Update on PCOS Research

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Conflicts

- Consultant: Euroscreen, AstraZeneca, Ferring, Clarus, Kindex, Takeda
- Funding: AstraZeneca, Ferring, NIH, Tobacco Settlement Funds

Off Label Uses

- Metformin, Other Anti-Diabetic drugs, and Aromatase Inhibitors are not FDA approved to treat infertility and/or PCOS
My Agenda for PCOS Research

- Cause of PCOS
  - Genetic
  - Imprinted (pregnancy)
  - Environmental

- Treatment of PCOS
  - Adolescents
  - Infertility
  - Obesity/Insulin Resistance

- Long term Health Effects
  - Cardiovascular and Cancer
  - Effects on Children
Evidence that PCOS is a genetic disease

- Familial clustering of PCOS and reproductive and metabolic abnormalities
- Persistence of abnormalities in long term culture of PCOS tissue
- Multi-system involvement
- Identification of genes that cause or modify the syndrome
How best to understand PCOS?

Hypothesis Driven Experiments

or

Discovery Driven - “omics”

Clinical Trials

Genome Wide Association Studies
The candidate gene approach to the genetics of PCOS has yielded few insights, whether from case control genetic association studies or from family studies.
Most if not all Candidate Genes do not hold up under replication studies.
To Identify PCOS Genes, We Want…

- Genome wide association study (GWAS)
  - Genetic Case/Control study
    - No Families Needed!
  - Large sample size
  - Full genome scan
  - Multi-stage design with replication built in to avoid false positives
Promise of Genome-Wide Association Studies

1. Discovery of Candidate Genes
2. Explore Pathophysiology/Treatment
   • Etiology
   • Pharmaceutical targets
3. Establish utility of genetic markers for risk prediction
   • For individual or public health decisions
   • For response to medication/adverse effects
Chinese GWAS in PCOS identifies 11 risk alleles

8,226 cases and 7,578 controls

Chen ZJ, Nature Genetics, 2012
The PCOS GWAS did not identify genes previously associated with Type 2 Diabetes or Obesity

Suggest: PCOS is a Unique Disorder and Environmental conditions may contribute to the association with Diabetes and Obesity in women with PCOS
GWAS Significant Associations with PCOS

<table>
<thead>
<tr>
<th>Fit with our understanding of PCOS</th>
<th>Novel Candidates of Unknown Action</th>
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<tbody>
<tr>
<td>FSHR (FSH Receptor)</td>
<td>DENND1A</td>
</tr>
<tr>
<td>LHCGR (LH/ hCG Receptor)</td>
<td>THADA</td>
</tr>
<tr>
<td>INSR (Insulin Receptor)</td>
<td>C9orf3</td>
</tr>
<tr>
<td></td>
<td>YAP1</td>
</tr>
<tr>
<td></td>
<td>RAB5B</td>
</tr>
<tr>
<td></td>
<td>TOX3</td>
</tr>
<tr>
<td></td>
<td>SUMO1P1</td>
</tr>
<tr>
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<td>HMGA2</td>
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Partial Replication of Chinese GWAS Findings in European Population

Esp DENND1A
What is DENND1A

- A gene which encodes the protein connecdenn 1
- Has a clathrin binding domain and is thought to facilitate endocytosis and receptor-mediated turnover
  - Three receptor genes associated with PCOS (LH, FSH, and INS)
- Expressed in androgen secreting tissues
  - Testes, Thecal cells, Adrenal cells
Use a human derived thecal cell system to explore the role of DENND1A in androgen production

There are two alternate transcripts from the gene

- Variant 1 (V1): A long one (>1,000 a.a.)-predominant form
- Variant 2 (V2): A short one (~ 500 a.a.)
DENND1A V2 Overexpressed in the PCOS Ovary

Mcallister et al, PNAS, 2014
Forced expression of DENND1A.V2 with an adenoviral vector in normal theca cells results in augmented androgen and progestin production.

Mcallister et al, PNAS, 2014
Silencing of DENND1A results in normal thecal cell androgen production and phenotype

- Knock down with both shRNA and with an IgG specific Antibody against DENND1A V2.

Mcallister et al, PNAS, 2014
DENND1A is Overexpressed in Exosomes from Urine in Women with PCOS

Mcallister et al, PNAS, 2014
Conclusion

- Our findings establish that increased DENND1A.V2 expression is sufficient to promote a PCOS phenotype in human theca cells
  - PCOS as a hyperandrogenic disorder
- This can inform development of diagnostic tests as well as novel therapeutic interventions.
Environmental Exposure Leading to PCOS

- In Utero
  - Birthweight
    - Low Birthweight (SGA)
  - Prenatal Androgenization
- Exposure During Infancy/Adolescence
  - Valproic Acid
  - Bisphenol A (BPA)
  - Polycyclic Aromatic Hydrocarbons or Ozone (Air Pollution)
The Barker Hypothesis: Thrifty Phenotype
Normal Birthweights in PCOS families compared to controls and U.S. Population Norms
Maternal Androgens are only mildly elevated in PCOS during Pregnancy

Sir-Petermann et al, Hum Reprod 2002
How to treat Infertility in PCOS?

Insulin Sensitizing Agents, GLP-1 Analogues, Weight loss, Exercise

Clomiphene, GnRH, Aromatase Inhibitor

FSH, Ovarian Surgery
Increasing Utilization of IVF for Ovulatory Dysfunction: More Multiple Pregnancies

- PCOS is the most common cause of anovulatory infertility (80%)
- In 2011 26% of ART pregnancies are twins and 1.3% are high order multiples

CDC Assisted Reproductive Technology National Summary

<table>
<thead>
<tr>
<th>Using own Eggs</th>
<th>2005</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of IVF cycles</td>
<td>89,385</td>
<td>101,213</td>
</tr>
<tr>
<td>Ovulatory Dysfunction (%)</td>
<td>8%</td>
<td>14%</td>
</tr>
</tbody>
</table>
Clomiphene Superior to Metformin for Live Birth: PPCOS I

P-value: <0.001

Legend:
- Green: Clomiphene
- Red: Metformin
- Blue: Combined

Legro et al, NEJM 2007
Does Ovulation = Live Birth?

- Ovulation
  - Fertilization
    - Implantation
      - Fetal Viability
        - Healthy Liveborn

Legro et al, Hum Reprod, 2004
Fecundity per Ovulation is Best with Clomiphene

* Significant compared to baseline

Legro et al, NEJM, 2007
All ovulations are not alike!!

Ovulation is a surrogate outcome for anovulatory infertility
## Live Birth Results of Double Blinded RCTs of Metformin and Clomiphene (N = 100)

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Treatments</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palumba et al, 2006, JCEM</td>
<td>100</td>
<td>Metformin vs CC</td>
<td>Metformin superior to CC</td>
</tr>
<tr>
<td>Moll et al, 2006, BMJ</td>
<td>225</td>
<td>CC vs Metformin/CC</td>
<td>No benefit of Metformin/CC</td>
</tr>
<tr>
<td>Legro et al, 2007, NEJM</td>
<td>626*</td>
<td>CC vs Metformin vs CC/ Metformin</td>
<td>No benefit of Metformin/CC AND Clomiphene superior to metformin</td>
</tr>
<tr>
<td>Mohd Zain et al, Fertil Steril 2008</td>
<td>125</td>
<td>CC vs Metformin vs CC/ Metformin</td>
<td>No benefit of Metformin/CC AND Clomiphene superior to metformin</td>
</tr>
<tr>
<td>Morin-Papunen et al, JCEM, 2012</td>
<td>320</td>
<td>Metformin/Placebo and then after 3 mos other treatments</td>
<td>No difference as single agent, Metformin beneficial as adjuvant in obese</td>
</tr>
</tbody>
</table>

*Adequately powered and designed to detect differences in live birth rates*
CONCLUSIONS: Therefore, the role of metformin in improving reproductive outcomes in women with PCOS appears to be limited.

Clomiphene Exacerbates Metabolic Dysfunction Relative to Metformin

* Significant compared to baseline

Legro et al, NEJM, 2007
Rationale for Aromatase Inhibitors (Letrozole) in Ovulation Induction

- Non steroidal
- Interferes with inappropriate estrogen feedback at the hypothalamus
  - Transient increase in androgens
- Shorter half life than clomiphene
- No adverse endometrial effects
  - i.e. “thin” endometrium
- Concerns about teratogenicity?
Primary Outcome: Live Birth

Rate Ratio for Live Birth (LTZ vs CC), 1.44; 95% confidence interval, 1.10 to 1.87

Legro et al, NEJM, 2014
Tertile Analysis by BMI

Lower Tertile

Mid Tertile

Upper Tertile

Legro et al, NEJM, 2014
Ovulation Rates Per Cycle Superior with Letrozole

* P < .01 vs CC

Legro et al, NEJM, 2014
Conclusions

- PCOS related anovulatory infertility can be treated as a reproductive disorder with treatments that impact the hypothalamic-pituitary-axis alone
  - Letrozole and Clomiphene as first line agents
- There is little *reproductive* benefit to treating PCOS with metformin or similar agents.
PCOS-Health Risks

- **Accepted**
  - Type 2 Diabetes
  - Endometrial Cancer

- **Uncertain**
  - Cardiovascular disease
  - Ovarian/Breast Cancer
High Prevalence of Glucose Intolerance in PCOS

**Study Site**
- University of Chicago**: 122
- Penn State Univ*: 144
- Mt Sinai*: 110
- Rezulin Collab Grp*: 408
- **TOTAL**: 784

**Percent**

- **Normal**
- **IGT**
- **Type 2 DM**

Reproductive Abnormal

Metabolic Abnormal

Birth  Puberty  Menopause  Death
## Diagnostic Criteria: No Menopausal Phenotype

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<tbody>
<tr>
<td>Oligomenorrhea</td>
<td>Yes</td>
<td>Maybe</td>
<td>Maybe</td>
</tr>
<tr>
<td>Hyperandrogenism (Biochemical and/or Clinical)</td>
<td>Yes</td>
<td>Maybe</td>
<td>Yes</td>
</tr>
<tr>
<td>Polycystic ovaries</td>
<td>No</td>
<td>Maybe</td>
<td>Maybe</td>
</tr>
</tbody>
</table>
Increased Cardiovascular Event Rate in Menopausal women with Androgen Excess and History of Irregular Menses

Shaw et al, JCEM 2008
No Change in CVD Risk Factors over 10 years in Women with and without PCOS in SWAN Cohort

N = 1166 women

Polotsky et al, JCEM, 2014
Lack of Increased CVD Hazard Ratio According to PCOS Phenotypes (compared to normal controls) in SWAN Cohort

Polotsky et al, JCEM, 2014
Summary Recommendation: Need long term epidemiologic studies to determine CVD and Cancer Risks
NIH Panel: Research Priorities

- Develop evidence based diagnostic criteria
  - Better androgen assays to diagnose androgen excess
  - Better tests of ovulatory function than history of irregular menses
  - Better ultrasound or imaging criteria for polycystic ovaries
  - Better name for the syndrome.
NIH Panel: Research Priorities

- Identify optimal therapies and best practices to achieve successful pregnancy
- Establish the prevalence of abnormal glucose tolerance in women wishing to conceive, and determine whether treatment of abnormal glucose tolerance prior to or early post-conception alters maternal-fetal outcomes.
NIH Panel: Research Priorities

- Conduct sufficiently large, well-controlled epidemiologic studies determining the prevalence, phenotypes, and morbidities of PCOS in multiethnic longitudinal studies to determine:
  - If it is associated with cardiovascular disease and cancer
  - If treatment of metabolic abnormalities reduces the risk of cardiovascular and diabetic complications and cancer.
Research is a Team Sport
Your Research Ideas and Questions