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*Precision Clinical Medicine*, 2025, **8**: pbaf012

DOI: 10.1093/pcmedi/pbaf012

**Correspondence**

# A multi-omic study profiles women with PCOS to reveal unique gut microbiome compositions

Loukia Lili,[1,*,‡] Laura J. Kunces,[1,‡] Cem Meydan,[1,2,‡] Sarah Pesce,[1] Evan E. Afshin,[1,2,5] Nate Rickard,[1] Theresa M. Stujenske,[1,3] Christopher R. D'Adamo,[4] Joel T. Dudley,[1] Bodi Zhang,[1] Christopher E. Mason[1,2,*]

[1]Department of Research and Development, Thorne HealthTech, Inc., 152W 57th St, New York, NY 10019, USA
[2]Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY 10021, USA
[3]Center for Fertility Awarness Education and Research, College of Osteopathic Medicine, Duquesne University, Pittsburgh, PA 15282, USA
[4]Department of Family & Community Medicine, University of Maryland School of Medicine, Center for Integrative Medicine, 520 W. Lombard Street, Baltimore, MD 21201, USA
[5]Department of Ophthalmology, SUNY Downstate Medical Center, Brooklyn, NY 11203, USA
*Corresponding authors: Loukia Lili, loukialili@gmail.com; Christopher E. Mason, christopher.e.mason@gmail.com
‡Loukia Lili, Laura J. Kunces, and Cem Meydan contributed equally to this work.

Dear Editor,

Polycystic ovary syndrome (PCOS) is a prevalent endocrine–metabolic disorder, impacting an estimated 6%–10% of reproductive-age women worldwide. Characterized by hyperandrogenism, oligomenorrhea or amenorrhea, and polycystic ovarian morphology, PCOS is associated with infertility, obesity, insulin resistance, and increased cardiovascular risk [1]. Despite its high prevalence and substantial clinical burden, PCOS remains underdiagnosed and heterogeneously managed, largely due to its multifactorial etiology and variable phenotypic expression [2].

To elucidate molecular underpinnings and identify candidate precision interventions, we conducted a comprehensive multi-omic study recruiting 60 women with self-reported PCOS and 25 age-matched controls. Our integrative analyses combined: (i) detailed blood biochemistry and steroid hormone profiling; (ii) low-coverage whole-genome sequencing of saliva samples with imputation to assay >5 million single-nucleotide polymorphisms (SNPs); and (iii) high-resolution fecal shotgun metagenomic sequencing with taxonomic and functional profiling. In addition, validated questionnaires captured dietary habits, gastrointestinal symptoms, and quality-of-life metrics, enabling correlative analyses across data modalities.

This prospective, observational cohort study (IRB HP-00 089 723) enrolled women aged 18–40 years. PCOS diagnosis was based on patient self-report and confirmed by biochemical and ultrasonographic criteria where available; participants were further subclassified into Rotterdam phenotypes A–D when specific features were reported. Exclusion criteria encompassed recent hormone therapy, other endocrine or metabolic disorders, major surgery, pregnancy, or cancer history. Controls were recruited to match PCOS participants by age and ethnicity. Informed, written consent was obtained from all subjects. Blood samples ($n = 71$; 47 PCOS, 24 controls) were drawn fasting on menstrual cycle days 10–15 to standardize hormonal status. Assays included glucose, insulin, HbA1c, high-sensitivity C-reactive protein (hsCRP), 25-hydroxyvitamin D, lipid panel [high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, total cholesterol], and a steroid hormone panel [follicle-stimulating hormone (FSH), lutenizing hormone (LH), progesterone, dehydroepiandrosterone suflate (DHEA-S), total and free testosterone, estradiol, sex hormone-binding globulin (SHBG), and thyroid-stimulating hormone (TSH)]. Clinical net effect sizes and statistical significance (Wilcoxon rank-sum, $P$ value $< 0.05$) were computed for group comparisons. Saliva for genomic DNA ($n = 61$; 40 PCOS, 21 controls) underwent low-coverage (0.5×) sequencing on a NovaSeq6000. Reads were aligned to GRCh38, and genotype imputation used an optimized Li–Stephens algorithm [3] against large reference panels to recover >5 million high-confidence SNPs. We focused on 117 SNPs previously implicated in PCOS through genome-wide association studies (GWAS), testing allele enrichment via Fisher's exact test. Fecal specimens ($n = 57$; 36 PCOS, 21 controls) were self-collected using standardized kits, with microbial DNA extracted via automated magnetic-bead protocols. Library preparation used Illumina's NexteraXT chemistry; sequencing yielded 5–6 million 150 bp paired-end reads per sample. Quality control (FastQC), human read removal (BWA-MEM), and taxonomic classification (KrakenUniq) against a curated database enabled species-level resolution [4]. Functional predictions included bile acid metabolite inference. Differential abundance analyses employed DESeq2 [5] with trimmed mean of M-values normalization, adjusting for age and race ($P < 0.01$). Predicted fecal metabolites from the microbiome data were taken from the Gut Health Test reports (Thorne HealthTech, Inc.)

Age-matched PCOS and control groups (means 31.8 vs. 32.7 years, $P = 0.49$ at Cohen's $d = 0.18$) displayed significant differences in body composition and metabolism. PCOS cases had higher BMI (29.4 vs. 24.0 kg/m$^2$; $P = 0.008$; $d = -0.65$) and exhibited pronounced hyperandrogenism: DHEA-S increased by 76 μg/dl, total testosterone by 14.5 ng/dl, and free testosterone by 2.5 pg/ml (all $P < 0.01$). SHBG levels declined by 19 nmol/l ($P < 0.05$), and 25-hydroxy vitamin D decreased by 5.9 ng/ml. Insulin was

Received 6 May 2025; revised 1 June 2025; accepted 3 June 2025. published 10 June 2025

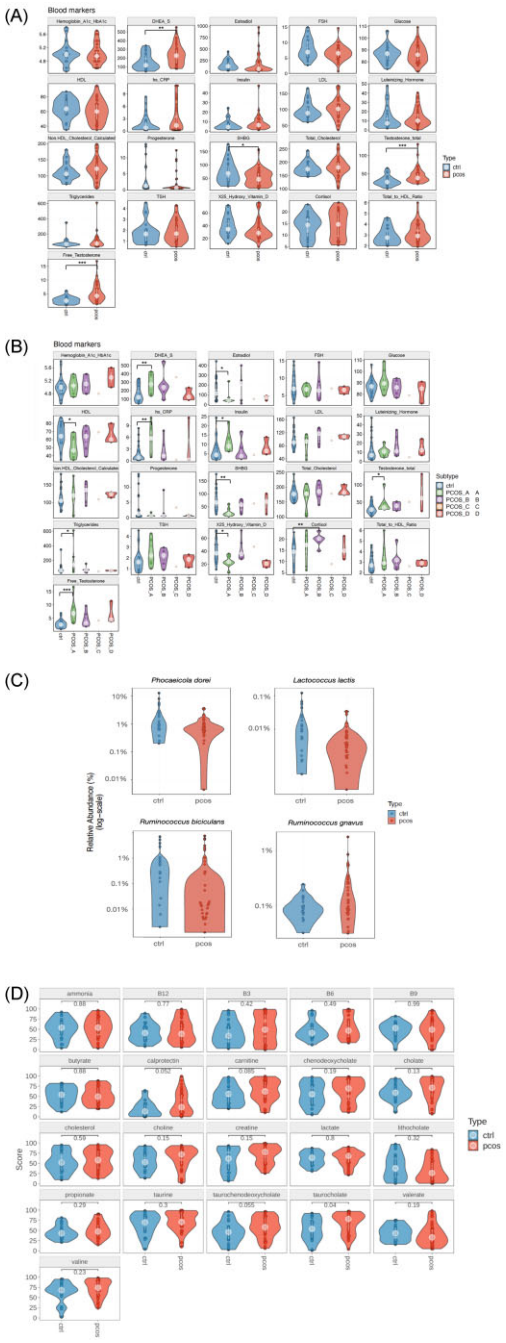


**Figure 1.** Multi-panel overview of key results. (**A**) Violin plots of overall blood markers highlighting significant differences in DHEA-S, total and free testosterone, and SHBG between PCOS and control participants. (**B**) Subtype-specific blood marker distributions for PCOS phenotypes A–D and controls, illustrating phenotype-dependent metabolic and hormonal shifts. (**C**) Relative abundances (log scale) of four gut bacterial species significantly altered in PCOS vs. controls: *Ruminococcus gnavus*, *Ruminococcus bicirculans*, *Phocaeicola dorei*, and *Lactococcus lactis* (DESeq2, FDR < 0.1). (**D**) Predicted fecal metabolite levels, showing elevated concentrations of taurocholate in PCOS (Wilcoxon rank-sum test, $P = 0.04$).

elevated (by 1.8 μIU/ml), and hsCRP rose by 1.2 mg/l, indicating insulin resistance and systemic inflammation (Fig. 1A and supplementary Table 1, see online supplementary material).

Within PCOS subtypes, phenotype A ($n$ = 8)—the fully expressed form with hyperandrogenism, anovulation, and polycystic ovaries—demonstrated the most extreme anomalies: triglycerides +108.2 mg/dl ($P < 0.05$), hsCRP +3.5 mg/l ($P < 0.01$), cortisol +2.2 μg/dl ($P < 0.01$); reduced HDL by 14.4 mg/dl ($P < 0.05$) and reduced 25-hydroxy vitamin D (−15.4 ng/ml, $P < 0.01$). Phenotype D ($n = 3$) had elevated free testosterone (+3.9 pg/ml) and lower vitamin D (–18.1 ng/ml). Phenotype B ($n = 7$) uniquely exhibited significant cortisol elevation (+6 μg/dl; $P < 0.01$). These results confirm that endocrine and metabolic dysregulation track with PCOS severity and phenotype [6] (Fig. 1B and supplementary Table 1).

Analysis of 117 GWAS-identified SNPs revealed significant enrichment of rs1159315, rs10818854, and rs10986105 ($P < 0.05$, supplementary Table 2, see online supplementary material). Each map to or near the DENND1A gene, a known regulator of androgen biosynthesis and insulin signaling. The presence of these alleles correlated with more pronounced hormonal and metabolic disturbances, suggesting a genetic predisposition that intersects endocrine and metabolic pathways.

Overall gut diversity did not differ significantly ($P > 0.05$, see online supplementary material) consistent with the findings of previous studies [7]. However, species-level analyses identified 45 taxa with differential abundance (FDR < 0.1). PCOS subjects exhibited increased *Ruminococcus gnavus* (also reported in human studies [8]) and decreased *Ruminococcus bicirculans*, *Phocaeicola dorei*, and *Lactococcus lactis* (Fig. 1C and supplementary Table 3, see online supplementary material). These organisms influence polysaccharide fermentation, mucosal integrity, and bile acid transformation [9, 10]. Correspondingly, predicted taurocholic acid levels were elevated in PCOS samples (median 152 vs. 98 mg/kg; $P = 0.04$), implicating disrupted bile acid–microbiome interactions that may drive metabolic endotoxemia and exacerbate insulin resistance (Fig. 1D).

Our multi-omic integration uncovers a self-reinforcing network in PCOS: genetic variants in DENND1A predispose to hyperandrogenism and insulin resistance, which in turn modulate gut microbiota composition and bile acid metabolism, fostering systemic inflammation. Targeted interventions could interrupt this cycle: (i) probiotic or prebiotic regimens to restore *L. lactis* and *R. bicirculans* populations; (ii) dietary or pharmacologic modulation of bile acid pools (e.g. bile acid sequestrants); and (iii) genotype-informed hormonal therapies to mitigate DENND1A-driven androgen excess.

This correspondence leverages comprehensive blood, genomic, and gut microbiome profiling to reveal coordinated disruptions across hormonal, genetic, and microbial domains in PCOS. Our findings support a precision-medicine framework, wherein multi-omic signatures guide stratification and personalized interventions, ultimately aiming to improve outcomes for women living with PCOS.

## Data availability

Demographic data and clinical measures are in supplemental Table 1; saliva SNPs in supplemental Table 2; differentially abundant taxa for PCOS vs. control samples in supplemental Table 3; detailed blood and questionnaire data are available upon request.

## Ethics approval and consent to participate

This study was conducted by an ethics approval IRB number HP-00089723 at the University of Maryland School of Medicine, IRB Approval Date of 8/23/2021. ClinicalTrials.gov ID: NCT04981275 (Registration Date 7/28/21). All participants in this study pro-

vided written consent that their data from this study may be published albeit in a way that personal information will remain confidential.

## Acknowledgments

This study was supported by the SBIR (small business innovation research program) grant of the National Institute of Health (grant No. 1R43HD103568-01 awarded to C.E.M.). We thank Thorne HealthTech, Inc. for providing at-home Gut Health kits.

## Author contributions

Loukia Lili (Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing), Laura J. Kunces (Data curation, Project administration), Cem Meydan (Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing), Sarah Pesce (Resources, Supervision), Evan E. Afshin (Project administration, Resources, Writing – review & editing), Nate Rickard (Funding acquisition, Resources, Supervision, Writing – review & editing), Theresa M. Stujenske (Conceptualization, Funding acquisition, Writing – review & editing), Christopher R. D'Adamo (Project administration, Resources, Supervision, Writing – review & editing), Joel T. Dudley (Conceptualization, Investigation, Resources, Supervision), Bodi Zhang (Conceptualization, Funding acquisition, Investigation, Resources), and Christopher E. Mason (Conceptualization, Funding acquisition, Investigation, Resources, Writing – review & editing)

## Supplementary data

Supplementary data is available at *PCMEDI* Journal online.

## Conflict of interest

Authors who were full-time employees at Thorne HealthTech, Inc. at the time of the grant award: L.L., L.J.K., S.P., N.R., T.M.S., and B.Z. Authors who were part-time employees at Thorne HealthTech, Inc. at the time of the grant award: C.M., E.A.E., J.T.D., and C.E.M. Authors who were shareholders of Thorne HealthTech, Inc. at the time of the grant award: L.L., C.M., N.R., J.T.D., B.Z., and C.E.M. The following author claims no conflicts of interest: C.R.D. The funding received for this work was exclusively supported by the NIH SBIR award. Thorne HealthTech, Inc. provided the at-home Gut Health kits. In addition, as an Editorial Board Member of *Precision Clinical Medicine*, C.E.M. was blinded from reviewing and making decisions on this manuscript.

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Received 6 May 2025; revised 1 June 2025; accepted 3 June 2025. published 10 June 2025