Fragile X Syndrome: New Advances and Targeted Treatments

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Fragile X: A Family Affair, all generations may be involved with fragile X mutations

Fragile X Syndrome: leading inherited disorder of ID and leading single gene associated with autism. 1 in 4,000 to 1 in 6,000 with FXS. 60% with ASD; 2-6% with autism have FXS.
Two different mutations in the same *FMRI* gene

<table>
<thead>
<tr>
<th>Typical (CGG) &lt; 45</th>
<th>Premutation (CGG) 55 - 200</th>
<th>Full mutation (CGG) &gt; 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/130-250 females</td>
<td>1/250-810 males</td>
<td>1/4000-6000</td>
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<tr>
<td>mRNA</td>
<td></td>
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<tr>
<td>FMRP</td>
<td></td>
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<tr>
<td>Clinical</td>
<td>normal</td>
<td>Fragile X syndrome (FXS)</td>
</tr>
<tr>
<td></td>
<td>Primary Ovarian Insufficiency (FXPOI)</td>
<td></td>
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<tr>
<td></td>
<td>Fragile X-associated</td>
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<tr>
<td></td>
<td>Tremor Ataxia Syndrome (FXTAS)</td>
<td></td>
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<tr>
<td></td>
<td>Depression and anxiety</td>
<td></td>
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<tr>
<td></td>
<td>ADHD and ASD</td>
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</table>
Fragile X-associated Disorders include more than FXS, FXPOI and FXTAS

- Premutation involvement includes a range of developmental, behavioral/emotional, endocrine and neurological problems. However the majority of carriers do not have developmental problems, they are bright, driven, and successful in life but OCD/anxiety features are common.

- Carriers are more common than the full mutation, however their problems are usually milder than FXS until aging.
Often many members in a family affected by FXS or premutation involvement
AGG interruptions influence risk for transmission of a full mutation

FMR1 gene

AGG interruptions influence expansion/stability during transmission

Yrigollen et al 2012
Pockets of Fragile X throughout the world including Ricaurte Columbia
Colombia Project of Hope
Different phenotype in young vs old
Emotional & Neurocognitive Features

- Hyperactivity, impulsivity and/or short attention span
- Executive function deficits: problems with organization, shifting set, planning, inhibition, tangential speech, perseveration
- Over reactivity to stimuli: enhanced electrodermal response to stimuli; enhanced cortisol release after stressors
- Anxiety
- Autism or ASD
- Mood instability: excessive outbursts, tantrums
FXS and ASD had a higher rate of seizures + med problems

<table>
<thead>
<tr>
<th></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>
Early life seizures displaces FMRP from dendritic puncta to perinuclear location

A: control with FMRP (green color and white arrows) in puncta of dendrites in rat hippocampus at 60d

B: After early life seizures FMRP shifts to perinuclear location (yellow arrow) and enhanced LTP With focal deficits of FMRP

Bernard, Costanos, Benke 2012 SFN
Bernard et al 2013
Anxiety Disorders Interview Scale (ADIS) for DSM IV

Cordiero et al 2011 JND

DSM-IV Anxiety Disorders: Males with Fragile X Syndrome

- Meets Criteria
- Features

N = 58, ages 5-26.71, M=13.07, SD=5.60
Sensory Modulation or Processing Problems in FXS

Enhanced electrodermal responses and lack of habituation to sensory stimuli correlate inversely with FMRP levels

(Miller et al 1999)
Lack of habituation in fMRI brain activation to gaze in FXS vs IQ and ASD matched controls (15 to 25yo)
Habituation correlated with FMRP levels in females with FXS

Bruno et al 2014 AJP
Lack of habituation correlated with ADOS score in females

(Bruno et al 2014 AJP)
Communication and Social Deficits are continuous in boys with FXS: 60% with ASD significant heterogeneity in the FXS-autism phenotype

DSM5: 60% of boys with FXS have ASD
Communication and Social Deficits are continuous in boys with FXS: 60% with ASD significant heterogeneity in the FXS-autism phenotype.

DSM5: 60% of boys with FXS have ASD
13 yo boy with XXY and FXS
Those with the PWP have a higher rate of autism (70%) than those with FXS alone and lower CYFIP levels (Nowicki 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 long term depression (LTD)
- Down regulation of GABA_A receptors
- Dysregulation of dopamine pathways
- Enhanced release of neurotransmitters
- Enhanced APP production
- Increased oxidative stress damage to neurons
- Enhanced release of neurotransmitters presynaptically
Dramatic Up-regulation of Proteins in the CNS without FMRP

Bassell and Gross 2008

mGluR theory of FXS
Bear et al 2004
Arbaclofen

Lovastatin
mGluR5 Antagonist Trials

• AFQ056 (malvoglurant Novartis)
  – Adult (2212) and adolescent (2214) trials finished failed to meet primary endpoint of improved behavior on the ABC during 3 month placebo-controlled trial
  – Extensions (2278 and 2279) ended (August 2014) and development plan for trial with reading intervention and trial with young kids stopped

• RO4927523 (Basimglurant Roche)
  – Large phase 2/3 adult study – age 14 and up
  – Small phase 2 child study – age 5-13
  – Both studies completed – primary endpoints not met
### Seaside Phase 3 FXS Arbaclofen Trials: Overall Efficacy Results

<table>
<thead>
<tr>
<th>301</th>
<th>ABC-FX Soc Avd</th>
<th>Vineland-Social</th>
<th>CGI-I</th>
<th>CGI-S</th>
<th>ABC-FX Irritability</th>
<th>ABC-FX Hyperactiv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>~7.7</td>
<td>~54.3</td>
<td>–</td>
<td>~4.6</td>
<td>~16.3</td>
<td>~12.1</td>
</tr>
<tr>
<td>Arbaclofen (flexible)</td>
<td>-2.3 ± 0.33</td>
<td>0.1 ± 1.17</td>
<td>3.2 ± 0.12</td>
<td>-0.5 ± 0.08</td>
<td>-4.2 ± 0.89</td>
<td>-3.6 ± 0.58</td>
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<tr>
<td>Placebo</td>
<td>-2.4 ± 0.32</td>
<td>2.5 ± 1.17</td>
<td>3.1 ± 0.12</td>
<td>-0.3 ± 0.08</td>
<td>-5.3 ± 0.88</td>
<td>-3.4 ± 0.57</td>
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<tr>
<td>p-value</td>
<td>0.974</td>
<td>0.151</td>
<td>0.587</td>
<td>0.063</td>
<td>0.421</td>
<td>0.811</td>
</tr>
</tbody>
</table>

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<th>CGI-I</th>
<th>CGI-S</th>
<th>ABC-FX Irritability</th>
<th>ABC-FX Hyper</th>
<th>Parent Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>~6.8</td>
<td>~65.1</td>
<td>–</td>
<td>~4.9</td>
<td>~29.0</td>
<td>~20.6</td>
<td></td>
</tr>
<tr>
<td>Arbaclofen 10mg TID</td>
<td>-3.7 ± 0.38</td>
<td>7.5 ± 2.73</td>
<td>3.0 ± 0.16</td>
<td>-0.5 ± 0.11</td>
<td>-9.7 ± 1.40</td>
<td>-6.0 ± 0.81</td>
<td>9.5 ± 16</td>
</tr>
<tr>
<td>Placebo</td>
<td>-2.8 ± 0.36</td>
<td>5.1 ± 2.46</td>
<td>3.3 ± 0.15</td>
<td>-0.4 ± 0.10</td>
<td>-5.5 ± 1.31</td>
<td>-4.0 ± 0.75</td>
<td>2.7 ± 15</td>
</tr>
<tr>
<td>p-value</td>
<td>0.085</td>
<td>0.510</td>
<td>0.119</td>
<td>0.909</td>
<td>0.031</td>
<td>0.081</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Both failed to meet primary endpoint for FDA but younger patients had a better Response overall (Berry-Kravis et al NFXF conference 2014)
Absence of FMRP leads to enhanced protein production
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.
- Utari et al 2010 surveyed 50 families with FXS Tx with minocycline found 70% positive response especially in language.
- Positive open trial in FXS in Toronto with age ≥ 13 years (Paribello et al 2010).
- Positive controlled trial of minocycline in 3.5 to 16yo with FXS (Leigh et al 2013 JDBP).
Controlled cross-over double-blind trial of minocycline, limited but significant improvement on CGI and VAS measures (Leigh et al 2013 JDBP)

Figure 1- Distribution of Clinical Global Impression-Improvement (CGI-I)

Intent to Treat Analysis

Minocycline $\bar{x} = 2.49 \pm 0.13$
Placebo $\bar{x} = 2.97 \pm 0.13$

$p = 0.0173$
Only 2 individuals had MMP9 levels done in each phase of study and both were responders to minocycline

Dziembowski et al 2013
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
SEROTONIN SYNTHESIS CAPACITY:
Autistic vs. Non-autistic Children

Chugani et al., 1999
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
**Controlled trial of sertraline in FXS**

- Children 2 to 6yo with FXS, controlled trial for 6 months. Analysis of first 30 patients (Hess et al 2014 NFXF). Data is being analyzed now for the 57 patients enrolled in the trial.

- $X^2 (3, N=30) = 11.52, p = .009$
**GABA_A** receptor expression is down-regulated 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 double blind cross-over trial (each arm 7 weeks) in children with FXS (6-18y) funded by DOD is ongoing at the MIND. Results will be available by end of 2015.
  - Targeting improvement in anxiety, behavior and seizure frequency
  - Frank Kooy has initiated the same trial in Belgium
Yoga and Mindfulness Meditation improves GABA inhibition
Metadoxine (Alcobra)

- Ion-pair salt of pyridoxine (vitamin B6) and 2-pyrrolidone-5-carboxylate (PCA or L-PGA)
- Short acting form approved for alcohol intoxication in other countries (not FDA approved)
- Long acting form being developed for ADHD – 2 positive studies
- Reversed multiple cognitive phenotypes in FXS mouse
- Binds to GABA transporter and stimulates GABAergic inhibitory transmission via presynaptic mechanism. GABA inhibition is deficient in FXS and in ASD
- Preliminary data are positive but not made public
Metadoxine (MG01CI) Phase 2 Placebo-Controlled Trial

- Males and females with FXS age 15-55  Enrolling now
- Primary outcome attention
  - Secondary is hyperactivity, behavioral, cognitive, biomarker outcomes
  - Some parent forms, many measures clinician administered or direct patient assessment
- ½ chance each placebo or metadoxine tablets – titration to low or high dose depending on tolerance
- Most meds allowed
- 6 visits over 2-3 months, 42 days of treatment, no extension
- Some blood, EKGs and cognitive tests
- Sites:  MIND (UCDavis), Rush (Chicago), Emory (Atlanta), U Mass, Southwest Autism Research (Phoenix), Suburban Research (Philadelphia), Denver, Kennedy-Krieger (Baltimore), Cincinnati Children’s, Baylor (Houston), U of Washington (Seattle), Israel
NNZ-2566 (Neuren)

- IGF1 analog
- Works on overactive ERK/AKT pathway in FXS mouse
- ERK/ARK in signaling pathway for mGluRs/other receptors
- Normalizes hyperactivity, social behavior, cognition, dendritic spines, ERK/AKT, even testis size in FXS mouse

ERK and AKT biomarkers
NNZ-2566 (IGF-1 analogue: Neuren) Phase 2 Placebo-Controlled Trial

- Males with FXS age 14-40
- Primary outcome is safety
  - Exploring different behavioral, phenotype, language, cognitive, biomarker outcomes
  - Some parent forms, many measures clinician administered or direct patient assessment
- 1/3 chance each placebo, low dose, high dose
- Strawberry liquid
- Most meds allowed
- 8 visits over 2-3 months (some weekly), 42 days of treatment, some travel support, no extension
- Lots of measures and blood tests, some days multiple PK samples
- Sites: MIND (UCDavis), Rush (Chicago), Emory (Atlanta), U Mass, Mt Sinai (NY), Suburban Research (Philadelphia), Denver (UCHSC)
Vitamins and antioxidants in Fragile X mouse and man

- Vit E (Alpha tocopherol) and N acetyl L cysteine (NAC) improved behavior and cognition in KO mouse (de Diego et al 2009)
- Controlled trial of Vit E and C in 6 to 18yo with FXS 10 mg/kg/day for 24 weeks (de Diego-Otero et al 2014)
- Omega 3 diet increased sociability in KO mouse and BDNF in DG of hippocampus (Pietropaolo et al 2014 Psychoneuroimmunology)
Treatment of KO mouse with Vitamin E, alpha tocopheral and N acetyl L cysteine (NAC) antioxidants

Treatment improved behavior, testicular size and oxidative stress

New study by de Diego-Otero et al 2014 is assessing a combination of alpha tocopherol and ascorbic acid both 10 mg/kg.day in FXS (6-18y) phase 2 controlled trial for 24 weeks
New treatments possibilities for FXS

- **Suramin**: purigenic antagonist
  - Many effects including decreasing PI3K and Akt levels, decrease of APP and TDP43 in mouse models of ASD and FXS (Naviaux et al 2015)

- **Bumetanide**: diuretic
  - Tyzio et al 2014: Chronic deficient chloride regulation in rodent models of autism. Giving bumetanide to mother rescues the GABA developmental sequence and autistic phenotype in rodents with FXS or ASD. Now in patient trials for ASD.

**Cannabinoids**: CBD trial in FXS
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, digital programs such as CogMed, Headsprout for reading, AT devices, iPAD apps.
Collaborators

UC Davis School of Medicine
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Chris Iwahashi  Anna Ludwig
Dolores Garcia-Arocena
Greg Mayeur  Chris Raske
Dept. Biostatistics
Danh Nguyen
Department of Pathology
Claudia Greco

University of Washington and UC Davis Fragile X Research Center NICHD Funded
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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

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