen synthesis through the p38 MAPK pathway, contributing to hyperandrogenism in PCOS patients[35–37].

From a clinical perspective, the discovery of these novel mutations emphasizes the importance of genetic screening in PCOS, especially for patients with atypical or severe phenotypes. While the identification of these mutations does not immediately alter clinical practice, it opens up new avenues for personalized medicine

in PCOS. Genetic profiling could potentially aid in tailoring treatments, particularly in cases where insulin resistance or hyperandrogenism predominates. One limitation of our study is the lack of segregation analysis due to the inability to reach family members after the February 2023 earthquake in Malatya. Future studies should include segregation analysis to further confirm the clinical significance of the identified mutations. Additionally, a broader population-based study is needed to determine whether these variants are specific to the Turkish population or have a broader relevance. In conclusion, our study introduces novel genetic variants that may contribute to PCOS pathogenesis. The findings underscore the potential role of the *RUNX2* and *MAPK14* genes in the development of PCOS, particularly in the context of autosomal dominant inheritance. Further research is needed to explore the functional impact of these variants and their relevance to PCOS diagnosis and treatment.

Methods

Participants

We selected 16 PCOS patients based on the AE-PCOS criteria. Informed and signed consent which is in accordance with the Declaration of Helsinki was obtained from all participants and patients who received genetic counseling. The local ethics committee (Local Ethics Committee of Yozgat Bozok University) approved this study with the number of KAEK-189_2021.02.24_07.

Biochemisrty-hormone

For hormonal and biochemical tests (Aeroset, Abbott Laboratories, Abbott Park, IL, Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA), venous blood samples were taken in the early follicular period (day 2–3 of menstruation), and such as after 8–10 h of fasting.

Genetics-targeted custom-made exome panel

Libraries were prepared by using ION AmpliSeqTM Custom Panel Library kit following manufacturer's instructions. Two different pools were prepared by using 92 primer couples (Supplemental Table 1). After barcoding and tagging process, sequencing was employed by using Ion TorrentTM Ion S5 530 chip (Thermofisher).

Within the scope of bioinformatics analysis, 'single-end' sequence raw data (*.fastq or UBAM) obtained from next generation sequencing platform was used. Files with bai and bam extensions were analyzed with IGV (Integrative Genome Viewer). Clinvar classifications, Polyphen and SIFT scores, UCSC common SNP lists, minor allele frequencies (85%) were used while filtering variations. We run hg19 (GRCh37, Genome Reference Consortium Human Build) when designating the chromosal localisations of candidates. Clinvar, OMIM, the Genome Aggregation Database (gnomAD v2.1.1), database of single nucleotide polymorphism (dbSNP), exome variant server and the mutation taster as in silico prediction tools. The American College of Medical Genetics and Genomics (ACMG) classification of variants by using varsome web page (https://varsome.com/) was also employed. When filtering the intronic candidates, it was taken into account that they are 10 bp away from the exon-intron junctions. Additional filtering steps were used to obtain more information about known variations, such as increasing MAF values if necessary. The phyloP scores that measure evolutionary conservation at individual alignment nucleotides were given when required.

Data availability

The data of current study are involved in the article and supplementary material.

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References

1. Franks, S. et al. The genetic basis of polycystic ovary syndrome. *Hum. Reprod.* https://doi.org/10.1093/humrep/12.12.2641 (1997).
2. Deswal, R., Narwal, V., Dang, A. & Pundir, C. The prevalence of polycystic ovary syndrome: a brief systematic review. *J. Hum. Reprod. Sci.* **13**, 261. https://doi.org/10.4103/jhrs.JHRS_95_18 (2020).
3. Cooper, H. E., Spellacy, W. N., Prem, K. A. & Cohen, W. D. Hereditary factors in the Stein-Leventhal syndrome. *Am. J. Obstet. Gynecol.* https://doi.org/10.1016/S0002-9378(15)33704-2 (1968).
4. Givens, J. R. et al. Familial ovarian hyperthecosis: a study of two families. *Am. J. Obstet. Gynecol.* https://doi.org/10.1016/0002-9378(71)90550-3 (1971).
5. Azziz, R. et al. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess Society Guideline. *J. Clin. Endocrinol. Metab.* **91**, 4237–4245. https://doi.org/10.1210/jc.2006-0178 (2006).
6. Franks, S. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: in defense of the Rotterdam criteria. *J. Clin. Endocrinol. Metab.* **91**, 786–789. https://doi.org/10.1210/jc.2005-2501 (2006).
7. Mumusoglu, S. & Yildiz, B. O. Polycystic ovary syndrome phenotypes and prevalence: Differential impact of diagnostic criteria and clinical versus unselected population. *Curr. Opin. Endocr. Metab. Res.* **12**, 66–71. https://doi.org/10.1016/j.coemr.2020.03.004 (2020).
8. Teede, H. J. et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome†‡. *Hum. Reprod.* **33**, 1602–1618. https://doi.org/10.1093/humrep/dey256 (2018).
9. Pusalkar, M., Meherji, P., Gokral, J., Chinnaraj, S. & Maitra, A. CYP11A1 and CYP17 promoter polymorphisms associate with hyperandrogenemia in polycystic ovary syndrome. *Fertil. Steril.* **92**, 653–659. https://doi.org/10.1016/j.fertnstert.2008.07.016 (2009).
10. Munawar Lone, N. et al. Association of the CYP17 and CYP19 gene polymorphisms in women with polycystic ovary syndrome from Punjab. *Pakistan Gynecol. Endocrinol.* **37**, 456–461. https://doi.org/10.1080/09513590.2020.1822803 (2021).
11. Witchel, S. F. & Aston, C. E. The role of heterozygosity for CYP21 in the polycystic ovary syndrome. *J. Pediatr. Endocrinol. Metab.* **13**, 1315–1317 (2000).
12. A.R.N. J.A, R. S. Investigating pathogenic SNPs in androgen receptor with direct influence on polycystic ovary syndrome (PCOS) in women. *Egypt. J. Med. Hum. Genet.* **23**, 1–10. https://doi.org/10.1186/s43042-022-00292-y (2022).

13. Martinez-Garcia, M. A. et al. Common variants in the sex hormone-binding globulin gene (SHBG) and polycystic ovary syndrome (PCOS) in Mediterranean women. *Hum. Reprod.* **27**, 3569–3576. https://doi.org/10.1093/humrep/des335 (2012).
14. Wu, C., Wei, K. & Jiang, Z. 5α-reductase activity in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Reprod. Biol. Endocrinol.* **15**, 21. https://doi.org/10.1186/s12958-017-0242-9 (2017).
15. Nazouri, A. S., Khosravifar, M., Akhlaghi, A. A., Shiva, M. & Afsharian, P. No relationship between most polymorphisms of steroidogenic acute regulatory (StAR) gene with polycystic ovarian syndrome. *Int. J. Reprod. Biomed.* **13** (12), 771–778 (2015).
16. Batista, M.C.P. et al. Trp28Arg/Ile35Thr LHB gene variants are associated with elevated testosterone levels in women with polycystic ovary syndrome. *Gene* **550**, 68–73. https://doi.org/10.1016/j.gene.2014.08.017 (2014).
17. Themmen, A. P. N. An update of the pathophysiology of human gonadotrophin subunit and receptor gene mutations and polymorphisms. *Reproduction* **130**, 263–274. https://doi.org/10.1530/rep.1.00663 (2005).
18. Kaur, M., Singh, S. & Kaur, A. Polymorphisms in FSHR modulating susceptibility to polycystic ovary syndrome: an updated meta-analysis. *J. Ovarian Res.* **16**, 183. https://doi.org/10.1186/s13048-023-01238-7 (2023).
19. Tong, Y., Liao, W. X., Roy, A. C. & Ng, S. C. Association of AccI polymorphism in the follicle-stimulating hormone beta gene with polycystic ovary syndrome. *Fertil. Steril.* **74**, 1233–1236. https://doi.org/10.1016/s0015-0282(00)01616-2 (2000).
20. Nectaria, X., Leandros, L., Ioannis, G. & Agathocles, T. The importance of ERα and ERβ gene polymorphisms in PCOS. *Gynecol. Endocrinol.* **28**, 505–508. https://doi.org/10.3109/09513590.2011.649811 (2012).
21. Gorsic, L. K. et al. Pathogenic anti-Müllerian hormone variants in polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* https://doi.org/10.1210/jc.2017-00612 (2017).
22. Hoyos, L. R. et al. Loss of anti-Müllerian hormone (AMH) immunoactivity due to a homozygous AMH gene variant rs10417628 in a woman with classical polycystic ovary syndrome (PCOS). *Hum. Reprod.* **35**, 2294–2302. https://doi.org/10.1093/humrep/deaa199 (2020).
23. Yun, J. H. et al. Association between INSVNTR polymorphism and polycystic ovary syndrome in a Korean population. *Gynecol. Endocrinol.* https://doi.org/10.3109/09513590.2011.650658 (2012).
24. Shi, X., Xie, X., Jia, Y. & Li, S. Associations of insulin receptor and insulin receptor substrates genetic polymorphisms with polycystic ovary syndrome: a systematic review and meta-analysis. *J. Obstet. Gynaecol. Res.* https://doi.org/10.1111/jog.13002 (2016).
25. Thangavelu, M., Godla, U. R., Paul Solomon, F. D. & Maddaly, R. Single-nucleotide polymorphism of INS, INSR, IRS1, IRS2, PPAR-G and CAPN10 genes in the pathogenesis of polycystic ovary syndrome. *J. Genet.* **96**, 87–96. https://doi.org/10.1007/s12041-017-0749-z (2017).
26. Liu, A. L. et al. Association between fat mass and obesity associated (FTO) gene rs9939609 A/T polymorphism and polycystic ovary syndrome: a systematic review and meta-analysis. *BMC Med. Genet.* **18**, 89. https://doi.org/10.1186/s12881-017-0452-1 (2017).
27. Batarfi, A. A. et al. MC4R variants rs12970134 and rs17782313 are associated with obese polycystic ovary syndrome patients in the western region of Saudi Arabia. *BMC Med. Genet.* **20**, 144. https://doi.org/10.1186/s12881-019-0876-x (2019).
28. Shi, Y. et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. *Nat. Genet.* **44**, 1020–1025. https://doi.org/10.1038/ng.2384 (2012).
29. Karakaya, C. et al. Further delineation of familial polycystic ovary syndrome (PCOS) via whole-exome sequencing: PCOS -related rare FBN3 and FN1 gene variants are identified. *J. Obstet. Gynaecol. Res.* **48**, 1202–1211. https://doi.org/10.1111/jog.15187 (2022).
30. Sharma, P., Jain, M. & Halder, A. An investigation of Steroid Biosynthesis pathway genes in women with polycystic ovary syndrome. *J. Hum. Reprod. Sci.* **15**, 240–249. https://doi.org/10.4103/jhrs.jhrs_86_22 (2022).
31. Liu, F. et al. Identification of a novel ESR1 mutation in a Chinese PCOS woman with estrogen insensitivity in IVF treatment. *Reprod. Biol. Endocrinol.* **20**, 157. https://doi.org/10.1186/s12958-022-01029-7 (2022).
32. Caburet, S. et al. A homozygous mutation of GNRHR in a familial case diagnosed with polycystic ovary syndrome. *Eur. J. Endocrinol.* **176**, K9–K14. https://doi.org/10.1530/EJE-16-0968 (2017).
33. Aydos, A. et al. Identification of polycystic ovary syndrome (PCOS) specific genes in cumulus and mural granulosa cells. *PLoS One*. https://doi.org/10.1371/journal.pone.0168875 (2016).
34. Arikan, Y. & Onat, T. INSR gene exome sequencing results in patients with PCOS, Celal Bayar Üniversitesi Sağlık Bilim. *Enstitüsü Derg.* **10**, 411–419. https://doi.org/10.34087/cbusbed.1358355 (2023).
35. Kempná, P. et al. Regulation of androgen biosynthesis – A short review andpreliminary results from the hyperandrogenic starvation NCI-H295R cell model. *Mol. Cell. Endocrinol.* **408**,124–132, https://doi.org/10.1016/j.mce.2014.12.015, (2025).
36. Zhu, W. et al. Oxidative stress increases the 17,20-lyase-catalyzing activity of adrenal P450c17 through p38α inthe development of hyperandrogenism. *Mol. Cell. Endocrinol.* **484**, 25–33,https://doi.org/10.1016/j.mce.2019.01.020 (2019).
37. Zhang, C., Wang, H., Yan, C., Gao, X. & Ling, X. Deregulation of RUNX2 by miR-320a deficiency impairssteroidogenesis in cumulus granulosa cells from polycystic ovary syndrome (PCOS) patients. Biochem. *Biophys. Res.Commun.* **482**, 1469–1476, https://doi.org/10.1016/j.bbrc.2016.12.059 (2017).

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Author contributions

YA: Conceptualization, investigation, methodology, analysis, writing-original draft TO: Investigation, analysis, material and data collection, writing-review & editing.

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Declarations

Competing interests

The authors declare no competing interests.

Patient consent

Informed consent was obtained from the patients

Additional information

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Correspondence and requests for materials should be addressed to Y.A.

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