The Promise of Human Genetics

Human disease → Gene discovery → Mutation analysis → Biochemistry → Disease models → Disease pathophysiology → Cell Biology → Novel therapeutics
FRAGILE X SYNDROME

X-linked dominant with reduced penetrance

Male Incidence 1/5,161

Intellectual Disability

Fragile Site @ Xq28
Clinical Features of Fragile X Syndrome

- **Facies**
  - Mild dolichocephaly
  - Head circumference (>50th %tile)
  - Prominent forehead & lower jaw
  - Large, protruding ears (>2σ for age)

- **Connective tissue**
  - Macroorchidism (>2σ for age)
  - Joint laxity with hyperextensibility
  - Pes planus
  - Mitral valve prolapse

- **Co-morbid**
  - Recurrent otitis media/sinusitus (common)
  - Gastroesophageal reflux (30%)
  - Epilepsy (20%)

- **Cognitive behavioral**
  - Intellectual Disability (average IQ 40)
  - Autism (25% males/ 6% females)
  - Impaired socio-emotional reciprocity
  - Tactile defensiveness
  - Gaze avoidance
  - Hyperarousal to sensory stimuli
  - Repetitive behaviors
  - Delayed Developmental Milestones

- **Delayed Developmental Milestones**
Fragile X Mental Retardation - 1 Gene (FMR1)

Identification of a Gene (FMR-1) Containing a CGG Repeat Coincident with a Breakpoint Cluster Region Exhibiting Length Variation in Fragile X Syndrome

Four Allelic Classes of FMR1

Common
6 - 44 repeats

Premutation
55 - 200 repeats

Intermediate
45 - 54 repeats

Full Mutation
>200 repeats

ACMG Technical Standards and Guidelines for Fragile X, 2001
FMR1 Phenotypes

Fragile X-Associated Primary Ovarian Insufficiency (FXPOI)

Fragile X-Associated Tremor Ataxia Syndrome (FXTAS)

Neither Found in Full Mutation Males or Females
Repeat Expansion into the Full Mutation Range Results in CpG Methylation and Chromatin Shifts to Heterochromatin

**FULL MUTATION**

- Full Mutation Males
- Active Full Mutation X in Females
  (~50 are penetrant; wide expressivity)

Coffee et al., Nature Genet 22:98, 1999
Coffee et al., Am J Hum Genet 71:293, 2002
Fragile X Mental Retardation Protein

Selective RNA Binding Protein (~3% brain mRNAs)

Target mRNAs have sequence motifs recognized by FMRP

Shuttles between Nucleus and Cytoplasm

Associates with large mRNP and Polyribosomes

FMRP Regulates Translation of Target mRNAs
FMRP Regulates Translation of Target mRNAs at the Synapse
<table>
<thead>
<tr>
<th>mRNA</th>
<th>Dendritic Localization</th>
<th>G-quartet-like structure</th>
<th>mGlur Stimulated</th>
<th>Method</th>
<th>References</th>
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<tbody>
<tr>
<td>App</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Co-IP</td>
<td>Westmark and Malter, 2007</td>
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<td>Arc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Co-IP</td>
<td>Braham and Wells 2007; Waung et al 2008; Park et al 2008</td>
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<td>CamKIIα</td>
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<td>+</td>
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<td>Co-IP</td>
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<td>eEF1A</td>
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<td>+</td>
<td>+</td>
<td>Co-IP; in vitro</td>
<td>Huang et al 2005; Sung et al 2003</td>
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<tr>
<td>Fmr1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in vitro</td>
<td>Weiler et al 1997; Antar et al 2004; Schaeffer et al 2001</td>
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<td>GluR1/2</td>
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<td>+</td>
<td>+</td>
<td>Co-IP</td>
<td>Muddashetty et al 2007</td>
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<td>Map1b</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Co-IP; in vitro; biophysical</td>
<td>Brown et al 2001; Darnell et al 2001; Antar et al 2005; Davidkova and Carroll, 2007; Hou et al 2006; Menon et al 2008</td>
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<td>Psd95</td>
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<td>+</td>
<td>Co-IP; in vitro; CLIP</td>
<td>Todd et al 2003; Zalfa et al 2007; Muddashetty et al 2007</td>
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<tr>
<td>Sema3F</td>
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<td>+</td>
<td></td>
<td>Co-IP; biophysical</td>
<td>Darnell et al 2001; Menon et al 2007</td>
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<tr>
<td>Rgs5</td>
<td>+</td>
<td>+</td>
<td></td>
<td>APRA; in vitro</td>
<td>Miyashiro et al 2003; Dictenberg et al 2008</td>
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<tr>
<td>Gaba-Aδ</td>
<td>+</td>
<td>+</td>
<td></td>
<td>APRA; in vitro</td>
<td>Miyashiro et al 2003; Dictenberg et al 2008</td>
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</tbody>
</table>
The molecular basis of Fragile X Syndrome involves several key events:

1. **Expansion to Full Mutation Alleles**
2. **Transcriptional Silencing of FMR1**
3. **Absence of the RNA-Binding Protein FMRP**
4. **Translational over-expression of select mRNAs**
5. **Altered synaptic function?**
6. **Fragile X Syndrome Phenotype**

These events lead to the characteristic phenotype associated with Fragile X Syndrome.
3p1 mGluR-dependent long-term depression is enhanced in Fmr1 KO mice

Wild-type:
- Requires protein synthesis

Exaggerated in Fmr1 Knock-out

mGluR5 Stimulation Results in AMPAR (GluR1) Internalization

Rat hippocampal primary culture neurons (18DIV)
DHPG induced AMPAR internalization is translation dependent (n=15 each)

**Surface/Total GluR1**

Translation inhibitors
ani: anisomycin
cyc: cycloheximide
pur: puromycin

Transcription inhibitor
act: actinomycin D
FMRP knockdown by siRNA leads to excessive AMPA receptor internalization

Rat hippocampal primary culture neurons (18DIV)

Normal FMRP

Low FMRP

AMPA receptor internalization

Surface AMPAR

Internalized AMPAR

FMRP

Rat hippocampal primary culture neurons (18DIV)
FMRP Phosphorylation Reveals an Immediate-Early Signaling Pathway Triggered by Group I mGluR and Mediated by PP2A

S6K1 Phosphorylates and Regulates Fragile X Mental Retardation Protein (FMRP) with the Neuronal Protein Synthesis-dependent Mammalian Target of Rapamycin (mTOR) Signaling Cascade

FMRP Proteins

Immediate-Early Translation

Synaptic Proteins

Maintenance Translation

PP2A

mTOR

Akt

PI3K

PIKE

Homer

mGluR1/5

glutamate

S6K1

< 1 min

< 5 min
FMRP loss results in excess translation

Immediate-Early Translation

Maintenance Translation

Weakened synaptic strength

Persistent Decrease in surface AMPARs

FMRP loss results in increased AMPAR internalization

Synaptic Proteins

PP2A

S6K1

mTOR

PI3K

PIKE

PDK1/2

Akt

Homer

mGluR1/5

glutamate
The mGluR theory of fragile X mental retardation

Mark F. Bear¹, Kimberly M. Huber² and Stephen T. Warren³

mGluR1/5

MPEP
2-Methyl-6-(phenylethynyl)-pyridine

mRNA translation

Modulate excess translation & downstream effects

FMRP
Fragile X syndrome

2-Methyl-6-(phenylethynyl)-pyridine
Can an mGluR antagonist rescue aberrant AMPA receptor trafficking in FMRP deficiency?

Control

![Images showing FMRP, Surface AMPAR, and Internalized AMPAR](image_url)
Pharmacological Rescue of Synaptic Plasticity, Courtship Behavior, and Mushroom Body Defects in a *Drosophila* Model of Fragile X Syndrome

Sean M.J. McBride,1,9 Catherine H. Choi,4,9 Yan Wang,3 David Liebelt,1 Evan Braunstein,1 David Ferreiro,1 Amita Sehgal,6 Kathleen K. Siwicki,7 Thomas C. Dockendorff,8 Hanh T. Nguyen,2 Thomas V. McDonald,3 and Thomas A. Jongens5,7

Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP

Q.J. Yan1, M. Rammal3, M. Tranfaglia3, R.P. Bauchwitz1,*

Rescue of behavioral phenotype and neuronal protrusion morphology in *Fmr1* KO mice

Femke M.S. de Vrij1, Josien Levenga1, Herma C. van der Linde1, Sebastiaan K. Koekkoek1, Chris I. De Zeeuw1, David L. Nelson,4 Ben A. Oostra1, and Rob Willemsen4,*

Correction of Fragile X Syndrome in Mice

Gül Dölen1,2 Emily Osterweil1, B.S. Shankaranarayana Rao,6 Gordon B. Smith,1 Benjamin D. Auerbach1, Sumantra Chattarji6, and Mark F. Bear1,*
First Drug Screen for Fragile X Syndrome using the Drosophila model
Selective food-mediated lethality in dFmr1 mutant

Viability of dFmr1 mutant on JAZMIX food

<table>
<thead>
<tr>
<th></th>
<th>dFmr1-/TM6C</th>
<th>dFmr1-+/dFmr1+</th>
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</thead>
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<tr>
<td>JAZMIX</td>
<td>124</td>
<td>2</td>
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<tr>
<td>Homemade</td>
<td>96</td>
<td>45</td>
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</table>

Expected ratio 2 : 1

Kevin Moses
# Excess Glutamate in Commercial Food

<table>
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<tr>
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<th>Absorbance for Glu</th>
<th>Relative amount</th>
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<tbody>
<tr>
<td>JAZMIX</td>
<td></td>
<td>1.8</td>
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<tr>
<td>Homemade</td>
<td></td>
<td>1</td>
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</table>
Glutamate Causes Dose-dependent Lethality on Lab Food

![Graph showing relative viability (%) against dose (µM) for different genotypes: W1118, dfmr1 +/-, and dfmr1 -/-. The graph indicates dose-dependent lethality with significant reductions at higher doses.](image-url)
mGluR Antagonist MPEP Rescues CF Lethality

Relative Viability (%) vs. Treatment

- LF
- CF
- CF + 10 μM MPEP

*Statistically significant difference
**Drug Screen Design**

$dfr1^3 / Kr: GFP,TM6C \times dfr1^3 / Kr: GFP,TM6C$

$dFmr1$ heterozygous & homozygous embryos

Embryo sorter

12 embryos per tube on CF
2,000 compounds at 40µM

<table>
<thead>
<tr>
<th>Drug name</th>
<th>dfmr1 viability (%) from screen</th>
<th>Relative dfmr1 viability (%) from confirmation</th>
<th>Optimized concentration from confirmation (µM)</th>
<th>General description</th>
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<tbody>
<tr>
<td>3-hydroxybenzylhydrazine dihydrochloride</td>
<td>67</td>
<td>False positive</td>
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<tr>
<td>Acetylcysteine</td>
<td>67</td>
<td>False positive</td>
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<tr>
<td><strong>Nipecotic acid</strong></td>
<td>58</td>
<td>84.2±20.2</td>
<td>40</td>
<td><strong>GABA reuptake inhibitor</strong></td>
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<tr>
<td><strong>Creatinine</strong></td>
<td>58</td>
<td>123.2±14.1</td>
<td>40</td>
<td><strong>Metabolism; GABAergic pathway</strong></td>
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<tr>
<td>Chloroxylenol</td>
<td>42</td>
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<tr>
<td>Ergonovine maleate</td>
<td>33</td>
<td>69.8±18.4</td>
<td>20</td>
<td>Serotonin pathway</td>
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<tr>
<td>Pilocarpine nitrate</td>
<td>33</td>
<td>68.5±6.1</td>
<td>5</td>
<td>Muscarinic agonist</td>
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<tr>
<td>Dienestrol</td>
<td>33</td>
<td>71.3±10.8</td>
<td>40</td>
<td>Sex hormone related</td>
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<tr>
<td>Clomiphene citrate</td>
<td>25</td>
<td>54.9±20.0</td>
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<td><strong>GABA</strong></td>
<td>25</td>
<td>83.1±19.2</td>
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<td><strong>GABAergic pathway</strong></td>
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<td>Kojic acid</td>
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<td>73.1±12.7</td>
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<td>Tyrosinase inhibitor</td>
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<td>Aminobenztropine</td>
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<td>66.4±8.3</td>
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<td>Muscarinic agonist</td>
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<td>Phenacylamine hydrochloride</td>
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<tr>
<td>Dopamine hydrochloride</td>
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<tr>
<td>Reserpine</td>
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<td>N/A</td>
<td>61.6±12.9</td>
<td>10</td>
<td>mGlurR pathway</td>
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</table>
Glu Toxicity is Rescued by GABA

CGP54626: a selective GABA\textsubscript{B} receptor antagonist
GABA Agonist (STX209) Rescues AMAP Trafficking Defect in *Fmr1* Knockout Neurons (n = 30)
mGluR5 Antagonists and GABA Agonists as Therapeutic Candidates in Fragile X Syndrome

**mGluR Antagonists**
- Fenobam (Neuropharm)
- STX107 (Seaside/Merck)
- AFQ056 (Novartis)
- NP22578 (Roche)

**GABAR Agonists**
- Ganaxolone (Marinus)
- Baclofen (Lioresal, Kemstro)
- STX209 (Arbaclofen - Seaside)

**Synaptic Translation**
- Fragile X Syndrome
- Negative Synaptic Translation
- Positive Synaptic Translation
- Negative Synaptic Translation
- Positive Synaptic Translation
The Promise of Human Genetics