NFX Foundation talk on Premutation Disorders and Conditions

Randi Hagerman MD
Professor and Endowed Chair in Fragile X Research
Medical Director MIND Institute
University of California at Davis Medical Center

Funding: NICHD, Azrieli Foundation, Zynerba, Anavex Life Sciences and NeuroNext

Disclosures: Consulting with Zynerba for Fragile X trials
Triumph of good over evil
be kind to your fellow man
2000 a seminal year

2000 was the year that Dr Flora Tassone in Paul’s lab discovered and reported the elevated $FMR1$ mRNA in premutation carriers (Tassone et al 2000 AJHG)

2000 was also the year that the NFXF international conference was in LA and we presented the first 5 carriers with tremor and ataxia later named FXTAS (Hagerman et al 2001 Neurology; Jacquemont et al 2003 AJHG and Jacquemont et al 2004 JAMA)

2000 was the year we moved to the MIND to push new treatments for ND disorders carrying a brain (Greco et al 2002)
Two different mutations in the same \textit{FMR1} gene

\begin{itemize}
\item Typical (CGG) $< 45$
\item Premutation (CGG) $55 - 200$
\item Full mutation (CGG) $> 200$
\end{itemize}

- mRNA
- FMRP
- Clinical: normal

\begin{itemize}
\item Primary Ovarian Insufficiency (FXPOI)
\item Fragile X-associated Tremor Ataxia Syndrome (FXTAS)
\item FX-associated Neuropsychiatric Disorders (FXAND or FXPAC): Depression, anxiety, ADHD, ASD, OCD, chronic fatigue, chronic pain
\end{itemize}

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

Fragile X syndrome (FXS)
Expression of the *FMR1* gene

![Graph showing the expression of the FMR1 gene with CGG repeat number on the x-axis and Relative FMR1 mRNA level and FMRP level on the y-axis.](image)

- Normal
- Gray
- Premutation
- Full mutation

- Unmethylated
- Partially methylated
- Hyper-methylated

**FXTAS, FXPOI**

**FXS**
Fragile X Disorders

- Fragile X syndrome (FXS) Julia Bell first X linked Pedigree 1949 and then Lubs 1969.
- Fragile X-associated tremor ataxia syndrome (FXTAS) (Hagerman et al 2001)
- Fragile X-associated primary ovarian insufficiency (FXPOI) (Cronister et al 1991)
- Fragile X-associated Neuropsychiatric Disorders: (FXAND or FXPAC) (Hagerman et al 2018) The list of significant involvement from the premutation is longer and if named then more research will take place: Also called FXPAC-fragile X-associated Conditions
  - In adulthood: psychiatric disorders including depression, anxiety, OCD, chronic fatigue, chronic pain, insomnia, fibromyalgia
Fragile X-associated Tremor/Ataxia syndrome (FXTAS)

• Most severe clinical problem in premutation carriers caused by the \textit{FMR1} gene and consequences of elevated mRNA

• Tremor, ataxia, neuropathy, cognitive deficits, progressive and severe end of spectrum

• Affecting approximately 40\% of males and 13 to 16\% of females and more frequent with age (Jacquemont et al 2004 JAMA)

• Described in 2001 (Hagerman et al Neurology)

• Named in 2003 (Jacquemont et al AJHG)
FXTAS - Males

- 40% overall rate of FXTAS over 50 but it increases with age
- Tremor, ataxia, cane, walker, wheelchair, bed ridden
- Disinhibition, mood lability, aggressiveness, sleep apnea
- Emotional difficulty in dealing with loss of function (Bacalman et al 2009)
  - Need for cane
  - No longer able to drive
  - No longer able to work
  - Inability to maintain cognitive level
- Possible concern re: surgery/anesthesia
- Recommendations
  - Vit B-12, D, E. folate, Omega 3, antioxidants, exercise
FXTAS - Females

- Female (8-16% risk)
  - May present as in males sometimes head tremor first
  - Increased immune related complaints (Coffey et al. 2008)
    - Thyroid, fibromyalgia, muscle pain, neuropathy, arthritis, central pain syndrome, chronic fatigue
  - Psychiatric problems including depression and anxiety common therefore named FX-associated Neuropsychiatric Disorders (FXAND)
  - Complaints often dismissed
  - May be considered to be hypochondriacs or exaggerating symptoms
Women have more pain with FXTAS:
Females vs males with FXTAS: 104 patients (41 females vs 63 males) (Johnson et al 2021 in press)

- women experience significantly more pain symptoms than men, particularly allodynia (20% vs. 2.0%, p=0.008), peripheral neuropathy pain (43.9% vs. 25.4%, p=0.0488), migraines (43.9% vs. 14.5%, p=0.0008), fibromyalgia (26.8% vs. 0%, p=0.0071) and back pain (48.5% vs. 23.4%, p=0.008).
- onset of peripheral neuropathy predicts the onset of ataxia (β=0.63±0.25, p=0.019) and tremor (β= 0.56±0.17, p=0.004) across gender.
- Women also report significant more anxiety (82.9% vs. 39.7%, p<0.001), which has implications for ideal pain treatment.
- depressive symptoms were present in a quarter of participants (24.4% female vs. 25.8% male)
- Females with FXTAS are significantly more likely to be taking any pain medication (58.54% females vs. 36.51% males, p=0.0271) as well as nerve pain medication (31.71% females vs. 14.29% males, p=0.331)
- FXTAS women are more likely to have thyroid problems (34.2% vs. 14.5%, p=0.0182).
Diagnostic Criteria for FXTAS updated

*Inclusion criterion:* 55 – 200 CGG repeats

<table>
<thead>
<tr>
<th>MRI</th>
<th>major</th>
<th><em>Middle cerebellar peduncle (MCP) lesions</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>minor</td>
<td>Cerebral white matter hyperintensity</td>
</tr>
<tr>
<td></td>
<td>minor</td>
<td>Moderate to severe generalized atrophy</td>
</tr>
<tr>
<td></td>
<td>minor</td>
<td>WMH in Splenium</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical exam</th>
<th>major</th>
<th><em>Intention tremor</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>major</td>
<td><em>Gait ataxia</em></td>
</tr>
<tr>
<td></td>
<td>minor</td>
<td>Parkinsonism</td>
</tr>
<tr>
<td></td>
<td>minor</td>
<td>Short term memory deficits</td>
</tr>
<tr>
<td></td>
<td>minor</td>
<td>Executive function deficits</td>
</tr>
<tr>
<td></td>
<td>minor</td>
<td>Neuropathy</td>
</tr>
</tbody>
</table>

**Diagnostic categories**

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

Inclusions (*post mortem*)
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 (Adams et al 2007), less dementia (Seritan et al 2008, 2016)

Inclusions reported in 2002 (Greco et al 2002, 2006)
-Inclusions first reported in women in the grandmother of Lorraine Ruiz RN, who died of FXTAS
Broad distribution of intranuclear inclusions in FXTAS (Hunsaker et al 2011)

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
Intranuclear inclusions
Neurons – Astrocytes in humans

Greco et al
2002, 2006 Brain

inclusions in anterior and posterior
RNA toxicity

Clogged proteosomes

- Normal nuclear proteasomal processing
- Ub-proteins for degradation
- Nuclear proteasome

A cellular stress response results in an increased load of damaged/oxidized proteins that exceeds the capacity of the nuclear proteasomal machinery, leading to macroscopic aggregate formation.

FXTAS nuclear proteasomal processing

- Excess Ub-proteins for degradation due to oxidative stress, increased DDR, etc.

FMRP and IQ (Kim et al 2019)

FSIQ

Holm et al 2021
LC MS/MS for composition of the FXTAS inclusions

More focused analysis of the highest-abundance inclusion-enriched proteins, those proteins which make up at least 0.5% of a sorted inclusion sample and those which were enriched by at least 50% were considered further

Ma et al. (2019)

<table>
<thead>
<tr>
<th>Proteins</th>
<th>FXTAS A Inclusions</th>
<th>FXTAS B Inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Over FXTAS A nuclear</td>
<td>Over control nuclear</td>
</tr>
<tr>
<td>Small ubiquitin-related modifier 2 (SUMO2)</td>
<td>5.5 (0.60/0.11)</td>
<td>60.0 (0.60/0.01)</td>
</tr>
<tr>
<td>p62/ SQSTM1</td>
<td>8.0 (0.08/0.01)</td>
<td>40.0 (0.08/0.002)</td>
</tr>
<tr>
<td>Myeloid leukemia factor 2 (MLF2)</td>
<td>9.3 (0.28/0.03)</td>
<td>93.3 (0.28/0.003)</td>
</tr>
<tr>
<td>Ubiquitin (RS27A)</td>
<td>3.6 (0.65/0.18)</td>
<td>5.9 (0.65/0.11)</td>
</tr>
<tr>
<td>Myelin basic protein (MBP)</td>
<td>3.5 (0.46/0.13)</td>
<td>1.2 (0.46/0.38)</td>
</tr>
<tr>
<td>Heat shock protein HSP 90-alpha (HSP90AA1)</td>
<td>1.6 (0.08/0.05)</td>
<td>4.0 (0.08/0.02)</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein L (HNRNPL)</td>
<td>1.9 (0.13/0.07)</td>
<td>6.5 (0.13/0.02)</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1)</td>
<td>1.7 (1.12/0.65)</td>
<td>2.7 (1.12/0.41)</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein A3 (HNRNPA3)</td>
<td>1.8 (0.95/0.54)</td>
<td>2.6 (0.95/0.36)</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoproteins C1/C2 (HNRNPC)</td>
<td>1.9 (0.28/0.15)</td>
<td>1.3 (0.28/0.22)</td>
</tr>
<tr>
<td>Beta-actin (ACTB)</td>
<td>2.2 (0.75/0.34)</td>
<td>1.2 (0.75/0.61)</td>
</tr>
<tr>
<td>Alpha-crystallin B chain (CRYAB)</td>
<td>1.9 (1.29/0.68)</td>
<td>1.1 (1.29/1.22)</td>
</tr>
<tr>
<td>Tubulin alpha-1B chain (TBA1B)</td>
<td>1.6 (1.01/0.63)</td>
<td>1.0 (1.01/0.98)</td>
</tr>
<tr>
<td>Tubulin beta-2A chain (TBB2A)</td>
<td>1.7 (0.64/0.37)</td>
<td>0.9 (0.64/0.71)</td>
</tr>
<tr>
<td>Calcium/calmodulin-dependent protein kinase type II subunit alpha (CAMK2A)</td>
<td>1.7 (0.43/0.25)</td>
<td>0.7 (0.43/0.61)</td>
</tr>
</tbody>
</table>
Other MRI Findings in FXTAS

- White matter disease in the pons
- Thinning of the corpus callosum and wmd in splenium
- Involvement of the insula

WMD and cognitive and motor deficits (Hocking et al 2019)

- 30 PM males 50 to 81 years and 17 with FXTAS
- UPDRS correlated best with infratentorial WMD
- Total WMD correlated with both motor (UPDRS, ICARS) and cognitive scores
- IQ, similarities, matrix reas, SDMT correlates with infratentorial WMD but Digit Span correlated with total WMD
Brainstem Volume Change: NC Vs. PNF (Wang et al 2017)

**Quadratic relationship**
PNF: \(-0.19 \pm 0.67 \text{ ml}, t = -0.29, p = 0.77\)
Age x group: \(-0.048 \pm 0.017 \text{ ml}, t = -2.82, p = 0.005\)

**Annual rate of change**
NC: \(-0.001 \times \text{age}^2 + 0.091 \times \text{age}\)
PNF: \(-0.001 \times \text{age} - 0.044 \times \text{age}\)

**Age of divergence in volume:** 4.1 years

**Linear relationship**
NC: \(4.13 \pm 1.10 \text{ ml}, t = 3.74, p = 0.0003\)
Age x NC: \(0.16 \pm 0.06 \text{ ml}, t = 2.4, p = 0.016\)
Age x PNF: \(0.31 \pm 0.08 \text{ ml}, t = 3.8, p = 0.0002\)

**Annual rate of change**
NC: \(-0.07 \pm 0.06 \text{ ml}\); PNF: \(0.08 \pm 0.08 \text{ ml}\)
PWF: \(-0.23 \pm 0.05 \text{ ml}\)

**PWF vs. PNF:** difference in volume occurs after age 50
Cerebellar Volume Change Begins in Childhood (Wang et al 2017)

Linear relationship
PNF: $-0.24 \pm 2.53$ ml, $t = -0.10, p = 0.92$
Age x group: $-0.14 \pm 0.06$ ml, $t = -2.24, p = 0.026$

Annual rate of change
NC: $-0.36 \pm 0.04$ ml
PNF: $-0.50 \pm 0.06$ ml

Age of divergence in volume: 6.4 years
Amazing discoveries by Jun Yi Wang every year (>13 papers/8 yrs)

- The brain changes in premutation carriers start in childhood
- The enlarged ventricles in FXTAS distort brain structure
- Hypergyrification and hypogyrification in pres (boys on left)
- The eye of the tiger sign in FXTAS
Veronica Martínez-Cerdeño is the Director of the FXS and FXTAS Brain Bank

- Maria Jimena Salcedo-Arellano and Bella McLennan are working with Veronica and making wonderful discoveries along with their team.
The Association of PD and Parkinsonism with FXTAS (Salcedo-Arellano et al 2020)

- Study of 40 patients with FXTAS who donated their brains to us. 7 dx with PD or parkinsonism and all 7 with dopaminergic neuronal cell loss in substantia nigra.

- 2 of 7 with Lewy bodies but 2 more without PD symptoms had Lewy bodies so 10% (4/40) total
Iron deposition within the putamen in FXTAS

Transport of iron into the brain is altered in FXTAS

Increased iron deposition in neuronal and glial cells in the putamen in FXTAS

Decrease in the amount of the iron-binding proteins transferrin and ceruloplasmin, and decreased number of neurons and glial cells that contained ceruloplasmin.

However, increased levels of iron, transferrin, and ceruloplasmin in microglial cells, indicating an attempt by the immune system to remove the excess iron.
53 female carriers with FXTAS vs 55 controls (Schneider et al 2020)

- Mean age 66.9 years; MCP sign in only 6 (9.1%); and 0% in controls
- Splenium sign is 61.5% vs 3.2% controls
- WMD in pons 30.8% vs 4.7% of controls
- Diffuse cerebral WMD in 35% vs 8% controls
- Higher CGG repeat, earlier onset of FXTAS same as Leehey et al 2008 and Tassone et al 2007
Splenium hyperintensity and cortical atrophy are most common MRI findings in females with FXTAS
(Schneider et al 2020)
Relationship of CGG repeat size and age of tremor onset (Schneider et al. 2020)
Spectrum of Premutation Involvement

**Cellular dysregulation**
- Calcium dysregulation
- Upregulation of heatshock proteins;
- RAN (repeat associated non AUG) translation, FMRpolyG
- Sequestration of DROSHA, DGCR8, Sam68
- Inclusion formation, WMD
- Mitochondrial dysfunction

**Environmental effects**
- Including
  - Alcoholism
  - Opioids
  - Chemotherapy
  - Toxins
  - Smoking
  - Stroke
  - CTE
  - Iron deposition

**Background gene effects**

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

**Psychiatric involvement (FXAND)**
- Anxiety
- Stress
- Depression

**Endocrine dysfunction**
- FXPOI

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

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

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

*Drs Guilivi and Ele Napoli and team have done the mitochondrial work*
**FXAND:** Depression and Anxiety can worsen with age in women with the premutation

<table>
<thead>
<tr>
<th>Lifetime DSM-IV Disorder</th>
<th>FMR1 Time 1 [subset of Roberts et al. (3)], % (n)</th>
<th>FMR1 Time 2, % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mood Disorders</td>
<td>51.81 (43)</td>
<td>60.24 (50)</td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>45.78 (38)</td>
<td>54.22 (45)</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>1.20 (1)</td>
<td>1.20 (1)</td>
</tr>
<tr>
<td>Bipolar disorder I or II</td>
<td>4.82 (4)</td>
<td>4.82 (4)</td>
</tr>
<tr>
<td>Anxiety Disorders</td>
<td>27.71 (23)</td>
<td>34.94 (29)</td>
</tr>
<tr>
<td>Panic disorder with agoraphobia</td>
<td>3.61 (3)</td>
<td>6.02 (5)</td>
</tr>
<tr>
<td>Panic disorder without agoraphobia</td>
<td>7.23 (6)</td>
<td>8.43 (7)</td>
</tr>
<tr>
<td>Agoraphobia without panic</td>
<td>3.61 (3)</td>
<td>3.61 (3)</td>
</tr>
<tr>
<td>Social phobia</td>
<td>8.43 (7)</td>
<td>9.64 (9)</td>
</tr>
<tr>
<td>Posttraumatic stress disorder</td>
<td>4.82 (4)</td>
<td>6.02 (5)</td>
</tr>
<tr>
<td>Specific phobia</td>
<td>4.82 (4)</td>
<td>7.23 (6)</td>
</tr>
<tr>
<td>Generalized anxiety disorder</td>
<td>6.02 (5)</td>
<td>10.84 (9)</td>
</tr>
<tr>
<td>Any Mood or Anxiety Disorder</td>
<td>59.04 (49)</td>
<td>66.27 (55)</td>
</tr>
</tbody>
</table>

NCS-R, National Comorbidity Survey Replication; SCID-I, Structured Clinical Interview for DSM-IV Axis I Disorders

Roberts et al 2016 Biological Psychiatry
Anxiety and Hippocampal Volumes in Females with the Premutation

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

Adams et al 2009
Marshfield study validates FXAND

Movaghar et al 2019 Sci Adv
Symptoms endorsed by 355 pre-menopausal women with and without FXPOI (Allen et al 2020 Genet Med)

<table>
<thead>
<tr>
<th>Condition</th>
<th>All PM (N = 355)</th>
<th>FXPOI (N = 87)</th>
<th>No FXPOI (N = 168)</th>
<th>P values for models comparing FXPOI and no FXPOI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Total</td>
<td>% Option 1* /% option 2 ²</td>
<td>% Total</td>
<td>% Option 1* /% option 2 ²</td>
</tr>
<tr>
<td>Anxiety</td>
<td>37.8%</td>
<td>15.2% / 22.5%</td>
<td>44.8%</td>
<td>12.6% / 32.2%</td>
</tr>
<tr>
<td>Depression</td>
<td>35.5%</td>
<td>8.4%  / 27.0%</td>
<td>33.3%</td>
<td>2.3% / 31.0%</td>
</tr>
<tr>
<td>Migraine headaches</td>
<td>33.2%</td>
<td>12.4% / 20.8%</td>
<td>33.3%</td>
<td>9.2% / 24.1%</td>
</tr>
<tr>
<td>Tension headaches</td>
<td>31.5%</td>
<td>21.7% / 9.9%</td>
<td>31.0%</td>
<td>17.2% / 13.8%</td>
</tr>
<tr>
<td>Sleep problems</td>
<td>28.7%</td>
<td>20.8% / 7.9%</td>
<td>34.5%</td>
<td>26.4% / 8.0%</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>20.3%</td>
<td>14.7% / 5.6%</td>
<td>22.1%</td>
<td>16.3% / 5.8%</td>
</tr>
<tr>
<td>IBS</td>
<td>19.7%</td>
<td>8.2% / 11.5%</td>
<td>19.5%</td>
<td>6.9% / 12.6%</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>19.1%</td>
<td>1.4% / 17.7%</td>
<td>26.4%</td>
<td>0% / 26.4%</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>17.5%</td>
<td>3.1% / 14.4%</td>
<td>23.0%</td>
<td>2.3% / 20.7%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>16.9%</td>
<td>0.6% / 16.3%</td>
<td>10.3%</td>
<td>1.1% / 9.2%</td>
</tr>
<tr>
<td>RLS</td>
<td>15.2%</td>
<td>11.3% / 3.9%</td>
<td>12.6%</td>
<td>9.2% / 3.4%</td>
</tr>
<tr>
<td>ataxia</td>
<td>13.5%</td>
<td>9.9% / 3.7%</td>
<td>9.2%</td>
<td>5.7% / 3.4%</td>
</tr>
<tr>
<td>Sleep apnea</td>
<td>13.0%</td>
<td>5.6% / 7.3%</td>
<td>14.9%</td>
<td>6.9% / 8.0%</td>
</tr>
<tr>
<td>Chronic muscle pain</td>
<td>11.9%</td>
<td>7.3% / 4.5%</td>
<td>14.9%</td>
<td>5.7% / 9.2%</td>
</tr>
<tr>
<td>Social phobia</td>
<td>11.8%</td>
<td>10.7% / 1.1%</td>
<td>20.7%</td>
<td>18.4% / 2.3%</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>11.5%</td>
<td>4.5% / 7.0%</td>
<td>16.1%</td>
<td>3.4% / 12.6%</td>
</tr>
<tr>
<td>CFS</td>
<td>11.3%</td>
<td>9.0% / 2.2%</td>
<td>14.9%</td>
<td>11.5% / 3.4%</td>
</tr>
<tr>
<td>TMJ</td>
<td>11.3%</td>
<td>1.4% / 9.9%</td>
<td>16.0%</td>
<td>4.6% / 11.5%</td>
</tr>
<tr>
<td>OCD</td>
<td>10.7%</td>
<td>8.4% / 2.2%</td>
<td>10.3%</td>
<td>5.7% / 4.6%</td>
</tr>
<tr>
<td>ADHD</td>
<td>10.7%</td>
<td>7.6% / 3.1%</td>
<td>12.6%</td>
<td>10.3% / 2.3%</td>
</tr>
<tr>
<td>LD</td>
<td>10.4%</td>
<td>7.6% / 2.6%</td>
<td>6.9%</td>
<td>5.7% / 1.1%</td>
</tr>
<tr>
<td>Tremor</td>
<td>10.1%</td>
<td>7.0% / 3.1%</td>
<td>8.0%</td>
<td>5.7% / 2.3%</td>
</tr>
</tbody>
</table>

*Model 1: endorsement of option 1 or option 2 was used to define affected individuals. Model 2: endorsement of option 2 only is used to define affected individuals. Endorsement of option 1 is grouped with option 0 as the unaffected population.

†ADHD attention deficit/hyperactivity disorder, CFS chronic fatigue syndrome, IBS irritable bowel syndrome, LD learning disability, OCD obsessive compulsive disorder, RLS restless leg syndrome, TMJ temporomandibular joint dysfunction.

*% of subjects who selected “I think I have this but have not been diagnosed by a medical professional.”

*% of subjects who selected “I have been diagnosed with this by a medical professional.”

Bonferroni-adjusted statistical significance p < 0.002 are bolded. Marginally significant models where significance was between 0.001 and 0.05 are underlined.
Clustering of conditions in pre women

<table>
<thead>
<tr>
<th>Heatmap showing frequencies of reported conditions within each cluster.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minimal health problems</strong></td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>Anxiety</td>
</tr>
<tr>
<td>Depression</td>
</tr>
<tr>
<td>Migraine</td>
</tr>
<tr>
<td>Tension headache</td>
</tr>
<tr>
<td>Sleep problems</td>
</tr>
<tr>
<td>Neuropathy</td>
</tr>
<tr>
<td>IBS</td>
</tr>
<tr>
<td>Osteoporosis</td>
</tr>
<tr>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Restless leg syndrome</td>
</tr>
<tr>
<td>Ataxia</td>
</tr>
<tr>
<td>Sleep apnea</td>
</tr>
<tr>
<td>Chronic muscle pain</td>
</tr>
<tr>
<td>Social phobia</td>
</tr>
<tr>
<td>Fibromyalgia</td>
</tr>
<tr>
<td>Chronic fatigue syndrome</td>
</tr>
<tr>
<td>TMJ</td>
</tr>
<tr>
<td>OCD</td>
</tr>
<tr>
<td>ADHD</td>
</tr>
<tr>
<td>LD</td>
</tr>
<tr>
<td>Tremor</td>
</tr>
</tbody>
</table>

Allen et al 2020
Comorbid Conditions in Pre Women related to Age, Smoking, BMI, Depression or Anxiety (Allen et al 2021)

Graphical representation of significant (p < 0.0023; shown in red) and marginally significant (p < 0.05; shown in black) odds ratios for age at interview (A), smoking (B), BMI (C), Depression (D), and Anxiety (E) for each comorbid condition tested.
Women with depression or anxiety self report an increased number of comorbid conditions (Allen et al 2021)
Health Profiles in mosaic and non-mosaic premutation women

(Mailer et al 2018 Frontiers in Genetics)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-mosaic PM (n = 45)</th>
<th>PM mosaic (n = 41)</th>
<th>PM/FM mosaic (n = 14)</th>
<th>F-value/ Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.8 (7.2)</td>
<td>58.1 (7.3)</td>
<td>54.8 (5.8)</td>
<td>2.77*</td>
</tr>
<tr>
<td>Marital status (1 = currently married)</td>
<td>0.77</td>
<td>0.78</td>
<td>1.00</td>
<td>ns</td>
</tr>
<tr>
<td>Education (1 = some college or higher)</td>
<td>0.91</td>
<td>0.83</td>
<td>1.00</td>
<td>ns</td>
</tr>
<tr>
<td>Employment status (1 = working)</td>
<td>0.67</td>
<td>0.63</td>
<td>0.64</td>
<td>ns</td>
</tr>
<tr>
<td>Number of biological children</td>
<td>2.51 (1.4)</td>
<td>2.33 (1.1)</td>
<td>2.14 (0.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Has more than 1 child with FXS</td>
<td>0.42</td>
<td>0.38</td>
<td>0.43</td>
<td>ns</td>
</tr>
<tr>
<td>CGG repeat length – long allele</td>
<td>88.2 (13.6)</td>
<td>92.3 (8.7)</td>
<td>121.4 (16.8)</td>
<td>39.5***</td>
</tr>
<tr>
<td>CGG repeat length – short allele</td>
<td>27.7 (5.1)</td>
<td>28.1 (7.2)</td>
<td>30.1 (3.6)</td>
<td>ns</td>
</tr>
<tr>
<td>AGG repeats (1 = zero AGG repeat)</td>
<td>0.93</td>
<td>0.98</td>
<td>1.00</td>
<td>ns</td>
</tr>
</tbody>
</table>

* p < 0.10, *** p < 0.001. aFor the two mosaic groups, the predominant CGG repeat on the long allele is reported.
Mosaic PM/FM women were healthier than non-mosaic women

(Mailick et al 2018)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Score</th>
<th>Non-mosaic PM (n = 45)</th>
<th>Mosaic PM (n = 41)</th>
<th>Mosaic PM/FM (n = 14)</th>
<th>F-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I felt anxious during the past week (POMS anxiety)</td>
<td>5.83</td>
<td>62.2%</td>
<td>80.5%</td>
<td>28.6%</td>
<td>7.00**</td>
</tr>
<tr>
<td>2. I had hot flushes/flushes during menopause</td>
<td>4.44</td>
<td>64.3%</td>
<td>70.3%</td>
<td>16.7%</td>
<td>6.04**</td>
</tr>
<tr>
<td>3. I feel worn out (SF-36)</td>
<td>3.89</td>
<td>80.0%</td>
<td>89.7%</td>
<td>85.7%</td>
<td>0.55</td>
</tr>
<tr>
<td>4. I am impulsive (BRIEF-A)</td>
<td>3.66</td>
<td>53.3%</td>
<td>53.7%</td>
<td>7.1%</td>
<td>5.74**</td>
</tr>
<tr>
<td>5. I expect my health to get worse (SF-36)</td>
<td>3.62</td>
<td>8.9%</td>
<td>27.5%</td>
<td>14.3%</td>
<td>3.19*</td>
</tr>
<tr>
<td>6. I start things at the last minute (BRIEF-A)</td>
<td>3.50</td>
<td>80.0%</td>
<td>56.1%</td>
<td>42.9%</td>
<td>5.47**</td>
</tr>
<tr>
<td>7. I have trouble finishing tasks (BRIEF-A)</td>
<td>3.42</td>
<td>64.4%</td>
<td>56.1%</td>
<td>14.3%</td>
<td>6.14**</td>
</tr>
<tr>
<td>8. I felt uneasy during the past week (POMS Anxiety)</td>
<td>3.24</td>
<td>42.2%</td>
<td>65.9%</td>
<td>35.7%</td>
<td>3.25*</td>
</tr>
<tr>
<td>9. I had trouble keeping my mind on what I was doing (CES-D)</td>
<td>3.20</td>
<td>55.6%</td>
<td>63.4%</td>
<td>42.9%</td>
<td>1.10</td>
</tr>
<tr>
<td>10. I have been a nervous person (SF-36)</td>
<td>3.11</td>
<td>66.7%</td>
<td>90.0%</td>
<td>64.3%</td>
<td>3.89*</td>
</tr>
<tr>
<td>11. My health limits me in walking several blocks (SF-36)</td>
<td>2.79</td>
<td>11.1%</td>
<td>35.0%</td>
<td>7.1%</td>
<td>4.92**</td>
</tr>
<tr>
<td>12. My health limits me in walking more than a mile (SF-36)</td>
<td>2.72</td>
<td>20.0%</td>
<td>42.5%</td>
<td>7.1%</td>
<td>4.62*</td>
</tr>
<tr>
<td>13. After having a problem, I don’t get over it easily (BRIEF-A)</td>
<td>2.63</td>
<td>62.2%</td>
<td>80.5%</td>
<td>42.9%</td>
<td>4.20*</td>
</tr>
<tr>
<td>14. I have trouble sitting still (BRIEF-A)</td>
<td>2.54</td>
<td>64.4%</td>
<td>43.9%</td>
<td>35.7%</td>
<td>3.63*</td>
</tr>
<tr>
<td>15. I felt nervous during the past week (POMS Anxiety)</td>
<td>2.42</td>
<td>42.2%</td>
<td>61.0%</td>
<td>21.4%</td>
<td>3.97*</td>
</tr>
<tr>
<td>16. I talk at the wrong time (BRIEF-A)</td>
<td>2.23</td>
<td>55.6%</td>
<td>51.2%</td>
<td>14.3%</td>
<td>4.37*</td>
</tr>
<tr>
<td>17. Lifetime diagnosis of anxiety, depression, or other emotional disorder</td>
<td>2.21</td>
<td>46.7%</td>
<td>43.9%</td>
<td>7.1%</td>
<td>3.94*</td>
</tr>
<tr>
<td>18. Lifetime diagnosis of arthritis, rheumatism, osteoporosis, or other bone or joint disease</td>
<td>1.72</td>
<td>37.8%</td>
<td>26.8%</td>
<td>0.0%</td>
<td>3.93*</td>
</tr>
<tr>
<td>19. Total number of prescription medications</td>
<td>1.27</td>
<td>2.28%</td>
<td>2.07</td>
<td>1.21</td>
<td>0.59</td>
</tr>
<tr>
<td>20. I had depression during menopause</td>
<td>1.18</td>
<td>31.0%</td>
<td>27.0%</td>
<td>0.0%</td>
<td>2.57†</td>
</tr>
<tr>
<td>21. I had feelings of pain, aches, tingling or cramps during the past week (MDS-UPDRS)</td>
<td>1.01</td>
<td>77.8%</td>
<td>78.0%</td>
<td>50.0%</td>
<td>2.49†</td>
</tr>
</tbody>
</table>

$^a p < 0.10$, $^b p < 0.05$, $^{**} p < 0.01$. $^a F$-value based on ANCOVA with age as a covariate.
64 yo woman with FXTAS rapid course related to psychiatric and medical problems
Decline over 7 mo in 64 y woman with FXTAS; 31, 85 repeats

- Well until last November at age 63 when her mother died from FXTAS, she was the caretaker and depressed with mother’s death and she was subsequently hospitalized for type 1 diabetes newly diagnosed.
- Panic attacks began in January so began fluoxetine and then switched to sertraline.
- Neuropathy problems with numbness and tingling for about a year.
- Balance problems began in January and began falling weekly and broke her hip in January, but did not require surgery.
- Intention tremor began bilaterally in Jan and she is dropping things. Ataxia has worsened and she is now using a walker regularly by June and seen for G-P study in July 2019.
64yo female with FXTAS now stage 4

Splenium sign and WMH spots
**Premutation involvement across the lifespan**

- **Infancy**
  - Elevated FMR1 mRNA/ lower FMRP expression levels
  - DNA damage
  - Additional genetic factors (CNV, mutations)
- **Childhood/Adolescence**
  - Visual motion processing deficits
  - Neurobehavioral disorders
  - FXAND
- **Young Adulthood**
  - FXPOI
  - Hypothyroidism, fibromyalgia, chronic fatigue, anxiety and depression, OCD, insomnia
  - FXAND
- **Adulthood**
  - Anxiety, stress, depression, psychosis
  - Neuropathy, migraine, memory problems, hypertension, erectile dysfunction
  - FXAND
- **Elderly**
  - FXTAS: Tremor, ataxia, parkinsonism, autonomic dysfunction, memory and cognitive decline
  - Environmental effects (toxic, insult, psychosocial trauma)
  - Additional medical problems (invasive surgeries)
Some get FXTAS and some do not

- Genetic factors can be protective or deleterious and environmental factors that may predispose to FXTAS
  - Toxins (Paul et al 2010 neurotoxicology)
  - Chemotherapy
  - Smoking - known association with FXPOI
  - Addiction to alcohol and drugs of abuse
  - Surgery and general anesthesia - often first symptoms after surgery in older patients
  - Depression and anxiety or stress which are all increased in carriers
  - Onset of autoimmune disease or cancer
  - Hypoxia from sleep apnea or bradycardia/arrythmias
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/mitochondrial dysfunction 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 Alzheimer, Parkinson disease, LBD or Multiple Sclerosis
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
Connective Tissue Problems in Carriers and in FXS

- Low FMRP leads to elastin abnormalities and MMP9 elevation leading to changes in the extracellular matrix and many of the physical features of FXS
  - Prominent and long ears, soft velvet like skin, joint dislocations, hyperextensible finger joints, high-arched palate, flat feet, mitral valve prolapse, aortic root dilation (Davids et al. 1990; Loehr et al. 1986, Sreeram et al. 1989)
- Some of these features can be seen in carriers more commonly than in age matched controls such as prominent ears (20.2% vs 6.4% of controls) Riddle at al. 1998
- Many carriers have back problems, disc protrusions and surgery
- Presumably those with the highest premutation levels would have lower FMRP and more connective tissue problems
Hypermobile Ehlers-Danlos Syndrome in Carriers (Tassankipjanich et al 2021)

- 49 y.o. female premutation carrier but diagnosed with EDS before testing for premutation
- 123 CGG repeats
- FXAND; migraines and diagnosis of Generalized Anxiety Disorder
- Chronic pain in muscles and joints; diagnosed with fibromyalgia
- IBS, oversensitive to sensory stimuli
- Autonomic dysfunction: intermittent hypertension and tachycardia

- 36 y.o. female premutation carrier diagnosed with EDS first
- 104 CGG repeats
- FXAND; anxiety, panic attacks, insomnia, migraines, OCD
- Hyperextensible finger joints, hips easily dislocate
- Chronic pain in joints, hands, and legs and chronic fatigue; diagnosed with fibromyalgia
- Orthostatic hypertension, vertigo, IBS
Spontaneous Coronary Artery Dissection (SCAD) seen in 2 female carriers (Forrest McKenzie et al 2020) Forrest is now in med school UCD

- SCAD is defined by tearing of the arterial wall from blood dissection and no hx of atherosclerotic heart disease
- Risk factors include intense physical exercise, emotional stress, fibromuscular dysplasia, high blood pressure, hormone replacement therapy, and pregnancy/giving birth
- Individuals with connective tissue disorders including Ehlers-Danlos and Marfan syndromes are at higher risk of developing SCAD
- First case in Korean medical journal Park H-Y et al 2017 of 45yo woman with premutation and now reported in our 2 additional cases from G-P study
SCAD Case 1: Clinical Background

- 56 y.o. premutation female; 88 CGG repeats
- No known cardiac risk factors or connective tissue problems
- Evidence of fibromuscular dysplasia in bilateral internal carotid arteries
- Fragile X-associated neuropsychiatric disorder (FXAND); anxiety and depression symptoms. She had been experiencing emotional stress for years due to behavioral problems in her son with FXS
- She has sudden crushing chest pain and in ER EKG suggested MI

Angiogram demonstrated SCAD at large circumflex artery OM1 branch
Marshfield study validates connective tissue problems

Movaghar et al 2019 Sci Adv
FXPOI: Curvilinear effect of CGG repeats and the age of menopause

Mailick et al 2012
FXPOI risk for carriers related to CGG repeats (2021 Allen et al.)

Fig. 1  Box plot of age at menopause distribution by repeat size group. Vertical lines within the box from left to right represent the lower quartile, the median, and the upper quartile, respectively. The horizontal lines represent the 5th and 95th percentiles, and the values beyond these lines, marked as dots, are considered outliers.
Risk to have a child with FXS relates to AGG anchors

Figure 16.3 Estimated risk for expansion to the full-mutation range of a transmitted, maternal premutation CGG repeat is a sensitive function of the number of AGG interruptions in the maternal allele, decreasing with increasing number of interruptions. The differential risk between zero and two AGG interruptions (0–2; blue dotted line) is highest between 75 and 80 total CGG repeats. Black solid line represents 0 AGG interruptions, (0); red dashed line is one interruption, (1); green dotted line is two interruptions, (2). Source: Adapted from Yrigollen et al. 2014.\textsuperscript{157}
Expansion of a premutation to a full mutation depends on mother’s repeat size and AGG interruptions

Nolin et al., 2014
Depienne and Mandel 2021 Repeat Disorders
AJHG
Treatments for premutation carriers

Polussa et al 2014
Brain Disorders and Therapy
Symptomatic treatment in FXTAS

• Tremor: can respond to primidone, beta blocker, anticonvulsant (Keppra) or DBS
• Tremor can be parkinsonian and respond to Sinemet
• Ataxia is difficult to treat could try Amantadine, Riluzole
• Pain: treat with CBD, Gabapentin, or pregabalin or duloxetine (Cymbalta)
• Depression/ Anxiety: treat with SSRI or SNRI
• sleep apnea study and treatment with CPAP if needed
Treatment studies of FXTAS

• Seritan et al 2014 J Cl 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

• Allopregnanolone study: IV once a week for 3 months: Helped neuropathy and neurocognitive measures (Wang et al 2018)
Sulforaphane (SFN) a dietary supplement that turns on the Nrf-2 antioxidant systems in cells

- SFN improved mitochondrial function in fibroblasts from patients with FXTAS (Napoli et al 2021 Neurobiol of Disease)
- Open label study of SFN in 15 patients with FXTAS to assess improvement in biomarkers and clinical symptoms is initiated using Avmacol regular strength 1 to 6 tablets per day with slow increase

Would metformin be helpful in carriers? Targeted treatment for FXS, protects against cancer, lowers blood sugar, protects against vascular dementia, lowers blood pressure, lowers inflammation
ANAVEX 2-73 study will be funded by Anavex Life Sciences: AV2-73 is a sigma1 agonist

- Reducing mitochondrial dysfunction
- Reducing protein misfolding
- Modulating Ca^{2+}

- Reducing oxidative stress
- Reducing inflammation
- Enabling neuroprotection
ANAVEX 2-73 history

• Clinical validation to enhance cognition in Alzheimer Disease in phase 2a and moving to phase 2/3 trials

• Preclinical validation in mouse models for depression, anxiety, epilepsy, infantile spasms, FXS, Rett syndrome, multiple sclerosis and Parkinson disease

• Demonstrated efficacy in Parkinson Disease Dementia

• Anavex 2-73 demonstrated efficacy in a controlled trial in Rett syndrome (MIND and in other centers) funded by Anavex Life Sciences Inc.
Treatment of KO mouse with Anavex 2-73 at 1mg/kg IP for 14 days normalized 3 behaviors and BDNF levels (Reyes et al 2021)
Curcumin and piperine with preclinical benefits for the premutation

Piperine Modulates Protein Mediated Toxicity in Fragile X-Associated Tremor/Ataxia Syndrome through Interacting Expanded CGG Repeat (r(CGG)exp) RNA

Arun Kumar Verma, Eshan Khan, Subodh Kumar Mishra, Neha Jain, and Amit Kumar*

Discipline of Biosciences and Biomedical Engineering, Indian Institute of Technology Indore, Simrol, Indore 453552, India

Supporting Information
Interested in joining the International Fragile X Premutation Registry?

Join now at fragilex.org/ifxpr
Collaborators

UC Davis School of Medicine

Dept. Biochem & Molec. Medicine
   Paul Hagerman   Flora Tassone
   Glenda Espinal
Department of Rehabilitation
   Veronica Martinez-Cerdeno

Colombia: Universidad del Valle: Wilmar Saldarriaga

Philippines: Angel Dy, Lourdes Tanchenco, Jeanne Dy, Melinda Tan

University of Colorado Health Sciences Center (Denver)
   Nicole Tartaglia   Maureen Leehey   James Grigsby; Karen Riley at DU

RUSH- (Chicago)
   Elizabeth Berry-Kravis   Deb Hall   Christopher Goetz

*Latrobe University, Melbourne Australia*
   Danuta Loesch

Canada: University of Alberta in Edmonton – Francois Bolduc
   St Justine Hospital Quebec _Sebastien Jacquemont

Indonesia: Diponegoro University, Samarang
   Sultana Hussein, Tri Indah Winarni, Agustini Utari

Support: NICHD, HRSA, DOD, NFXF, Azrieli Foundation, Anavex Life Sciences Corp