Thinking outside the brain: Maternal autoantibodies in autism

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Overarching Goals of Our Research at UCD

• Examine the roles of environmental factors, genes, and the immune system as they relate to ASD susceptibility.

• Through both population studies and animal models, address how environmental triggers and immune dysregulation affect brain development.

• Determine how biological markers, such as those related to immune system dysfunction, play a role in ASD
  – May clarify why some children develop neurodevelopmental disorders.

• Gestational immune environment in ASD
  – How changes in immune signaling molecules affect neurodevelopment
  – Maternal autoantibodies to fetal brain proteins and their potential role in ASD.
Maternal Autoantibody Related (MAR) Autism

Autoantibodies present in the circulation of mothers during pregnancy that recognize proteins in the developing fetal brain
Our Model

A. Maternal antibody Production - trigger unknown

B. Placental transfer of IgG to fetus

C. IgG interferes with CNS development

Initial finding: Some mothers who have children with autism produce anti-brain antibodies

Maternal antibodies present during pregnancy result in Maternal Antibody Related (MAR) autism
Maternal Anti-Brain Antibodies and ASD: The studies behind the novel immune biomarker for autism risk

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

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

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

**Basic Science**
- The antigenic targets of the antibodies have been identified

**Animal Models**
- Animal models (2 monkey, 2 mouse) show behavioral changes after prenatal exposure to the antibodies; monkey model has also reported increased brain volume

**Brain Tissue Studies**
- Animal models provide tissue to explore brain pathology (ongoing)

**Epidemiology**
- Large population studies to identify potential risk factors for ASD

**Initial finding: Immunology**
- Some mothers who have children with autism produce anti-brain antibodies

### Translational Potential:
- Identify kids with this subphenotype and develop tailored behavioral treatment
- Screen women at risk and develop preventative strategies
- Define pathophysiology associated with these antibodies and develop therapeutic interventions
Maternal antibodies to fetal brain proteins- MAR autism


- These antibodies are highly specific for autism, and have demonstrated pathology in animal models (Martin, 2008, Singer, 2009 and Braunschweig, 2012).
What are the antigenic targets?

- A targeted 2-D gel electrophoresis/mass spectrometric approach was used to determine the identity of the antigenic targets based on their original fetal monkey brain band patterns.

- To verify specificity, maternal plasma samples were absorbed overnight with purified antigen or with purified control protein.

- Reactivity to the target band was removed with purified antigen.
Several Ab combinations are only found in mothers of ASD children

All specific combinations combined identify an association of MAR antibodies in 23% of mothers

<table>
<thead>
<tr>
<th>Antigen/Antigen Combinations</th>
<th>AU/ASD (n=246)</th>
<th>TD (n=149)</th>
<th>P-value†</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactivity to any antigen‡</td>
<td>89% (n=218)</td>
<td>70% (n=105)</td>
<td>&lt;0.0001</td>
<td>3.26 (1.92-5.53)</td>
</tr>
<tr>
<td><strong>INDIVIDUAL PROTEINS</strong></td>
<td></td>
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</tr>
<tr>
<td>LDH</td>
<td>28% (n=68)</td>
<td>13% (n=20)</td>
<td>0.0012</td>
<td>2.5 (1.4-4.3)</td>
</tr>
<tr>
<td>Cypin</td>
<td>25% (n=62)</td>
<td>19% (n=29)</td>
<td>0.22</td>
<td>1.4 (0.8-2.3)</td>
</tr>
<tr>
<td>STIP1</td>
<td>59% (n=145)</td>
<td>36% (n=53)</td>
<td>&lt;0.0001</td>
<td>2.6 (1.7-4)</td>
</tr>
<tr>
<td>CRMP1</td>
<td>32% (n=78)</td>
<td>18% (n=27)</td>
<td>0.0034</td>
<td>2.1 (1.3-3.4)</td>
</tr>
<tr>
<td>CRMP2</td>
<td>18% (n=44)</td>
<td>7% (n=11)</td>
<td>0.004</td>
<td>2.7 (1.4-5.5)</td>
</tr>
<tr>
<td>YBX1</td>
<td>31% (n=78)</td>
<td>23% (n=34)</td>
<td>0.065</td>
<td>1.57 (0.98-2.5)</td>
</tr>
<tr>
<td><strong>SIGNIFICANT COMBINATIONS</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LDH + STIP1</td>
<td>16% (n=40)</td>
<td>6% (n=9)</td>
<td>0.0026</td>
<td>3.0 (1.4-6.4)</td>
</tr>
<tr>
<td>LDH + CRMP1</td>
<td>9% (n=22)</td>
<td>3% (n=4)</td>
<td>0.0196</td>
<td>3.6 (1.2-10.5)</td>
</tr>
<tr>
<td>Cypin + STIP1</td>
<td>18% (n=45)</td>
<td>8% (n=12)</td>
<td>0.0049</td>
<td>2.6 (1.3-5)</td>
</tr>
<tr>
<td>Cypin + CRMP1</td>
<td>9% (n=22)</td>
<td>3% (n=5)</td>
<td>0.038</td>
<td>2.8 (1-7.6)</td>
</tr>
<tr>
<td>STIP1 + CRMP1</td>
<td>18% (n=45)</td>
<td>7% (n=10)</td>
<td>0.0014</td>
<td>3.1 (1.5-6.4)</td>
</tr>
<tr>
<td><strong>SPECIFIC COMBINATIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Combinations of 2 antigens</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LDH + YBX1</td>
<td>2% (n=4)</td>
<td>0% (n=0)</td>
<td>0.16</td>
<td>3.1 (0.37-124)**</td>
</tr>
<tr>
<td>LDH + CRMP2</td>
<td>1% (n=2)</td>
<td>0% (n=0)</td>
<td>0.5</td>
<td>2.18 (0.15-67)**</td>
</tr>
<tr>
<td>YBX1 + CRMP2</td>
<td>5% (n=12)</td>
<td>0.6% (n=1)</td>
<td>0.037</td>
<td>7.6 (0.97-59)**</td>
</tr>
<tr>
<td>CRMP1 + CRMP2</td>
<td>1% (n=2)</td>
<td>0% (n=0)</td>
<td>0.5</td>
<td>2.18 (0.15-67)**</td>
</tr>
<tr>
<td>Combinations of 3 or more antigens</td>
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<tr>
<td>LDH + Cypin + STIP1</td>
<td>5% (n=12)</td>
<td>0.6% (n=1)</td>
<td>0.0371</td>
<td>3.8 (0.96-29.1)**</td>
</tr>
<tr>
<td>LDH + STIP1 + CRMP1</td>
<td>5% (n=13)</td>
<td>0% (n=0)</td>
<td>0.0025</td>
<td>8.3 (1-293)**</td>
</tr>
<tr>
<td>LDH + Cypin + STIP1 + CRMP1</td>
<td>2% (n=5)</td>
<td>0% (n=0)</td>
<td>0.16</td>
<td>3.1 (0.37-124)**</td>
</tr>
<tr>
<td>LDH + Cypin + YBX1 + STIP1</td>
<td>2% (n=4)</td>
<td>0% (n=0)</td>
<td>0.16</td>
<td>3.1 (0.37-124)**</td>
</tr>
<tr>
<td>LDH + Cypin + YBX1 + STIP1 + CRMP1</td>
<td>0.4% (n=1)</td>
<td>0% (n=0)</td>
<td>1^</td>
<td>1.82 (0.07-45)**</td>
</tr>
<tr>
<td>LDH + YBX1 + STIP1 + CRMP1 + CRMP2</td>
<td>0.4% (n=1)</td>
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<td>0.0025</td>
<td>5.8 (1.5-43.9)**</td>
</tr>
<tr>
<td>Cypin + YBX1 + STIP1 + CRMP1</td>
<td>2% (n=4)</td>
<td>0% (n=0)</td>
<td>0.16</td>
<td>3.1 (0.37-124)**</td>
</tr>
<tr>
<td>Cypin + YBX1 + STIP1 + CRMP2</td>
<td>1% (n=2)</td>
<td>0% (n=0)</td>
<td>0.5</td>
<td>2.18 (0.15-67)**</td>
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<td>1% (n=2)</td>
<td>0% (n=0)</td>
<td>0.5</td>
<td>2.18 (0.15-67)**</td>
</tr>
<tr>
<td>All specific combinations combined</td>
<td>23% (n=56)**</td>
<td>1% (n=2)</td>
<td>&lt;0.0001</td>
<td>21.7 (5.2-90) **</td>
</tr>
<tr>
<td># of Antigens</td>
<td>Antigen</td>
<td>% ASD N=241</td>
<td>N</td>
<td>% TD N=147</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------------------------</td>
<td>-------------</td>
<td>---</td>
<td>------------</td>
</tr>
<tr>
<td>2</td>
<td>LDH + CRMP2</td>
<td>7%</td>
<td>17</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>STIP1 + CRMP2</td>
<td>10%</td>
<td>24</td>
<td>1%</td>
</tr>
<tr>
<td>2</td>
<td>CRMP1 + CRMP2</td>
<td>7%</td>
<td>16</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>LDH + STIP1 + CRMP1</td>
<td>5%</td>
<td>12</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>Cypin + YBX1 + CRMP1</td>
<td>2%</td>
<td>5</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>Cypin + STIP1 + CRMP1</td>
<td>7%</td>
<td>18</td>
<td>1%</td>
</tr>
<tr>
<td>3</td>
<td>Cypin + STIP1 + CRMP2</td>
<td>3%</td>
<td>8</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>YBX1 + STIP1 + CRMP2</td>
<td>3%</td>
<td>7</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>LDH + Cypin + YBX1 + STIP1</td>
<td>2%</td>
<td>5</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>LDH + Cypin + STIP1 + CRMP1</td>
<td>2%</td>
<td>5</td>
<td>0%</td>
</tr>
</tbody>
</table>

- Statistical significance was determined through a permutation analysis.
- Evidence provided by a data-driven selection that antibody combinations are associated with case status.
7 Maternal Antibodies Bind to Protein Targets Critical to Normal Brain Development*

YBX-1- 
Neural tube formation, cell division

CRMP1- 
Cell migration

Embryonic Cross-section

STIP1- 
Neuritogenesis

Cypin (GDA)- 
Dendritic branching

CRMP1- 
Growth cone collapse

CRMP2- 
Axon outgrowth 
Growth cone collapse 
Basal dendrite patterning

YBX-1- 
Transcription regulation

LDH- 
Metabolism

What about the intracellular targets?

- Human MAR Ag specific biotinylated IgG binds specifically to early developing neurons. Intracellular staining (live uptake in D14 mouse embryo).
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>0.006</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDH + Cypin (n=4)</td>
<td>n.s.</td>
<td>0.041</td>
<td>n.s.</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>0.028</td>
<td>0.058</td>
<td>0.047</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
Autism severity score associated with MAR

- **Girls**
  - MAR+ (n=4): 5.50 ± 1.29
  - MAR- (n=21): 7.10 ± 1.48
  - MAR- (n=172): 7.16 ± 1.51

- **Boys**
  - MAR+ (n=40): 7.68 ± 1.73
  - MAR- (n=21): 7.10 ± 1.48
  - MAR- (n=172): 7.16 ± 1.51

*p = 0.05*
What are the effects of these specific maternal autoantibodies? Do they have pathologic significance?
• 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 do animal models tell us?

Hypothesis: Antibodies from maternal circulation can affect brain development and produce a behavioral outcome relevant to autism.

– We now have several animal studies to address this question.

– Our first monkey model pilot study demonstrated behavioral changes in offspring following passive transfer of maternal IgG.

Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey

MD Bauman1,2,3, A-M Iosif4, P Ashwood5, D Braunschweig6, A Lee1,3, CM Schumann1,3, J Van de Water5 and DG Amaral1,2,3,7

Work of colleague Dr. Melissa Bauman at the U.C. Davis M.I.N.D. Institute

Using a passive transfer model to examine the pathologic significance of IgG from mothers with reactivity to the 37/73 kDa bands
**Methods**

Purified human maternal IgG

Injected into pregnant rhesus monkeys 6 time points beginning late 1\(^{st}\) trimester

165 Day Gestation

<table>
<thead>
<tr>
<th></th>
<th>ASD-IgG (n=8)</th>
<th>Control-IgG (n=7)</th>
<th>Untreated (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaque Offspring</td>
<td>4 males 4 females</td>
<td>2 males 5 females</td>
<td>4 males 5 females</td>
</tr>
<tr>
<td>Untreated (n=9)</td>
<td>5 males 4 females</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Outcome Measures

Brain Development

Behavioral Development
Summary of Behavioral Changes

Mothers of the IgG-treated monkeys are “overprotective”.

As they get older, the IgG-treated offspring show evidence of abnormal social behavior with both familiar and unfamiliar peers.

The IgG-treated monkeys fail to engage in species-typical reciprocal social interactions.
Brain Changes

IgG-treated monkeys have larger brains.
Maternal Antibody Model Summary

Prenatal exposure of gestating monkeys to autism-specific maternal antibodies is associated with two characteristics of ASD: atypical social development and enlarged brain volume.
Mouse model of MAR: Changes in Behavior Consistent with ASD in Social and Stereotypic Behavior Domains

- Maternal autism-associated IgG antibodies delay development, reduce social interaction, and produce anxiety in a mouse gestational transfer model (Braunschweig, et al. JNI, 2012)
Second mouse study:

Intraventricular injections of Purified 1 ug IgG *in vivo* (E14 and E16)
→ Stem Cells + Behavior
A) A non-significant trend for treatment was observed for total time spent grooming, with MAU offspring grooming significantly longer than MTD offspring ($^p = 0.0794$).

B) MAU offspring groomed significantly longer on average than MTD offspring (*$p = 0.0135$).

In: Brain Behavior Research, 2014
Extended Social Interaction Behaviors Amongst Offspring

A) MAU offspring spent more time sniffing the novel object (*p = 0.001) and less time sniffing the stranger mouse (^p = 0.073) than MTD offspring.

B) MAU offspring groomed more often than MTD offspring (*p = 0.001).

C) MAU offspring spent more time grooming than MTD offspring (*p = 0.026).

D) A significant treatment x offspring sex interaction indicated that MAU females groomed more frequently than MTD females (*p = 0.023), but the same effect was not seen in males (*p < 0.05).
Conclusions - the big picture

• Gestational immune dysregulation may contribute to risk of altered neurodevelopment
  • Inappropriate inflammatory response to immune challenge
  • Genetic predisposition plays a role (MET polymorphism)
• Maternal autoantibodies are specific for ASD
  • Evidence that these antibodies are causing symptoms associated with ASD
Conclusions and Future Directions

Identification of MAR antibodies, their target proteins, and their epitopes

Prospective studies to determine predictive value

Create true animal model

MAR peptide epitopes

Expand to non-human primate model

Behavior

Biomarker test: Identify women at risk—
The UC Davis M.I.N.D. Institute Team

Dr. Judy Van de Water
Dr. Paul Ashwood
Dr. David Amaral
Dr. Christine Wu Nordahl
Dr. Melissa Bauman
Dr. Veronic Martinez-Cerdeno
Dr. Daniel Braunschweig
Robert Boyce
Elizabeth Fox
Lauren Matelski
Marjannie Eloi

Lori Haapanen

UCD Center for Children’s Environmental Health, C.H.A.R.G.E. and MARBLES

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Dr. Irva Hertz-Picciotto
Sally Ozonoff
Dr. Robin Hansen
Dr. Pam Lein
Melissa Rose

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.
Evaluation

Please complete the evaluation for this session.