How Fragile X and Anxiety Impact our family

Robin Blackwood, Greenville, SC
6 Months Old
Sam Blackwood, 23 months old
Analysis indicates an abnormal male pattern upon Southern Blot analysis with evident of Trinucleotide repeat amplification within FMRI. The pattern observed was a full mutation in FMRI with approximately **600-1300 repeats** (estimated from the Southern blot), a pattern found in males who have Fragile X Syndrome.

Robin Blackwood, 35 years old
One expanded premutation allele considering of approximately **100 repeats** was detected by PCR analysis is. Southern analysis also indicated an abnormal female pattern with evidence of trinucleotide repeats expansion with FMRI.
<table>
<thead>
<tr>
<th>Fragile X Syndrome</th>
<th>Autism Spectrum Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Social Anxiety</td>
<td>• Social Indifference</td>
</tr>
<tr>
<td>• Friendly and sociable, but shy and socially anxious</td>
<td>• Gaze Indifference</td>
</tr>
<tr>
<td>• Gaze Aversion</td>
<td>• Lack of understanding of facial expression</td>
</tr>
<tr>
<td>• Moderate to severe Intellectual Disability</td>
<td>• High IQ possible, mild, moderate and severe Intellectual</td>
</tr>
<tr>
<td></td>
<td>Disability</td>
</tr>
<tr>
<td>• Delayed symbolic play</td>
<td>• Distorted imitative &amp; symbolic play</td>
</tr>
<tr>
<td>• Language impairments – delayed, echolalia with</td>
<td>• Variable language impairments – usually affecting</td>
</tr>
<tr>
<td>repetitive, rapid &amp; cluttered speech</td>
<td>receptive more than expressive language</td>
</tr>
<tr>
<td>• Understanding of facial expression</td>
<td>• Absent Theory of Mind</td>
</tr>
<tr>
<td>• Distorted Theory of Mind (Not absent)</td>
<td>• Aloof, passive, active eccentric, over pedantic</td>
</tr>
<tr>
<td>• Self injury - often hand biting in response to</td>
<td>• A Self injury - variable in cause and in how it manifests</td>
</tr>
<tr>
<td>anxiety &amp; excitement</td>
<td></td>
</tr>
</tbody>
</table>

**How Fragile X And Autism Are Different and Overlap**

Professor Cornish, Professor Kau, Professor Wing and Assoc.

UK Fragile X Society
10 Years Old
Some people make your laugh a little louder, your smile a little brighter and your life a little better.
Thank You.

Robin Blackwood

YouTube: SCFragileX
Tumblr: fragilexworld
Twitter: FragileXSC
Phone: 864-905-2444
Email: robinbblackwood@gmail.com
Early biobehavioral risk markers of anxiety in high-risk infants

Abigail L. Hogan, Conner Black, Kayla Smith, Nicolas Poupore, and Jane E. Roberts

University Of South Carolina
March 1, 2019
What is fragile X syndrome (FXS)?
Characteristics of FXS

Prevalence:
- 1 in 4,000 males
- 1 in 6,000 females

Most common inherited cause of intellectual disability (ID)

Females less severely affected

“Portal disorder”
- Anxiety
- Autism spectrum disorder (ASD)
- AD/HD
Social anxiety disorder

Fear or anxiety about, or avoidance of, social interactions and situations that involve the possibility of being scrutinized
- Eating in front of others
- Public speaking/performance
- Circle time/show-and-tell
- 50% are diagnosed by 13 years of age

7% of typically-developing (TD) children and adolescents will be diagnosed with social anxiety

1Beedso, Knappe, & Pine (2011)
Social anxiety in FXS

Prevalence of social anxiety in FXS:
- 37% of males and females, aged 5-27 years (DSM-IV criteria)²
- 13% of males, aged 13-24 years (DSM-5 criteria)³

Increased social avoidance across the lifespan in FXS, but this seems to be linked more closely with ASD symptoms than anxiety symptoms⁴,⁵

²Cordeiro et al. (2011); ³Ezell, Hogan, et al. (2018); ⁴Roberts et al. (under review); ⁵Roberts et al. (under review)
Social anxiety in ASD

Prevalence of social anxiety in children with autism spectrum disorder (ASD): ~50%

Prevalence of social anxiety in siblings of children with ASD (non-ASD ASIBs): ???

MUSC Medical University of South Carolina
Changing What’s Possible | MUSC.edu
White & Schry (2013)
Challenges to assessing social anxiety in children with ID

Diagnosis of social anxiety requires the child to be able to understand the concept social evaluation and describe symptoms, both of which are difficult for children with ID.

Most anxiety symptoms are assessed via parent reported measures. Although parent insight is helpful, direct measurement provides increased strength in assessing anxiety.
The present study

Do high-ASD-risk infants demonstrate more social anxiety risk markers than low-risk controls (LRCs) at 12 months?

- This study utilizes a multi-method approach to characterize the early behavioral and physiological markers of social anxiety in infants with FXS and infant siblings of children with ASD (who do not end up with ASD themselves).

- Prospective studies of high-risk infants provide unique opportunities for identifying early anxiety risk markers in infants who are more likely to later develop social anxiety than infants in the general population.
Early predictors of childhood social anxiety in TD infants

Infancy/Toddlerhood

Behavioral Inhibition\textsuperscript{7,8}

Respiratory Sinus Arrhythmia\textsuperscript{8}

Childhood/Adolescence

Changing What’s Possible | MUSC.edu

\textsuperscript{7}Mian et al. (2016); \textsuperscript{8}Brooker et al. (2013)
Behavioral inhibition (BI)

Temperament characteristic:
- Negative affect and reactivity
- Excessive fear responses
- Gaze avoidance
- Withdrawal

Relationship to later social anxiety:
- One of the strongest developmental predictors of anxiety disorders\(^7\text{-}^9\)
- Early BI in response to unfamiliar adults predicts later social anxiety specifically\(^8\)

\(^7\)Mian et al. (2016); \(^8\)Brooker et al. (2013); \(^9\)Wichstrøm et al. (2013)
Respiratory sinus arrhythmia (RSA)

Index of parasympathetic nervous system function:
• Variability in heart rate during respiration
• Higher baseline RSA associated with appropriate emotional reactivity, good attentional abilities
Reactivity in RSA during environmental challenges (e.g., novelty) mediates behavioral responses to the environment\textsuperscript{10,11}

Relationship to later social anxiety:
• Attenuated RSA suppression to a stranger’s approach at 6 months \(\rightarrow\) more markers of social anxiety at 36 months\textsuperscript{8}

\textsuperscript{4}Brooker et al. (2013); \textsuperscript{10}Bahzenova et al. (2001); \textsuperscript{11}Porges et al. (1996)
### Participants

<table>
<thead>
<tr>
<th></th>
<th>LRC (n = 38)</th>
<th>FXS (n = 17)</th>
<th>ASIB (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronological Age, M (SD)</strong></td>
<td>12.40 (0.59)</td>
<td>12.55 (0.55)</td>
<td>12.28 (0.90)</td>
</tr>
<tr>
<td><strong>Mullen Early Learning Composite, M (SD)</strong></td>
<td>99.16 (12.51)</td>
<td>80.41 (20.37)</td>
<td>95.81 (17.56)</td>
</tr>
<tr>
<td><strong>Sex, n (%) male</strong></td>
<td>30 (78.9%)</td>
<td>10 (58.8%)</td>
<td>22 (68.8%)</td>
</tr>
</tbody>
</table>

Participants drawn from a larger longitudinal study of emergent ASD symptoms
- **FXS**: Confirmed via genetic testing
- **ASIBs**: Younger siblings of children with ASD
- **LRCs**: No family history of ASD or related disorders

ASIB and LRC infants were excluded from the present study if diagnosed with ASD themselves later in development (≥ 24 months)
Behavioral inhibition (BI)

Behavioral Inhibition during Stranger Approach:

1. Gaze avoidance (away from Stranger)
2. Escape Behavior*
3. Distress Vocalizations*

*Composite score taking into account proportion of time spent at each intensity level (higher = more intense)
Respiratory Sinus Arrhythmia (RSA)

- Baseline Period: Three-minute video
- Stranger Approach
- RSA Suppression (Baseline– Stranger Approach)
Gaze Behavior

![Graph showing proportion of looking time for different conditions and groups.](image)
Escape Behavior
Distress Vocalizations

$p = .014$

$p = .001$

Composite Score

<table>
<thead>
<tr>
<th></th>
<th>LRC</th>
<th>FXS</th>
<th>ASIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escape Behavior</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distress Vocalizations</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MUSC Medical University of South Carolina
Changing What’s Possible | MUSC.edu
RSA Suppression

![Heart rate variability graph]

- LRC: Lower RSA suppression in Baseline compared to Stranger condition with $\Delta = 0.58$ and $p = 0.009$
- FXS: Slight decrease in RSA with $\Delta = 0.27$ and $p = 0.293$
- ASIB: Stable RSA across conditions with $\Delta = 0.02$ and $p = 0.916$
Conclusions

• Non-ASD ASIB infants exhibit more biobehavioral risk markers (e.g., elevated behavioral inhibition, attenuated RSA suppression), but infants with FXS do not

• Social anxiety and associated risk markers are salient features that need to be further characterized in non-ASD ASIBs throughout development

• Findings corroborate other studies on temperament and social avoidance, which suggest that that social avoidance in FXS is more closely tied to ASD symptoms than to anxiety symptoms

• Follow-up studies relating later ASD and anxiety symptoms in FXS to these earlier biobehavioral profiles are needed
  • Is it possible that infants with FXS are not interpreting the stranger approach as “threatening” because of cognitive or developmental delay?

• Identification of at-risk children → targeted intervention and treatment
Thank You!

Dr. Jane Roberts
Neurodevelopmental Disorders Lab Students and Staff
Nicolas Poupore
Kayla Smith
Conner Black
Jenna Smith

Families who have participated in this study!

Funding Sources
R01MH90194; R01MH107573 (PI: Roberts)
ASPIRE Track II-B (PI: Hogan)
Evaluating nicotinamide riboside supplementation in a mouse model of autism

Freeman Lab
Furman University
Autism Spectrum Disorder

Diagnostic Criteria (DSM-5):

- Persistent deficits in social communication and social interaction across multiple contexts
- Restricted, repetitive patterns of behavior, interests, or activities
- Symptoms must be present in the early developmental period
- Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning
- These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay
Models of Autism

1) Neuropeptide abnormalities
   i.e. oxytocin or vasopressin

2) Models that mimic epigenetic marks that increase autism risk in humans
   i.e. VPA or thalidomide

3) Models of neonatal lesions on brain areas affected in individuals with autism
   i.e. maternal immune activation

4) Models with genetic diseases associated with autism
   i.e. neuroligin, shank3, Foxp2, Oxtr, Mecp2, BTBR

Models of Autism

1) Neuropeptide abnormalities
   i.e. oxytocin or vasopressin

2) Models that mimic epigenetic marks that increase autism risk in humans
   i.e. VPA or thalidomide

3) Models of neonatal lesions on brain areas affected in individuals with autism
   i.e. maternal immune activation

4) Models with genetic diseases associated with autism
   i.e. neuroligin, shank3, Foxp2, Oxtr, Mecp2, BTBR

Valproic Acid

Anti-epileptic drug
Voltage-gated sodium channel blocker
HDAC inhibitor
Attenuates calcium mediated T current
Augments release of GABA
The valproic acid model of autism

Construct validity
› VPA model effectively mimics an aspect of its etiology

Face validity
› VPA model: behavioral endpoints and comorbidities (ex. GI problems, sleep disturbances)

Predictive validity
› Pharmacological treatments, such as donepezil, ciproxifan, resveratrol, atomoxetine reduce symptoms in the VPA model

Intersection of epigenetic regulation and mitochondrial function in autism

Specific Aim 3.1 – Can nicotinamide riboside (NR) reverse behavioral abnormalities in the VPA mouse model of ASD?
Specific Aim 3.2 – Does treatment with NR alter cellular composition in the developing brain?
Preliminary Data – Dr. Luigi Boccuto (Greenwood Genetics Center)

Figure 1 – Histogram of energy metabolism in the ASD cells vs. control cells. The bar height of the histogram indicates the mean of measurements of 50 cell lines for each group; vertical bars are standard errors of the means. Star symbol (*) denotes level of statistical significance determined by a t test (***P <0.001). The substrates were ordered according to their individual P values with the lowest on the left (published in [19]).
Study Design

- VPA or Saline i.p.
- NR or control
- Wean
- Behavioral Battery Begins
- Serum and Livers collected

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>E12.5</td>
<td>Saline + Control</td>
<td>Saline + Control</td>
</tr>
<tr>
<td></td>
<td>Saline + NR</td>
<td>Saline + NR</td>
</tr>
<tr>
<td></td>
<td>VPA + Control</td>
<td>VPA + Control</td>
</tr>
<tr>
<td></td>
<td>VPA + NR</td>
<td>VPA + NR</td>
</tr>
</tbody>
</table>

n=10/group
Open Field Testing

1st day of behavioral battery
10 minutes of testing
Measure of anxiety

Preliminary Results:

- Female VPA spend less time in center compared to Female Saline
- VPA and VPA NR-treated mice are more active
Open Field Traces

Female Saline

Female VPA

Female VPA + NR
Sociability Testing

2nd day of behavioral testing
10 minutes: social interaction evaluation

Preliminary Results

› VPA-treated mice spend less time investigating pencil cup and stranger mouse compared to control
› NR treatment increases interaction time in males
Elevated Plus Maze

4th day of behavioral testing
5 minutes of testing
Measure of anxiety
Preliminary Results:
  › Female VPA spend the least time in uncovered arms
  › NR rescues this effect
Conclusions

Our preliminary data reveals increased anxiety in the VPA-treated mice, especially for females.

Males with ASD are found to show more externalizing behavior problems than females, such as aggressive behavior, hyperactivity, reduced prosocial behavior, and increased repetitive/restricted behaviors and interests.

Females with ASD show greater internalizing symptoms than boys, including anxiety, depression, and other emotional symptoms as reported by parents.

Werling and Geschwind. Curr Opin Neurol. 2013
Acknowledgments

EPSCoR SRP:
Lead PI: Dr. Jeffery Twiss (Claflin)
Co-PI: Dr. Sofia Lizarraga
Co-PI: Dr. Luigi Boccuto Program
Co-PI: Dr. Kevin Champaigne
Co-PI: Dr. Omar Bagasra
Co-I: Dr. Jill Turner
Co-I: Dr. Norma Frizzell
Co-I: Dr. Sajish Mathew

Undergraduate Students:
Morgan Reese
Skylar Lambert
Olivia Larner – SC LEND

(Leadership Education in Neurodevelopmental and Related Disabilities)
High Frequency Repetitive Transcranial Magnetic Stimulation (rTMS) in Adults with Autism Spectrum Disorder (ASD) and Depression: A Pilot Study

Melanie G. Wiley
Medical Science Training Program
Medical University of South Carolina
Conflicts of Interests

rTMS is not a FDA approved treatment for Autism Spectrum Disorders.
Major Depressive Disorder

- **rTMS treatment**: *FDA approved*
- **TMS device**: Neotonus NeoPulse solid focal figure 8-coil
  - 10 Hz, 3000 pulses/session
  - 120% of Motor Threshold
- **Target**: Left dorsolateral prefrontal cortex (DLPFC)
  - Executive functioning, motivation, and memory
  - High connectivity
  - Limbic system
  - Ventromedial PFC

**References**
- Spronk et. al Neurofeedback and Neuromodulation Techniques and Applications, 2011
- Veenstra-Vanderweele J et.al. Neuropsychopharmacology Reviews (2012) 37, 196-212
Depression in Autism is Different

- Higher risk for suicidal ideation and suicide attempts
- First-line treatments for depression less effective
- Standard treatments can worsen autism symptoms
- Greater number of symptoms and increased severity

References:
Specific Aims

• **Aim 1**: Determine the safety and therapeutic efficacy of left-sided DLPFC high frequency rTMS on *depression symptoms* in patients with ASD
  • Hypothesis: 25 sessions of 10Hz rTMS at 120% motor threshold is a safe treatment that will decrease depression symptoms in patients with ASD

• **Aim 2**: Determine the effect of left DLPFC rTMS on *core symptoms of ASD*
  • Hypothesis: rTMS will improve the core ASD symptoms
Research Design

Enrollment
- Clinical Interview
- IQ Testing
- 13 adults

Pre-TMS
- Motor Threshold
- Clinical Assessments

rTMS Treatment
- 5x/week
- 25 sessions

Post-rTMS
- Clinical Assessments
- 1 month follow-up
- 3 month follow-up
Clinical Assessments

• Depression Assessment
  • 17 item HAM-D: Hamilton Depression Scale

• Autism Assessments
  • Self-reporting
    • SRS-2: Social Responsiveness Scale
    • RAADS-R: Ritvo Autism Asperger Diagnostic Scale
  • Informant-reporting
    • ABC: Aberrant Behavioral Checklist
    • RBS-R: Repetitive and Restricted Behavior
Results: Safe Treatment in Adults with ASD

• No adverse events using FDA approved depression protocol
  • 25 sessions
  • High frequency (10Hz) rTMS
  • 120% motor threshold

• 11/13 participants completed 5 weeks of rTMS
  • One dropout due to anxiety
  • One dropout due to irritability/anxiety
Results: Improved Depression Assessment

17 item-Hamilton Depression Rating Scale
- Depressed mood
- Feelings of guilt
- Suicide
- Insomnia
- Anxiety
- Somatic symptoms
- Weight loss

n=10
p=0.005
Results: No change in **Self-reporting** ASD Assessment

Social Responsiveness Scale (SRS-2)
- Social awareness
- Social cognition
- Social communication
- Social motivation
- Restricted interests and repetitive behavior

n=8
p=0.575
Results: No change in **Self-reporting** ASD Assessment

Ritvo Autism Aspergers Diagnostic Scale - Revised (RAADS-R)
- Social relatedness
- Language
- Communication
- Sensorimotor deficits
- Stereotypies

n=9  
p=0.553
Results: Improved Informant-reporting ASD Assessment

Aberrant Behavior Checklist (ABC)
- Irritability
- Lethargy/Social Withdrawal
- Stereotypy
- Hyperactivity
- Inappropriate Speech

n=9
p=0.034
Results: Improved Informant-reporting ASD Assessment

Repetitive behavior scale-revised (RBS-R)
- Stereotypy
- Self-injury
- Compulsion
- Tics

n=9  
p=0.014
Conclusions

- **Aim 1:** Determine the safety and therapeutic efficacy of left-sided DLPFC high frequency rTMS on depression symptoms in patients with ASD
  - Results support safety of high frequency (10 Hz) rTMS in adults with autism
  - Improved depressive symptoms after completion of a standard depression rTMS treatment series in adults with autism

- **Aim 2:** Determine the effect of left DLPFC rTMS on core symptoms of ASD:
  - Self-rating scales show no improvement
  - Informant-rating scales show improvement

**Proposed next study** → Efficacy of rTMS for depression and Autism symptoms in a double blinded Randomized Controlled Trial
Acknowledgements

• Erin Henneberry, M.D.
• Danielle Lowe M.D./Ph.D.
• Philipp Summers
• Hussam Alsarraf, M.D.
• Greg Sahlem, M.D.
• Laura Lohnes
• Sarah Russo M.D./Ph.D.
• Baron Short, M.D.
• Brain Stimulation Lab
Selective axonal translation of prenylated Cdc42 mRNA isoform supports axon growth

Matthew Zdradzinski
University of South Carolina
Subcellular mRNA localization in polarized cells

A: Budding yeast Ash1 mRNA
B: Drosophila embryo bicoid mRNA, oskar mRNA, nanos mRNA
C: Xenopus oocyte Vg1 mRNA

D: Fibroblast β-actin mRNA
E: Immature neuron β-actin mRNA, CamKIIa mRNA
F: Oligodendrocyte MBP mRNA

Lamellipodium, Growth cone, Dendrites, Myelin lamellae
Dendrites and Axons Synthesize Proteins Locally

Functions of Axonal Protein Synthesis
1. Axon growth (development & regeneration)
2. Axon pathfinding
3. Retrograde signaling
4. Axon maintenance/survival
5. Neuron Survival

Links for Axonal Transcriptome in Neurological Disorders
1. Neuropathic pain
2. Viral infection
3. Motor neuron disease
4. Alzheimer’s disease

Cdc42 as a positive regulator of axon growth

Axon length

Hall A. (2014)
Rajnicek A. M. (2006)
Cdc42 gene codes for 2 distinct isoforms

Pre-mRNA

Cdc42
1 2 3 4 5

Short UTR
6

Long UTR
7

mRNA

Palm-Cdc42
1 2 3 4 5 6

TQPKRCCIF

Prenyl-Cdc42
1 2 3 4 5 7

PKKSRCVLL

Protein

Cdc42

Palmitoylation

Differential Localization

Cdc42

Prenylation
Alternatively spliced Cdc42 isoforms differentially localize within neurons

Adapted from Zhang et al. 2001

Priyanka Patel, Elizabeth Thames, Seung Joon Lee
Cdc42 RNA localization is conserved in vivo

In Vitro
Which Cdc42 splice variant is required for axon growth?

Prenyl-Cdc42 mRNA is required for normal axon growth.
Is prenylation of CDC42 protein required for axon localization?

- Prenyl-Cdc42 CDS only (restricted to cell body)
- Prenyl-Cdc42 CDS + Long UTR (localizes to axon)
- Palm-Cdc42 CDS + Long UTR (localizes to axon)

Prenylation targets axonally translated CDC42 to growth cone periphery
Prenyl-Cdc42 mRNA selectively localizes down axons *in vitro* and *in vivo*.

Prenyl-Cdc42 is necessary for normal axonal growth.

Localization of Prenyl-Cdc42 mRNA and prenylation of its protein product are necessary for localization of CDC42 to the leading edge of the growth cone.
Palm-Cdc42 mRNA localizes into dendrites

Adapted from Yao K. (2016)
adapted from Bassell & Twiss (2006)
Acknowledgements

**Twiss Lab**
Matthew Zdradzinski
Amar Kar
Seung Joon Lee
Priyanka Patel
Sean McGill
Sharmina Miller-Randolph
Pabitra Sahoo
Terika Smith
Elizabeth Thames

**Collaborators**
Mike Fainzilber, Weizmann Inst.
AI Burlingame, UCSF
Nora Perrone-Bizzozero, Univ. New Mexico
Giovanni Coppola, UCLA
Qun Lu, East Carolina Univ.
Michael Shtutman, USC

**Center for Childhood Neurotherapeutics**
at the **University of South Carolina**

**Undergraduates:**
Jeremy Money
Blake Jones
Katherine Kenny
Jane Marryat
Devon Cassidy

**Funding from:** ASPIRE (Univ. of SC/SJL-2015), Wings for Life (PP-2018), NIH (RO1)
NSF, Dr. Miriam & Sheldon G. Adelson Medical Research Fnd., and South Carolina SmartState Endowment Program (Twiss Lab).