Quantitative Trait Meta-Analysis Identifies Rare Noncoding Variants Associated with Altered Hormone Levels in Polycystic Ovary Syndrome

Matthew Dapas[1], Ryan Sisk[1], Richard S. Legro[2], Margrit Urbanek[1,3,4], Andrea Dunaif[5,*], M. Geoffrey Hayes[1,3,6,*]

[1] Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL; [2] Department of Obstetrics and Gynecology, Penn State College of Medicine, Hershey, PA; [3] Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL; [4] Center for Reproductive Science, Northwestern University Feinberg School of Medicine, Chicago, IL; [5] Division of Endocrinology, Diabetes and Bone Disease, Icahn School of Medicine at Mount Sinai, New York, NY; [6] Department of Anthropology, Northwestern University, Evanston, IL; * These authors jointly supervised this work.

Background

- Polycystic ovary syndrome (PCOS) is a complex genetic disorder characterized by hyperandrogenism, chronic anovulation, and polycystic ovarian morphology.
- PCOS affects up to 15% of premenopausal women worldwide [1].
- Common risk alleles identified to date confer modest increases in disease risk and account for a small proportion of the estimated genetic heritability of PCOS.

Hypothesis

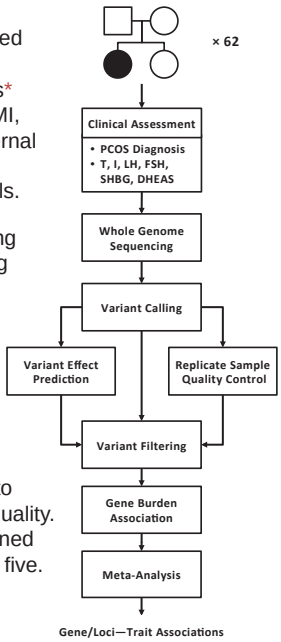
Rare variants contribute to the pathogenesis of PCOS.

Methods

Subjects: 62 two-generation families (avg. size = 4.2) with one or more affected daughters. All probands fulfilled NIH criteria for PCOS [2]. The following traits* were recorded for each subject: age, BMI, T, I, DHEAS, SHBG, LH, and FSH. Paternal hormone levels were excluded from all association tests, except for insulin levels.

Sequencing: Whole genome sequencing and variant calling were performed using the Complete Genomics pipeline on GRCh37. The Scripps Wellderly Genome Resource served as the control population [3].

Variant Selection: Rare variants (MAF ≤ 2%) were filtered for predicted, gene-specific deleterious effects [4-5]. Variants were filtered further according to Mendelian inheritance and variant-call quality. Optimal quality thresholds were determined using replicate samples from a family of five.



Association Testing: Variants in gene regions (including 3' UTR, introns, and 7.5kb upstream of 5' TSS) and sliding windows were tested for association with PCOS and its hormonal traits using a family-based variance component association test [6]. Age and BMI were included as covariates. Residuals were corrected for normality using an inverse-normal-transformation. Variants were weighted according to their predicted levels of deleteriousness [4]. Coding and noncoding variants were tested independently. Coding variant associations were further adjusted by relative variation intolerance [7].

Meta-Analysis: Associations between gene regions and distinct quantitative traits were combined into a single meta-statistic using a modified Fisher's combined probability test for correlated traits [8]. Results were corrected for multiple testing and genomic inflation, including all groupings with at least one variant. Only genes with rare, deleterious variants in at least 10% of cases were considered.

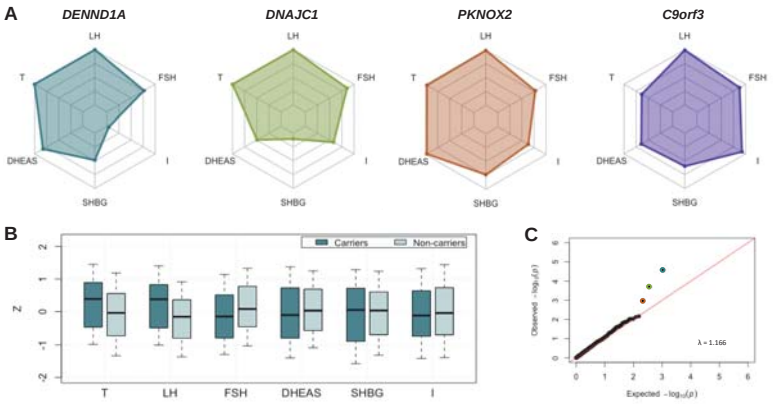
* T: testosterone; I: Insulin; LH: luteinizing hormone; FSH: follicle-stimulating hormone; SHBG: sex hormone-binding globulin; DHEAS: dehydroepiandrosterone

Results

Noncoding Quantitative Trait Association Meta-Analysis

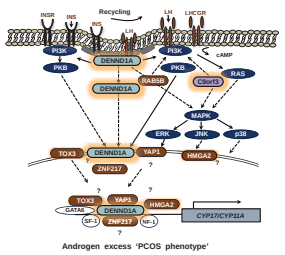
Chr	Gene / Locus	Length	Variants	Families[1]	Aff : Unaff OR[2]	*P*	P_{adj}
9	***DENND1A*** ***(q33.3)***	**550kb**	**30**	**47%**	**1.29** **[0.78 – 2.22]**	**2.66×10^{-5}**	**0.017**
10	***DNAJC1*** ***(p12.31)***	247kb	6	11%	1.40 [0.89 – 2.40]	1.95×10^{-4}	0.12
11	***PKNOX2*** ***(q24.2)***	269kb	18	39%	1.54 [0.91 – 2.81]	1.07×10^{-3}	0.67
9	***C9orf3***[3] ***(q22.32)***	420kb	9	19%	1.79 [0.74 - 5.04]	1.45×10^{-2}	1.00

1. Number of families with ≥1 gene variant present in multiple generations
2. Odds ratio estimates based on number of variants in affected vs. unaffected subjects
3. C9orf3 included for reference as next highest-ranking PCOS GWAS gene in meta-analysis results (#12 / 542)



A) Relative ranks of individual trait associations for top genes and *C9orf3*
B) Residual trait differences in subjects with ≥1 rare variant in *DENND1A*
C) P-values for meta-analysis of family-based SKAT trait associations

Hypothesized Androgen Signaling Cascade [9]



- Multiple association studies have found associations between PCOS and common, noncoding variants in both *DENND1A* and *C9orf3* [10-13].
- PCOS theca cells have higher levels of a *DENND1A* isoform, DENND1A.V2 [14].
- Overexpression of DENND1A.V2 in theca cells leads to increased androgen biosynthesis [14].

Conclusions

- Our findings suggest that rare, noncoding variants in *DENND1A* contribute to elevated androgen and LH levels in PCOS.
- The *DENND1A* and *C9orf3* variant associations further strengthen the evidence for their integral involvement in PCOS pathogenesis.
- A family-based, quantitative trait meta-analysis can be a powerful approach to rare variant association testing.

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