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

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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 Wellelry 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 normally 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 variance 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.

Results

Noncoding Quantitative Trait Association Meta-Analysis

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene / Locus</th>
<th>Length</th>
<th>Variants</th>
<th>Families</th>
<th>Aff : Unaff</th>
<th>OR</th>
<th>P</th>
<th>PD</th>
<th># Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>DENND1A</td>
<td>559kb</td>
<td>30</td>
<td>47%</td>
<td>1.29</td>
<td>0.76 – 2.32</td>
<td>2.66 x 10^-4</td>
<td>0.017</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>DNAJC1</td>
<td>247kb</td>
<td>6</td>
<td>11%</td>
<td>1.40</td>
<td>0.91 – 2.81</td>
<td>1.95 x 10^-4</td>
<td>0.12</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>PKNOX2</td>
<td>269kb</td>
<td>18</td>
<td>39%</td>
<td>1.54</td>
<td>0.91 – 2.81</td>
<td>1.07 x 10^-4</td>
<td>0.67</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>C9orf3</td>
<td>430kb</td>
<td>9</td>
<td>19%</td>
<td>1.79</td>
<td>0.74 - 5.04</td>
<td>1.45 x 10^-2</td>
<td>1.00</td>
<td>3</td>
</tr>
</tbody>
</table>

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 [11/542]

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.

References

3. Erikson, 2016, Cell 165:1002
5. Huang, 2017, Nat Genet 49:618
7. Petrovska, 2013, FCS Genet 9
8. Yang, 2016, BMC Bioinformatics 17:19
9. McAllister, 2015, TEM 26
12. Welt, 2012, J Clin Endocrinol Metab 97
14. Overexpression of DENND1A.V2 in theca cells leads to increased androgen biosynthesis [14].

Hypothesized Androgen Signaling Cascade

• 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].