Fragile X-associated Disorders: Premutation Carrier Involvement and Treatment

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Benefits of the Premutation

• A subgroup have an exceptionally high IQ probably related to high FMRP levels
• Great verbal abilities combined with high drive for achievement and obsessive thinking can lead to high education levels, many MD, PhDs, lawyers, clergy, very successful business people, pilots etc with the premutation
Reaction time in women with the premutation was better than controls on a magnitude estimation task (p=0.05)
IQ in adults throughout the CGG repeat range
Expression of the *FMR1* gene

<table>
<thead>
<tr>
<th>CGG repeat number</th>
<th>0</th>
<th>45</th>
<th>55</th>
<th>200</th>
<th>&gt;1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative <em>FMR1</em> mRNA level</td>
<td>normal</td>
<td>gray</td>
<td>premutation</td>
<td>full mutation</td>
<td>FXTAS and FXPOI</td>
</tr>
<tr>
<td>FMRP level</td>
<td>unmethylated</td>
<td>partially methylated</td>
<td>hyper-methylated</td>
<td>FXS</td>
<td></td>
</tr>
</tbody>
</table>
Expanded CGG repeat inhibits DGCR8/DROSHA activity and Dysregulates miRNAs

predicted decrease in miRNA levels

Observed decreases in miRNA levels

Sellier C … Charlet N 2013 and 2014 JND
Enhanced cell death in premutation neurons in culture is a reflection of their vulnerability

- To environmental toxins
- Trauma as in seizures
- Oxidative stress
- Second genetic hits

Decreased cell survival by 21 days

Chen et al 2009 HMG
Genetic and Environmental Interactions

Genetic and environmental factors:
- Genetic susceptibility
- Pathological states (e.g. anxiety disorders or depression)
- Physical and psychological stress

Toxins:
- Substance abuse
- Diet and exercise
- Aging

Mitochondrial dysfunction:
- ROS ↑
- mtDNA damage ↑
- Ca²⁺ ↑
- Membrane potential ↓
- ATP production ↓

Altered neuronal signaling:
- Glutamate ↑
- GABA ↓
- Receptor modulation
  - Ca²⁺ ↑
  - NOS and NO ↑

Inflammation:
- Pro-inflammatory cytokines ↑
- Pro-inflammatory signaling ↑
  - NFκB activity ↑
  - CREB activity ↓
- NOS, COX-2 and NADPH oxidase ↑

Oxidative stress:
- Increased generation of reactive species
  - ROS, RNS and NO ↑
- Impaired oxidative defences
  - Antioxidant enzyme activities ↓
  - Antioxidants ↓

Damage to macromolecules:
- Protein carbonylation
- Protein oxidation
- DNA damage
- Lipid peroxidation

Inhibition of neurogenesis:
- Antioxidant protection

Accelerated telomere shortening
- Antioxidant protection

Apoptosis

Changes in plasticity
Neurodegeneration
Brain damage

Iiris et al 2010 Neurosciences Research
Age of menopause in carriers
Fragile X-associated Primary Ovarian Insufficiency (FXPOI)

Maitlick et al 2014
Boys with the premutation are at high risk for ADHD and autism or ASD: A developmental form of RNA toxicity

- ADHD (CGI≥15 and DSM-IV)
  - 93% (13/14) of probands
  - 38% (6/13) of nonprobands
  - 13% (2/16) of controls
- ASD (DSM-IV and ADOS/ADI)
  - 73% (11/14) of probands*
    - 29% (4/14) Full autism
    - 50% (7/14) PDDNOS
  - 8% (1/13) of nonprobands
    - 8% (1/13) Full autism
  - None of controls

Farzin et al, 2006 J Dev Beh Pediatrics

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.
Follow-up after 4 years brothers with the premutation

- Youngest brother with ADHD, anxiety and social deficits responded well to sertraline: “miracle drug” now in normal classroom, friends and normal IQ
- Oldest brother with autism and ADHD did not do well with sertraline (activation so D/C), developed seizures, now with severe autism and low verbal abilities
In premutation boys with seizures there is a strong association with autism and ID.

Chonchiaya et al 2011 Human Genetics
TABLE III. Frequency of Reported Developmental Delay and Other Conditions for Premutation Males Compared With a Sample of Normal Males Matched on Age and Family Income

<table>
<thead>
<tr>
<th>Condition</th>
<th>Premutation males (n = 57)</th>
<th>Non-FX males (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental delay</td>
<td>33.0***</td>
<td>1.8</td>
</tr>
<tr>
<td>Attention problems</td>
<td>41.1*</td>
<td>21.4</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>28.1</td>
<td>14.0</td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>19.3*</td>
<td>5.3</td>
</tr>
<tr>
<td>Self-injury</td>
<td>8.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Autism</td>
<td>19.3*</td>
<td>5.3</td>
</tr>
<tr>
<td>Seizures</td>
<td>11.3**</td>
<td>1.2</td>
</tr>
<tr>
<td>Anxiety</td>
<td>33.3**</td>
<td>8.8</td>
</tr>
<tr>
<td>Depression</td>
<td>10.7</td>
<td>10.7</td>
</tr>
</tbody>
</table>

*P < 0.05  
**P < 0.01  
***P < 0.001
Reasons for Premutation Involvement

- Upper end of the premutation has low FMRP
- Elevated mRNA leading to RNA toxicity
- Chronic DNA damage repair response
- RAN (repeat associated non AUG) translation
- Environmental Toxicity (smoking, general anesthesia, inhalants, opioids, alcohol)
- 20% of those with the pre and autism or ID have a second genetic hit or CNV (Lozano et al 2014 JND)
The newborn-screening process

- Early intervention
- Genetic and reproductive counseling
- Access to treatment and services
- Long term follow-up

PCR
Blood spot collection
Capillary electrophoresis analysis
Cascade testing

Tassone 2014
Decreased contrast sensitivity (CS) for second-order dynamic stimuli in premutation and full mutation babies

In fulls: Farzin et al 2008
Gallego et al 2014 JND

Average contrast threshold for TD, premutation and FXS infants.
Psychiatric symptoms in carriers

- Johnston et al 2001: Women with >100 repeats have higher rates of depression and interpersonal sensitivity
- Hessl et al 2005: higher rates of OCD and psychopathology correlated with mRNA levels
- Roberts et al 2009: SCID in 93 pre women; elevated MDD 43% & panic disorder 8.6%
- Bourgeois et al 2009: Lifetime mood disorder in 65% of FXTAS and 42% of non FXTAS pres; lifetime anxiety disorder in 52% of FXTAS and 47% of non FXTAS pres
Anxiety and Hippocampal Volumes in Females with the Premutation

Circles with FXTAS, triangles without \( (r=-0.634; \ p<0.001) \)

Adams et al 2009
### 146 female carriers medical history

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control N=69</th>
<th>Non FXTAS N=128</th>
<th>Control N=39</th>
<th>FXTAS N=18</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXTAS</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>POI</td>
<td>5.6%</td>
<td><strong>19.0%</strong></td>
<td>5.9%</td>
<td><strong>13.3%</strong></td>
</tr>
<tr>
<td>Thyroid Problems</td>
<td>10.1%</td>
<td>17.3%</td>
<td>15.4%</td>
<td><strong>50.0%</strong></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.0%</td>
<td>3.9%</td>
<td>0%</td>
<td>11.1%</td>
</tr>
<tr>
<td>Lupus</td>
<td>0.0%</td>
<td>2.4%</td>
<td>0%</td>
<td>5.9%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10.1%</td>
<td>16.4%</td>
<td>18%</td>
<td><strong>61.1%</strong></td>
</tr>
<tr>
<td>MS</td>
<td>0.0%</td>
<td>4.8%</td>
<td>0%</td>
<td>17.7%</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>5.0%</td>
<td>8.3%</td>
<td>9.4%</td>
<td><strong>43.8%</strong></td>
</tr>
<tr>
<td>Muscle Pain</td>
<td>8.9%</td>
<td><strong>25.6%</strong></td>
<td>10.7%</td>
<td><strong>76.5%</strong></td>
</tr>
<tr>
<td>Tremor</td>
<td>1.5%</td>
<td><strong>11.7%</strong></td>
<td>0%</td>
<td><strong>89%</strong></td>
</tr>
<tr>
<td>Problems</td>
<td>1.5%</td>
<td>8.6%</td>
<td>2.6%</td>
<td><strong>83.3%</strong></td>
</tr>
<tr>
<td>Walking/Balance</td>
<td>11.9%</td>
<td><strong>45.2%</strong></td>
<td>18.9%</td>
<td><strong>83.3%</strong></td>
</tr>
</tbody>
</table>

**Fisher’s exact test for 2×2 contingency table analysis p<0.05

Coffey et al 2008 AJMG
Spectrum of Premutation Involvement

**FMR1 CGG-repeat toxic RNA “trigger”**

- Upregulation of heatshock proteins
- ASFMR1 splice isoforms in FXTAS
- Kinase activation
- Sequestration of DROSHA,DGCR8 Sam68
- Mitochondrial dysfunction
- RAN translation
- Inclusion formation
- Neuropathology

**Background gene effects**

- Neurodevelopmental problems
  - Social anxiety → ASD
  - ADHD
  - Cognitive deficits

- Psychiatric involvement
  - Anxiety
  - Stress
  - Depression

- Endocrine dysfunction
  - FXPOI

- Immune dysregulation
  - Hypothyroidism
  - Fibromyalgia, central pain syndrome
  - Lupus- MS features

- Neurological problems
  - Neuropathy
  - Migraine, sleep apnea, RLS
  - Memory problems, foggy thinking
  - Hypertension, chronic fatigue

- FXTAS
  - tremor, ataxia, Parkinsonism
  - autonomic dysfunction, EF deficits,
  - memory and cognitive decline
There are fMRI, volumetric and DTI changes in premutation carriers before the onset of FXTAS.

Hashimoto et al 2011 J Psych Res

Gray matter density decreases in FXTAS & non-FXTAS pres

Hashimoto et al 2010 Brain
Progressive CNS dysfunction

Neural cell dysfunction

Additional (genetic) factors

Environmental (toxic) insults

Additional medical problems (e.g., surgery, diabetes)

Non-degenerative degenerative

FXTAS

Progressive CNS dysfunction

Also highlights possible approaches for intervention
CNS and PNS Distribution of the Intranuclear Inclusions in FXTAS

Strong parallels in human and mouse

Broad distribution throughout brain and spinal cord
- in brain, exclusively in nuclei of neurons and astrocytes

Also found in numerous peripheral tissues
- ganglion cells of adrenal medulla
- dorsal root ganglia
- paraspinal sympathetic ganglia
- myenteric ganglia of the stomach/intestine
- subepicardial autonomic ganglia of the heart
- testicular (Leydig) cells
- anterior and posterior pituitary
- thyroid
- ovarian stromal cells

Greco et al., 2002 Brain; Willemsen et al., 2003 Hum Mol Genet; Greco et al., 2006 Brain
Greco et al., 2007 J Urology; Brouwer et al., 2008 Psychoneuroendocrinology
Godken et al., 2009 Neuropathology; Hunsaker et al., 2011
Features of Premutation Involvement

*Means significantly higher in FXTAS vs controls

Many problems start far earlier such as hypertension, anxiety, depression, fibromyalgia, hypothyroidism, hearing loss

<table>
<thead>
<tr>
<th>Features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autonomic problems</td>
<td></td>
</tr>
<tr>
<td>Erectile dysfunction</td>
<td>Greco et al (2007)</td>
</tr>
<tr>
<td>Orthostatic hypotension</td>
<td>Leehey et al (2007)</td>
</tr>
<tr>
<td>Cardiac arrhythmia</td>
<td>Leehey et al (2011)</td>
</tr>
<tr>
<td>Dizzy spells or vertigo</td>
<td>Leehey et al (2011)</td>
</tr>
<tr>
<td>Sensory</td>
<td></td>
</tr>
<tr>
<td>Olfactory dysfunction</td>
<td>Juncos et al (2012)</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>Juncos et al (2011)</td>
</tr>
<tr>
<td>Sleep problems</td>
<td></td>
</tr>
<tr>
<td>Insomnia</td>
<td>Chonchaiya et al (2010)</td>
</tr>
<tr>
<td>Sleep apnea</td>
<td>Hamlin et al (2011)</td>
</tr>
<tr>
<td>Daytime sleeping</td>
<td>Hamlin et al (2011)</td>
</tr>
<tr>
<td>Motor symptoms</td>
<td></td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>Leehey et al (2007)</td>
</tr>
<tr>
<td>Psychiatric</td>
<td></td>
</tr>
<tr>
<td>Depression, ; anxiety</td>
<td>Bourgeois et al (2011); Seritan et al (2013)</td>
</tr>
<tr>
<td>Chronic pain</td>
<td></td>
</tr>
<tr>
<td>Neuropathic pain</td>
<td></td>
</tr>
<tr>
<td>Immune-mediated disorder</td>
<td></td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Established and expanded features of FXTAS

Hagerman and Hagerman 2013 Lancet Neurology; Wheeler et al 2014 JND
Premutation Involvement across the lifespan

- Infancy
- Childhood
- Young Adulthood
- Middle aged
- Elderly

- Executive Dysfunction-ADHD
- Autism Spectrum
- FXPOI
- FXTAS
- Psychopathology
- Fibromyalgia/Hypothyroidism
- Hypertension/Migraines/Autoimmune
- Perceptual Aberration
- Autism Spectrum
- FXPOI
- Psychopathology
- Fibromyalgia/Hypothyroidism
- Hypertension/Migraines/Autoimmune
- Perceptual Aberration
Treatment of FXTAS

• Seritan et al 2014 J Clinical Psychiatry: Controlled trial of memantine was not helpful for tremor, ataxia or executive function deficits in patients with FXTAS

• Subgroup of FXTAS patients underwent event related potential (ERP) studies (n=41) and significant benefits in cued recall memory and N400 repetition effects were seen with memantine compared to placebo (J-C Yang et al 2014 Neuropsychopharmacology)
Treatments for premutation carriers

- SSRIs
- Mitochondrial protection
- Antioxidants
- Treat high blood pressure, obesity, diabetes type 2
- Treat anxiety, depression
- Treat hypothyroidism
- Avoid substance abuse (alcohol, smoking, marijuana, opioids)
- Avoid general anesthesia
- Meditation
- Biofeedback for stress reduction

- Exercise
- Treat vitamin deficiency
- Boost folate & vitamin B12
- Treat sleep apnea
- Treat immune dysfunction
- Immune boosters, probiotics
- Avoid pesticides, high mercury in fish, Bisphenol A (BPA)

- Reduce oxidative stress
- Alleviate factors that lead to CNS deterioration
- Avoid toxins if possible
- Cognitive Stimulation / Decrease Stress

Polussa et al 2014
Brain Disorders and Therapy
Health Maintenance—perhaps prevention

• Check your blood pressure with every physician visit, at least yearly and more frequently as you age; treat hypertension; thyroid disease, sleep apnea, migraines

• Avoid toxins (excessive ETOH, opioids, smoking, methamphetamine, general anesthesia)

• Treat depression, anxiety with meds &/or counseling and exercise daily

• Take a multiple vitamin and get vit D, folate and B12 levels checked. Deficiency is common

• Take antioxidants: Vit C,E, NAC, omega 3s, berries etc. Folate and B12 supplementation slows brain atrophy with age by lowering homocysteine

• Avoid weight gain, metabolic syndrome and type 2 diabetes.
Exercise Benefits

- Exercise stimulates neurogenesis in the hippocampus (Speisman 2013)
- Exercise increases the size of the hippocampus, improves memory and boosts BDNF levels in hippocampus (Erickson et al 2011)
- Exercise blunts the HPA axis response to stress (Traudstadottir et al 2005)
- Exercise improves inflammation, depression, neuroimmune dysfunction and gliosis (Eyre et al 2012; Shanely et al 2013)
- Exercise stimulates telomerase activity so lengthening of telomeres (Wolf et al 2011) that are shortened in carriers (Jenkins et al 2012)
- Exercise improves mitochondrial function and biogenesis in CNS and muscle (Little et al 2010)
- Exercise reduces vascular endothelial oxidative stress (Pierce et al 2011)
Treatment of stress and emotional problems such as anxiety or depression

- Trial of SSRI or SNRI
- Duloxetine (Cymbalta) for pain symptoms
- Counseling or psychotherapy
- Biofeedback programs that are computerized
- Jogging or another exercise regimen
- Massage therapy
- Mindfulness meditation
- yoga
Yoga and Mindfulness Meditation improves GABA inhibition
Allopregnanolone: a natural neurosteroid

GABAA agonist

- **AlloP** is being studied in traumatic brain injury and in Alzheimer’s Disease (Brinton et al 2013)
- **AlloP** reduced spike frequency and duration in premutation neurons (Cao et al 2012 HMG)
- Stimulates neurogenesis
- open label trial in FXTAS approved by FDA for 12 weeks
Conclusions

- There can be lifelong involvement of premutation carriers but many of the changes (i.e. MRI) are subclinical
- Mitochondrial dysfunction and oxidative stress are key biochemical features of premutation involvement
- Genetic and environmental and lifestyle factors are important for CNS involvement and can be modified with clinical and treatment recommendations
- The brain is dynamic and can respond to new lifestyle changes and stimulation
Collaborators

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Elizabeth Berry-Kravis  Deb Hall  Christopher Goetz

Waisman Center-University of Wisconsin
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*Latrobe University, Melbourne Australia*
Danuta Loesch  Richard Huggins

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