The Immune and Nervous Systems

**Immune System**
- Body's natural defense mechanism
- Detection of wide variety of foreign agents

**Nervous System**
- Transmit signals between different regions of the body
- Interactions between complex neural pathways
- CNS: brain and spinal cord
- PNS: sensory neurons

**Neuroimmunology**
- Complex interactions between the two systems during homeostasis, response to injuries, and development.
Astroglia

Microglia

Neuron

Perivascular macrophage

B Cell or Plasma cell

Cytokines IL-1, IL-6, TNF-α

ICAM-1

Neuroimmune Cross-Talk

ACTH Receptor

CRH Receptor

CRH

GABA

Glutamate Receptor

Serotonin

ACh

Opioid Receptor

Cytokines IL-1, IL-6, TNF-α

BBB

Monocyte/Macrophage

Dendritic Cell

T-Cell

B Cell or Plasma cell

Cytokine Receptors

CRH

5-HT Receptor

GABA(A)-R

Glutamate Receptor

ACTH

ACTH Receptor

CRH Receptor

GABA(A)-R

ACh Receptor

IFN-α

Glutamate

IL-1, IL-6, and TNF-α
Autism and the Immune Response

What we know now

• Various immune system abnormalities have been reported in children with autistic disorders by a number of different laboratories.

• Both enhanced autoimmunity and reduced immune function have been shown.

• Development of ‘autism’ animal models with immune basis
Current evidence for immune dysfunction in autism comes from many avenues.

Evidence for immune dysfunction in ASD

- Immune - genetics
- Brain and CNS Immunity
- Systemic Immunity - Cells and antibodies
- Animal Models
Causes of ASD

- Not well understood

Genetics

Environmental Factors

Immune System

Nervous System
There are two types of immune responses: Innate and Adaptive
• Innate immunity refers to antigen non-specific defense mechanisms that a host uses immediately or within several hours after exposure to almost any antigen.

• This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.
Adaptive Immunity

- Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a particular antigen.

- The response can be long lasting and result in “memory cells”

- There are two major branches of the adaptive immune responses: humoral immunity (antibodies) and cell-mediated immunity.

- The adaptive immune response involves B cells and T cells.
Overview of the State of Cellular Immunity—Cytokine/Chemokine Analysis in Autism
Evolution of Studies on Immune Cells in Autism


- More recently, an increase in expression of NK cell associated genes was noted in ASD (Gregg et al., 2008).

- Lower NK cell activity found in about 45% of a subset of children with ASD (Vojdani et al., 2008).

- An imbalance between inhibitory and activating NK cells has been implicated autism (Enstrom et al., 2009; Schleinitz et al., 2010).
NK Cells

- Large granular non-T and non-B cells that kill virally infected cells and some tumor cells.
- Important in innate immunity to viruses and other intracellular pathogens.
NK Cells in Autism

Functionally, following stimulation, children with ASD had a decrease in NK cell cytotoxic activity compared to age-matched controls (Enstrom et al., 2009b).

What does this mean?

- There are enough cells there
- Functionally they cannot do their job efficiently
- First line defense for viral infection
Macrophages/Monocytes in Autism

- Significantly higher monocyte count with no difference in the absolute leukocyte counts (Sweeten et al., 2003b).

- TLR-2 activated monocytes had an increase in IL-1b, IL-6 and TNF-a.

- TLR-4 activation gave an increase in IL-1b.

- TLR-9 activation resulted in a decrease in IL-1b, IL-6, GMCSF and TNF-a (Enstrom et al., 2010).

- Children with ASD have a dysfunction in monocyte signaling that may lead to long-term problems in response to infection.
Children with gastrointestinal problems in conjunction with ASD had lower production of the pro-inflammatory cytokines, IL-6, IL-1b, IL-12, IL-23, and the counter-regulatory cytokine IL-10, when monocytes were stimulated (Jyonouchi et al., 2011).

This impaired signaling was in response to Toll-like receptor agonists for TLR2/6 and TLR 7/8, which are intracellular receptors for ssRNA.
Several studies have indicated abnormalities in T cell immunity in children with autism compared to healthy controls.

First noted in 1977, lymphocytes cultured from children with autism and challenged with the T cell mitogen PHA had a depressed proliferation compared to controls (Stubbs and Crawford, 1977).

A similar study of children with autism ages 7-15, cultured PHA-challenged T cells showed a decrease in T helper cells, and a lower suppressor (now called regulatory cells) cell ratio as determined by flow cytometry (Denney et al., 1996).
T cells in Autism - more recently

- PBMCs challenged with PHA or tetanus, showed a significant decrease in the expression of CD3, CD4, and CD8 on T cells (Ashwood et al., 2011).

- CD4+ T lymphocytes from children with autism showed a decreased expression of CD95, the Fas ‘cell death’ receptor.
  - Children with autism might have poor regulation of the cellular immune response (Stranges et al., 2007).

- Lower frequency of Treg cells children with autism compared to controls (Mostafa et al., 2010).
  - a correlation with allergy and family history of autoimmunity.
T cells in Autism - more recently

• When peripheral blood T cells were stimulated, GM-CSF, TNFα, and IL-13 were significantly increased whereas IL-12p40 was decreased in ASD relative to TD controls.

• Increased pro-inflammatory or TH1 cytokines were associated with greater impairments in core features of ASD as well as aberrant behaviors.

• In contrast, production of GM-CSF and TH2 cytokines were associated with better cognitive and adaptive function.

Cytokines- Master regulators of the immune system

- The immune response is controlled by mediators known as cytokines that are responsible for cell-cell communication.
- Cells produce cytokines in response to a stimulus. They direct the function of the cell that produces them and the cells nearby if they have appropriate cytokine receptors.
- They are produced by many cell types including including T cells, B cells and macrophages.
Immune-Neuro Interface

CNS Environment

- Neurotoxicity
- Sleep, Fever

Almost all aspects of neural development

Synaptic Transmission & Plasticity

- Oligodendrocyte function
- Interneuron migration
- Neuromodulation

Immune Environment

- Pro-inflammatory pathways
- Anti-inflammatory pathways
- Leukocyte trafficking

IL-1β, TNF-α, IL-6, TGF-β, CCL1, GRO-α, CXCL12, SDF1, CCL2, MCP1
Regulatory T cells/Cytokines

- Significantly lower frequency of CD4(+)CD25( high) regulatory T cells in the blood of 30 AU and 30 age- and sex-matched TD children (Mostafa, 2010). Were not examined for Foxp3, a more definitive marker.

- Children with autism (n=75) had significantly lower plasma TGFβ1 levels compared with typically developing general population controls (n=36) (p=0.0017) (Ashwood, 2008).
  - A significant positive correlation of measures of social interaction and TGFβ1 levels in children with regression (n=42) based on ADOS scores (p=0.0048).

- Decreased serum TGF-β in small groups of ASD subjects compared to matched healthy controls (Okada, 2007).
Plasma TGF-β levels

N=96
N=36
N=32
N=28
<table>
<thead>
<tr>
<th>Study Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated levels of IL-1b, IL-6, IL-8 and IL-12p40. Associated with regression</td>
<td>(Ashwood, et al., 2011b)</td>
</tr>
<tr>
<td>Increase in chemokine <strong>MCP-1</strong>, <strong>Rantes</strong> and <strong>Eotaxin</strong> levels in ASD subjects compared to age-matched typically developing controls. An association between increases chemokines levels with aberrant behaviors.</td>
<td>(Ashwood et al., 2011c)</td>
</tr>
<tr>
<td>In male ASD subjects, an increase in cytokines <strong>IL-1beta</strong>, <strong>IL-1RA</strong>, <strong>IL-5</strong>, <strong>IL-8</strong>, <strong>IL-12(p70)</strong>, <strong>IL-13</strong>, <strong>IL-17</strong> and <strong>GRO-alpha</strong>.</td>
<td>(Suzuki et al., 2011)</td>
</tr>
<tr>
<td>Increase in <strong>leptin</strong> levels in ASD subjects compared to age-matched controls.</td>
<td>(Ashwood et al., 2008b)</td>
</tr>
<tr>
<td>Increase in <strong>macrophage migration inhibitory factor (MIF)</strong> in ASD subjects compared to age-matched controls.</td>
<td>(Grigorenko et al., 2008)</td>
</tr>
<tr>
<td>Decrease in <strong>TGF-beta</strong> in subjects with ASD compared to controls.</td>
<td>(Ashwood et al., 2008a; Okada et al., 2007)</td>
</tr>
<tr>
<td>Increase in <strong>IL-12</strong> and <strong>IFN-gamma</strong> in ASD subjects compared to age-matched controls.</td>
<td>(Singh, 1996)</td>
</tr>
<tr>
<td>Study Description</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>In isolated PBMCs stimulated with PHA, increase in <strong>GM-CSF</strong>, <strong>TNF-alpha</strong> and <strong>IL-13</strong>. A decrease in <strong>IL-12(p40)</strong> in ASD subjects vs. controls.</td>
<td>(Ashwood et al., 2011d)</td>
</tr>
<tr>
<td>Stimulation of TLR on monocytes - ASD vs. to age-matched controls. Increase in <strong>IL-1beta</strong>, <strong>IL-6</strong>, <strong>TNF-alpha</strong>, with stimulation of TLR2. Increase in <strong>IL-1beta</strong>, with stimulation of TLR4. Decrease in <strong>IL-1beta</strong>, <strong>IL-6</strong>, <strong>GMCSF</strong>, <strong>TNF-alpha</strong> with TLR9.</td>
<td>(Enstrom et al., 2010)</td>
</tr>
<tr>
<td>Increase in <strong>IFN-gamma</strong> in NK cells from subjects with ASD.</td>
<td>(Enstrom et al., 2009b)</td>
</tr>
<tr>
<td>Increase production of cytokines from Th1 and Th2 cytokines in ASD subjects vs age-matched controls.</td>
<td>(Molloy et al., 2006)</td>
</tr>
<tr>
<td>Increase in <strong>IL-12</strong> and <strong>TNF-alpha</strong> in ASD subject with GI symptoms.</td>
<td>(Jyonouchi et al., 2005)</td>
</tr>
<tr>
<td>Increase in <strong>IFN-gamma</strong> and <strong>TNF-alpha</strong> in isolated PBMCs from ASD subjects compared to age-matched controls stimulated with LPS.</td>
<td>(Jyonouchi et al., 2002)</td>
</tr>
<tr>
<td>Unstimulated whole blood from ASD vs. age-matched controls – increase in <strong>IFN-gamma</strong> and <strong>IL-1RA</strong> with -higher <strong>IL-6</strong> and <strong>TNF-alpha</strong>.</td>
<td>(Croonenberghs et al., 2002)</td>
</tr>
<tr>
<td>Unstimulated PBMC- ASD subjects: higher levels of <strong>TNF-alpha</strong>, <strong>IL-1beta</strong>, and <strong>IL-6</strong> vs. controls. PBMCs stimulated with LPS, PHA and tetanus produced increase levels of <strong>IL-12</strong> and <strong>IL-1beta</strong>.</td>
<td>(Jyonouchi et al., 2002)</td>
</tr>
</tbody>
</table>
Plasma levels of IL-6, IL-8, IL-1β and IL-12p40 are significantly higher in the ASD (n=97) group when compared to TD (n=87) and DD (n=39) controls.
### Onset status – relationship with cytokines

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>Typically Developing (n=87)</th>
<th>Early Onset (n=53)</th>
<th>Regression (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>62.8</td>
<td>61</td>
<td>144.3*‡</td>
</tr>
<tr>
<td>IL-2</td>
<td>8</td>
<td>17.7</td>
<td>19.3</td>
</tr>
<tr>
<td>IL-4</td>
<td>36.7</td>
<td>28.8</td>
<td>39.4</td>
</tr>
<tr>
<td>IL-5</td>
<td>9.8</td>
<td>9.2</td>
<td>11.5</td>
</tr>
<tr>
<td>IL-6</td>
<td>11.8</td>
<td>15.1</td>
<td>32.6*</td>
</tr>
<tr>
<td>IL-8</td>
<td>3.9</td>
<td>6.8*</td>
<td>14.5*</td>
</tr>
<tr>
<td>IL-10</td>
<td>16.4</td>
<td>7.5</td>
<td>15.6</td>
</tr>
<tr>
<td>IL-12 p40</td>
<td>171.7</td>
<td>192.3*</td>
<td>198.6*</td>
</tr>
<tr>
<td>IL-13</td>
<td>20.9</td>
<td>14.1</td>
<td>29.4</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>54.2</td>
<td>51.3</td>
<td>101.2*‡</td>
</tr>
<tr>
<td>IFNγ</td>
<td>62.8</td>
<td>51.2</td>
<td>94.1</td>
</tr>
<tr>
<td>TNFα</td>
<td>63.9</td>
<td>56.2</td>
<td>111.1*</td>
</tr>
<tr>
<td>Cytokine</td>
<td>AUT Age 4 yrs</td>
<td>Age 3 yrs</td>
<td>AUT</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------</td>
<td>-----------</td>
<td>-----</td>
</tr>
<tr>
<td>IFN gamma</td>
<td>5.27</td>
<td>7.52</td>
<td>212.8</td>
</tr>
<tr>
<td>IL-2</td>
<td>OOR &lt;</td>
<td>OOR &lt;</td>
<td>49.05</td>
</tr>
<tr>
<td>IL-4</td>
<td>190</td>
<td>498.49</td>
<td>80.64</td>
</tr>
<tr>
<td>IL-6</td>
<td>14.6</td>
<td>19.24</td>
<td>114.33</td>
</tr>
<tr>
<td>IL-7</td>
<td>173.4</td>
<td>6.7</td>
<td>946.5</td>
</tr>
<tr>
<td>IL-8</td>
<td>39.4</td>
<td>80.42</td>
<td>77.22</td>
</tr>
<tr>
<td>IL-10</td>
<td>70.2</td>
<td>129.56</td>
<td>32.89</td>
</tr>
<tr>
<td>IL-12 (p70)</td>
<td>1.4</td>
<td>*0.99</td>
<td>10.69</td>
</tr>
<tr>
<td>IL-13</td>
<td>16.6</td>
<td>22.61</td>
<td>45.67</td>
</tr>
<tr>
<td>MCP-1</td>
<td>286.5</td>
<td>275.41</td>
<td>247.2</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>84.7</td>
<td>126.7</td>
<td>63.2</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>835</td>
<td>1329.72</td>
<td>497.9</td>
</tr>
<tr>
<td>IL-1a</td>
<td>119</td>
<td>153.42</td>
<td>434.7</td>
</tr>
<tr>
<td>IL-1B</td>
<td>15.4</td>
<td>25.96</td>
<td>15.13</td>
</tr>
<tr>
<td>IL-12 (p40)</td>
<td>401</td>
<td>488.02</td>
<td>268.2</td>
</tr>
<tr>
<td>IL-17</td>
<td>0.95</td>
<td>1.97</td>
<td>194.6</td>
</tr>
<tr>
<td>IP-10</td>
<td>86.4</td>
<td>82.1</td>
<td>122.6</td>
</tr>
<tr>
<td>MIP-1a</td>
<td>62.1</td>
<td>101.56</td>
<td>519.1</td>
</tr>
<tr>
<td>TNFα</td>
<td>3.2</td>
<td>3.09</td>
<td>3.96</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>142</td>
<td>289.7</td>
<td>127.2</td>
</tr>
<tr>
<td>MIP-1B</td>
<td>59.7</td>
<td>64.73</td>
<td>724.5</td>
</tr>
</tbody>
</table>
Immune Dysregulation

Dysregulation of the immune system can lead to autoimmunity, for which autoantibodies are one hallmark feature.
Autoantibodies when the immune system gets it wrong

Is this a good guy or a bad guy???
Several investigators have proposed an autoimmune-based etiology for a subset of children with autism. The autoantibodies are directed against various brain components including:

- serotonin receptors, heat shock proteins, glial filament proteins and myelin basic protein, as well as other proteins with significant neurological relevance (Connolly et al., 2006; Connolly et al., 1999; Goines et al., 2011a; Wills et al., 2007; Wills et al., 2009; Wills et al., 2011).
Autoantibodies from children with ASD

Immunohistochemical and Western blot analysis of autoantibody localization in cerebellum of Rhesus monkeys (Wills, 2009).
Western blot - Human cerebellum

- The presence of the ~45 kDa band corresponds to Golgi staining by IHC (p=0.04).
- Children with these antibodies had lower adaptive and cognitive function.
- Increased aberrant behaviors when compared to children without these antibodies.

Goines, et al. BBI, 2011
# Autoantibody Specificity

## Child Autoantibody Targets in Cerebellum

<table>
<thead>
<tr>
<th>Band</th>
<th>ASD</th>
<th>AU</th>
<th>AU/ASD</th>
<th>TD</th>
<th>ASD vs.</th>
<th>AU vs.</th>
<th>AU/ASD vs.</th>
<th>AU vs. ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=70</td>
<td>n=207</td>
<td>n=277</td>
<td>n=189</td>
<td>TD</td>
<td>TD</td>
<td>TD</td>
<td>vs. TD</td>
<td>ASD</td>
</tr>
<tr>
<td>45</td>
<td>5 (7.1%)</td>
<td>2 (9.7%)</td>
<td>25 (9%)</td>
<td>7 (3.6%)</td>
<td>NS</td>
<td>0.017</td>
<td>0.025</td>
<td>NS</td>
</tr>
<tr>
<td>62</td>
<td>17 (8.2%)</td>
<td>29</td>
<td>16</td>
<td>12 (16%)</td>
<td>(10%)</td>
<td>(8.2%)</td>
<td>0.043</td>
<td>NS</td>
</tr>
<tr>
<td>45 + 62</td>
<td>0 (0%)</td>
<td>6 (3%)</td>
<td>6 (2%)</td>
<td>0 (0%)</td>
<td>NS</td>
<td>0.03</td>
<td>0.05</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Maternal antibodies to fetal brain proteins


- These antibodies are highly specific for autism, and have demonstrated pathology in animal models (Martin, 2008, Singer, 2009 and Braunschweig, 2012).
Prenatal Growth of the Human Brain

The human brain consists of approximately 100 billion neurons (which is as many cells as there are stars in the Milky Way).

During the last trimester, neurons form at a rate of around 580,000 per minute.

After Cowan 1979
Maternal Anti-Brain Antibodies and ASD:
The studies behind the novel immune biomarker for autism risk

- Does prenatal exposure cause changes in brain and behavior relevant to ASD?
- Is prenatal exposure to maternal anti-brain antibodies associated with specific subtypes of ASD?
- Is prenatal exposure associated with abnormal brain growth?
- Why do some women produce these anti-brain antibodies?
- How might the antibodies impact fetal brain development?

**ASD Behavior**
- Antibodies have been associated with regression and language deficits, and stereotypic behavior

**MRI**
- Enlarged brain volume in male children prenatally exposed to the antibodies

**Genetics**
- MET genetic variant associated with production of the anti-brain antibodies

**Basic Science**
- Efforts are underway to verify the antigenic targets of the antibodies

**Brain Tissue Studies**
- While there are no known postmortem cases, animal models provide an opportunity to explore brain pathology

**Animal Models**
- Animal models show behavioral change following prenatal exposure to the antibodies; monkey model has also reported increased brain volume

**Translational Potential:**
- Identify children with this subphenotype and develop tailored behavioral treatment
- Screen women at risk and develop preventative strategies
- Establish the pathophysiology associated with these antibodies and develop therapeutic interventions
### MAR Antibody Presence in Maternal Blood

<table>
<thead>
<tr>
<th>MAR Ab Groupings</th>
<th>Incidence in Autism</th>
<th>Incidence in Normal Pop.</th>
<th>Clinical Utility/Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only one Ab</td>
<td>89%</td>
<td>70%</td>
<td>not clinically significant</td>
</tr>
<tr>
<td>Significant Ab doublets</td>
<td>70%</td>
<td>27%</td>
<td>3X AU risk; counsel caution and early intervention</td>
</tr>
<tr>
<td>Specific Ab doublets</td>
<td>4%</td>
<td>0%</td>
<td>mothers w/specific combinations have 99%+ risk; counsel early diagnosis and intervention</td>
</tr>
<tr>
<td>Specific Ab triplets</td>
<td>19%</td>
<td>0.6%*</td>
<td></td>
</tr>
<tr>
<td>All specific MAR combinations</td>
<td>23%</td>
<td>0.6%</td>
<td></td>
</tr>
</tbody>
</table>

*1 Typically Developing (TD) child with score abnormally high score of 22 on ABC subscale for hyperactivity

We Identified 8 MAR Autoantibodies That Bind to Protein Targets* Critical to Normal Brain Development

- Antibodies interfere with normal protein function
- The more antibodies, the more points of developmental interference…
- And the likelihood of autism increases
## MAR Patterns Correlate with ASD Behaviors

### P-values for Significant Behavior Correlations (P<.05)

<table>
<thead>
<tr>
<th>Antibody Status</th>
<th>Irritability</th>
<th>Lethargy</th>
<th>Stereotypy</th>
<th>Hyperactivity</th>
<th>Moods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any LDH (n=63)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.024</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Any Cypin (n=24)</td>
<td>n.s.</td>
<td></td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDH + Cypin (n=4)</td>
<td>n.s.</td>
<td></td>
<td>0.041</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDH + STIP1 (n=36)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.015</td>
<td>n.s.</td>
<td>0.062</td>
</tr>
<tr>
<td>LDH + CRMP1 (n=21)</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
<td>0.028</td>
<td>0.058</td>
</tr>
<tr>
<td>LDH + STIP1 + CRMP1 (n=12)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.007</td>
<td>0.057</td>
<td>0.061</td>
</tr>
<tr>
<td>LDH + STIP1 + CRMP1 or LDH + Cypin (n=15)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.013</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

- Presence of LDH antibodies appear to contribute heavily to stereotypic behavior, a core feature of ASD
- Antibodies to LDH in combination with STIP1 and CRMP1 are highly significantly associated with stereotypic behavior
- Antibodies to Cypin alone is highly significantly associated with lethargic behavior
Short Communication

Maternal autoantibodies are associated with abnormal brain enlargement in a subgroup of children with autism spectrum disorder

Christine Wu Nordahl\textsuperscript{a,b,*}, Daniel Braunschweig\textsuperscript{c}, Ana-Maria Iosif\textsuperscript{d}, Aaron Lee\textsuperscript{b}, Sally Rogers\textsuperscript{a,b}, Paul Ashwood\textsuperscript{a,c}, David G. Amara\textsuperscript{a,b}, Judy Van de Water\textsuperscript{a,c}

- Studied 181 2-4 YO male children (131 ASD, 50 typically developing (TD) controls) and evaluated total brain volume using structural magnetic resonance imaging (MRI).
- The ASD MAR group exhibited a more extreme 12.1\% abnormal brain enlargement relative to TD controls.
- The remaining ASD children had a smaller 4.4\% abnormal brain enlargement relative to TD controls.
- Lobar and tissue type analyses revealed that the frontal lobe is selectively enlarged
- MAR autoantibodies may impact brain development leading to abnormal enlargement.
What is the susceptibility factor for the production of these antibodies?

Where genetic susceptibility and immune function converge…..
A genetic variant that disrupts the MET transcription is associated with autism

The MET receptor tyrosine kinase is a key negative regulator of immune responsiveness, controlling the degree of activation of antigen presenting cells (APCs; e.g., dendritic cells, monocytes, and B cells).

The ‘C’ allele with polymorphism rs1858830 increases relative risk for ASD approximately 2.25-fold in children.
What is the relationship between MET and anti-fetal brain antibodies?

- We have found a higher incidence of the MET ‘C’ allele in the blood of mothers who have antibodies to fetal brain proteins (Heuer et al, 2011).
- This allele confers a functional reduction in the receptor MET production.

<table>
<thead>
<tr>
<th>37/73 kDa bands</th>
<th>Diagnosis Groups</th>
<th>MET Genotype</th>
<th>Allelic Chi-square p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C/C</td>
<td>C/G</td>
</tr>
<tr>
<td>Positive</td>
<td>(All have ASD) n=19</td>
<td>11 (58%)</td>
<td>7 (37%)</td>
</tr>
<tr>
<td>Negative</td>
<td>ASD + TD (n=346)</td>
<td>101 (29%)</td>
<td>154 (45%)</td>
</tr>
<tr>
<td></td>
<td>ASD Only (n=183)</td>
<td>51 (28%)</td>
<td>79 (43%)</td>
</tr>
</tbody>
</table>
The functional MET promoter variant alters expression of the MET receptor in immune cells.
- This may predispose to the development of antibodies to fetal brain proteins in some mothers whose children develop autism.

Further, this genetic susceptibility may lead to loss of immune regulation during gestation, which may, even in the absence of autoantibody production, have an effect on neurodevelopment.
What is the role of the environment in the immune dysregulation noted in ASD?
Polybrominated Diphenyl Ethers (PBDEs)

- Persistant Organic Pollutants (POPs)
- Flame-retardants
  - Textile, building and manufacture of electronic appliances
- Widely dispersed in global environment
- May interfere with normal immune and or neurological development (Lawler et al., 2004)
- Varies routes of exposure

Dingemans et al., 2011 *Environmental Health Perspectives Supplements*

- Increased production of inflammatory cytokines in ASD compared to age matched typically developing (TD) controls
- Children with ASD have differential immune sensitivity to some environmental toxicants
- Do children with ASD have a genetic susceptibility to PBDE effects?

Ashwood et al., 2009 Brain Behav Imm
LPS Challenged BDE-49 exposed AU and TD children:
Conclusions

• We see changes in immune function in several branches of the immune system including altered antibody production, altered NK cell function, autoantibodies, and differential cellular responses to various stimuli.
  • Maternal autoantibodies are specific for ASD

• Plasma cytokines can be informative
  ▪ Fairly stable over time with multiple samples

• Very variable from subject to subject both ASD and controls

• See several profiles within autism
  ▪ Elevated inflammatory
  ▪ Reduced profile
  ▪ Normal profile
  ▪ What appears to be a somewhat ASD-specific profile
  ▪ Onset status driven
Immune & CNS systems interactions in disease

ENVIRONMENT
Infections, toxins, Maternal factors…

Immunogenetic background:
MET, Cytokines, HLA (MHCI)

GENES

Systematic Cytokines & Chemokines
Autoantibodies

CNS Cytokines & Chemokines

ASD Schizophrenia Neurodev Disorders

Brain Development Connectivity & Plasticity

Therapeutics
This work was supported by grants NIEHS 1 P01 ES11269-01, the U.S. Environmental Protection Agency (U.S. EPA) through the Science to Achieve Results (STAR) program (Grant R829388), Autism Speaks, the JBJ Foundation, and the UC Davis M.I.N.D. Institute.
Polychlorinated Biphenyls (PCBs): Environmental Risk Factors for ASD?

Pamela Lein, Ph.D.
Department of Molecular Biosciences
Center for Children's Environmental Health
UC Davis School of Veterinary Medicine
What is the evidence that environmental factors contribute to ASD risk?

1. Rapid increase in ASD prevalence
2. Genetic studies
3. Clinical heterogeneity of ASD
4. Systemic and CNS pathophysiology
   - Oxidative stress
   - Immune dysfunction (including neuroinflammation)
   - Mitochondrial dysfunction

These pathophysiological outcomes known to be exacerbated by environmental factors:

- Air pollution, organophosphorus pesticides, heavy metals
PCB Developmental Neurotoxicity

- Human epidemiological data suggest a negative association between developmental exposure to environmental PCBs and cognitive function in infancy or childhood
  - Decreased IQ, impaired learning and memory, attentional deficits, lowered reading comprehension, psychomotor problems

- Comparable cognitive and behavioral deficits observed in primate and rodent models following developmental PCB exposures
  - Developmental neurotoxic effects of PCBs have been observed at relatively low exposure levels corresponding to between 1 and 10x the background levels observed in humans
PCB developmental neurotoxicity mediated primarily by non-dioxin-like PCB congeners

Non-dioxin-like congeners

- 2, 2’, 3, 5’, 6-pentachlorobiphenyl (PCB 95)

Dioxin-like congeners

- 2, 3’,4,4’-tetrachlorobiphenyl (PCB 66)

<table>
<thead>
<tr>
<th></th>
<th>Non-dioxin-like congeners</th>
<th>Dioxin-like congeners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental Neurotoxicity</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>Carcinogenic</td>
<td>+/-</td>
<td>+++</td>
</tr>
<tr>
<td>Arylhydrocarbon Receptor (AhR)</td>
<td>Low to no affinity</td>
<td>High affinity</td>
</tr>
</tbody>
</table>
Cell and molecular mechanism(s) of PCB developmental neurotoxicity unknown

- Decreased dopamine content
- Interference with thyroid hormone signaling
- Increased levels of intracellular calcium \( \text{Ca}^{2+} \)
  - Sensitization of the ryanodine receptor (RyR)
Does developmental exposure to non-dioxin-like PCBs alter dendritic growth?

- Dendritic branching patterns influence the number, types and distribution of synaptic inputs
- Structural plasticity of dendrites is thought to be the cellular substrate of learning and memory
- Altered patterns of dendritic growth and plasticity are associated with ASD and other neurodevelopmental disorders
Hypothesis:

Developmental exposures to non-dioxin-like PCBs cause behavioral deficits via altered pattern of dendritic growth and plasticity
Experimental Design of *In Vivo* Studies Using Aroclor 1254

-14d G0 P0 P21 P24 P31

- Dams exposed to Aroclor 1254 in the diet 0, 1 or 6 mg/kg/d
- Pups tested in Morris water maze

- Tissue samples collected from weanlings
- **Tissue samples collected from maze trained pups and untrained littermates**
Environmental risk factors for ASD

- Rubella infection during the first trimester of pregnancy
- *In utero* exposure to thalidomide or valproic acid
- Paternal age
- Environmental chemicals (?)
  - Heavy metals (lead, methylmercury)
  - Pesticides
    - Organophosphorus pesticides (OPs), e.g., chlorpyrifos, diazinon
    - Organochlorine pesticides (OCs), e.g., DDT, dieldrin, lindane
  - Persistent organic pollutants (POPs)
    - Polychlorinated biphenyls (PCBs)
    - Polybrominated diphenyl ethers (PBDEs)
    - Polycyclic aromatic hydrocarbons (PAHs)

*However, efforts to identify specific environmental risk factors for ASD have produced a number of candidates but few definitive hits*
Morris Water Maze

Spatial Test:
1. subject is placed on the platform for 20 sec
2. subject is placed in start quadrant
3. subject is allowed to swim for 45 sec or until the platform is found
4. subject is placed on the platform for 20 sec before being removed from pool

Average time to find the platform (escape latency) provides a measure of learning over successive trials
Morris Water Maze

- In early trials subjects tend to swim around perimeter
- After several trials animals use searching behavior
- Once task is learned, subjects swim directly to platform

thigmotaxis  searching  learned
Developmental Aroclor 1254 exposure causes deficits in spatial learning

**Morris Water Maze**

**Probe Test**
1. platform removed from pool
2. subject placed in a start quadrant
3. subject allowed to swim for 45 sec
4. time spent in platform quadrant is used as a measure of learning/memory

**Cue Test** *(motivation/vision/motor function)*
1. platform moved and marked with a flag
2. subject placed in a start quadrant
3. subject allowed to swim for 45 sec
4. time to platform gives measure of motor function, motivation and visual function
Developmental Aroclor 1254 exposure causes deficits in spatial memory

Developmental Aroclor 1254 exposure alters dendritic growth in cerebellar Purkinje cells

Developmental Aroclor 1254 exposure alters dendritic growth in cortical pyramidal neurons

Developmental PCB exposure alters RyR expression \( \text{\textit{(data collected by Kyungho Kim, Pessah lab)}} \)

Conclusions from this study:

- Developmental PCB exposure enhanced basal dendritic growth but decreased experience-dependent dendritic plasticity.
- Effects of PCBs on dendritic arborization correlated with altered RyR expression.
Hypothesis:

Non-coplanar PCBs disrupt neuronal connectivity via RyR-mediated mechanisms that modulate Ca\(^{2+}\)-dependent signaling pathways linked to activity-dependent dendritic growth and plasticity.
PCB 95 alters dendritic growth in primary cultures of hippocampal neurons
SAR and pharmacological RyR blockade suggest dendrite-promoting activity of PCBs is RyR-dependent.
RyR activity required for PCB effects on dendrites

A

Vehicle  PCB 95  siRyR1/PCB 95  siRyR2/PCB 95

B

C

Vehicle  PCB 95

Total dendritic length (μm)

Control  siRNA control  siRyR1  siRyR2

**  **

Total dendritic termini

Control  siRNA control  siRyR1  siRyR2

**  **
Experimental approaches for investigating $\text{Ca}^{2+}$-dependent signaling pathways in PCB-induced dendritic growth
PCB 95 increases Ca^{2+} in primary cultured hippocampal neurons
(data collected by Diptiman Bose, Pessah lab)
Experimental approaches for investigating Ca\(^{2+}\)-dependent signaling pathways in PCB-induced dendritic growth
PCB-induced dendritic growth requires CREB activation.
Experimental approaches for investigating Ca\textsuperscript{2+}-dependent signaling pathways in PCB-induced dendritic growth
PCB-induced dendritic growth requires Wnt signaling
Non-dioxin-like PCBs hijack the signaling pathway that controls normal activity-dependent dendritic growth.
Exposure of rat pups to PCBs in the maternal diet throughout gestation and lactation interferes with normal patterns of dendritic growth in the hippocampus of weanling rats.
Are these findings relevant to ASD?

- Increased dendritic arborization and altered plasticity are associated with ASD.

- Experimental evidence suggests that developmental PCB exposure causes effects that mimic some aspects of ASD.
  - Developmental exposure to PCB 95 causes an imbalance between excitation and inhibition in the auditory cortex of weanling rats (Kenet et al., 2007).
  - Perinatal exposure to a mixture of PCB 47 and 77 alters social behaviors in rats (Joulous-Jamshidi et al. 2010).
Effects of activity on dendritic growth are mediated primarily by Ca\textsuperscript{2+}-dependent signaling

A significant number of the candidate genes for autism encode proteins whose primary role is to generate intracellular calcium signals or are themselves tightly regulated by local fluctuations in calcium levels.

How might non-dioxin-like PCBs influence ASD susceptibility?

environmental exposures $\times$ genetic susceptibility $\times$ timing

non-dioxin-like PCBs heritable defects in Ca$^{2+}$ signaling

↓

risk, severity and treatment outcome

One fundamental way by which heritable genetic vulnerabilities could amplify adverse effects triggered by environmental exposures is if both factors (genes and environment) converge to dysregulate the same signaling system at critical times of development.
PCBs may also influence neurodevelopment via effects on the immune system.
PCB 95 exposure decreases baseline immune activity

T cell growth factor

![Graph of IL-2 levels](image1)

T cell cytokine

![Graph of IL-13 levels](image2)

Chemokine

![Graph of MCP-1 levels](image3)
PCB 95 exposure increases innate immune activity
Do PCB effects on immune activity contribute to PCB effects on neurodevelopment?

Relevance to ASD?
# Acknowledgements

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Isaac Pessah, UC Davis  
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<table>
<thead>
<tr>
<th>Lein Laboratory</th>
<th>Funding Sources</th>
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<tbody>
<tr>
<td>Angela Howard</td>
<td>CROET, OHSU</td>
</tr>
<tr>
<td>Dongren Yang</td>
<td>NIH</td>
</tr>
<tr>
<td>Donald Bruun</td>
<td>USEPA</td>
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<tr>
<td>Christopher Barnhart</td>
<td>M.I.N.D. Institute, UC Davis</td>
</tr>
</tbody>
</table>
FRAGILE X SYNDROME 
TARGETED TREATMENTS AND AUTISM

Reymundo Lozano MD
Fragile X Research
MIND Institute

University of California at Davis Medical Center
Genes and Autism. 06/01/13
Causes of ASD and Fragile X Syndrome

- Genetics (30-40%)
- Environmental Factors
- Immune System
- Multifactorial

FXS 2-6%

Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions
G. Bradley Schaefer, MD1 and Nancy J. Mendelsohn, MD2; for the Professional Practice and Guidelines Committee
A new paradigm for psychopharmac drug development in fragile X syndrome

- Martin-Bell (fragile X) syndrome
- 1943
- FMR1 gene silenced by CGG repeat
- 1991
- Fmr1 knockout mouse
- 1994
- Rescue of Fmr1 KO by reducing mGluR5
- 2007
- Excessive mGluR5 function
- 2002
- Basic neurobiology
- mGluR5 NAMs in phase 2 clinical trials
- 2010
A NEW AGE
TARGETED TREATMENTS

- Advances in the last 3 years or so have ushered in a new age of targeted treatments to reverse the neurobiological abnormalities for neurodevelopmental disorders

- Fragile X syndrome: mGluR5 antagonists, GABA agonists, minocycline, Arbaclofen

- Autism has similarities in GABA and glutamate imbalances, common pathways ie mTOR, miRNA dysregulation, mitochondrial abnormalities, oxidative stress, synaptic plasticity deficits and environmental toxicity
## Targeted treatment research in other conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Animal model / Drug target</th>
<th>Drug / Effect in animal</th>
<th>Human trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF</td>
<td><em>Nf1</em> mouse / increased RAS/ERK signaling</td>
<td>Statins / improved attention and spatial cognition</td>
<td>Possible improvement in spatial skills</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td><em>MeCP2</em> mouse / reduced BDNF signaling</td>
<td>IGF-1 fragment / rescue of lethality, neuropathology, autonomic abnormalities</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Down syndrome</td>
<td><em>Ts65Dn</em> mouse / excessive inhibitory neurotransmission</td>
<td>GABA-A negative modulators / improved cognition</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Tuberous sclerosis (TSC2)</td>
<td><em>TSC2</em> mouse / elevated mTOR signaling</td>
<td>Rapamycin / improved spatial learning &amp; contextual discrimination</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>
FRAGILE X AS A MODEL OF AUTISM

- Many children with FXS have autism (30%) or PDDNOS (30%) and many children with autism (2-6%) have fragile X.

- Both disorders have big heads and rapid brain growth early in childhood.

- FXS has problems with hyperarousal and anxiety so it models this subtype of autism.

- Both disorders have problems with facial processing i.e., avoiding looking at the eyes which overactivates the amygdala.

- Those with FXS and autism have lower IQ than FXS alone.

- FMRP regulates the translation of many genes associated with autism - latest estimate 30% to almost 50% of autism genes (Darnell 2011 Cell; Iossifov et al 2012 Neuron).
Communication and Social Deficits are continuous in boys with FXS: 30% with autism and another 30% with PDDNOS but significant heterogeneity in the FXS-autism phenotype.
Description of the secondary medical/genetic problems

Percentage of patients

FXS alone (n=33) FXS+ASD (n=57)

<table>
<thead>
<tr>
<th>Description of the secondary medical/genetic problems</th>
<th>FXS Alone (n = 33)</th>
<th>FXS+ ASD (n = 57)</th>
<th>Chi-Square Test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>percentage</td>
<td>n</td>
</tr>
<tr>
<td>Full mutation</td>
<td>19</td>
<td>57.6</td>
<td>37</td>
</tr>
<tr>
<td>Mosaic</td>
<td>14</td>
<td>42.4</td>
<td>20</td>
</tr>
<tr>
<td>No medical problems</td>
<td>27</td>
<td>81.8</td>
<td>35</td>
</tr>
<tr>
<td>Seizures</td>
<td>4</td>
<td>12.1</td>
<td>16</td>
</tr>
<tr>
<td>MRI Abnormalities</td>
<td>1</td>
<td>3.0</td>
<td>2</td>
</tr>
<tr>
<td>Genetic Abnormalities</td>
<td>1</td>
<td>3.1</td>
<td>4</td>
</tr>
<tr>
<td>Total Medical Problems</td>
<td>6</td>
<td>18.2</td>
<td>22</td>
</tr>
</tbody>
</table>

Garcia-Nonell et al 2007
FMRP has many functions and its absence causes dysregulation of several systems known to be associated with autism

- Transporter of mRNAs to the synapse
- Controls (usually suppression of) translation of many mRNAs related to synaptic plasticity
- Absence of FMRP causes increased protein production throughout the brain
- Up regulation of mGluR5 pathways leading to LTD
- Down regulation of GABA_A receptors
- Dysregulation of dopamine pathways
- Enhanced APP production
- Increased oxidative stress damage to neurons
LOWERED BRAIN FMRP LEVELS IN PSYCHIATRIC DISORDERS

FMRP lower in adult autism brains

Fatemi et al schizophrenia research 2010
Proteins Controlled by FMRP (Darnell et al 2011 Cell)
mTOR activity plays a role in many cellular processes such as transcription, autophagy, cytoskeletal rearrangement, protein turnover, and translation initiation.
Fragile X Syndrome

- 1 in 3,600
- Leading inherited of ID leading
- Single gene associated with autism
- 2-6% with autism have FXS
- Anxiety disorders, mood instability. ..
FRAGILE X SYNDROME AND THE EXPRESSION OF THE FMR1 GENE

FXS is the most common form of intellectual disabilities and the leading known heritable form of autism.

... is caused by a large CGG-repeat expansion in a non-coding portion of the FMR1 gene.

<table>
<thead>
<tr>
<th>DNA</th>
<th>mRNA</th>
<th>FMRP</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical</td>
<td>(CGG) &lt; 45</td>
<td>Normal</td>
<td>Fragile site Xq27.3</td>
</tr>
<tr>
<td></td>
<td>Gray/intermediate alleles 45-54 CGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premutation</td>
<td>(CGG) 55 - 200</td>
<td>Primary ovarian insufficiency (FXPOI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tremor/ataxia syndrome (FXTAS)</td>
<td></td>
</tr>
<tr>
<td>Full</td>
<td>(CGG) &gt; 200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1/130-250 females
1/250-810 males
1/3600-4000
Expression of the *FMR1* gene

- **CGG repeat number**
  - 0
  - 2
  - 4
  - 6
  - 8
  - 10

- **Relative FMR1 mRNA level**
  - Normal
  - Gray
  - Premutation
  - Full mutation

- **FMRP level**
  - Unmethylated
  - Partially methylated
  - Hyper-methylated

- **CGG repeat number vs. FMRP level**

- **Graph**
  - FXS
  - FXTAS and POI

- **X-axis**: CGG repeat number
  - 0
  - 45
  - 55
  - 200
  - >1000

- **Y-axis**: Relative FMR1 mRNA level
  - 0
  - 1
  - 2
  - 4
  - 6
  - 8
  - 10
AGG interruptions are normally present in normal CGG repeats. 0, 1, 2, or 3 interruptions are common.

They typically occur around 9-10 CGG repeats.

(Eichler et al. 1994; 1996)
Risk Model

0 AGG interruptions
1 AGG interruption
2 AGG interruptions
Differential risk

Yrigollen et al., 2012
FRAGILE X SYNDROME AND RELATED DISORDERS

Genetic Testing and Counseling

- **Premutation**: Individuals with a single copy of the expanded FMR1 gene (60-200 CGG repeats) are considered premutation carriers and have a 50% chance of passing the expanded allele to their offspring.

- **Full Mutation**: Individuals with more than 200 CGG repeats are classified as having a full mutation. They usually have fragile X syndrome and typically have severe developmental delays, learning disabilities, and cognitive impairment.

- **FMR1 Negative**: Individuals with fewer than 60 CGG repeats are considered normal and do not have fragile X syndrome.

Genetic Counseling and Testing Options

- **Cytogenetic Testing**: This involves examining the chromosomes for any visible abnormalities. It's a straightforward test but may not detect all cases of fragile X syndrome.

- **Molecular Testing**: This involves analyzing the DNA for specific genetic changes. It's more sensitive than cytogenetics and can detect premutations and full mutations.

- **FMR1 Testing**: This is the gold standard for detecting fragile X syndrome. It involves specifically analyzing the FMR1 gene for the presence of the expanded CGG repeat.

- **Fetal Testing**: This can be done during pregnancy to determine if the fetus has fragile X syndrome. It can be done via amniocentesis or chorionic villus sampling.

Support and Resources

- **Support Groups**: Joining a support group can provide emotional support and practical advice from others who understand the challenges of living with fragile X syndrome.

- **Educational Resources**: There are numerous resources available online that provide information on fragile X syndrome, including websites, articles, and videos.

- **Professional Advice**: Consulting with a genetic counselor or a neurologist can help navigate the complexities of fragile X syndrome and provide personalized advice.

- **Research Opportunities**: Participating in research studies can contribute to the understanding of fragile X syndrome and may offer access to new treatments or therapies.
### Spectrum of Premutation Involvement

#### Cellular dysregulation
- **FMR1** CGG-repeat toxic RNA “trigger”
- Up-regulation of *heatshock* proteins
- **Kinase** activation
- Sequestration of **DROSHA/DGCR8**
- **miRNA** dysregulation
- **Mitochondrial** dysfunction
- **Inclusion formation**

#### Background gene effects

#### Environmental effects

#### Neurodevelopmental problems
- Social anxiety → ASD
- ADHD; Cognitive deficits
- Seizures

#### Psychiatric involvement
- Anxiety
- Stress
- Depression

#### Endocrine dysfunction
- FXPOI

#### Immune dysregulation
- Hypothyroidism
- Fibromyalgia; chronic fatigue
- Lupus- MS features

#### Neurological problems
- Neuropathy
- Migraine
- Memory problems, foggy thinking
- Hypertension, erectile dysfunction

#### FXTAS
- Tremor, ataxia, Parkinsonism
- Autonomic dysfunction, EF deficits,
  memory and cognitive decline

#### Neuropathology
Boys with the premutation are at high risk for ADHD and autism or ASD: A developmental form of RNA toxicity?

- ADHD (CGI>15 and DSM-IV)
  - 93% (13/14) of probands
  - 38% (6/13) of nonprobands
  - 13% (2/16) of controls
- ASD (DSM-IV and ADOS/ADI)
  - 73% (11/14) of probands*
    - 29% (4/14) Full autism
    - 50% (7/14) PDDNOS
  - 8% (1/13) of nonprobands
    - 8% (1/13) Full autism
  - None of controls

Farzin et al, 2006 J Dev Beh Pediatrics

Two brothers with the FMR1 premutation ages 6 and 7. Boy on right presented as proband with autism and ADHD and his brother has anxiety and ADHD.
A NEW COHORT OF PREMUTATION BOYS COMPARED TO CONTROLS

Most of the patients with seizures developed ASD

\[ *= p < 0.01 \]
\[ ** = p < 0.05 \]

Chonchiaya et al 2011 Human Genetics
Abnormal synaptic plasticity in FMR1 KO mice

(b)

Activation of Gp1 mGluRs (exaggerated in fragile X)

AMPA receptor
NMDA receptor

Bear et al., 2004
A Model for FMRP function

The mGluR hypothesis

Gradual loss of synapses

Too many “immature” spines

mGluR mediated signaling is directly coupled to the regulation of translation initiation in neurons.
The Role of FMRP: binds and transports mRNAs
And regulates translation usually through inhibition

FMRP transports mRNAs
to the synapse and regulates translation.

FMRP inhibits protein translation with mGluR5 stimulation

Oostra 2006
Dramatic Up-regulation of Proteins in the CNS without FMRP

Bassell and Gross 2008

mGluR theory of FXS
Bear et al 2004
A therapeutic approach for FXS

Diagram showing pre-synaptic and post-synaptic regions with GABA-B and mGluR5 receptors.
Arbaclofen Control Clinical Trial in FXS from 5-25YO

Pre-synaptic

GABA-B

Post-synaptic

Proteins

Arbaclofen

mGluR5

mGluR3

Seaside Therapeutics
MMP-9 synthesis, release and activation
Minocycline
Mechanisms for Neuroprotection

Minocycline

- Antiapoptotic
  - Decreases Caspase activity.
  - Decreases cytochrome C release.

- Antioxidant
  - Increases phosphorylation of GluR1 receptors.
  - Decreases cyclooxygenase.

- Anti-inflammatory
  - Inhibits microglial activation.
  - Decreases p38 MAPK

MMP9

Weak synaptic connections in FXS

Strong synaptic connections with minocycline treatment

MMP9

Lowers MMP9
mGluR5 antagonists for FXS

- **Fenobam**: improvement in PPI and behavior in single dose with 12 adults with FXS at MIND and Rush (Berry-Kravis et al 2009 JMG)

- **Roche mGluR5** antagonist RO4917523 currently in controlled trials at multiple centers including MIND (16yo and older with FXS). Initiated 5-12 childhood PK 3 mo studies


- **STX 107** an mGluR5 antagonist licensed by Seaside Therapeutics.
Study Measures

– Baseline:
  • Cognitive Assessment: Stanford Binet V, WISC IV, Leiter-R or Mullen Scales of Early Learning
  • Autism Assessment: Autism Diagnostic Observation Schedule (ADOS), DSMIV Criteria for Autism Checklist

– Primary Outcome Measures
  • Clinical Global Impression Scale-Improvement (CGI-I)
  • Visual Analogue Scale for Severity of Behavior (VAS)

– Secondary Outcome Measures
  • Vineland Adaptive Behavior Scale-II (VABS-II)
  • Aberrant Behavior Checklist- Community Edition (ABC-C)
  • Expressive Vocabulary Test-II (EVT-II)
ONLY THE FULL MUTATIONS FULLY METHYLATED RESPONDED TO AFQ056
JACQUEMONT ET AL 2011 SCI TRANS MED
R-BACLOFEN= ARBACLOFEN: STX209

• Baclofen is racemic

• Both isomers are selective GABA-B agonists
  • GABA-B: R:S potency ratio 15:1
  • in vivo: R:S potency ratio 10-100:1

• R-Baclofen kinetics comparable when given alone or as part of racemic mixture (with S-baclofen)

• R-Baclofen is more potent in blocking presynaptic release of glutamate and therefore may be helpful in FXS and perhaps in autism
• Double-blind, randomized, placebo-controlled, 2-period crossover
• Endpoints
  o Global: CGI-I; CGI-S; blinded treatment preference
  o Focused: Aberrant Behavior Checklist - Irritability (ABC-I) scale; ABC-Total & other subscales; Vineland Adaptive Behavior Scale; Visual Analog Scale of top 3 problem behaviors; other
• Down titration after completion of 4 week period
• Ages 6 to 40 years and maximum dose was 10 mg tid
• Published now Berry-Kravis et al 2012 Science Translational Medicine
CGI-I (IMPROVEMENT) RESULTS IN ARBACLOFEN (STX209) TRIAL

“Responders”
35% vs. 18%
p = 0.11

44% vs. 6%
(p < 0.05)

58% vs. 19%
p < 0.01

Berry-Kravis et al 2012
ARBACLOFEN FOR ASD

- Autistic Disorder or PDD-NOS
- ABC-Irritability ≥ 16 at baseline
- n = 32; age 6 – 17 years
- Concomitant meds: ≤ 2 psychoactives; no antipsychotics
- Treatment period: 8 weeks
<table>
<thead>
<tr>
<th></th>
<th>Baseline (mean ± SD)</th>
<th>Week 8 (mean ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC-Irritability</td>
<td>27.0 ± 7.6</td>
<td>17.7 ± 10.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ABC-Social Withdrawal</td>
<td>17.3 ± 8.2</td>
<td>12.6 ± 9.3</td>
<td>= 0.001</td>
</tr>
<tr>
<td>ABC-Total</td>
<td>90.3 ± 29.4</td>
<td>64.0 ± 35.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CGI-I</td>
<td>–</td>
<td>2.5 ± 0.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CGI-S</td>
<td>5.1 ± 0.9</td>
<td>4.4 ± 1.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ADHD-IV Rating Scale</td>
<td>34.2 ± 11.4</td>
<td>26.1 ± 13.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CY-BOCS</td>
<td>14.8 ± 4.1</td>
<td>11.6 ± 5.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CASI-Anxiety</td>
<td>20.4 ± 10.6</td>
<td>16.5 ± 13.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Social Responsiveness</td>
<td>117.0 ± 33.8</td>
<td>103.0 ± 29.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Vineland-Communication</td>
<td>61.4 ± 10.5</td>
<td>65.4 ± 9.5</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
MINOCYCLINE STUDIES IN FXS OR AUTISM

• Bilousova et al 2009 demonstrated that minocycline lowers MMP9 levels in FXS and improved behavior and cognition in the FX mouse

• Agustini Utari MD surveyed 50 families whose child was Tx with minocycline for >2wks and found 70% positive response especially in language and limited side effects (Utari et al 2010 AJIDDD).

• Positive open trial in FXS in Toronto with age ≥ 13 years (Paribello et al 2010)
MINOCYCLINE HYDROCHLORIDE

- Semisynthetic tetracycline derivative
- Commonly used in treatment of acne vulgaris
- Found to have neuroprotective effects
- Investigated in Huntington’s Disease, ALS, multiple sclerosis
STUDY DESIGN

- Randomized
- Double blind Placebo controlled trial 3.5-16y
- Crossover : 3 months for each arm
- Voluntary recruitment from UC Davis MIND Institute Fragile X Research and Treatment Center 66 entered 48 completed

www.clinicaltrials.gov

<table>
<thead>
<tr>
<th>Weight</th>
<th>Minocycline Daily Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25kg</td>
<td>25mg</td>
</tr>
<tr>
<td>25-45kg</td>
<td>50mg</td>
</tr>
<tr>
<td>&gt;45kg</td>
<td>100mg</td>
</tr>
</tbody>
</table>
CONTROLLED TRIAL OF MINOCYCLINE

- Minocycline
- Placebo

Screening

Visit 1
- Cognitive Assessment
- Autism Assessment
- VABS-II
- ABC-C
- CGI
- EVT-II
- VAS
- Hx & PE
- Con Meds
- AEs

Visit 2
- VABS-II
- ABC-C
- CGI
- EVT-II
- VAS
- Hx & PE
- Con Meds
- AEs

Visit 3
- VABS-II
- ABC-C
- CGI
- EVT-II
- VAS
- Hx & PE
- Con Meds
- AEs

Study Period: January 2010-December 2011

Monthly Phone Calls

Visit 1

Visit 2

Visit 3
CONTROLLED CROSS-OVER DOUBLE-BLIND TRIAL OF MINOCYCLINE, SIGNIFICANT IMPROVEMENT ON CGI

Intent to Treat Analysis

Minocycline $\bar{x} = 2.49 \pm 0.13$
Placebo $\bar{x} = 2.97 \pm 0.13$
$p = 0.0173$
RESULTS: VISUAL ANALOGUE SCALE

<table>
<thead>
<tr>
<th>VAS Behavior Category</th>
<th>Minocycline Mean</th>
<th>Minocycline SE</th>
<th>Placebo Mean</th>
<th>Placebo SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggression/ADHD (n=46)</td>
<td>4.47</td>
<td>0.35</td>
<td>4.25</td>
<td>0.32</td>
<td>0.5355</td>
</tr>
<tr>
<td>Anxiety/Mood (n=26)</td>
<td>5.26</td>
<td>0.46</td>
<td>4.05</td>
<td>0.46</td>
<td>0.0488</td>
</tr>
<tr>
<td>Language/Cognition (n=38)</td>
<td>4.99</td>
<td>0.36</td>
<td>4.70</td>
<td>0.34</td>
<td>0.5345</td>
</tr>
<tr>
<td>Other (n=11)</td>
<td>6.02</td>
<td>0.58</td>
<td>3.45</td>
<td>0.34</td>
<td>0.0175</td>
</tr>
</tbody>
</table>

Significant change in VAS for Anxiety/Mood and for “other” category including diverse problems such as toilet training and social interactions.

No influence of FSIQ & ADOS total score on response to minocycline.
ADVERSE EVENTS

78% of participants reported AE; 49% on minocycline, 51% on placebo

<table>
<thead>
<tr>
<th>Category</th>
<th>Minocycline</th>
<th></th>
<th>Placebo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>%</td>
<td>Count</td>
<td>%</td>
</tr>
<tr>
<td>Diarrhea/Loose Stools</td>
<td>15</td>
<td>21.13</td>
<td>15</td>
<td>20.55</td>
</tr>
<tr>
<td>GI Upset/Vomiting/Loss of Appetite</td>
<td>9</td>
<td>12.68</td>
<td>15</td>
<td>20.55</td>
</tr>
<tr>
<td>Dizziness/Unsteadiness</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.37</td>
</tr>
<tr>
<td>Headaches</td>
<td>4</td>
<td>5.63</td>
<td>5</td>
<td>6.85</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>2</td>
<td>2.82</td>
<td>3</td>
<td>4.11</td>
</tr>
<tr>
<td>Skin Rash/Itching/Swelling</td>
<td>12</td>
<td>16.9</td>
<td>7</td>
<td>9.59</td>
</tr>
<tr>
<td>Fever/chills/URI symptoms/Sore Throat</td>
<td>6</td>
<td>8.45</td>
<td>11</td>
<td>15.07</td>
</tr>
<tr>
<td>Blue-grey/grey hue to teeth or other tissues</td>
<td>3</td>
<td>4.23</td>
<td>1</td>
<td>1.37</td>
</tr>
<tr>
<td>Dark colored urine/changes in urination</td>
<td>1</td>
<td>1.41</td>
<td>2</td>
<td>2.74</td>
</tr>
<tr>
<td>Sunburn/sun sensitivity</td>
<td>4</td>
<td>5.63</td>
<td>1</td>
<td>1.37</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
<td>21.13</td>
<td>12</td>
<td>16.44</td>
</tr>
</tbody>
</table>

No difference in AEs on minocycline vs placebo

p=0.551

Sanchez 2004
Severe involvement from FXS
Autistic, non-verbal, aggressive, would not tolerate clothes could not go outside

After 2 years on minocycline
He can talk and dress
He drinks from a cup
He walks with his social worker
Aggression is gone
He can come to clinic
Looks at magazines and TV
ONLY 2 INDIVIDUALS HAD MMP9 LEVELS DONE IN EACH PHASE OF STUDY AND BOTH WERE RESPONDERS TO MINOCYCLINE

Tassone et al unpublished
Ed Weeber carried out trials of minocycline in AS mouse model with positive effects and is carrying out a controlled trial of minocycline in AS children and has preliminary positive results.

Clinical use of minocycline in AS at the MIND has shown improvements in language and motor abilities in 5 children with AS.
GABA_A RECEPTOR EXPRESSION IS DOWN IN FXS

- **GABA_A** expression is down regulated in the KO mouse (D’Hulst et al 2007; Kooy et al 2005)
- **GABA_A agonists**: Ganaxolone
  - Investigational medication with efficacy in infantile spasms and other types of epilepsy: A controlled trial in children with FXS (6-18y) funded by DOD is in progress at the MIND Institute; Marinus to supply ganaxolone
  - Targeting improvement in anxiety, behavior and seizure frequency
GANAXOLONE TREATMENT TIMELINE DOUBLE-BLIND CROSSOVER CONTROLLED TRIAL

**Period 1**
- Screening
- Baseline
- Week 3
- Week 6
- Week 8
- Week 7
- Ganaxolone
- Placebo

**Period 2**
- Week 11
- Week 14
- Ganaxolone
- Placebo
- Week 7
- Week 15-16
In autism serotonin synthesis is reduced frontally. This may be true for FXS since clinically they respond to early sertraline Tx.
SERTRALINE TREATMENT IN EARLY CHILDHOOD IN FXS

A RETROSPECTIVE STUDY OF 45 CHILDREN FOLLOWED 12 TO 50 MONTHS AND 11 TREATED WITH SERTRALINE: SIGNIFICANT DIFFERENCES IN EXPRESSIVE AND RECEPTIVE LANGUAGE IN TX VS NON TREATED (P=0.0001 AND P=0.0071 RESPECTIVELY)

Winarni et al 2012 Autism Treatment and Research
LOVASTATIN AND LANGUAGE INTERVENTION

- Relationship between MIF, MMP-9, Ca2+ signaling, and the MEK/ERK pathway in inflammation.
Pollution, Environmental Toxicity
Microenvironment
Genes
Social Interactions
Environmental Changes
LOVASTATIN AND LANGUAGE INTERVENTION

- Relationship between MIF, MMP-9, Ca2+ signaling, and the MEK/ERK pathway in inflammation.
TARGETED TREATMENTS MUST BE COMBINED WITH INNOVATIVE EDUCATIONAL PROGRAMS

• If synaptic connections are improved with targeted treatment we must enhance these connections with educational interventions

• Combine treatment trials with educational interventions, computer programs such as CogMed, AT devices, iPad apps.
There is little empirical research on the key factors that promote or hinder:

- language improvement
- social communication progress and
- learning acquisition

in children with ASD and FXS or both, by using an iPad®-centered intervention approach.

PI: María Díez-Juan, M.A. Clinical Psychologist. ARTP Research Scholar. MIND Institute. UC Davis
MIND APPs Objective

We aim to demonstrate the efficacy of the iPad®-centered intervention on social communication, language development and academic gains in children with FXS & ASD.

Methods

- iPad-centered intervention by collecting data on young children (2 to 5 years) and children (6 to 12 years) with ASD and/or FXS - 6 weeks.
- randomized clinical trial (RTC)
- 30 enrolled subjects
- Crossover

We will use Care Circles application as a platform to coordinate the whole plan and guidelines. Also to track data through parents’ reports.
HOW WILL CLINICAL PRACTICE CHANGE

• All individuals would be treated – need clinic resources to accommodate management, patient education and monitoring – FXCRC

• Early diagnosis and treatment imperative – newborn screening

• Dosing may be tricky, combinations with different pathway targets may work best – need practitioners with FXS experience to assess response

• Likely stepwise improvement in treatments – need ongoing clinical trials network (FXCRC) to keep building best treatment protocols (like cancer tx model)
Collaborators

UC Davis School of Medicine
Dept. Biochem & Molec. Medicine
  Paul Hagerman  Flora Tassone
  Chris Iwahashi
  Anna Ludwig
  Dolores Garcia-Arocena
  Greg Mayeur  Chris Raske
Dept. Biostatistics
  Danh Nguyen

University of Washington and UC Davis Fragile X Research Center NICHD Funded
Charles Laird  Mike Guralnick  Gwen Glew

University of Colorado Health Sciences Center (Denver)
Nicole Tartaglia  Maureen Leehey  James Grigsby  Karen Riley at DU

RUSH-Presbyterian-St. Luke’s Medical Center (Chicago)
  Elizabeth Berry-Kravis  Deb Hall  Christopher Goetz

Waisman Center-University of Wisconsin
  Len Abbeduto has come to the MIND

*Latrobe University, Melbourne Australia*
  Danuta Loesch  Richard Huggins

Support: NICHD, NINDS, NIA, NFXF, CDC, NFXF Neuropharm, Roche, Novartis, Seaside Therapeutics