Recent Studies on the genetics of NAS

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OB/GYN grand rounds
• I have no conflicts associated with these studies

• The studies were funded by NIH grants 2U54GM104942-02, 5P20GM103434, and 1P20GM121299
Objectives

1. Discuss issues associated with NAS
2. Outline the importance of studying genetics for various aspects of OUD.
3. Describe recent genetic studies with the MARC program
Neonatal Abstinence Syndrome (NAS)

- Passive exposure of the newborn occurs when a mother uses a neuroactive drug during her pregnancy

- When the infant is deprived of these substances through the birthing process, a withdrawal syndrome may develop

- Classic NAS consists of a wide variety of CNS signs of irritability, GI problems, autonomic signs of dysfunction, and respiratory symptoms

- The hallmark of neonatal withdrawal is a striking disorder of movement, most aptly termed “jitteriness”

- Autonomic over-reactivity is typically exhibited by yawning, sneezing, mottling and fever

- Cerebral irritation results in an irritable and hypertonic infant
NAS Prevalence

NAS incidence rates 2012–2013


NAS Births per 1000 at CHH

p<0.0001 for trend
Neonates treated for NAS with Methadone at CHH

<table>
<thead>
<tr>
<th>Year</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Births</td>
<td>2436</td>
<td>2494</td>
<td>2641</td>
<td>2801</td>
<td>2755</td>
<td>2810</td>
</tr>
<tr>
<td>Prenatal Exposures</td>
<td>71</td>
<td>79</td>
<td>111</td>
<td>459</td>
<td>547</td>
<td>522</td>
</tr>
<tr>
<td>NAS per 1000</td>
<td>29.1</td>
<td>31.7</td>
<td>42.0</td>
<td>163.9</td>
<td>198.5</td>
<td>185.8</td>
</tr>
<tr>
<td>Pharmacologically Treated NAS</td>
<td>83</td>
<td>102</td>
<td>135</td>
<td>214</td>
<td>224</td>
<td>265</td>
</tr>
<tr>
<td>Treated NAS per 1000</td>
<td>34.1</td>
<td>40.9</td>
<td>51.1</td>
<td>76.4</td>
<td>81.3</td>
<td>94.3</td>
</tr>
<tr>
<td>%multi-Substance</td>
<td>46%</td>
<td>47%</td>
<td>58%</td>
<td>54%</td>
<td>52%</td>
<td>53%</td>
</tr>
</tbody>
</table>
From a state perspective
Drug combinations alter NAS profile

% of neonates still receiving medical treatment at CHH for NAS for years 2014-2015. Black line represents total NAS babies over this period, while the red is neonates that had exposure to gabapentin in utero. The curves are significantly different ($p<.0001$ Chi Square), with median LOS of 27 (NAS) and 54 (gabapentin) days.
What are the potential long term consequences of inter uterine exposure
So what are we doing in Biomedical Sciences?

• Two primary research projects
  • Opioids + gabapentin in neuronal development
    • Animal model
  • Genetic markers
How does prenatal drug exposure affect synaptic development *in vivo*?

Taylor Boggess

Drug treatments:
- 5mg/kg buprenorphine,
- 30mg/kg gabapentin,
- or both

Drug/Vehicle
Condensed Milk Mixture (Daily from E7 to P11)

Pregnant Dam α2δ-1 +/-

(Bred with α2δ-1 +/- Male)

α2δ-1 +/+  
α2δ-1 +/-  
α2δ-1 -/-  
M / F

P21
Pups sacrificed, brains isolated and fixed in paraformaldehyde

Sectioning, immunohistochemical (IHC) staining, and confocal microscopy

Belzung, Turiault & Griebel, *Pharm Biochem Behav* 2014
Addiction-related changes in astrocytes and gene transcription

NeuN
ΔFosB
GFAP

Lasting (?) increases in gliosis and addiction-associated gene expression pathways
How does prenatal drug exposure affect synaptic development \textit{in vivo}?

PND 21 α2δ-1 +/- male and female mice

Taylor Boggess

Prenatal drug exposure increases excitatory connectivity in reward-associated brain regions
Are inhibitory synapses also impacted by drugs of abuse?

PND 21 α2δ-1 +/- male and female mice

Taylor Boggess

Decreased inhibitory connectivity in (some) reward-associated brain regions

*(overall hyperexcitability)*
Making a Brain in a Dish
CD34 → hPSC → Organoid Workflow

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Ania Mazur
Taylor Boggess
Shanai Brown
Jesse Stevens
Ean Bills

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Rationale:

- Only ~50% of exposed infants have pharmacologically treatable NAS, but we can't predict which
  - Fetal development and pregnancy outcomes are regulated by a complex interplay between both mother and fetus.
  - Alterations in the expression patterns of genes in either the neonate or mother can have profound effects on development.
  - It is thus important to consider the maternal-neonate dyad as a unit as well as individually.

Hypothesis

NAS intensity is in part due to variants in genes involved in maternal and neonate metabolism of opioids and in genes related to brain and placental development.
Study design

• Patients recruited at 36 weeks of pregnancy from WVU and Marshall MAT programs.
• DNA collected by cheek swab and isolated. Whole exomes sequenced by the WV-CTSI genomic core at 100x coverage using an Illumina HiSeq.
• Once the sequences were obtained the dyads’ exomes were analyzed using a logistic regression model for needing or not needing treatment at each genomic locus. Maternal dominant model is being presented.
• Network analysis of various aspects of OUD using STRING via Cytoscape.
## Patients in Cohort

<table>
<thead>
<tr>
<th></th>
<th>No treatment (+/- sem)</th>
<th>Neaded treatment (+/- sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Dyads</td>
<td>14 (61%)</td>
<td>9 (39%)</td>
</tr>
<tr>
<td><strong>Mother</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age years</td>
<td>29.3 (0.76)</td>
<td>29.3 (1.4)</td>
</tr>
<tr>
<td>BUP dose mg</td>
<td>10.4 (1.03)</td>
<td>12.9 (1.3)</td>
</tr>
<tr>
<td># of previous treatment programs</td>
<td>1 (0.3)</td>
<td>1.6 (0.6)</td>
</tr>
<tr>
<td>Weeks of treatment</td>
<td>23.4 (3.1)</td>
<td>24.9 (3.4)</td>
</tr>
<tr>
<td><strong>Neonate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Age weeks</td>
<td>38.9 (0.4)</td>
<td>38.1 (0.4)</td>
</tr>
<tr>
<td>Weight g</td>
<td>3368 (155)</td>
<td>2985 (179)</td>
</tr>
<tr>
<td>Length cm</td>
<td>49.4 (1.0)</td>
<td>47.9 (1.3)</td>
</tr>
</tbody>
</table>

Comparison of length of stay for neonates that did or did not require treatment with an opioid to control their NAS. Scatter plot for each group with associated mean and SD. LOS was significantly longer in neonates that required opioid therapy (*p<0.01 Students t-test).

Comparison of head circumference at birth in neonates that did or did not require treatment with an opioid to control their NAS. Scatter plot for each group with associated mean and SD. Head circumference was significantly smaller in neonates that required opioid therapy (*p<0.05 Students t-test).
No difference between our groups in SNPs for Opioid receptors or metabolizing enzymes!

SNPs linked to altered OPRM1 function associated with either pain response or addiction
### Polymorphisms more common in mothers of treated or non-treated neonates

**Maternal treated neonates**

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs#</th>
<th>OR</th>
<th>Gene</th>
<th>rs#</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR4Q2</td>
<td>12587697</td>
<td>5.5</td>
<td>FTCD</td>
<td>61735841</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>80024306</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12586792</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN1B</td>
<td>67701503</td>
<td>10</td>
<td>RNF21S</td>
<td>5749088</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>67486287</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNN2</td>
<td>2304260</td>
<td>7</td>
<td>DSE</td>
<td>10485183</td>
<td>2</td>
</tr>
<tr>
<td>APOL4</td>
<td>6000173</td>
<td>5.1</td>
<td>PHIP</td>
<td>9350797</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2227168</td>
<td>5.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNF212</td>
<td>60035268</td>
<td>4.1</td>
<td>ANKDD1A</td>
<td>34988193</td>
<td>10.3</td>
</tr>
<tr>
<td>PRSS55</td>
<td>61743179</td>
<td>15</td>
<td>NPSR1</td>
<td>6972158</td>
<td>12.6</td>
</tr>
<tr>
<td>C5orf38</td>
<td>62333235</td>
<td>10</td>
<td>NUP210</td>
<td>7628051</td>
<td>4.9</td>
</tr>
<tr>
<td>TANC1</td>
<td>4664277</td>
<td>7</td>
<td>NIPSNAP3B</td>
<td>3739741</td>
<td>12.6</td>
</tr>
<tr>
<td>SLC26A8</td>
<td>743923</td>
<td>4.6</td>
<td></td>
<td></td>
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</table>

**Maternal non-treated neonates**

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs#</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PITPNM3</td>
<td>3809835</td>
<td>0.05</td>
</tr>
<tr>
<td>SLC2A14</td>
<td>36141788</td>
<td>0.07</td>
</tr>
<tr>
<td>FANCA</td>
<td>2239359</td>
<td>0.10</td>
</tr>
<tr>
<td>MAP2K3</td>
<td>56020453</td>
<td>0.17</td>
</tr>
<tr>
<td>ATF7IP</td>
<td>2231909</td>
<td>0.27</td>
</tr>
<tr>
<td>ZNF773</td>
<td>67054060</td>
<td>0.40</td>
</tr>
<tr>
<td>ACE</td>
<td>4363</td>
<td>0.27</td>
</tr>
<tr>
<td>NLRP8</td>
<td>306496</td>
<td>0.04</td>
</tr>
<tr>
<td>DCAF8L2</td>
<td>5926895</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Total 21 SNPs in 17 genes more common in mothers of neonates that required opioid treatment for NOWS
Total of 9 SNPs in 9 genes more common in mothers of neonates that did not require opioid treatment for NOWS
Gene networks involved in opioid abuse, dependence, and withdrawal

Total number of genes that have been associated with abuse, dependence, or withdrawal (216) based on Cytoscape analysis of String disease networks.

Associated genes based on merged network analysis. Sixteen genes found in all three String pathways. NPSR1 potential risk gene form our study interacts with this network.

Oxytocin

Gene in our study

- from our study
- opioid receptors
- neuroendocrine
- dopamine related
- neuropeptide precursors
- growth factor
Neuropeptide S receptor 1 (NPSR1)

- rs6972158 in our study OR of 12.6
- G-coupled receptor for neuropeptide S
  - Regulator of neuroendocrine and autonomic function
  - Expressed in areas of brain associated with various aspects of OUD
  - Regulation of oxytocin
    - Important neuropeptide for pregnancy, lactation, and attachment
- Polymorphisms have been linked to:
  - Anxiety and depression
  - GI motility
  - Pain
  - Addiction
  - Specifically rs6972158 – risk factor for somatization of trauma and anxiety
### Polymorphisms seen on NPSR1 in our study population.

Allelic rates for mothers and also the 1000 genome rate for the American population.

<table>
<thead>
<tr>
<th>rs#</th>
<th>change</th>
<th>AA</th>
<th>1000 genome</th>
<th>Our Population (# Mothers)</th>
<th>Neomate no opioid treatment</th>
<th>Neonate needed opioid treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2530547</td>
<td>C-T</td>
<td>Non cod</td>
<td>0.42</td>
<td>0.38 (4)</td>
<td>0.83 (3)</td>
<td></td>
</tr>
<tr>
<td>324981</td>
<td>A-T</td>
<td>N107I</td>
<td>0.41</td>
<td>0.47 (14)</td>
<td>0.33 (9)</td>
<td></td>
</tr>
<tr>
<td>34705969</td>
<td>G-T</td>
<td>C197S</td>
<td>0.02</td>
<td>0.03 (14)</td>
<td>0.00 (9)</td>
<td></td>
</tr>
<tr>
<td>727162</td>
<td>C-G</td>
<td>R241S</td>
<td>0.35</td>
<td>0.29 (13)</td>
<td>0.22 (9)</td>
<td></td>
</tr>
<tr>
<td>6972158</td>
<td>A-G</td>
<td>Q344R</td>
<td>0.23</td>
<td>0.27 (14) *</td>
<td>0.56 (9) *</td>
<td></td>
</tr>
<tr>
<td>7809642</td>
<td>C-T</td>
<td>R360Ter</td>
<td>0.1</td>
<td>0.12 (12)</td>
<td>0.44 (9) *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-A</td>
<td>R360R</td>
<td>0.06</td>
<td>0.04 (12)</td>
<td>0.06 (9)</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates a significantly different allelic frequency when compared to 1000 genome data by Chi-Squared analysis.

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**Maternal expression of NPSR1 SNPs within our patient population**

**Fig. 1** Two-dimensional localization of human NPSR1 and NPSR2. The most conserved residues in the transmembrane domains are shaded in red. Residues that have naturally occurring mutations or polymorphisms identified by Exome Aggregation Consortium database are shaded in gray.
Why could maternal NPSR1 SNPs be important for long term effects of opioid exposure in utero?

Stimulation of NPSR1 leads to a downstream increase in oxytocin, thus a reduction of NPSR1 activation will lead to a reduction in OXT levels.
If you change oxytocin levels in the maternal brain, it will impact maternal mental health and attachment, and thus potentially impact neonate development.
Summary and future studies

Summary:
• In a preliminary whole exome study we found:
  • 21 SNPs in 17 genes more common in mothers of neonates that required opioid treatment for NAS.
  • 9 SNPs in 9 genes more common in mothers of neonates that did not require opioid treatment for NAS.
• Network analysis using Cytoscape indicates that one of our candidate genes of interest NPSR1 involved in multiple aspects of OUD, thus a change in function could impact our population outcomes.
• Analysis of NPSR1 SNPs indicate that several SNPs with known links to behavioral health and addiction are differentially expressed in our cohort.

Future studies:
• Validate NPSR1 SNP profiles as a biomarker for NAS intensity, using a candidate gene approach.
• Investigate if NPSR1 SNPs lead to changes in maternal-neonate attachment due to oxytocin regulation.
• Animal modeling.

This research was funded by the WV-CTSI pilot project core and supported by, Clinical Research Resources, Marshall and WVU IRB, the genomics core, and the bioinformatics and Biostatistics core.
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