Fragile X: Lessons From Aging

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Fragile X Syndrome

Leading heritable form of mental retardation

One in ~260 females and one in ~800 males are carriers
One-third of all X-linked mental retardation
One in ~4,000 in general population

Leading (known) single gene associated with autism

3-6% of all children with autism
Approximately 30% of young children with fragile X syndrome have autism
Normal: 5 to 44 CGG repeats
Gray Zone: 45 to 54
Premutation: 55 to 200
Full mutation: >200
Handbiting 60%

Handflapping 80%

Poor eye Contact 90%

Tactile 80%

defensiveness

Unusual sensory responses to stimuli

Perseverative speech or behavior in almost all-routines

One brother with autism and disinterested in people and the other without autism but both have the sensory hyperarousal and autistic features
Enhanced electrodermal responses to sensory stimuli correlate inversely with FMRP levels (Miller et al 1999)
Emotional & Neurocognitive Features

- Hyperactivity, impulsivity and/or short attention span
- Executive function deficits: problems with organization, shifting set, planning, inhibition tangential speech, schizotypal features, ADHD, perseveration
- Overreactivity to stimuli: enhanced electrodermal response to stimuli; enhanced cortisol release after stressors
- Anxiety
- Autism or ASD
- Mood instability: excessive outbursts, tantrums

Anti-FMRP antibody stains lymphocytes + for FMRP (red)
Genomics approach to the FXS Phenotype

- **epilepsy**
  - mRNAs of GABA a receptor subunits bind to FMRP
- **mental retardation**
  - cytoskeletal and dendritic structural gene mRNAs ie cadherins involved in synapse structure and plasticity bind to FMRP
  - mGluR5 enhanced LTD
- **Anxiety**
  - mRNA for glucocorticoid receptor binds to FMRP
- **Autism**
  - Microarray studies in progress comparing FXS+autism to FXS alone looking for genes working epistatically with FMR1 whose expression is altered with autism (Nowicki et al 2004)
Classification of Glutamate Receptors

**Metabotropic (mGluRs)**

- **Group I**
  - mGluR1,-5

- **Group II**
  - mGluR2,-3

- **Group III**
  - mGluR4,-6,-7,-8

**Ionotropic (iGluRs)**

- NMDA,
- AMPA,
- Kainate
Neurobiology of FXS

• KO mouse has excessive hippocampal long term depression (LTD) mediated by the glutamate system (mGluR5)

• Enhanced LTD interferes with the formation and maintenance of synaptic strength needed for learning

  » Huber et al 2002; Snyder et al 2001; Willemsen et al 2004
mGluR5 stimulation leads to LTD; FMRP puts the breaks on this. So in FXS there is dramatically increased LTD

They cannot recruit the extended neural network to solve more difficult tasks

These deficits correlate inversely with FMRP

The more FMRP, the better the activation
Specific Psychopharmacological Interventions

- **Ampakines**: CX516 ampakine trial underway at the MIND Institute and at Rush in Chicago

  ![Chemical Name of CX516: 1-(quinoxalin-6-ylcarbonyl) piperidine](image)

- **mGluR5 antagonists**: MPEP studies are helpful for seizures in KO mice and there is some enhancement of memory and cognition (Bauchwitz et al 2004)
Lithium down regulates the mGluR5 pathway by blocking IP3 (inositol triphosphate) turnover. Controlled trials are taking place in the KO mouse and the fly.

Lithium has been helpful in fragile X (anecdotal) for treatment of mood instability and aggression (Hagerman 2002). Careful controlled studies assessing behavioral and cognitive benefits are now warranted in FXS. A collaborative study is in the planning phase: UCLA, UC Davis and Chicago.

Mc Bride et al 2004
Time delay between first concerns and diagnosis of fragile X syndrome

Bailey et al 2003
Newborn Screening with blood spots analyzed by PCR
Neuroimaging findings in FXS:

Enlargement of
overall brain
caudate
hippocampus
ventricles

Smaller:
posterior cerebellar vermis
Superior temporal gyrus
Study of 80 children with FXS with and without ASD

Figure 1. Head Circumference Percentile Means in Children 0-5 years

Jenkins et al
Fragile X and Autism

- Approximately 3 to 6% of children with autism have fragile X syndrome (Brown et al. 1986, Hagerman 1996, Bailey et al. 1993)
- Autistic-like features are seen in the majority of patients with FXS
- Boys with FXS and autism have a lower IQ than those with FXS or those with autism (Bailey et al. 1998, 1999). Autism does not correlate with FMRP.
Fig. 2. Mean Battelle Developmental Inventory developmental age scores for boys with FXS (n = 13), boys with autism (n = 13), and boys with FXS and autism (n = 13).

Bailey et al 2000, J Aut Dev Disord 30:49
Studies of young children with FXS with and without autism compared to autism and DD controls

- Philofsky et al 2004 and Rogers et al 2003: FXS+autism have lower cognitive scores on the Mullens and lowered expressive language than autism or FXS alone. Receptive language and imitation skills are a strength in FXS alone.

- Rogers et al 2003: Children with FXS and children with autism had more sensory sxss on SSP than children with DD or MA matched typicals. Children with FXS had lowest scores on low energy/muscle weakness scale
Autism Evaluation of Boys with FXS

Use of the ADI-R alone will label 40 to 50% autistic because of the number of autism features at age 4

- Of 69 boys with FXS assessed using the ADI-R, ADOS-G and DSM IV clinical criteria:
  - 29% (n=20) met classification criteria for Autism
  - 16% (n=11) met classification criteria for PDDNOS
  - 55% (n=38) did not meet criteria for either autism or PDDNOS

- Correlations between ADOS scores and FMRP, CGG repeats or FMR1-mRNA were not significant
Autism and Fragile X may be caused by genes that are epistatic with FMRP

Autism with no interest in social interactions

Autism with limited interest in social interactions but anxiety interferes with interactions
Obvious Second Hits Leading to Autism

- Down Syndrome
- Birth trauma or Cerebral Palsy
- Seizures in 13 to 22% of males and 4 to 5% of females (Musumeci et al 1999; Berry-Kravis et al 2002)
  - including generalized or partial or partial complex
  - centrotemporal spikes are most common and predict resolution of seizures in childhood
  - onset of seizures typically 2 to 15 years
- Prader-Willi Phenotype
History of birth trauma with subsequent CP.
Seizures, Severe MR, Strabismus, Autism.
The Prader-Willi Phenotype of Fragile X Syndrome

Bardoni & Mandel, 2002

Current Opinion in Genetics & Development
Autism is also seen in some premutation carriers.

**Gene**

- **Promoter**
- 

**Carrier** (premutation)
- 55 to 200 CGG repeats

**Fragile X syndrome** (full mutation)
- > 200 CGG repeats

Aziz et al 2003, Tassone et al 2000
Beth Goodlin-Jones et al 2004;
Fragile X protein levels decrease in upper premutation range

Why? the FMR1 gene is unmethylated in this range

Tassone et al 2000.
FMR1 mRNA (gene activity) is elevated in the premutation range.

Tassone et al. 2000 AJHG.
Expression of the \textit{FMR1} gene

- **Normal**
  - (CGG) $n < 55$
  - transcription
  - mRNA
  - translation
  - FMRP
  - Clinical phenotype: Normal

- **Premutation**
  - (CGG) $55 \leq n \leq 200$
  - transcription
  - mRNA
  - translation
  - FMRP
  - Clinical phenotype: Emotional problems, Premature ovarian failure

- **Full mutation**
  - (CGG) $n \geq 200$
  - transcription
  - mRNA
  - translation
  - FMRP
  - Clinical phenotype: Fragile X syndrome
Study of probands vs non probands with premutation

- ADHD (CGI≥15 and DSM-IV)
  - 73% (11/15) of probands*
  - 50% (6/12) of nonprobands
  - 12% (2/17) of controls*
  p<.01

- ASD (SCQ≥15)
  - 67% (10/15) of probands*
  - 8% (1/12) of nonprobands
  - None of controls*
  p<.05

- ASD (DSM-IV)
  - 73% (11/15) of probands*
    - 33% (5/15) Full autism
    - 40% (6/15) PDDNOS
  - 17% (2/12) of nonprobands
    - 8% (1/12) Full autism
    - 8% (1/12) PDDNOS
  - None of controls*
  p<.05

- Medication (Stimulants, SSRIs and/or atypical antidepressants)
  - 93% (14/15) of probands*
  - 17% (2/12) of nonprobands
  - 6% (1/17) of controls*
  p<.01

Two brothers with the FMR1 premutation ages 6 and 7. Boy on right presented as proband with autism and ADHD and his brother has anxiety and ADHD.
Case 1: DR. 63 GF with 89 CGG repeats

• Onset of tremor in right hand at age 54
  – Involved left hand within two years
  – Retired early as an electrician at age 58
  – Writing illegible at age 58
  – 2 handled cup for drinking and wife cuts meat
  – Has not driven for over 1 year
  – Gait lists to left and frequent falls improved with Amantidin
  – Atenolol not helpful
  – VIQ-93, PIQ-73, FSIQ-83
Fragile X–associated Tremor/Ataxia Syndrome - FXTAS

- Intention tremor that is progressive
- Ataxia and/or frequent falling
- Parkinsonian features: masked facies, intermittent resting tremor, increased tone or response to L-dopa
- Cognitive deficits: memory problems & executive function deficits – decrease in PIQ first
- Psychological features: anxiety, mood liability, outbursts or reclusive behavior
- Peripheral neuropathy: decreased sensation in lower extremities
- MRI global brain atrophy
- MRI – deep cerebellar white matter hyperintensities
# Diagnostic Criteria

**Inclusion criteria:** CGG repeat 55-200

<table>
<thead>
<tr>
<th>MRI</th>
<th>Major</th>
<th>Middle cerebellar peduncles lesions</th>
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<tbody>
<tr>
<td>Minor</td>
<td>Cerebral white matter hyperintensity</td>
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<td>Minor</td>
<td>Moderate to severe generalized atrophy</td>
<td></td>
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<tr>
<td><strong>Clinical</strong></td>
<td><strong>Major</strong></td>
<td>Intentional Tremor</td>
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<tr>
<td></td>
<td><strong>Major</strong></td>
<td>Gait Ataxia</td>
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<tr>
<td>Minor</td>
<td>Parkinsonism</td>
<td></td>
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<tr>
<td>Minor</td>
<td>Short term memory deficits</td>
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<tr>
<td>Minor</td>
<td>Executive function deficits</td>
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## Diagnostic Categories CGG repeat 55-200

<table>
<thead>
<tr>
<th>Definite</th>
<th>Probable</th>
<th>Possible</th>
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<tr>
<td>1 MRI <strong>Major</strong> +</td>
<td>1 MRI <strong>Major</strong> +1clin</td>
<td>1 MRI Minor +</td>
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<tr>
<td>1 Clinical <strong>Major</strong></td>
<td>minor or 2 clin <strong>Major</strong></td>
<td>1 Clinical <strong>Major</strong></td>
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</table>
Intranuclear inclusions
Neurons – Astrocytes in humans

Greco et al 2002 Brain

Anti-ubiquitin antibody
Intranuclear inclusions

<table>
<thead>
<tr>
<th>Region</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 1</th>
<th>Case 2</th>
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<tbody>
<tr>
<td>Frontal cortex</td>
<td>6</td>
<td>3</td>
<td>45</td>
<td>15</td>
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<td>Temporal cortex</td>
<td>4</td>
<td>2</td>
<td>44</td>
<td>11</td>
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<tr>
<td>Putamen</td>
<td>4</td>
<td>4</td>
<td>45</td>
<td>7</td>
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<tr>
<td>Globus Palidus</td>
<td>4</td>
<td>1</td>
<td>42</td>
<td>13</td>
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<tr>
<td>Hippocampus</td>
<td>38</td>
<td>43</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Dentate nucleus</td>
<td>3</td>
<td>3</td>
<td>49</td>
<td>17</td>
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</table>

Neurons | Astrocytes
The CGG repeat – as RNA – stimulates formation of inclusions

Mouse *Fmr1* gene with ~100 CGG repeats
(Willemsen et al., 2003)

Fly with ~90 CGG repeats placed in an unrelated reporter gene
(Jin et al., 2003)
White matter Spongiosis
Neuropsychiatric Phenotype

• It presents as a frontal, subcortical dementia with deficits in executive function and memory initially and relative sparing of verbal abilities initially (Bacalman et al 2005)
• Behavior problems are mainly dysinhibition initially associated with inappropriate behavior. Anxiety and depression may be long term problems for many
• Levels of mRNA correlate with anxiety and OCD symptoms on the SCL-90 (Hessl et al 2005)
California Family Study of the prevalence of FXTAS

- Jacquemont et al JAMA 29:460, 2004:
  - 123 families with FXS in the Northern and Southern Fragile X Associations
  - in 192 individuals who are >50 and either premutation carriers or controls the penetrance in male carriers was 17% in the 50s; 38% in the 60s; 47% in the 70s; 75% in the 80s
  - some may be stable for decades and others have a more rapid progression; one case with rapid progression had both Alzheimers and FXTAS
## Females with FXTAS

<table>
<thead>
<tr>
<th></th>
<th>CASE 1</th>
<th>CASE 2</th>
<th>CASE 3</th>
<th>CASE 4</th>
<th>CASE 5</th>
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<tr>
<td>Age</td>
<td>67</td>
<td>57</td>
<td>85</td>
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<td>FSIQ</td>
<td>126</td>
<td>99</td>
<td>100</td>
<td>111</td>
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<td>VIQ</td>
<td>130</td>
<td>103</td>
<td>104</td>
<td>110</td>
<td>88</td>
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<tr>
<td>PIQ</td>
<td>116</td>
<td>94</td>
<td>94</td>
<td>111</td>
<td>86</td>
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<tr>
<td>Age of onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tremor</td>
<td>42</td>
<td>30</td>
<td>82</td>
<td>52</td>
<td>71</td>
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<tr>
<td>Age of onset</td>
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<tr>
<td>ataxia</td>
<td>59</td>
<td>37</td>
<td>79</td>
<td>60</td>
<td>71</td>
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<tr>
<td>CGG repeat</td>
<td>18, 90</td>
<td>29, 93</td>
<td>29, 87</td>
<td>18, 90</td>
<td>30,78</td>
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<tr>
<td>FMRP level*</td>
<td>89</td>
<td>96</td>
<td>80</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>Activation ratio**</td>
<td>0.51</td>
<td>0.35</td>
<td>0.53</td>
<td>0.5</td>
<td>0.21</td>
</tr>
<tr>
<td>mRNA level</td>
<td>3.25 ± 0.55</td>
<td>4.6 ± 0.29</td>
<td>1.40 ± 0.07</td>
<td>2.52 ± 0.27</td>
<td>2.6 ± 0.04</td>
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<tr>
<td>MRI</td>
<td>+ MCP sign</td>
<td>No MCP sign</td>
<td>pacemaker</td>
<td>pacemaker</td>
<td>No MCP sign</td>
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<tr>
<td>FXTAS diagnosis</td>
<td>Definite</td>
<td>Probable</td>
<td>Definite</td>
<td>Probable</td>
<td>Probable</td>
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</tbody>
</table>
Neuronal and astrocytic inclusions - also in females with FXTAS

Case 3
Isolation of inclusions

Isolate nuclei from frozen cortical tissue

Disruption of nuclei and preparative flow sorting to yield purified inclusions (~10^6 inclusions /gram brain tissue)

αB-crystallin  ubiquitin  merge

αB-crystallin  ubiquitin  merge
Another puzzle:
Myelin Basic Protein appears to be in the inclusions

Ubiquitin  MBP  merge

Non-fluorescent Anti-MBP staining
4 sisters (50 to 36 years) with the premutation; the oldest two have FXTAS and the younger ones have intermittent tremor and ataxia; all have anxiety and mood problems.

One sister with FXTAS also has autoimmune problems, including a lupus-like rash, joint pain and muscle pain; others have presented with MS sx's.
RNA gain-of-function model for FXTAS

Premutation allele > 54 CGG repeats

Hypothetical: DM-type model
- Depletion of protein pool
- Excess binding
- Reduced FMRP levels
- Abnormal regulation of other cell functions
- Other proteins
- HSPs
- Ubiquitin

Clinical involvement on the fragile X spectrum
- Neurological dysfunction
- Inclusion formation
- FXTAS
Charcot Marie Tooth and FXTAS
Research directions for FXTAS

Successful growth of neural stem cells from the brains of adults who have died with FXTAS or with fragile X syndrome

Schwartz et al 2004
Neural stem cells from a boy with FXS

Schwartz et al in press
The National Fragile X Foundation has Lesson Plans on line

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FXS and FXTAS

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