Overview of Premutation Disorders

Fragile X Association of Germany 10-1-16

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MIND Institute

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Conflicts: Ovid, Zynerba
Two different mutations in the same *FMR1* gene

1/130-250 females
1/250-810 males
1/3600-5000

Typical

(CGG) < 45

Premutation

(CGG) 55 - 200

Full mutation

(CGG) > 200

mRNA

FMRP

Clinical normal

Primary Ovarian Insufficiency (FXPOI)
Fragile X-associated
Tremor Ataxia Syndrome (FXTAS)
Depression and anxiety
ADHD, OCD, seizures and ASD

Fragile X syndrome (FXS)
Age- and generational involvement in fragile X-associated disorders
Spectrum of Premutation Involvement

**Cellular dysregulation**
- Upregulation of heatshock proteins
- ASFMR1 splice isoforms in FXTAS
- Kinase activation
- Sequestration of DROSHA, DGCR8, Sam68
- Mitochondrial dysfunction
- Inclusion formation
- FMRpolyG

**Background gene effects**

**Environmental effects**

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

**Psychiatric involvement**
- Anxiety
- Stress
- Depression

**Endocrine dysfunction**
- FXPOI

**Immune dysregulation**
- Hypothyroidism
- Fibromyalgia
- Lupus-MS features

**Neurological problems**
- Neuropathy-chronic pain
- Migraine, sleep apnea, RLS
- Memory problems, foggy thinking
- Hypertension, erectile dysfunction

**FXTAS**
- Tremor, ataxia, Parkinsonism
- Autonomic dysfunction, EF deficits, memory and cognitive decline

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

- Neurodevelopmental problems
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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, vertigo, tinnitus, insomnia

<table>
<thead>
<tr>
<th>Table 2: Established and expanded features of FXTAS</th>
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<tr>
<td><strong>Autonomic problems</strong></td>
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<tr>
<td>Constipation/irritable bowel syndrome</td>
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<td>Erectile dysfunction</td>
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<td>Problems swallowing</td>
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<tr>
<td>Gastrointestinal reflux</td>
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<tr>
<td>Orthostatic hypotension</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Urinary urgency &amp; incontinence</td>
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<tr>
<td>Cardiac arrhythmia</td>
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<td>Dizzy spells or vertigo</td>
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<tr>
<td><strong>Sensory</strong></td>
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<td>Offactory dysfunction</td>
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<td>Hearing loss</td>
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<td>Neuropathy</td>
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<td><strong>Sleep problems</strong></td>
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<td>Insomnia</td>
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<tr>
<td>Sleep apnea</td>
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<tr>
<td>Daytime sleeping</td>
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<tr>
<td><strong>Motor symptoms</strong></td>
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<tr>
<td>Tremors; ataxia;</td>
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<td>Muscle weakness</td>
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<td>Parkinsonism</td>
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<tr>
<td><strong>Psychiatric</strong></td>
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<tr>
<td>Depression; anxiety;</td>
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<tr>
<td>Irritability</td>
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<tr>
<td><strong>Chronic pain</strong></td>
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<tr>
<td>Fibromyalgia</td>
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<tr>
<td>Neuropathic pain</td>
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<td><strong>Immune-mediated disorder</strong></td>
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<tr>
<td>Hypothyroidism</td>
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<tr>
<td>Fibromyalgia</td>
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Hagerman and Hagerman 2013 Lancet Neurology; Wheeler et al 2014 JND
Fragile X-associated Primary Ovarian Insufficiency (FXPOI) in 16-20%
Enhanced cell death in premutation neurons

Oxidative stress
Mitochondrial dysfunction
Kaplan et al 2012

Decreased cell survival by 21 days

Chen et al 2009 HMG
Molecular Mechanisms of Toxicity in Premutation Carriers

A. Protein sequestration

- (CGG)_{55-200}
- DROSHA:DGCR8 - Aberrant miRNA
- Purα - Stress and Immune effects
- SAM68 - abnormal RNA splicing, apoptosis & signal transduction
- hnRNP A2/B1 - loss of RNA chaperone function, mislocalization of BC1 RNA, etc.

B. Repeat-associated non-AUG (RAN) translation

- (CGG)_{55-200}
- FMRP ORF
- GUG 5' UTR
- AUG
- generation of toxic proteins
- polyG-containing protein
- polyA-containing protein

Berman et al 2014 JND
Mechanisms of premutation involvement

- DNA damage response
- Transient creation and dissolution of R-loops
- FMR1 transcription
- Protein sequestration
- Mitochondrial dysfunction
- Chronic DNA damage repair
- Sequestration
- FMRpolyG
- FMRP
Expanded CGG repeat inhibits DGCR8/DROSHA activity

Observed decreases in miRNA levels

Predicted decrease in miRNA levels

Sellier C ... Charlet et al 2013
Other symptoms

- Cardiac arrhythmia; bradycardia, pacemaker
- Back pain and disc problems: perhaps connective tissue changes may relate
- Foggy thinking memory problems
- Workup for Lupus ANA+ comes and goes
- Internal tremor
- Non-epileptic seizures
- Pulmonary fibrosis
- Myelodysplasia
- CHF and Kidney failure may be late changes
Inclusions first reported by Greco et al. 2002, 2006 in neurons and astrocytes and in other tissues (Hunsaker et al. 2011) and in Purkinje cells (Ariza et al. 2016)
Broad distribution of intranuclear inclusions in FXTAS

in brain, exclusively in nuclei of neurons and astrocytes
Also present in numerous peripheral tissues
- anterior and posterior pituitary
- pancreas, adrenal
- thyroid, kidney, heart
- dorsal root ganglia
- paraspinal sympathetic ganglia
- subepicardial autonomic ganglia of the heart
- ganglion cells of adrenal medulla
- myenteric ganglia of the stomach/intestine
- ovarian stromal cells
- testicular (Leydig) 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 Acta Pathologica
Progressive CNS dysfunction

Non-degenerative degenerative

Additional (genetic) factors  Environmental (toxic) insults  Additional medical problems (e.g., surgery, diabetes, hypoxia)

Neural cell dysfunction

Progressive CNS dysfunction

Also highlights possible approaches for intervention
Changes in cerebellum and brain stem in controls and carriers

A A 68-year-old control

B A 69-year-old at stage 1

C A 68-year-old at Stage 4

322 males (age 8-81 years), 142 controls, 109 asymptomatic carriers, 72 FXTAS+ carriers

Jun Yi Wang et al 2016 in review
Cerebellar Volume

Green = controls
Blue = carriers
Purple = FXTAS

Jun Yi Wang et al 2016
Brainstem Volume

Green = controls
Blue = carriers
Purple = FXTAS

Jun Yi Wang et al 2016
Ventricular Volume

Ventricle volume (ml) vs Age

Ventricle volume (ml) vs Age

Ventricle volume (ml) vs Age
Anxiety and Hippocampal Volumes in Females with the Premutation

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

Adams et al 2009
Health Maintenance

• Check your blood pressure regularly, CBC and thyroid function yearly; treat hypertension; treat thyroid disease, anemia or hypoxia w/sleep apnea

• Treat migraines, avoid toxins in environment

• Treat depression, anxiety with SSRIs &/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,D,E, NAC, omega 3s berries, green tea etc. Folate and B12 supplementation slows brain atrophy with age, lowers homocysteine

• Avoid weight gain, metabolic syndrome and type 2 diabetes.
Treatments for premutation carriers

SSRIs
Mitochondrial protection
Antioxidants
Reduce oxidative stress
Treat vitamin deficiency
Boost folate & vitamin B12
Treat sleep apnea
Treat immune dysfunction
Immune boosters, probiotics
Avoid substance abuse (alcohol, smoking, marijuana, opioids)
Avoid general anesthesia
Avoid toxins if possible
Avoid pesticides, high mercury in fish, Bisphenol A (BPA)
Cognitive Stimulation / Decrease Stress
Meditation
Biofeedback for stress reduction
Cognitive training
Polussa et al 2014
Brain Disorders and Therapy
Antioxidants

Lipid Peroxyl Radical Scavengers

- Tirilazad
- Melatonin
- Resveratrol
- Lipoic Acid
- Curcumin
- OPC-14117
Where is the delineation between FXTAS and other premutation problems?

- The fragile X-associated tremor ataxia syndrome (FXTAS) was defined in 2003 before we knew about many other problems.
- It has a phenotype well entrenched with minor changes to definition such as adding neuropathy and wmd in splenium of the corpus callosum.
- However, there are life long changes to the CNS that start in early adulthood.
### Diagnostic Criteria for FXTAS

*put together with what we knew in 2003*

**Inclusion criterion:** 55 – 200 CGG repeats

<table>
<thead>
<tr>
<th>MRI</th>
<th>major</th>
<th>Middle cerebellar peduncle (MCP) lesions</th>
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<tbody>
<tr>
<td></td>
<td>minor</td>
<td>Cerebral white matter hyperintensity</td>
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<tr>
<td>Clinical exam</td>
<td>minor</td>
<td>Moderate to severe generalized atrophy</td>
</tr>
<tr>
<td>Clinical exam</td>
<td>major</td>
<td>Intention tremor</td>
</tr>
<tr>
<td>Clinical exam</td>
<td>major</td>
<td>Gait ataxia</td>
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<tr>
<td></td>
<td>minor</td>
<td>Parkinsonism</td>
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<td></td>
<td>minor</td>
<td>Short term memory deficits</td>
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<tr>
<td></td>
<td>minor</td>
<td>Executive function deficits</td>
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</tbody>
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### Diagnostic categories

<table>
<thead>
<tr>
<th>Definite</th>
<th>Probable</th>
<th>Possible</th>
</tr>
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<tbody>
<tr>
<td>1 clinical <strong>major</strong> AND 1 MRI <strong>major</strong></td>
<td>2 clinical <strong>major</strong> OR 1 MRI <strong>major</strong> AND 1 clinical minor</td>
<td>1 clinical <strong>major</strong> AND 1 MRI minor</td>
</tr>
</tbody>
</table>

Inclusions *(post mortem)*

Jacquemont et al 2003 AJHG
A different course in women with FXTAS

Women with FXTAS were not described until 2004 (Hagerman et al 2004 AJHG) and only 13% have the MCP

Findings on MRI studies (Adams et al 2007), less dementia (Seritan et al 2008, 2016)

Inclusions reported in 2002 (Greco et al 2002, 2006)
Other MRI Findings in FXTAS

- White matter disease in the pons
- Thinning of the corpus callosum and wmd in splenium
- Involvement of the insula
Insula Sign in some premutation carriers with FXTAS

early involvement of insula may influence pain perception
FXTAS in unmethylated full mutation carrier

- Loesch et al 2011 Clin Genet: 65yo truck driver (240-480 repeats) with history of alcoholism and smoker 30 yrs; age 54 heavy daily ETOH and disorientation episodes; 58y cholesteectomy and subsequent tremor and ataxia; aggression; hallucinations; urinary incontinence

- Additional cases now reported Pretto et al 2013, Santa Maria et al 2014.
Mild symptoms should be differentiated from FXTAS

Most of the symptoms of premutation carriers are secondary to changes in the brain related to low level RNA toxicity influenced by background genetic effects and environmental influences ie depression, anxiety, tingling, migraines, mood instability etc. This is not FXTAS

FXTAS is a quantum leap in neuronal problems ie neurodegeneration associated with white matter disease and more brain atrophy and it can progress faster when combined with alzheimers, Parkinson’s disease, LBD multiple sclerosis
Life course of aging in carriers

Foggy thinking, poor memory, numbness, tingling, intermittent tremor, balance problems, dizzy episodes - NOT FXTAS

FXTAS
Neurodegeneration
Rapid course often combined with Alzheimers, LBD or other

Mild neurological symptoms
FXTAS mild course

Normal aging
100th birthday with FXTAS
Treatment of premutation problems including FXTAS

- Seritan et al 2014 J Clin 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 treatment benefits in cued recall memory and N400 repetition effects were seen compared to placebo
Allopregnanolone: a natural neurosteroid GABA<sub>A</sub> 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, and neuroregenerative
  Open label trial (12 weeks) in FXTAS approved by FDA
Allopregnanolone

- Endogenous neurosteroid
- Produced by cortical and hippocampal pyramidal neurons
- Positive allosteric modulator
- GABA(A) Receptor
- Enhance inhibitory effect of GABA
- Neuroprotection (prevention of apoptosis)
- Neurogenesis
Cognitive improvements with Allopregnanolone treatment

- BDS-2 measures executive function, working memory
- Improvement in scores in all patients
- Frontal cortex involved

- Majority show improvements in scores post infusion
- No change in JN’s scores; SG slight decrease
- Compare against a control for natural progression of disease
Collaborators

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- Chris Iwahashi
- Anna Ludwig
- Dolores Garcia-Arocena
- Greg Mayeur
- Chris Raske

Dept. Biostatistics
- Danh Nguyen

University of Washington and UC Davis Fragile X Research Center

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