The Refractory Brain: Designing Drugs to Treat Challenging Disorders of the Central Nervous System

Genes to Drugs: The evolution of the effective treatment of Fragile X Syndrome and associated comorbid disorders

Jacquelyn Bainbridge, PharmD, FCCP
Department of Clinical Pharmacy
Molly Huntsman, PhD
Departments of Pharmaceutical Sciences and Pediatrics

Disclosure Statement – financial relationships to disclose

Molly Huntsman, PhD
FRAXA Research Foundation:
Grant/Research Support
Sage Therapeutics:
Consultant

Jacci Bainbridge, PharmD
NIH, UCB Pharma:
Grant/Research Support
Teva, UCB Pharma:
Consultant

Dr. Bainbridge will discuss off-label uses of statins

Circuit Changes in NDD

- In NDDs circuit changes occur in development
- Protein expression of critical genes does not occur in these disorders
- Leads to circuit and connectivity alterations in these disorders that are not present in the normally developing brain (the canvas is different not blank)
- Fragile X syndrome is the leading genetic cause of Autism
- The core problem associated with these disorders occurs at the synapse which can be treated with therapeutic rescue (targeting synapse and circuits)
  - In Rett MECP2 protein missing
  - In Fragile X FMRP protein missing
  - In Epilepsy hereditary alterations in ion channels (potassium and sodium)

Pathogenesis of Seizures

- Excitatory/Inhibitory imbalances
- Pharmacological rescue
- Excitatory: Glutamatergic
  - Glutamate agonists: Aspartate, Kainic acid
- Inhibitory: GABAergic
  - GABA agonists: Benzodiazepine's and Barbiturate's

The goal of treating E/I imbalances through pharmacological rescue

NDDs with hyper excitable phenotypes: FXS, RETT, Tuberous sclerosis
NDDs with hypo excitable phenotypes: Down's Syndrome

Wetmore et al., 2010
The effective control of inhibitory and excitatory connections through pharmacological manipulation

Fragile X Syndrome

- X-linked disorder - leading known inherited cause of intellectual disability - incidence rate is ~1 in 3600-4000 males and 1:6000-8000 in females.
- Connective tissue abnormalities.
  - Long narrow face, big ears, eye shape
- Neurological phenotypes of FXS include:
  - Impaired cognitive and executive function
  - Attention deficit and hyperactivity
- Large proportion (~75%) show autistic behavior and 1/3 are diagnosed with autism by DSM-IV criteria.
- Abnormal EEG patterns and elevated incidences of seizure disorders (20-25%, 15% epilepsy).

Benign focal seizures are prevalent in FXS

Pharmacotherapy Principles

- Goal of Therapy: Minimize specific negative target behaviors so that other education/behavior/social interventions possible
- ASD children may require lower doses than doses recommended for a primary indication
- Titrate slow to lowest effective dose
- Symptoms worsen with higher doses
- Within classes, responses are drug specific (i.e., fail one drug, try another drug within same class)

<table>
<thead>
<tr>
<th>Target Symptom</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitive behavior, behavioral rigidity, obsessive compulsive symptoms</td>
<td>SSRI Atypical Antipsychotics (rare) • risperidone, aripiprazole Valproate (MPEP) glutamate antagonist</td>
</tr>
<tr>
<td>Hyperactivity, impulsivity, inattention</td>
<td>Stimulants (mild) Alpha-2-agonists • clonidine, guanfacine Atypical Antipsychotics (rare)</td>
</tr>
<tr>
<td>Aggression, explosive outbursts, irritability, self-injury</td>
<td>Atypical Antipsychotics Alpha-2-agonists Anticonvulsants (AEDs)/mood stabilizers Beta-blockers</td>
</tr>
</tbody>
</table>

Adapted from Pediatr 2007;120:1162-82

<table>
<thead>
<tr>
<th>Target Symptom</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td>SSRI Buspirone Mirtazapine</td>
</tr>
<tr>
<td>Depressive or Bipolar</td>
<td>SSRI +/- AED Mirtazapine Lithium Atypical Antipsychotics (rare)</td>
</tr>
<tr>
<td>Sleep Dysfunction</td>
<td>Melatonin • ramelteon (Rozerem) melatonin receptor agonist Antihistamines Alpha-2-agonists AEDs</td>
</tr>
</tbody>
</table>
Epilepsy

<table>
<thead>
<tr>
<th>First Line</th>
<th>Adjunctive</th>
<th>Avoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Lamotrigine</td>
<td>Phenobarbital (dental problems)</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Oxcarbazepine</td>
<td>Phenobarbital (exacerbation of behavioral problems)</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>Gabapentin (exacerbation of behavioral problems)</td>
<td>Levetiracetam</td>
</tr>
</tbody>
</table>

Seizures in FXS are relatively easy to manage and are generally controlled with monotherapy.


Genetic Controls Balancing Excitatory and Inhibitory Synaptogenesis in Neurodevelopmental Disorder Models

- Fragile X Syndrome (FXS) results from loss of mRNA-binding FMRP, which regulates synaptic transcript trafficking, stability and translation in activity-dependent synaptogenesis and plasticity mechanisms.
- Genetic models of FXS exhibit striking excitatory and inhibitory synapse imbalance, associated with impaired cognitive and social interaction behaviors.
- The use of genetic mouse models has begun to shed light on the mechanistic bases of excitation/inhibition imbalance for a range of neurodevelopmental disease states.

FXS has a complex phenotype with the addition comorbidities

- Initially, the fragile or marker X chromosome was not clearly associated with any other phenotype but mental retardation. However, once the laboratory conditions necessary for the reproducible expression of the cytogenetic fragile site were established, a number of affected families were detected and the associated phenotype emerged.
- Mental retardation is the most prominent phenotype, with IQ values typically between 20 and 70.
- The cognitive dysfunction particularly affects short-term memory for complex information, visuospatial skills, and speech.
- FXS patients show hyperactivity, hypersensitivity to sensorial stimuli, and attention deficit, and between 15–50% of affected individuals show some autistic behavior such as poor visual contact, tactile defensiveness, and repetitive behaviors.
- A significant proportion of patients exhibit seizures (30-35%) during their childhood and 10-15% will be diagnosed with epilepsy.

1943 Martin-Bell (fragile x) syndrome
1991 Fmr1 gene silenced by CGG repeat
1994 Fmr1 knockout Mouse
2002 Excessive mGluR5 signaling
2007 Validation of mGluRs as a therapeutic target
2012 mGluRs inhibition in Phase II/III clinical trials

FXS: From genes to treatment in 20 years

- Adapted from Bhakar et al., 2012 Ann Rev Neurosci v 35

Fragile X Syndrome is inherited


How do we start to compare?

Human
Mouse

“Not actual size”
**Fmr1 mutant mice are a well characterized and highly valuable animal model of FXS**

**Mouse model of FXS**
- Mild cognitive defects
- Hyperactivity
- Anxiety
- Macroorchidism
- Increased sensitivity to epileptic seizures (audogenic and limbic)
- Immature dendritic spines
- Decreased # of inhibitory neurons

**Human Fragile X Syndrome**
- Mental impairment, ranging from learning disabilities to mental retardation
- Attention deficits and hyperactivity
- Anxiety
- Macroorchidism
- Epilepsy in 15% of fragile X patients with full mutation
- Immature dendritic spines (post-mitotic studies)
- Autistic behavior
- Hypersensitivity to sensory stimuli

Absence of FMRP

**Neuroscience research in Fmr1 KO mice identifies a mechanism of enhanced neuronal excitability**

- When FMR1 was cloned in 1991, nothing was known about the function of its protein product until the KO mutant mice were available.
- Research from the past 20 years has led to the understanding that FMRP plays a critical role in synaptic connectivity.
- It is now believed that many symptoms can arise from modest changes in synaptic signaling - changes that can be corrected with targeted therapies such as those that are now in clinical trials

**Compounds in FXS/ASD drug development**

<table>
<thead>
<tr>
<th>Drug Target</th>
<th>Compound</th>
<th>Safety harness</th>
<th>Clinical trial phase</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>mGluR5 inhibitors</td>
<td>RO25-4893</td>
<td>Fully safe/absence</td>
<td>Phase 1 (2014)</td>
<td>NIMH/NIH</td>
</tr>
<tr>
<td></td>
<td>Acamprosate</td>
<td>Safe</td>
<td>Phase 1 (2013)</td>
<td>NIH/ULtraVU</td>
</tr>
<tr>
<td>Ras/RTK/ERK</td>
<td>Resaddin</td>
<td>NLR</td>
<td>Phase I (2013)</td>
<td>FDA</td>
</tr>
<tr>
<td>Akt/Rho</td>
<td>Eleidolide</td>
<td>Fully safe/absence</td>
<td>Phase 1/IIIA/B</td>
<td>NIH/NIH</td>
</tr>
<tr>
<td></td>
<td>Ganexolone</td>
<td>Fully safe/absence</td>
<td>Phase 1/IIIA/B</td>
<td>NIMH/NIH</td>
</tr>
<tr>
<td>mGluR5 inhibitors</td>
<td>Truxedide</td>
<td>NLR</td>
<td>Phase I</td>
<td>FDA</td>
</tr>
<tr>
<td>Endocannabinoids</td>
<td>Panulukast</td>
<td>NLR</td>
<td>NLR</td>
<td>NLR</td>
</tr>
</tbody>
</table>

Source: clinicaltrials.gov

**The early days of discovery**

- In 1943, Martin-Bell (fragile x) syndrome
- In 1991, Fmr1 gene silenced by CGG repeat
- In 1994, Knockout Mouse

**“You can’t solve the phenotype without a pathway”**

- Loss of function of Fmr1 gene leads to discovery of FMRP function.
- mGluR Theory of FXS
- Excessive glutamate receptor mediated signaling and receptor trafficking.
- Neuronal structure changes that
- E/I ratio imbalances through defective GABA-mediated inhibition.
- Protein kinases inhibitors that mediate cytoskeleton formation.
- Cell growth signaling cascades - MEK/Ras inhibitors

**“FOUND in translation”**

- FMRP is a selective RNA-binding protein; it binds to mRNA in the mammalian brain.
- FMRP controls the synthesis of many different types of proteins associated with excitatory and inhibitory synapses.
- Selective control of synaptic proteins results in hyperexcitability.
The “mGluR Theory” of FXS

- The mGluR theory is based on the assumption of enhanced signaling cascades through specialized excitatory receptors.
- This is due to based on the assumption that the fragile X mental retardation protein (FMRP) is synthesized in response to mGluR activation and functions as a translational repressor.
- This molecular change alters cellular shape. The net loss of synaptic receptors promotes the elongation of dendritic spines.

Validation of the mGluR theory

- Dolen et al., 2007 Neuron
- Chuang et al., 2005 J Neuroscience

Rescue of defective inhibition

- Paluszkiewicz, Martin and Huntsman, 2011 Dev Neurosci

Targeting specific types of GABA_A receptors can rescue cellular hyper-excitability

- Olmos-Serrano and Paluszkiewicz et al., 2010 J Neuroscience

Controlling protein synthesis:
Lovastatin decreases seizures in Fmr1 KO mice.


Both FTS (farnesyl thiosalicylic acid) and Lovastatin reduce Ras signaling

Rescue of Cellular Structure decreases seizure activity in Fmr1 KO mice

- Dolan et al., 2013 Proc Natl Acad Sci
Therapeutic strategies for FXS/ASD

- MPEP
- AFQ056
- Acamprosate
- mGluR
- mGluR
- GABA<sub>B</sub>
- GABA<sub>B</sub>
- GABA<sub>A</sub>
- GABA<sub>A</sub>
- Arbaclofen
- Lovastatin
- FRAX486
- ganaxolone

FXS: From genes to treatment in 20 years

- 1943: Martin-Bell (fragile x) syndrome
- 1991: Fmr1 gene silenced by CGG repeat
- 1994: Fmr1 Knockout Mouse
- 2002: Basic Neuroscience research
- 2007: Validation of mGluR5 as a therapeutic target
- 2012: mGluR5 inhibitors in Phase II/III clinical trials

Adapted from Bhakar et al., 2012 Ann Rev Neuroscience 35.