Defining the Role of Sex Hormones in PAH: Science and Career Development

Eric D. Austin, MD MSCI
Assistant Professor of Pediatrics
Director, Vanderbilt Pediatric PH & Pulmonary Vascular Disease Program
Vanderbilt University School of Medicine
eric.austin@vanderbilt.edu
No relationships to disclose

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- Personal relationships with tobacco industry entities: none
- Off-Label Disclosure: not needed
- I am still on this career development journey
Successful Physician-Scientist Dev’t

• Be **Proactive** in carving out your career path
• *Seek and Cultivate* Mentors
• Establish an area of **Expertise**
• Medical research is a **Team Sport**
• Aggressively pursue **Funding Opportunities**
• Navigate the diverse missions of academic medicine with **Self-Discipline**

Proactive & Mentorship
Focus on PH, specifically PAH

1. PULMONARY ARTERIAL HYPERTENSION (PAH)
   - Idiopathic PAH
   - Heritable PAH (Family and/or gene mutation)
   - Drug- and toxin-induced
   - Associated with:
     - Connective tissue diseases
     - Congenital heart diseases
     - HIV
     - Portal hypertension
     - Schistosomiasis

1'. PULMONARY VENO-OCCULSIVE DZ (PVOD) AND/OR PULMONARY CAPILLARY HEMANGIOMATOSIS (PCH)

1''. PPHN

2. PH DUE TO LEFT HEART DISEASE
   - Systolic dysfunction
   - Diastolic dysfunction
   - Valvular disease

3. PH DUE TO LUNG DISEASES AND/OR HYPOXIA
   - Bronchopulmonary dysplasia (BPD), COPD
   - Interstitial Lung Disease (ILD)
   - Other lung dz’s w/ mixed restrictive/obstructive defects
   - Sleep-disordered breathing
   - Alveolar hypoventilation disorders
   - Chronic exposure to high altitude
   - Developmental lung abnormalities

4. CHRONIC THROMBOEMBOLIC PH (CTEPH)

5. PH UNCLEAR MULTIFACTORIAL MECHANISMS
   - Hematologic d/o’s: hemolysis, myeloproliferative, splenectomy
   - Systemic d/o’s: sarcoidosis, LCH, LAM, NF, vasculitis
   - Metabolic d/o’s: glycogens storage dz, Gaucher’s
   - Thyroid
   - Others: tumurol obstruction, fibrosing mediastinitis, CR

Simonneau et al, JACC 2013
Established Risk Factors for PAH

- Genetic susceptibility
  - BMPR2 gene mutation (TGFβ genes, CAV1, KCNK3)
- Female
- Connective tissue disease
- Hereditary hemorrhagic telangiectasia (HHT)
  - ALK1 & ENG gene mutations
- Portal Hypertension
- Drug & toxin exposures
  - aminorex, fenfluramine, dexfenfluramine
**BMPR2 HPAH:**
reduced penetrance and variable expressivity

A Large HPAH Family: 36 Confirmed PAH
29 Female
7 Male

Updated Summer 2011 (Pulm Cir)—initial pub by Loyd, Primm, Newman *Am Rev Resp Diseases* 1984
BMPR2 HPAH: penetrance higher females

A Large HPAH Family: 36 Confirmed PAH
29 Female
7 Male

K12 Funding (2006): Genetic Modifiers of PAH

Updated Summer 2011 (Pulm Circ)—initial pub by Loyd, Primm, Newman Am Rev Resp Diseases 1984
Female predominance suggests a role for Sex Hormones

- Penetrance of *BMPR2* mutations not equal for females (higher) and males
- Most forms of PAH are female predominant
- Gene expression data suggestive that Sex Hormone Metabolism is different in PAH patients
  - *CYP1B1* expression
- Conflicting data about estrogens and PH in animal models
- Survival
**Pursuit: Estrogens**

ERα ERβ → GPR30 → Kinases → Rapid Nongenomic Effects
1. Vasodilation
   - increase eNOS activity
   - increase PGI2
2. Promote angiogenesis
3. Modify injury response

**Genomic Effects**
Alter gene expression
- pro-proliferative
- pro-migratory

SERMs modify these effects, as do certain Estrogen Metabolites

Sex Hormone Metabolism

- Estrone (E₁)
  - 17β-HSD1
  - CYP1B1, CYP1A1
  - COMT
  - 2-Hydroxyestrogens (2-OHE₁/₂)
  - 2-Methoxyestrogens (2-MeOE₁/₂)
  - COMT
  - 4-Hydroxyestrogens (4-OHE₁/₂)
  - 4-Methoxyestrogens (4-MeOE₁/₂)

- 17β-Estradiol (Estradiol, E₂)
  - CYP1B1, CYP1A1
  - CYP3A4, CYP1A1
  - 16α-Hydroxyestrone (16α-OHE₁)
  - 17β-HSD

- Testosterone
  - CYP19A1 (Aromatase)

- Estriol (E₃)
  - COMT
  - 17β-HSD1

CYP1B1, CYP1A2, COMT, 17β-HSD, Aromatase
Sex Hormone Metabolism

- Estrone (E₁)
  - 17β-HSD1
  - CYP1B1, CYP1A1
  - COMT

- 17β-Estradiol (Estradiol, E₂)
  - CYP1B1
  - CYP1A2
  - CYP1A1

- Testosterone
  - CYP19A1 (Aromatase)

- 2-Hydroxyestrogens (2-OHE₁/₂)
  - 2-Methoxyestrogens (2-MeOE₁/₂)

- 4-Hydroxyestrogens (4-OHE₁/₂)
  - 4-Methoxyestrogens (4-MeOE₁/₂)

- 16α-Hydroxyestrone (16α -OHE₁)
  - Estriol (E₃)

- 2-, 4- Estrogens Antagonize ER
  - ‘anti-proliferative’
  - ‘apoptotic’

- 16-Estrogens ER Agonists
  - ‘pro-proliferative’
  - ‘anti-apoptotic’
**CYP1B1 N453S : N/N genotype associated with lower CYP1B1 activity**

<table>
<thead>
<tr>
<th>Females</th>
<th>BMPR2-PAH n (%)</th>
<th>BMPR2-Healthy n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/N</td>
<td>46 (74%)</td>
<td>10 (42%)</td>
<td>0.005</td>
</tr>
<tr>
<td>N/S or S/S</td>
<td>16 (26%)</td>
<td>14 (58%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Males</th>
<th>BMPR2-PAH n (%)</th>
<th>BMPR2-Healthy n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/N</td>
<td>18 (60%)</td>
<td>17 (71%)</td>
<td>0.407</td>
</tr>
<tr>
<td>N/S or S/S</td>
<td>12 (40%)</td>
<td>7 (29%)</td>
<td></td>
</tr>
</tbody>
</table>

Females with N/N genotype:
Unadjusted OR = 4.1

* Hannah, *Cancer Res* 2000
Austin, *Eur Resp J* 2009
Sex Hormone Metabolism

- **Estrone (E₁)**
- **Beta-Estradiol (E₂)**
- **Testosterone**

**Enzymes Involved**
- CYP1B1
- CYP1A2
- COMT
- 17β-HSD1
- 17β-HSD
- CYP19A1 (Aromatase)

**Metabolites**
- Estrone (E₁)
- Beta-Estradiol (E₂)
- Testosterone
- Estriol (E₃)

**2-Hydroxyestrogens (2-OHE₁/₂)**
- Estrone (E₁)
- Beta-Estradiol (E₂)

**4-Hydroxyestrogens (4-OHE₁/₂)**
- Estrone (E₁)
- Beta-Estradiol (E₂)

**2-Methoxyestrogens (2-MeOE₁/₂)**
- Estrone (E₁)
- Estriol (E₃)

**4-Methoxyestrogens (4-MeOE₁/₂)**
- Estrone (E₁)
- Beta-Estradiol (E₂)

**Effects**
- 2-, 4- Estrogens Antagonize ER 'anti-proliferative' 'apoptotic'
- 16-Estrogens ER Agonists 'pro-proliferative' 'anti-apoptotic'

**Pathways**

Make more 16-estrogens
Sex Hormone Metabolism

Global Hypothesis:
A highly estrogenic milieu promotes PAH

- Epidemiologic exposures
- Hormone levels
- In vitro and animal model studies

2-Hydroxyestrogens (2-OHE₁/₂)

16-α-Hydroxyestrone (16α-OHE₁)

2-Methoxyestrogens

K23, CTSA, foundation, & P01 (Loyd) Funding:
Sex Hormones in PAH

2-, 4- Estrogens Antagonize ER
‘anti-proliferative’ ‘apoptotic’

2-Methoxyestrogens

Testosterone

CYP1B1

CYP1A2

COMT

17-β-HSD

17-β-HSD1

Aromatase (CYP19A1)

[Image]

Sex Hormone Metabolism

2-, 4- Estrogens Antagonize ER
‘anti-proliferative’ ‘apoptotic’

2-Methoxyestrogens

Testosterone

CYP1B1

CYP1A2

COMT

17-β-HSD

17-β-HSD1

Aromatase (CYP19A1)
Ratio of ‘2-estrogens’ / ‘16-estrogens’: Lower in PAH Patients

Female Data Shown, but similar in males and IPAH cases ($P = 0.05$)
Team Sport: James West’s Bmpr2R899x transgenic murine model:
2ME versus 16αOHE

- Male mice
  - 1.25 mg/hr x 4 weeks
  1. vehicle
  2. 2ME
  3. 16αOHE
  4. 2ME + 16αOHE

Hypothesis: 2-estrogens (2ME) protective while 16-estrogens (16αOHE) detrimental

16αOHE increases penetrance

A

RVSP (mmHg)

B

PVR (dyn*s/cm^5)

RVSP mmHg

PVR dyn*s/cm^5

Conclusions

• **Proactive & Mentored** pursuit of a pressing question in the PAH field w/ **Team** approach
  
• Sex Hormone contributes to PAH
  – Skew toward ‘16-estrogens’ in humans

• ‘16-estrogens’ amplify Bmpr2 murine model penetrance

• Estrogen antagonism may be protective
  – Long term effects unknown, incl. RV function

• Precise mechanisms active area investigation
  – Pulmonary vasculature
  – RV

• R01 application exploring the interplay between Sex Hormones, Cellular Metabolic Defects, and PAH
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