The Rapidly Changing Landscape of Fragile X

Elizabeth Berry-Kravis MD PhD
Rush University Medical Center

Disclosures: EBK has received funding from Neuropharm LTD, Seaside Therapeutics, Novartis and Roche Pharmaceuticals to consult on trial design and conduct clinical trials in FXS
Features of Fragile X Syndrome

- **Physical**: large prominent ears, long face, large head, prominent jaw and forehead, midfacial hypoplasia, hyperflexible joints, large testis
- **Intellectual Disability or LD**
- **Behavior problems**: hyperactivity, distractibility, anxiety, perseveration
  - **Autism**: 18-36% AD, 43-67% ASD
- **Seizures** – 15%
- **Strabismus** – 30%
- **Medical**: otitis, sinus, MVP, reflux, sleep apnea, loose stools, allergies
FXS Treatment in Clinic - Supportive

- Early intervention
- Intensive speech therapy
- OT with sensory integration
- Inclusion in school as much as possible
- Educational curriculum, environment, teaching style matched to FXS cognitive profile
- Socialization program
- Behavior plan
- Behavior medications for ADD/anxiety

Rush FXS Clinic since 1992  > 450 patients

- Aggressive tx of otitis – tubes/audiology
- Manage sleep apnea – T&A
- Treat sleep dysregulation – melatonin/medications
- Yearly eye exams – patching, surgery, glasses
- Control seizures
- Orthopedics if needed
- Monitor for MVP/heart
- Genetic counseling
- Discuss reproductive options
Seizures in Fragile X Syndrome – Recent and Largest Study

- National Fragile X Survey
  - 1394 FXS full mutation (1090 M, 304 F)
  - 173 (12%) seizures: 154 (14%) M, 19 (6%) F

- Rush FX Clinic – Chart Data
  - 352 FXS full mutation (268 M, 84 F)
  - 46 (13%) seizures, 39 (14.5%) M, 7 (8%) F

Berry-Kravis et al, 2011 AJIDDD
Number of Current Seizure Medications

- None
- One
- Two or more

Effectiveness of Medications

- Not at all
- A little
- Somewhat
- A lot
Co-Occurring Conditions/Symptoms: Seizure Cohort Vs. Matched No Seizure Cohort

<table>
<thead>
<tr>
<th>Condition</th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seizures (%)</td>
<td>No Seizures (%)</td>
<td>N</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Attention Problems</td>
<td>88.1</td>
<td>82.1</td>
<td>134</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>62.9</td>
<td>68.2</td>
<td>132</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>48.9</td>
<td>34.6</td>
<td>133</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Self-Injury</td>
<td>52.2</td>
<td>43.3</td>
<td>134</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Autism</td>
<td>63.6</td>
<td>41.1</td>
<td>129</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>75.2</td>
<td>57.9</td>
<td>133</td>
<td>0.0038</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>12.6</td>
<td>8.7</td>
<td>127</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Developmental Delay</td>
<td>98.5</td>
<td>95.6</td>
<td>135</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Poor Verbal Ability</td>
<td>30.6</td>
<td>18.2</td>
<td>121</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>Poor Reading Ability</td>
<td>64.5</td>
<td>66.9</td>
<td>124</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Fair-Poor Thinking</td>
<td>89.5</td>
<td>87.3</td>
<td>134</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Fair-Poor Quality of Life</td>
<td>13.2</td>
<td>13.9</td>
<td>136</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Fair-Poor Overall Health</td>
<td>14.1</td>
<td>6.7</td>
<td>135</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

- Seizure frequency in FXS: 14-14.5% males, 6-8% females.
- Seizure characteristics similar in survey and clinic cohorts, suggests parent data for this is reliable.
- Seizures in FXS are often partial onset, present frequently in mid-childhood, and are generally infrequent and easily controlled with minimal AEDs.
- Learning, reading not associated with seizures but diagnosis of autism is associated with seizures, and aggression, anxiety, and poor verbal skills may also co-occur with seizures.
- Seizures and autism may share mechanisms based on defects of synaptic plasticity and glutamatergic signaling due to absence of FMRP in FXS.
Principles of Seizure Management in FXS

1. Start medicines after 2 or more clinical seizures, do not need to treat abnormal EEG
2. Stop 2 years after last seizure
3. Single drug regimen, lowest effective dose best
4. Dose guide is effectiveness and toxicity
5. Match drug to seizure type, patient characteristics
6. Use drugs with less effect on cognition and behavior
7. EEG is adjunct for deciding which drug, how much, and how long
8. More drug is not necessarily more effective
Intellectual Disability in FXS

- Present with motor (some), language (most), behavior (high functioning)
- Males - average adult IQ about 40 and mental age 5-6y, range severe ID to normal (mosaics)
- IQ scores higher when young, decline with age
- Specific cognitive profile
- Achievement and Adaptive skills higher
Fragile X Syndrome
Characteristic cognitive pattern with prominent executive function deficits

• Strengths
  – Receptive vocabulary
  – Syntax
  – Imitation
  – Grammatical structure
  – Visual memory
  – Simultaneous processing
  – Experiential learning

• Weaknesses
  – Auditory processing
  – Sequencing
  – Abstraction
  – Short-term memory
  – Topic maintenance/"connectedness"
  – Mathematics
  – Working memory
  – Coordination/praxis
General Components of the Educational Approach

- Early intervention
- Speech therapy - work on fluency, vocab, length of utterance, pragmatics
- OT/sensory integration therapy – work on fine motor and writing problems
- Structure/routines
- Schedule/message boards - visual cues (pictures of normal day events) to help understand schedule, token boards
- Inclusive program when possible to maximize imitation of normal behaviors
General Components of the Educational Approach

• Classroom modification
  • Environmental - seat away from distraction, quiet area in room, natural lights, help with initiation
  • Instruction - co-operative learning in small groups, peer tutoring, high teacher-to-student ratio, one-on-one instruction, indirect instruction
  • Curriculum - appropriate task complexity, text enlargement, high interest/daily life topics

• Behavior management - behavior modification, calming, medications
• Aide to deliver specialized curriculum, carry out behavior and socialization interventions
• DO NOT FORCE EYE CONTACT
FXS Curriculum Matched to Cognitive Profile

• Learning based on visual memory - visual cues to all instructions
• Use of whole language, logos or picture-word association rather than phonics for reading (Edmark)
• Use of computer-assisted writing
• Concrete, hands-on math with visual representation/manipulatives (eg. TouchPoint, number lines)
• Functional life-math: eg. money, time
Social Interventions in School

- Individual socialization therapy
- Group socialization therapy (modeling social interactions with normal peers)
- Videos of appropriate social behaviors for behaviors that are problematic
- Social stories
- Peer tutoring
- Circle of Friends
Behavior Problems in FXS

- Hyperactivity/fidgety (90%)
- Short attention span (~100%)
- Anxiety (~100%)
- Tactile defensiveness (80%)
- Eye (gaze) aversion (>90%)
- Perseverative speech/thinking (>80%)
- Hand flapping (60%)
- Hand biting (50%)-self regulatory
- Mood swings
- Outbursts/aggression

THE BIGGEST PROBLEM FOR MANY FAMILIES

Behavior in fragile X out-of-proportion to cognitive level
Difficult Behavior in FXS

- Problems communicating
  - Cannot express what is bothering them
  - Only alternative may be aberrant behavior
- Lack of cognitive sophistication about interpreting problem and coming up with a solution
- Social misinterpretation (lack of understanding about when others are teasing etc)
- Poorly modulated emotional state
- Overarousal and hypersensitivity to everything: noises, smells, facial expressions, temperature, flashing visual stimuli, something different in room, routine change, “on the spot”
Difficult Behavior in FXS

• Anxiety
  – Aberrant behavior may be only strategy FXS individual has to deal with anxiety
  – Anxiety about novel exposures, separation, being unable to do something, not doing it right, other specific phobias, no particular thing
  – FXS individual learns what is difficult and becomes increasingly anxious about success and novelty
  – Anxiety increases with age; fear learning is enhanced in FXS – fear without threat
  – Fight or flight reactions over minimal stimuli – can see visual signs – red ears, increased pulse, sweating, pacing, increased perseveration

• Need behavior plan - figure out antecedents and use behavior/environment modification
Decision to Use Behavioral Medication

• Individual engaging in dangerous behaviors
• Individual is dysfunctional from behavior
• Individual could accomplish more or be higher functioning if specific behavior is managed
  – Increase ability to participate
  – Able to be in more inclusive setting
• Not necessarily “when all else fails”
• ALWAYS an adjunct to behavioral, environmental measures
Psychopharmacology in Fragile X Syndrome

- Targets behavior to improve functioning
- Supportive, does not target underlying cognitive problem
- Only one prior controlled trial in FXS (N=15) shows Ritalin effective in 2/3 of boys
- Therapeutic decisions based on target largest problem symptom complex(es) – trial and error
- May need to treat multiple behavioral domains
<table>
<thead>
<tr>
<th>Problem Behavior Cluster</th>
<th>Medication Class</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distractibility/ Hyperactivity</td>
<td>Stimulants</td>
<td>Methylphenidate, Adderall, Provigil</td>
</tr>
<tr>
<td>Overarousal/ Hypersensitivity</td>
<td>Alpha-agonists</td>
<td>Clonidine, Tenex</td>
</tr>
<tr>
<td>Anxiety/OCD/ Perseverative</td>
<td>SSRIs</td>
<td>Prosac, Zoloft, Celexa, Lexapro</td>
</tr>
<tr>
<td>Mood Lability</td>
<td>Antidepressants, AEDs, Lithium</td>
<td>Tricyclics, Effexor, Li, SSRIs, VPA, TPX, CBZ</td>
</tr>
<tr>
<td>Aggression/Self abusive</td>
<td>Atypical Antipsychotics</td>
<td>Risperdal, Abilify, Seroquel, Geodon</td>
</tr>
</tbody>
</table>
Percent of Individuals With FXS Taking Medications for Specific Symptoms

Males, N=1064

Females, N=299

From National Survey
Bailey et al. 2011 in press, JDBP
Percent of Individuals with FXS Using Medication By Symptom and Age

Males

Females

- Anxiety
- Attention
- Hyperactive
- Anger
- Mood
- Sleep
- Self-injury
- Seizures
- Depression
Perceived Efficacy of Medications for Individuals with FXS by Symptom

Males

Females

- Anxiety
- Attention
- Hyperactivity
- Anger
- Mood
- Sleep
- Self-Injury
- Seizures
- Depression

Legend:
- Green: A lot
- Red: Somewhat
- Blue: Little or none

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Supportive Psychopharmacology is Helpful in FXS...

...but treating the underlying disorder would be better

<table>
<thead>
<tr>
<th>Condition</th>
<th>Successfully Treated</th>
<th>Failed</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attention Hyperactivity</td>
<td>65%</td>
<td>30%</td>
<td>5%</td>
</tr>
<tr>
<td>Anxiety Mood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperarousal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oversensitivity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abilify in FXS

- Abilify Responce (n=64)
  - Successfully Treated: 65%
  - Failed: 30%
  - Unknown: 5%

- Females (trial) 50%
- Females (patient) 50%
- Males (trial) 50%
- Males (patient) 50%
- Total (trial) 50%
- Total (patient) 50%

208 trials 136 patients
231 trials 123 patients
100 trials 58 patients
52 trials 52 patients
Fragile X Syndrome: an Unmet Need

- **Behavior**: attention, hyperactivity, anxiety, sensory over-responsiveness, aggression, perseveration, rigidity, mood lability/irritability
  - Currently available medications help, do not fully treat
- **Cognition**: low IQ, impaired social/executive cognition
  - No medications to treat this
- **Therapy**: behavioral, cognitive training help, do not normalize
- **Unmet medication needs**:
  - Improved medications to target specific behaviors
  - Medications to target underlying brain disorder directly (behavioral and cognitive treatment)
**FMR1 the Fragile X Gene Causes 3 Diseases**

<table>
<thead>
<tr>
<th>Normal</th>
<th>FMR1</th>
<th>10-45 CGGs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premutation</td>
<td>55-200 CGGs</td>
<td>“Carrier”</td>
</tr>
<tr>
<td>FXTAS</td>
<td>FXPOI</td>
<td></td>
</tr>
<tr>
<td>FXS</td>
<td>&gt;200 CGGs (Full mutation)</td>
<td></td>
</tr>
</tbody>
</table>

- **Carrier state passed through many generations before FXS**
- **Fragile X Syndrome**
- **Simple DNA test to diagnose FXS – measures repeat size**
- **Tremor, neurological disease**
- **Synaptic defects Intellectual compromise**

- FMR1 inclusions disrupt nuclear processes transcription splicing
- No FMR1 mRNA No FMRP
- CH₃ CH₃
FMRP Expression and Disability

Social anxiety/shyness

Distractibility/hyperactivity

Executive deficits

Spatial perceptual deficits

NVLD

Intellectual Disability
The Fragile X Mouse (Knockout; K/O)

- \textit{Fmr1} gene inactivated
- No active FMRP
- Subtle cognitive problems
- Audiogenic seizures
- Good neurobiological model to answer question: WHAT DOES FMRP DO?
What Does FMRP Do?

FMRP is in dendrites and regulates proteins translation in dendritic spines – needs to be regulated precisely for synaptic maturation and connectivity.

Both FXS Mouse and Human Brains:
Dendritic spines are abnormal in FXS with immature long spines.

McKinney et al, AJMG, 2005

FMRP regulates maturation of brain synapses

Picture courtesy of Gary Bassell
**mGluR Theory of Fragile X: The Basic Concept**

**Aberrant dendritic translation and synaptic plasticity in FXS**

- **Normal Mature connection**
  - LTD
  - AMPA
  - mGluR1/5
  - FMRP
  - Ribosome

- **Fragile X Immature connection (too weak)**
  - Excessive LTD – due to mGluR system overactivity
  - AMPA
  - mGluR1/5
  - FMRP
  - Ribosome
Treatments aimed at many of these targets reverse phenotypes in the fmr1 K/O mouse

FMRP mechanism

Targets for FXS treatment based on mechanism

Treatments aimed at many of these targets reverse phenotypes in the fmr1 K/O mouse
Potential Mechanisms for New Treatments of FXS – mGluRs and Beyond

Dendrite
Excessive LTD – due to mGluR system overactivity

Fragile X Immature connection (too weak)
mGluR Theory Hat
Equation for Fragile X Research to Lead to Treatment

FMR1

\[ \text{Find Gene} + \text{Missing Protein} + \text{Mouse model} = \text{Targeted treatment} \]

Knowledge of brain cell mechanisms abnormal in FXS
Mechanism 1A: mGluR5 Blockers in FXS Models Reverse Phenotypes at all Ages

- Audiogenic seizures
- Epileptiform bursts
- Open field behavior
- Dendritic spine shape
- AMPA receptor internalization
- Excessive LTD
- Excessive protein synthesis
- Behavioral phenotypes

Many phenotypes in the FXS fly (eg. courtship and odor-shock memory) reversed by mGluR5 blockers

Phenotypes in the fmr1 K/O mouse also all reversed by crossing fmr1 K/O to mGluR5 heterozygous mutant (half the mGluR5 receptors)

Bauschwitz, 2006

Wong et al. 2006
AMPAR Receptor Rescue by MPEP

Nakamoto et al. 2007

siRNA to block FMRP translation

Control

siRNA and MPEP

GluR1 = AMPA receptors that drive strength of neural connections
WE CAN “CURE” THE FXS MOUSE

But Mouse is not Man...
Human brain a lot more wiring time but cautious optimism for treatment — amount of reversal possible at given age in human unclear.
Translational Challenges: Scientific Validity and Technical Feasibility

FXS 2002
- Validated targets and pathways
- Identify multiple druggable targets
- Validated screening assays
- Validated biomarkers: surrogate endpoints and susceptibility

FXS 2011
- Validated predictive animal models
- Early predictors of safety
- Secured IP
- Multiple Proof-of-Concept trials
- Defined regulatory, reimbursement, and adoption pathways

13 years, 5 years in trials
9 drugs to get 1
$$$$$$$$
Lack of Models for Trials in FXS

• Before 2002 lack of significant moderate/large clinical trial experience in FXS with any “standard” drug
• No defined measure of behavioral improvement
• No “gold standard” outcome measure
• No template from any developmental disability about measuring cognitive outcomes when attempting to treat underlying disorder

Basic science has targets but mismatch in trial design/outcome measure development
Issues for Each FXS Trial

- Type of design – how long placebo period?
- Dosing – same as when no disorder?
- Safety assessments – can’t always report AEs
- Age of subjects – younger might respond better
- Formulation – can’t always swallow pills
- Gender/mosaics – better higher or lower function
- Other medications – allow or not; pure signal vs improvement over available treatment
- Efficacy outcome measures – strong placebo effects; not well developed for FXS
- Distance and travel for rare disease
Mechanism 3: AMPA Activators

- Mouse – increase BDNF, reverse hippocampus LTP (connection strengthening) deficits
- Human - Phase II Double-blind, placebo-controlled Ampakine CX516 (Cortex Pharmaceuticals) trial at RUSH/MIND, 49 participants
  - Drug too weak, only improved those on antipsychotics
  - Good safety, 94% completion rate (shows individuals with FXS can do intensive trial)
- Waiting for a better ampakine

*Berry-Kravis et al. JCAP 2006*
Mechanism 2: Block Specific Overactive Protein

- **Minocycline** – blocks MMP9, overactive in FXS mouse
- **Mouse** – reverse dendrite spine shape and behaviors
- **Observational study with patient report**– improved some obsessive and mood symptoms (Hagerman)
- **Open-label 8 week study**: 20 patients age 13-32, improved behavior on ABC, CGI, VAS (Paribello)
- **Double blind placebo-controlled study ongoing at MIND (Hagerman)** – age 5 and up
Mechanism 4: Other Systems Impacting mGluR Pathway

- **Arbaclofen (Seaside)** - GABA-B agonist, decrease glutamate release – less mGluR5 activation
  - Mouse/fly – reverse audiogenic seizures and other phenotypes, GABA system abnormal
  - Placebo-controlled crossover trial with 4 weeks of treatment each arm – 63 subjects (Seaside – 12 sites)
  - Good safety/side effect profile
  - Efficacy best in irritable/autistic/socially-impaired patients
  - Extension in progress, further trials planned for 2011

Other: mGluR2/3 agonists, ganaxolone (GABA-A), acamprosate (GABA activator, glu blocker?), donazepil (cholinergic)
Mechanism 4: Correct Connectivity Through Systems Effect: R-baclofen – Decrease Excessive Glutamatergic Transmission

Pre-synaptic

Post-synaptic

Model Courtesy of Seaside Therapeutics
CGI-I (Improvement) Results in Arbaclofen trial (Seaside)

"Responders" 35% vs. 18%  
\( p = 0.11 \)

44% vs. 6%  
\( p < 0.05 \)

Per Protocol  
N=52

58% vs. 19%  
\( p < 0.01 \)

Autism  
N=18

Low Social  
N=27
Efficacy scores: “Lower sociability” subgroup

<table>
<thead>
<tr>
<th></th>
<th>STX209 n=27 (mean ± SD)</th>
<th>Placebo n=27 (mean ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGI-I</td>
<td>2.7 ± 1.1</td>
<td>3.5 ± 1.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CGI-S</td>
<td>-1.0 ± 1.1</td>
<td>-0.3 ± 0.9</td>
<td>= 0.01</td>
</tr>
<tr>
<td>Treatment preference (clinician)</td>
<td>63%</td>
<td>19%</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Treatment preference (parent)</td>
<td>67%</td>
<td>19%</td>
<td>= 0.001</td>
</tr>
<tr>
<td>ABC-Social Withdrawal</td>
<td>-4.3 ± 6.3</td>
<td>-0.4 ± 7.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Vineland Socialization domain (raw score)</td>
<td>14.2 ± 19.0</td>
<td>4.6 ± 10.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Responders (CGI-I =1 or 2, and ABC-SW improvement ≥ 25%)</td>
<td>42%</td>
<td>7%</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
Mechanism 1B (Inside Cell): Block Excessive Signaling in mGluR-Activated Translational Pathway

• Not receptor-specific, cover all receptors activating pathway, more off-target effects on cellular metabolism

• **Lithium** – blocks PI and GSK3β in signaling pathway
  – Mouse/fly – behavior/spines phenotypes reversed
  – open label 2 month trial, 15 patients

• behavior, adaptive, ERK biomarker, 1 cognitive task better

• Other pathway targets
  – PAK inhibitors (Afraxis), PI3K blockers, GSK3β blockers

*Berry-Kravis et al, JDBP 2008*
Open-Label Lithium Trial in FXS

13/15 better at 2 mo, little toxicity

ABC Total (range 0-174)
13 improved, p=0.005

Also significant improvement in CGI, VAS, and Vineland Personal Daily Living Skills and Maladaptive Behavior

RBANS List Learning (range 0-40)
8 of 10 improved, p=0.028
CX516 placebo change: 0.0

Sustained improvement in
9/11 treated for 1 year
Lithium Study
Biomarker - ERK Activation in Lymphocytes

ERK phosphorylation slower in K/O and FXS

N=11
Baseline mean: 4.87 min

2 Month Treatment mean: 4.11 min (p=0.007)

(about 1 min is difference FXS and control)

1 Year Treatment mean: 3.56 min (P=0.00006)
Mechanism 1A (Outside Cell): mGluR5 Negative Modulators

- Receptor specific – correct one activation route but not all, less off target effects
- **Fenobam** (Neuropharm) – Rush/MIND - single dose in 12 patients, safe/PPI improved  
  *Berry-Kravis et al. JMG 2009*
- **STX107** (Seaside) - In Phase I, FXS trials 2011
- **AFQ056** (Novartis) – Phase II, placebo-controlled 28 day (30 M >18) trial in Europe, multinational phase III trial with USA sites 160 adults, 160 age 12-17 – recruiting now and has 2 year extension
- **RO4917523** (Roche) – Phase II placebo-controlled 6 week (68 M & F >18) trial in USA, phase III trial about to begin
Trials of mGluR5 Blockers in FXS: Fenobam

RUSH and UC Davis (Neuropharm and FRAXA) safety trial of 1 dose (50-150 mg), 12 adult FXS (6M, 6F), age 18-38, IQ 36-85

- PPI improved 20% in 6/12 subjects (control test-retest group 2/13, p=0.03)
- Positive behavioral changes in 9/12 subjects
- No fenobam-related AEs
- Erratic PK

Berry-Kravis et al. JMG 2009
AFQ056 mGluR5 blocker most effective in subgroup of fully methylated subjects in small exploratory trial – methylation status may define treatment response to targeted treatments

Jacquemont et al. presented at ASHG meeting 2010
Outcome Measures in FXS – A Problem

• First clinical trials – CX516 (2002-4), lithium (2005-6) - many outcome measures found not to be not useful in FXS
• Some reliable and reproducible – mostly behavior forms
  ➢ But none of these covers spectrum of behaviors characteristic of FXS well
  ➢ Need FXS-specific empirically validated behavioral outcome measure

• Profiles for many cognitive tests piloted
  ➢ many have problems with floor effects, motivation issues, developmental issues, repeatability, not clear if important things measured, how change sensitive

RBANS List Learning
N = 28
7% refusals (N = 3), ICC = 0.7
Mental Age cut-off > 3

Berry-Kravis et al. JADD 2008
What We Need to Know to Have a Usable Measure for FXS Trials

• **Feasibility**
  – Age range
  – Functional range
  – Floor and ceilings
  – Distribution

• **Reproducibility**
  – Test-retest
  – Time frame for retest
  – Learning effects

• **Validity**
  – Abnormal in condition
  – Targeted to disease phenotype as assessed in other ways
  – Measure something important for quality of life/function

• **Intervention responsiveness**
  – Drugs
  – Behavioral
  – Placebo effect
  – Time of treatment
Objective Novel Phenotype-Based Outcome Measures

**PROS**
- More scientifically based, translate from models
- Tap into specific features of underlying disorder

**CONS**
- Unclear if predict better function in life
- Not likely useful for FDA approval

**KiTAP**
- Executive function, attention
- 6 Measures- feasible, min ceiling/basal, reproducible in test-retest, correlate with behavior ratings

**PPI**
- Sensory overresponsiveness

**Hessl et al AJMG (2008)**

**Test-retest ICC=0.88**
Eye Tracking

Feasibility
- Age 5 and up
- Most FXS kids/adults can do

Validity
Clearly abnormal
Phenotype: eye aversion
??? no functional correlate yet

Reproducibility
1-2 week retest, 16 FXS, 20 controls

Time looking at eyes
P<0.001 all face types

Number fixations to eyes
P<0.001 all face types

Validity

Feasibility

Intervention response

Proportion LT Eyes
Control α = 0.684
FXS α = 0.935

No data
?????
but placebo effect unlikely
Biomarkers for FXS Trials

APP and Aβ Metabolites

Matrix Metalloproteinase – 9 (MMP-9)

% MMP-9 active in plasma

Pilot in 10 FXS non-mosaic full mutation males and 8 similar aged control males

* p<0.004 Malter, Westmark, Berry-Kravis, unpublished data
Pupillometry

Feasibility

- Age 5 and up
- Most FXS kids/adults can do

Validity

Happy

Fear

Clearly abnormal in FXS, phenotype: social anxiety

No functional correlate yet????

Reproducibility

ICC 0.74 Happy

ICC 0.58 Fear

Validity

16 FXS, 20 controls, 1-2 week retest

Intervention response

Unknown???

Placebo effect unlikely
Conclusions of NIH Study Group on FXS Outcome Measures

- Adaptive/maladaptive behavior forms – ABC: 5 subscales cover many behaviors in FXS but not all - very reproducible in FXS (ICC=0.8-0.9), somewhat sensitive to change in lithium minocycline, arbaclofen, AFQ056 studies, factor structure not right for FXS – need re-factoring (done by 5 site collaboration)
- Anxiety – PARS, feasible and valid? (Hessl and B-K validating)
- Executive/inhibition – KiTAP, ?
- Social cognition - Eye tracker, ?
- Memory – RBANS, ?, Working memory - ?
- Learning - ?
- Language – coded conversation session (length of utterance perseveration, verbal fluency) – (B-K/Abbeduto validating)
- Motor – stereotypies, motor learning, coordination, hyperkinesis? (Tartaglia validate coord measures, EBK/Hall stereotypies)
- Biomarkers (PPI, ERK, APP, others?), fMRI?
- **Need FXS-specific scale covering entire phenotype** (Hessl)
Re-Factored ABC for FXS

- Project based on recommendations from 2008-2009 NIH FXS Outcome Measure Meetings (led by Hessl)
- ABC factor structure not felt right for FXS – re-evaluated based on over 600 ABC ratings entered into NDAR from 5 sites, refactored scale using two independent methods of factor analysis (same result)
- Items eliminated and moved, social withdrawal, SIB questions regrouped, 6 factors emerged, in review
- May show improvements in FXS behavior areas better
- Evidence: Seaside study – Social Withdrawal subscale on new FXS ABC - sigt improvement across entire per protocol group.... Emphasize need to validate measure in disorder being studied
Conclusions From PARS Study

- PARS is feasible with FXS families (N=49, 33 M, 16 F)
- PARS has good range of scores in FXS and mean scores and standard deviations similar at first and second administration
- Scores may be higher in younger individuals
- High IRR and cross site reliability (43 Rush, 6 UC Davis)
- Good reproducibility for both 5- and 7-item scale – ICC for number items endorsed and severity scores all >0.72
- Good correlation with both clinician and parent-report of anxiety (ADAMS) – all p<0.01
- ABC correlations – anxiety manifests more as irritability and hyperactivity in kids, social withdrawal becomes strongest correlate in adults
Observational Expressive Language Task

- 36 FXS Subjects (all verbal) – 25M 11F – age 5-35
- Video- and audiotape Narrative and Conversation (picture book story then standardized conversation, 10 min each) - test-retest
- Enter into SALT and measure # utterances, mean length of utterance, # word roots, # intelligible utterances
- Excellent reproducibility
  – Most measures ICC>0.9
- Correlate with Vineland
  – Expressive language score
The most common movement was rubbing him/herself, followed by (in no particular order): rocking, waving arms, body movement, repetitive movement, yelling, sniffing the body, hand movements, manipulating objects, gazing at objects, and bizarre postures.

Less common or absent movements included: sniffing objects, spinning the body, rolling the head, whirling, pacing, twirling, bouncing, spinning object, running, finger movements, clapping, grimacing, and waving hands

Videos from Language study, rate 10 min on SBS
Clinical Validity of SBS Ratings

• ABC-C Total Score and Stereotyped Behavior Scale (SBS) score from first session was correlated with SBS (Pearson 0.3, \( p=0.01 \))

• ABC-C Stereotyped Behavior Subscore was strongly correlated with SBS (Pearson 0.4, \( p=0.0007 \))

• IQ was strongly negatively correlated with SBS Score (Pearson -0.48, \( p=0.006 \))
Conclusions of Pilot Stereotypy Study

• Very feasible as no demands on patient
• Clinician-rated SBS is consistent with parent reported stereotypic behavior and as expected, stereotypies are more prevalent in FXS individuals with more impaired cognitive function and general behavioral function
• Analysis of SBS reproducibility in progress
• Creation and validation of new FXS SBS focused on the stereotypes seen in FXS (Hall)
• Eventually correlate stereotypies with perseverative language from same audio.videotapes
Funded by cooperative agreement with CDC, AUCD and IBR (NFXF, NYSPI)

- Facilitate new clinics and standardize care
- Better understand FXS-associated problems
- Allow adequate recruitment for FXS studies
- Facilitate clinical trial network to allow sufficient recruitment to test new mechanistically based treatments for FXS for FDA approval
- FXCRC Registry and Database to help achieve above goals- can join through any FXCRC clinic
Potential Pathway Overlap in Autism – May Have Treatment Overlap

A. Proteins involved in other forms of autism may be in signaling cascade for translational regulation

B. FMRP may regulate proteins involved in different forms of autism

C. May be convergence of glutamate/GABA pathways involved in both disorders

Wang, Berry-Kravis, Hagerman; Neurotherapeutics 2010
Acknowledgements/Disclosures

• FRAXA Research Foundation
• NIH – NINDS
• Kiwanis Spastic Paralysis Foundation
• National Fragile X Foundation
• Collaborators
  – Sue Ellen Krause PhD
  – Sandy Block MS
  – Steve Guter MS
  – Ed Cook MD
  – Randi Hagerman MD
  – Maureen Leehey MD
  – Deborah Hall MD PhD
  – Paul Hagerman MD PhD
  – Chris Goetz MD
  – Don Bailey PhD
  – Glenn Stebbins PhD
  – Pete Van der Klish PhD
  – Jim Malter MD PhD
  – Cara Westmark PhD
  – Nicole Russo PhD
  – Iryna Ethell PhD
  – Steve Porges PhD
  – Mina Johnson PhD
  – Isabel Boutet PhD
  – Cary Kogan PhD
  – Steve Hooper PhD
  – Ivan Jean Weiler PhD
  – Bill Greenough PhD
  – Mark Bear PhD
  – Emily Osterweil PhD
  – David Hessl PhD
  – Mike Nelson PhD
  – Faraz Farzin PhD
  – Christina Gross PhD
  – Sue Leurgans PhD
  – Joanna Wuu MS
  – Joanne O’Keefe PhD
  – Molly Losh PhD
  – Jeannie Aschkenasi PhD

• Lab Research Associate
  – Lili Zhou MD
  – Victor Kaytser

• Study Co-ordinators
  – Kristina Potanos
  – Dahlia Weinberg
  – Rebecca Lara
  – Foster Lewin
  – Allison Sumis
  – Crystal Hervey
  – Kristine Urban
  – Christina Prescott
  – Anna DeSonia

• Students
  – Ok-Kyung Kim
  – Elizabeth Churcinski
  – Andrew Knox
  – Christina Tran
  – Felicia Scaggs
  – Emily Doll
  – Jessica Yesinsky

• Cortex Pharmaceuticals
• Asuragen

• Clinical Trial Funding
  – Seaside Therapeutics
  – Neuropharm
  – Roche
  – Novartis