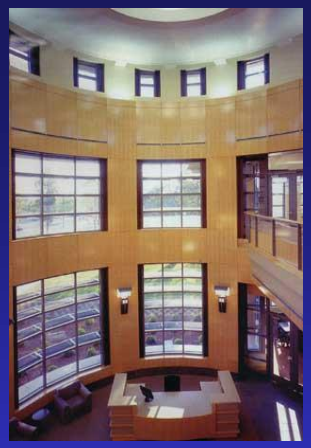


Premutation Disorders and Conditions

Fragile X Association of Australia lecture Dec 10, 2021

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

2000 a seminal year

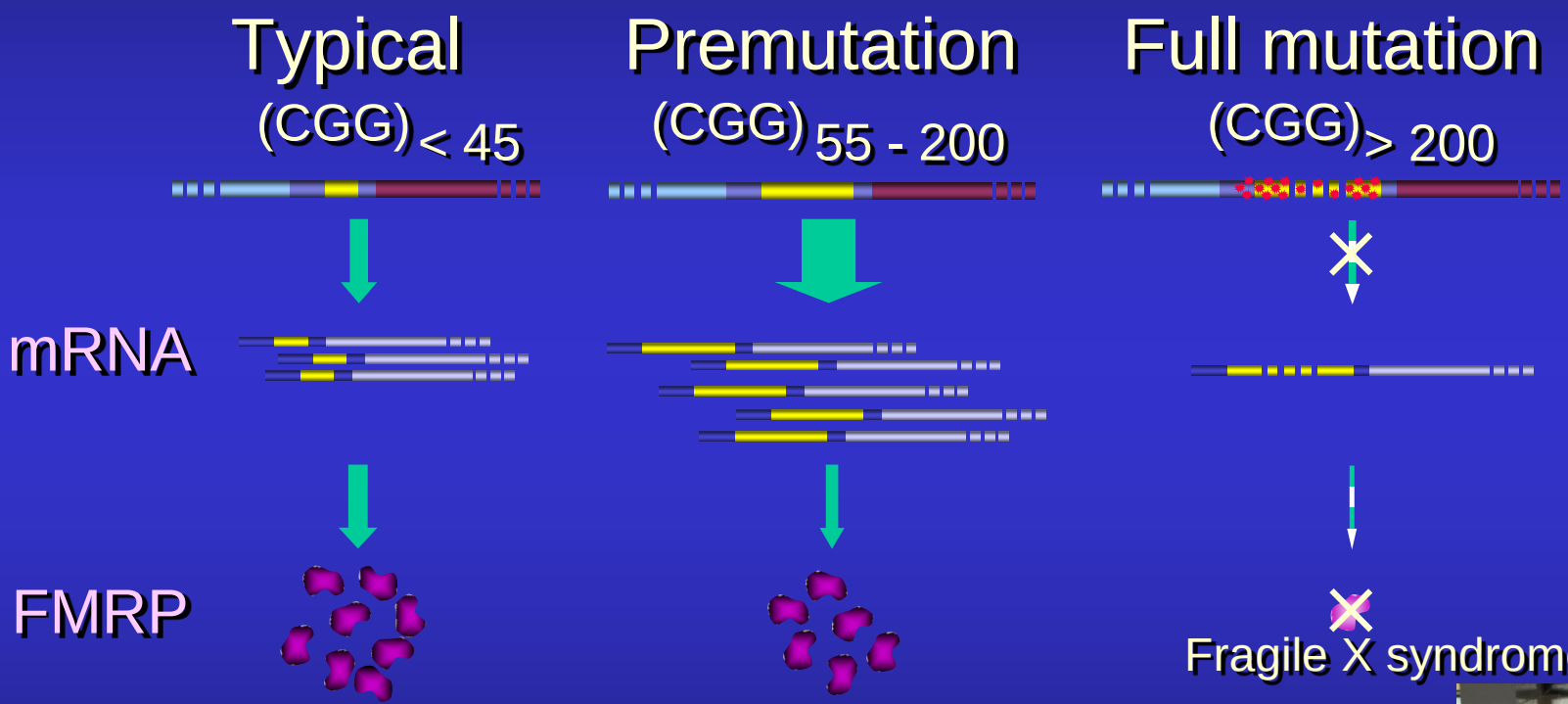
2000 was the year that Dr Flora Tassone in Paul's lab discovered and reported the elevated *FMR1* mRNA in premutation carriers (Tassone et al 2000 AJHG)

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 *FMR1* gene

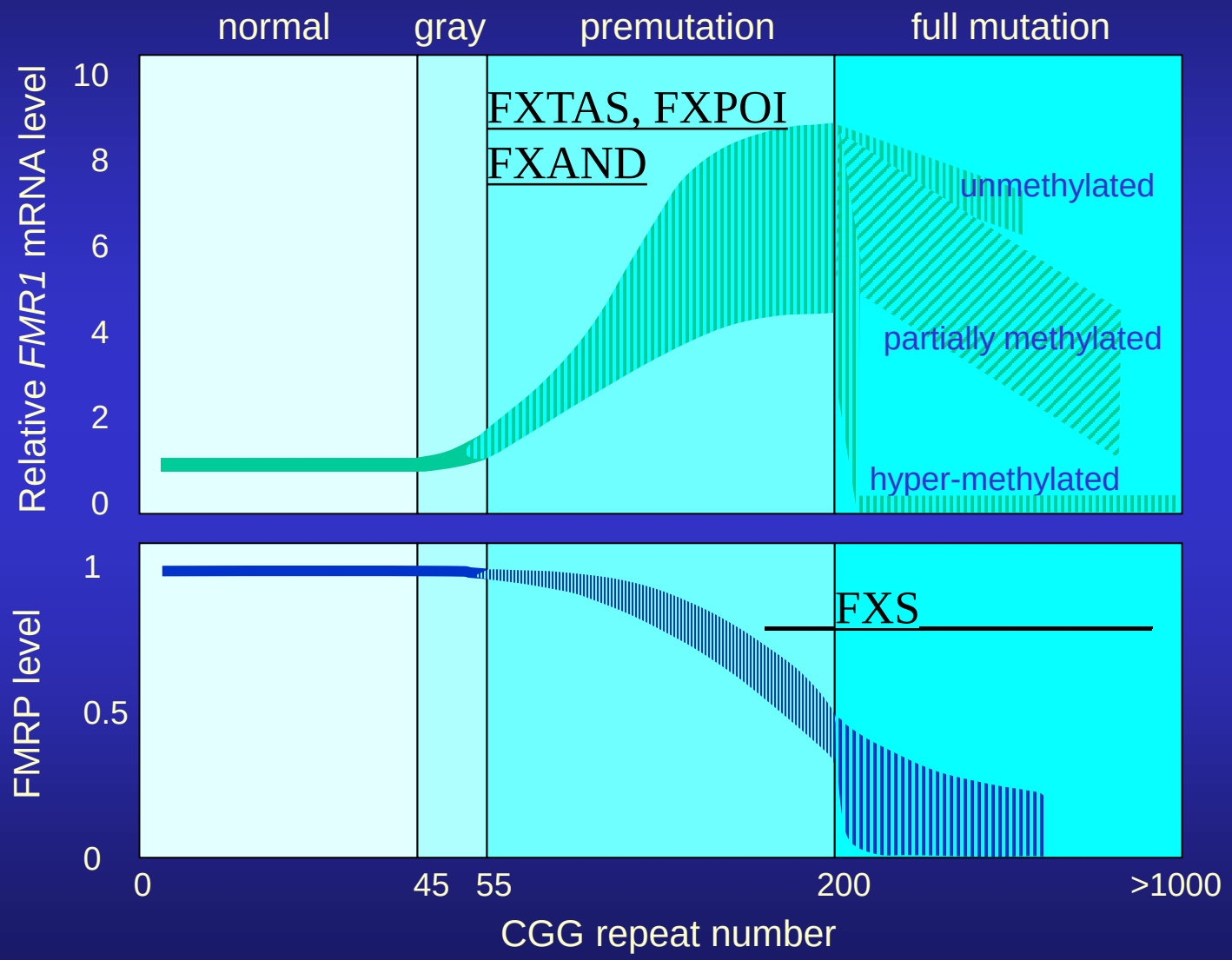
1/130-250 females
1/250-810 males **1/3600-5000**



Clinical **normal**

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

Expression of the *FMR1* gene



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 childhood: ADHD, autism, ASD, anxiety disorders (Aziz et al 2003; Goodlin-Jones et al 2004; Farzin et al 2006; Bailey et al 2008)
- 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 *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 medications for tremor and psychiatric symptoms

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)

- Danuta Loesch data 2021 demonstrates faster progression of psychiatric problems in females vs males but males progress faster with motor symptoms

Diagnostic Criteria for FXTAS updated

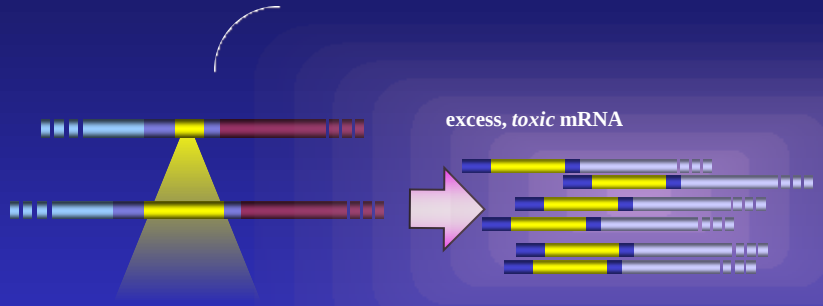
***Inclusion criterion*: 55 – 200 CGG repeats**

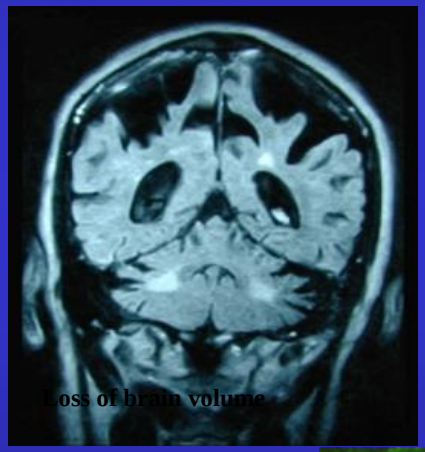
MRI	*major*	*Middle cerebellar peduncle (MCP) lesions*
	minor	Cerebral white matter hyperintensity
	minor	Moderate to severe generalized atrophy
	minor	WMH in Splenium
Clinical exam	*major*	*Intention tremor*
	major	*Gait ataxia*
	minor	Parkinsonism
	minor	Short term memory deficits
	minor	Executive function deficits
	minor	Neuropathy

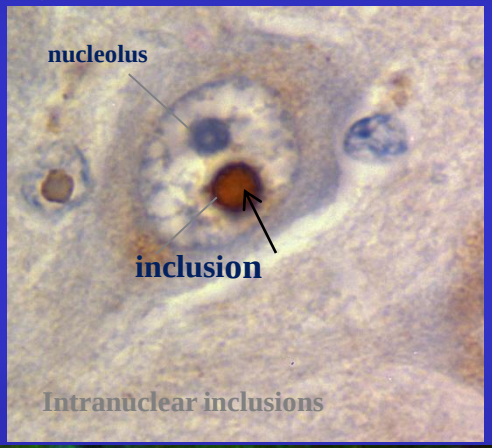
Diagnostic categories

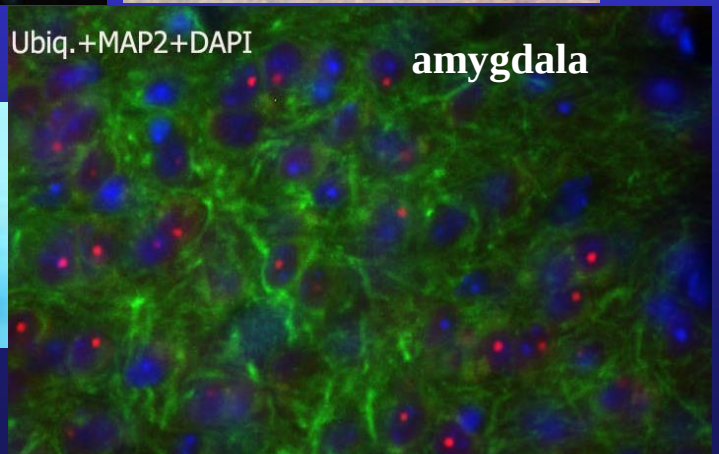
Definite	Probable	Possible
1 clinical *major* AND 1 MRI *major* Inclusions (*post mortem*)	2 clinical *major* OR 1 MRI *major* AND 1 clinical minor	1 clinical *major* AND 1 MRI minor

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

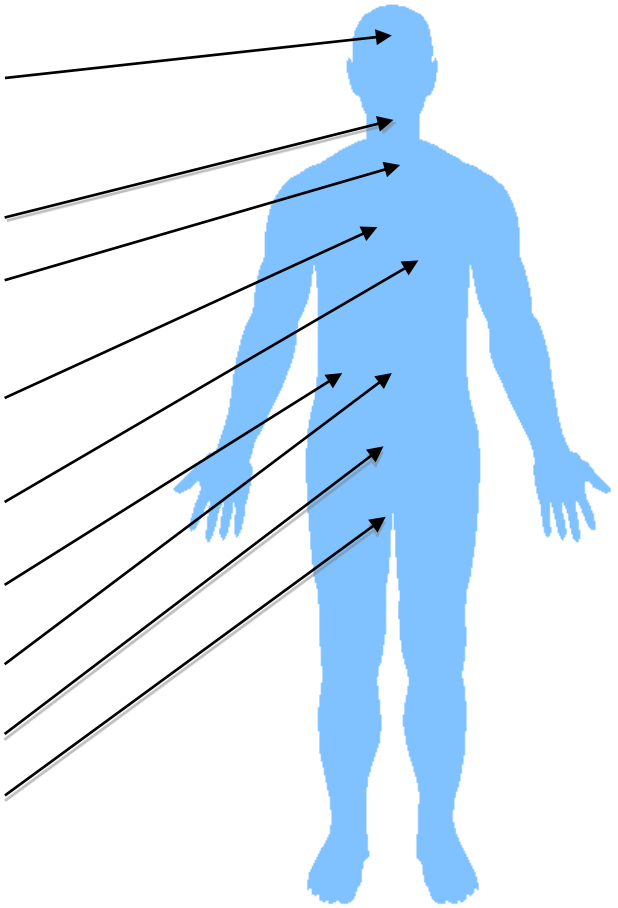
subepicardial autonomic ganglia of the heart

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 a 2011 Acta Pathologica

Spectrum of Premutation Involvement

FMR1 CGG-repeat toxic RNA "trigger"

Background gene effects

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

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

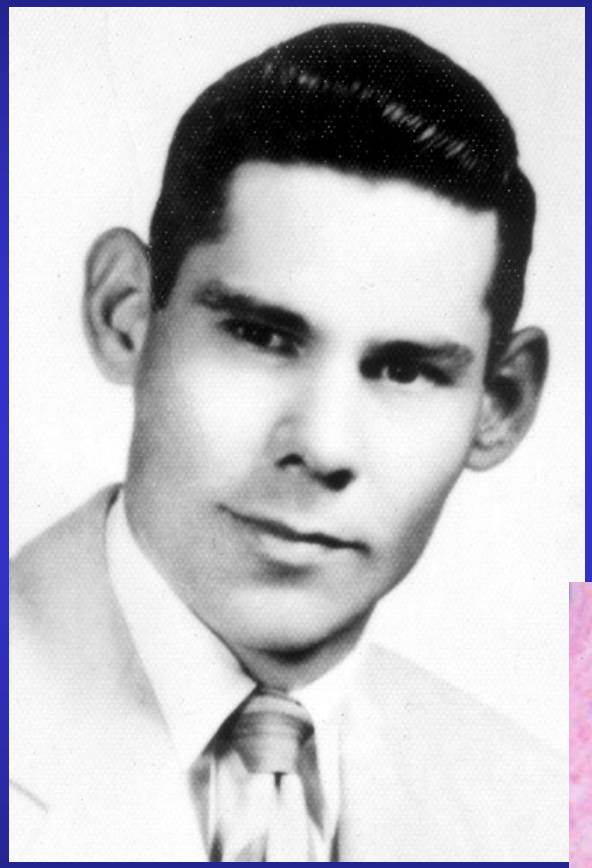
FXTAS

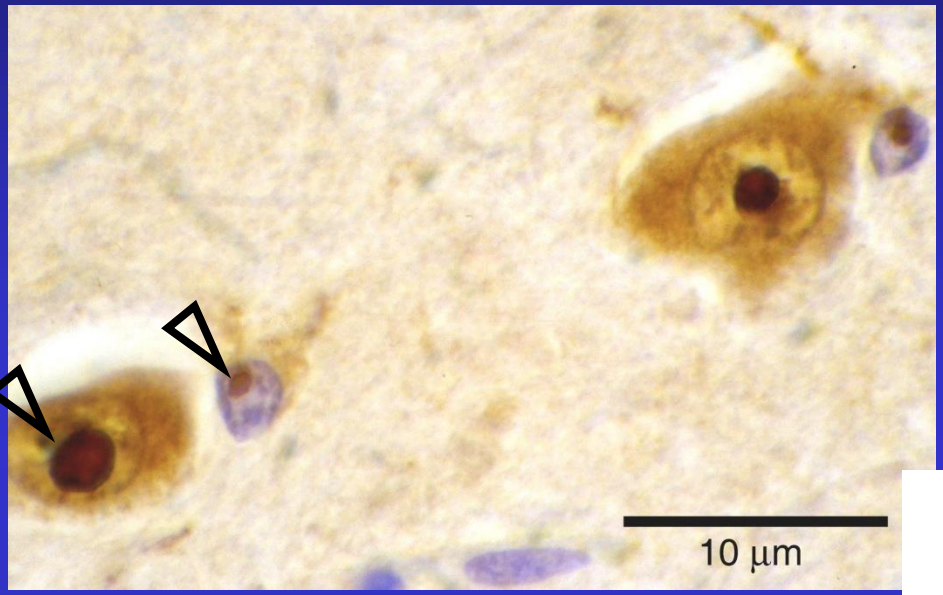
tremor, ataxia, Parkinsonism
autonomic dysfunction, EF deficits, memory and cognitive decline

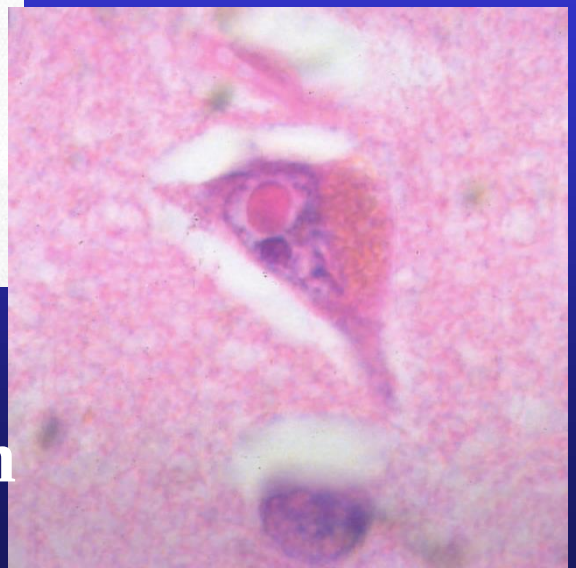
Drs Guilivi and Ele Napoli and team have done the mitochondrial work

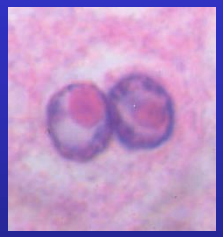
Intranuclear inclusions

Neurons – Astrocytes in humans









Greco et al
2002, 2006 Brain

LC MS/MS for composition of the FXTAS inclusions

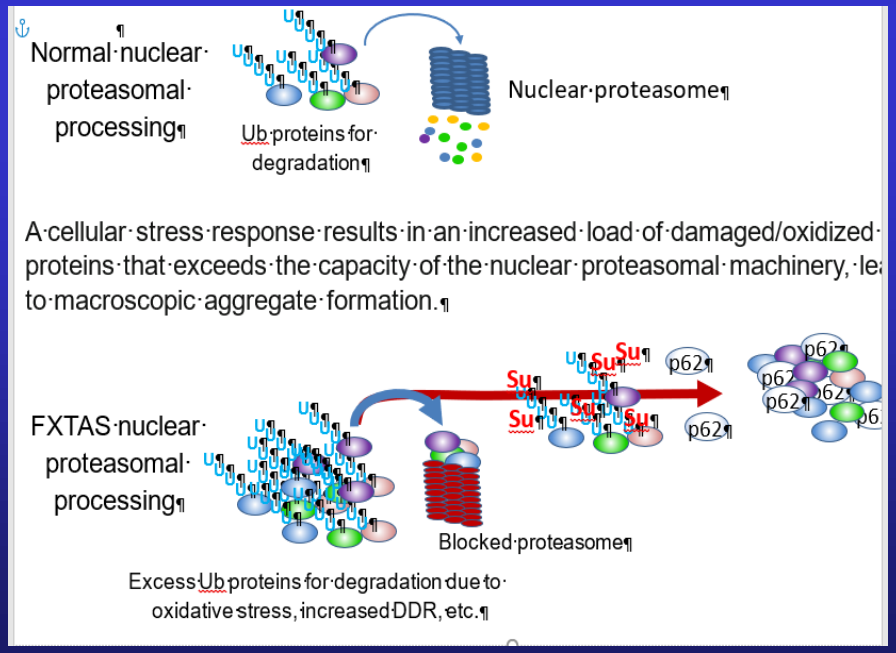
More focused analysis of the highest-abundance inclusion-enriched proteins, those proteins which make up at least 0.5% of a sorted inclusion sample and those which were enriched by at least 50% were considered further

Ma et al. (2019)

Proteins	FXTAS A Inclusions		FXTAS B Inclusions	
	Over FXTAS A nuclear	Over control nuclear	Over FXTAS B nuclear	Over control nuclear
Small ubiquitin-related modifier 2 (SUMO2)	**5.5 (0.60/0.11)**	**60.0 (0.60/0.01)**	**9.1 (4.37/0.48)**	**437.0 (4.37/0.01)**
p62/ SQSTM1	**8.0 (0.08/0.01)**	**40.0 (0.08/0.002)**	**30.0 (0.60/0.02)**	**300.0 (0.60/ 0.002)**
Myeloid leukemia factor 2 (MLF2)	**9.3 (0.28/0.03)**	**93.3 (0.28/0.003)**	**27.3 (0.82/0.03)**	**273.3 (0.82/ 0.003)**
Ubiquitin (RS27A)	**3.6 (0.65/0.18)**	**5.9 (0.65/0.11)**	**6.7 (5.17/0.77)**	**47.0 (5.17/0.11)**
Myelin basic protein (MBP)	**3.5 (0.46/0.13)**	**1.2 (0.46/0.38)**	**15.2 (0.76/0.05)**	**2.0 (0.76/0.38)**
Heat shock protein HSP 90-alpha (HSP90AA1)	1.6 (0.08/0.05)	4.0 (0.08/0.02)	1.7 (0.67/0.39)	33.5 (0.67/0.02)
Heterogeneous nuclear ribonucleoprotein L (HNRNPL)	1.9 (0.13/0.07)	6.5 (0.13/0.02)	1.6 (0.52/0.32)	26.0 (0.52/0.02)
Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1)	1.7 (1.12/0.65)	2.7 (1.12/0.41)	1.6 (2.16/1.32)	5.3 (2.16/0.41)
Heterogeneous nuclear ribonucleoprotein A3 (HNRNPA3)	1.8 (0.95/0.54)	2.6 (0.95/0.36)	1.6 (1.59/0.97)	4.4 (1.59/0.36)
Heterogeneous nuclear ribonucleoproteins C1/C2 (HNRNPC)	1.9 (0.28/0.15)	1.3 (0.28/0.22)	2.0 (0.59/0.30)	2.7 (0.59/0.22)
Beta-actin (ACTB)	2.2 (0.75/0.34)	1.2 (0.75/0.61)	1.8 (1.33/0.75)	2.2 (1.33/0.61)
Alpha-crystallin B chain (CRYAB)	1.9 (1.29/0.68)	1.1 (1.29/1.22)	1.7 (2.66/1.53)	2.2 (2.66/1.22)
Tubulin alpha-1B chain (TBA1B)	1.6 (1.01/0.63)	1.0 (1.01/0.98)	1.6 (0.81/0.50)	0.8 (0.81/0.98)
Tubulin beta-2A chain (TBB2A)	1.7 (0.64/0.37)	0.9 (0.64/0.71)	1.5 (0.58/0.39)	0.8 (0.58/0.71)
Calcium/calmodulin-dependent protein kinase type II subunit alpha (CAMK2A)	1.7 (0.43/0.25)	0.7 (0.43/0.61)	1.6 (0.59/0.38)	1.0 (0.59/0.61)

RNA toxicity

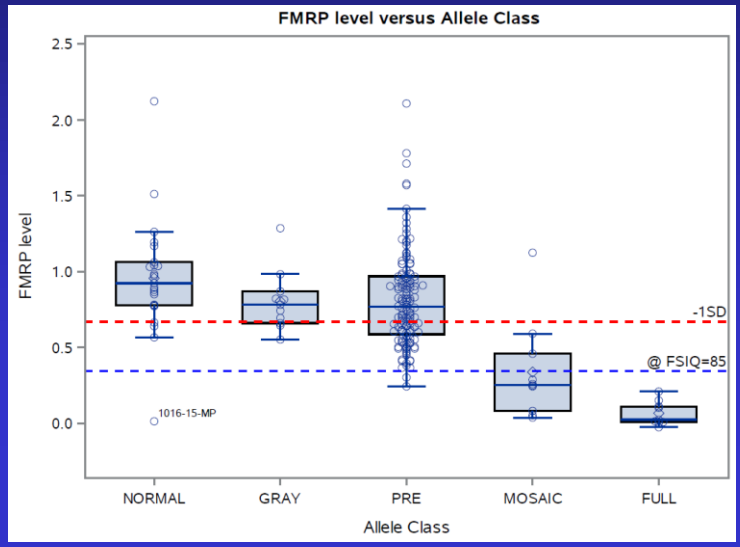
Clogged proteosomes

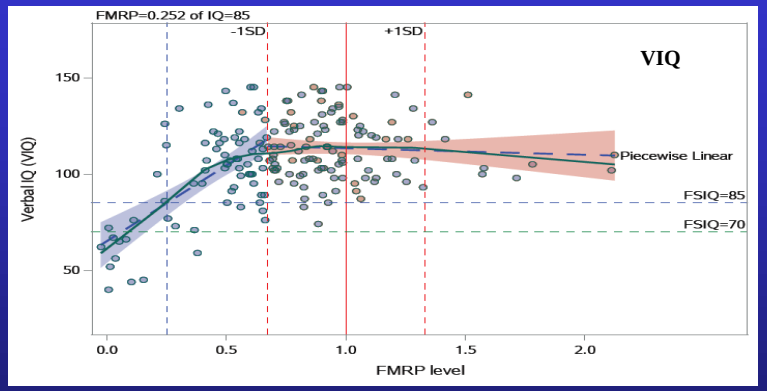


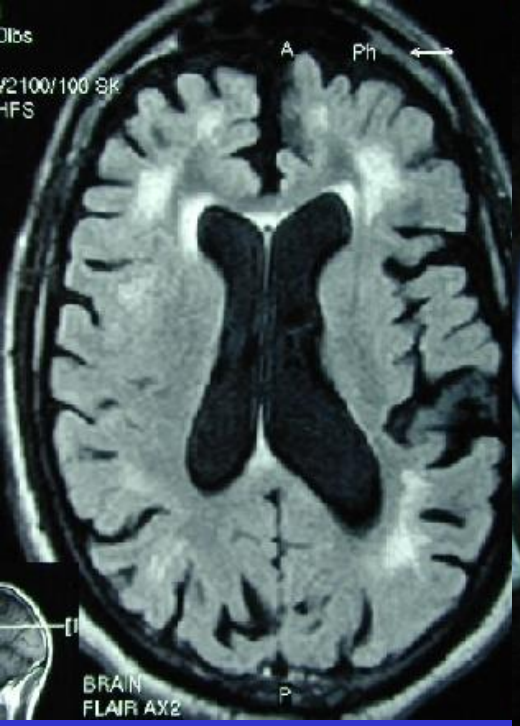
Holm et al 2021

FMRP and IQ (Kim et al 2019)

FSIQ



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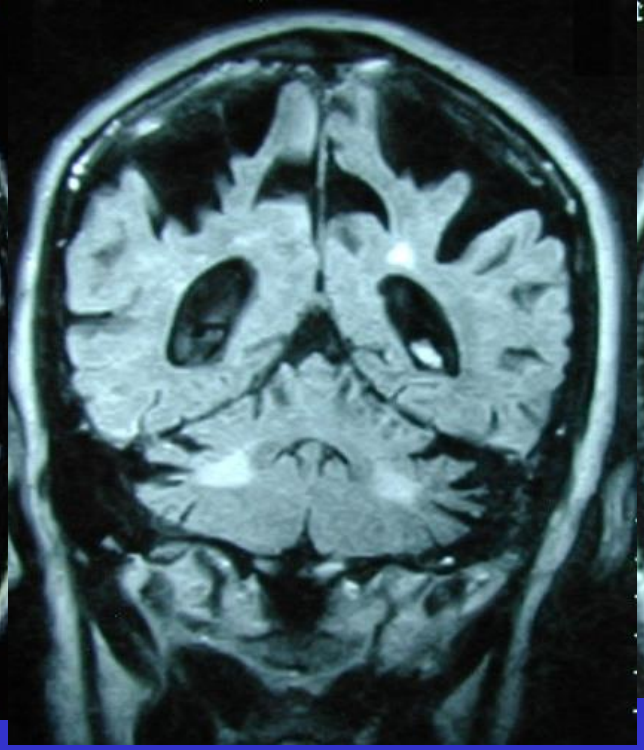
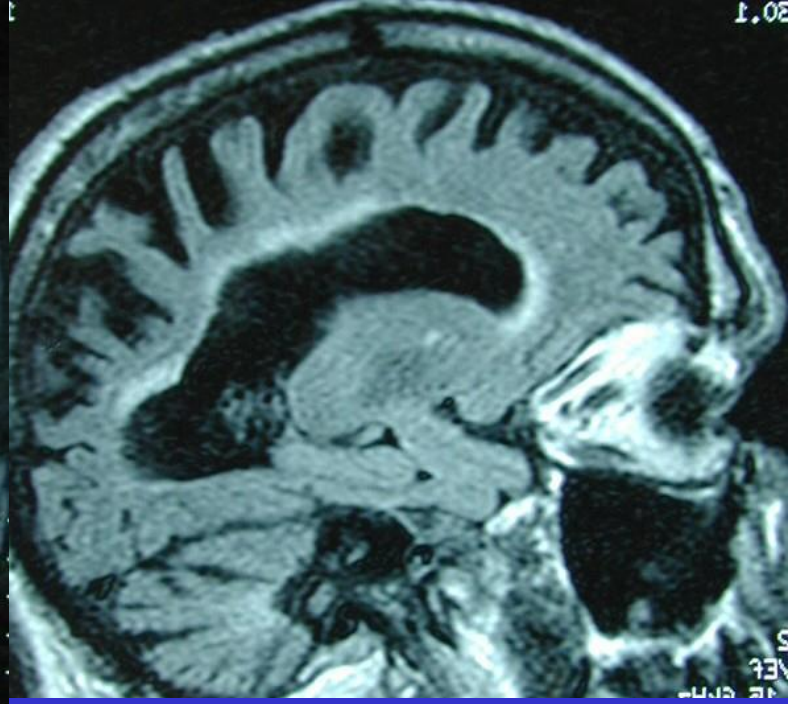
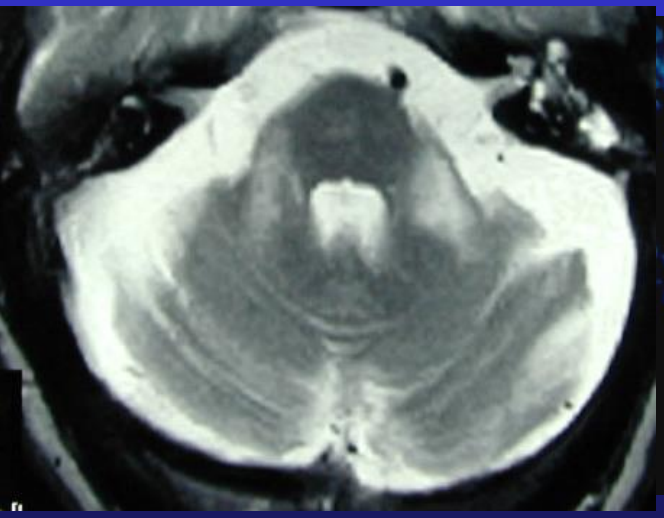
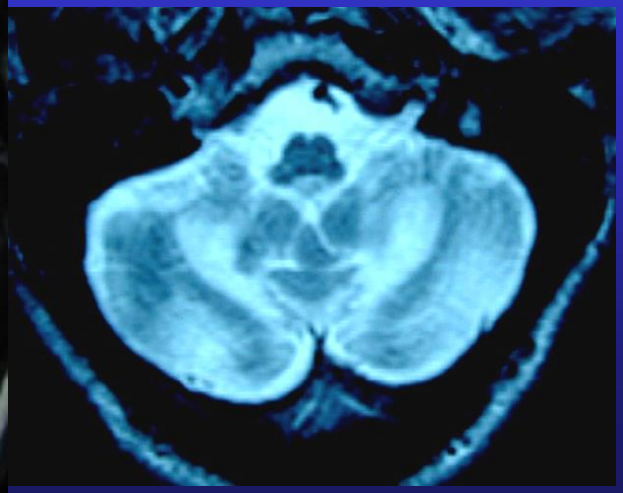
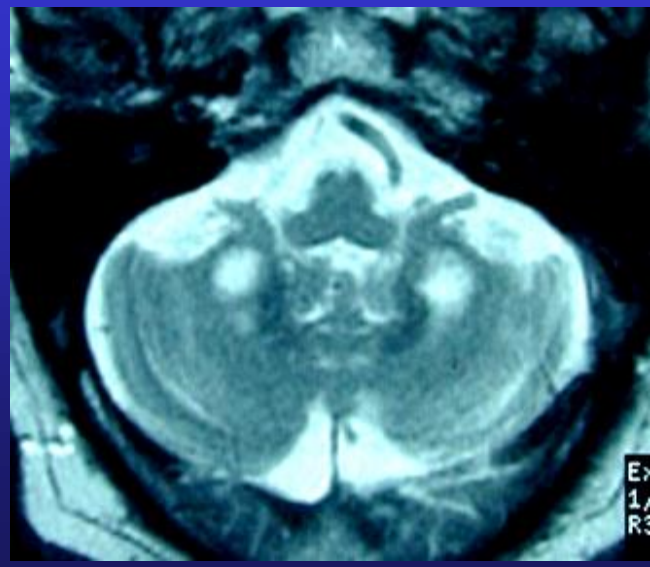




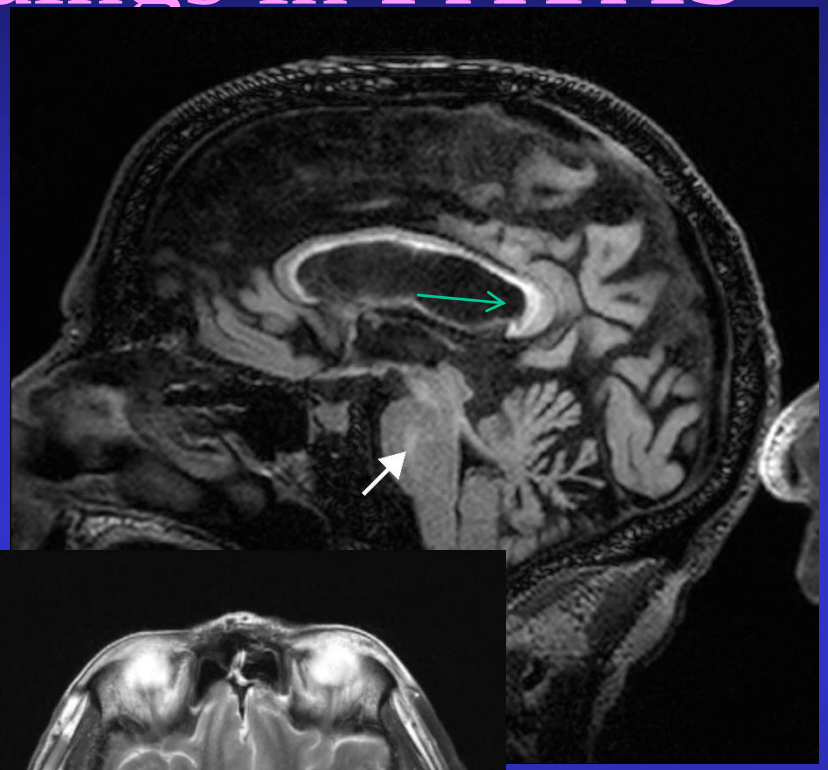


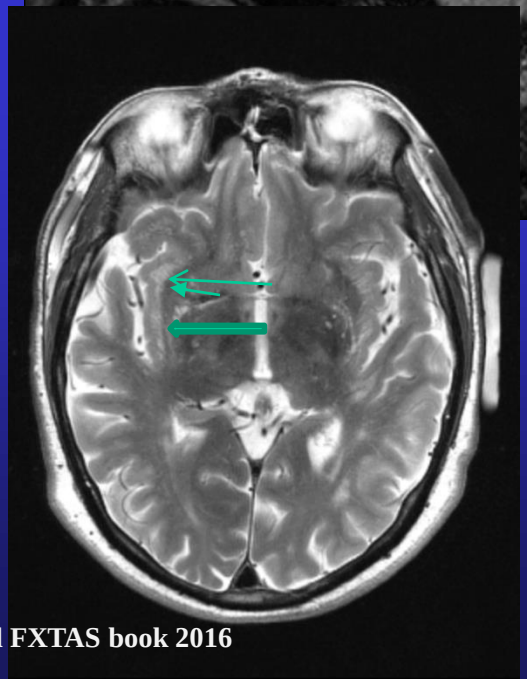




Other MRI Findings in FXTAS

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





Adams et al 2012 ; Apartis et al 2012; Hall et al 2014, Tassone and Hall FXTAS book 2016

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

- 30 PM males 50 to 81years 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

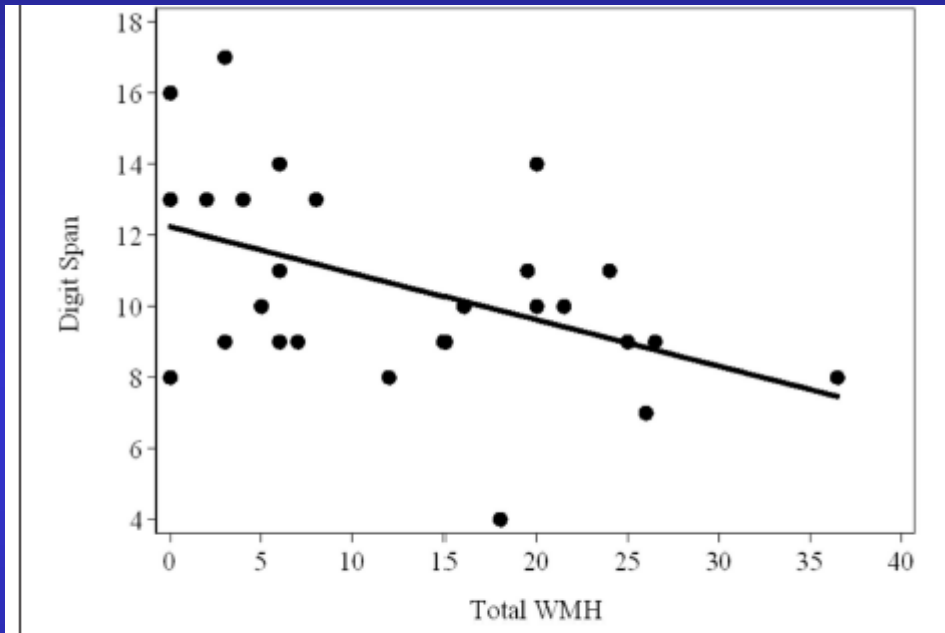
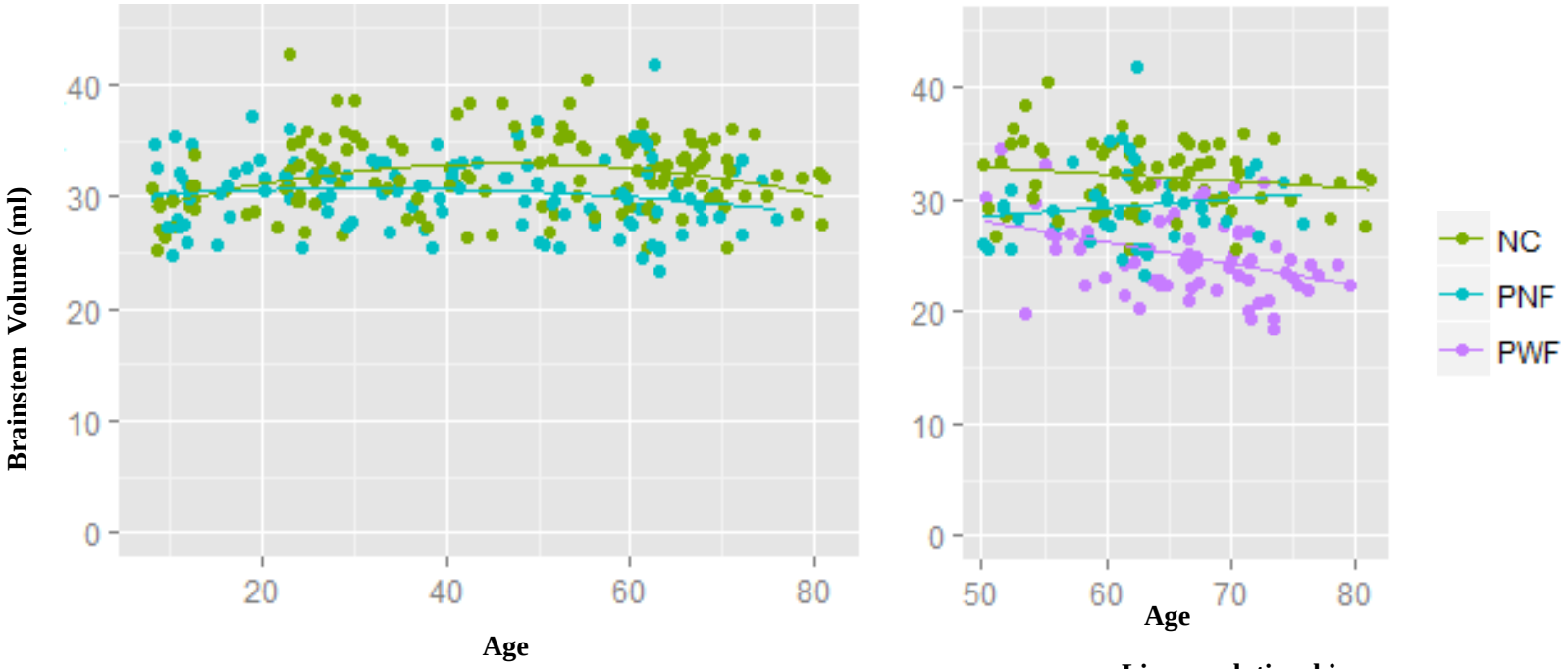


FIGURE 2 | Scatterplot showing the (raw) Total Digit Span scores against the WMH scores in the total sample (FXTAS and Non FXTAS combined) of PM carriers.

Brainstem Volume Change: NC Vs. PNF (Wang et al 2017)



Quadratic relationship

PNF: –0.19± 0.67 ml, t = –0.29, p = 0.77

Age x group: –0.048 ± 0.017 ml, t = –2.82, p = 0.005

Annual rate of change

NC: $-0.001 \times age^2 + 0.091 \times age$

PNF: $-0.001 \times age^2 - 0.044 \times age$

Age of divergence in volume: 4.1 years

Linear relationship

NC: 4.13 ± 1.10 ml, t = 3.74, p = 0.0003

Age x NC: 0.16 ± 0.06 ml, t = 2.4, p = 0.016

Age x PNF: 0.31 ± 0.08 ml, t = 3.8, p = 0.0002

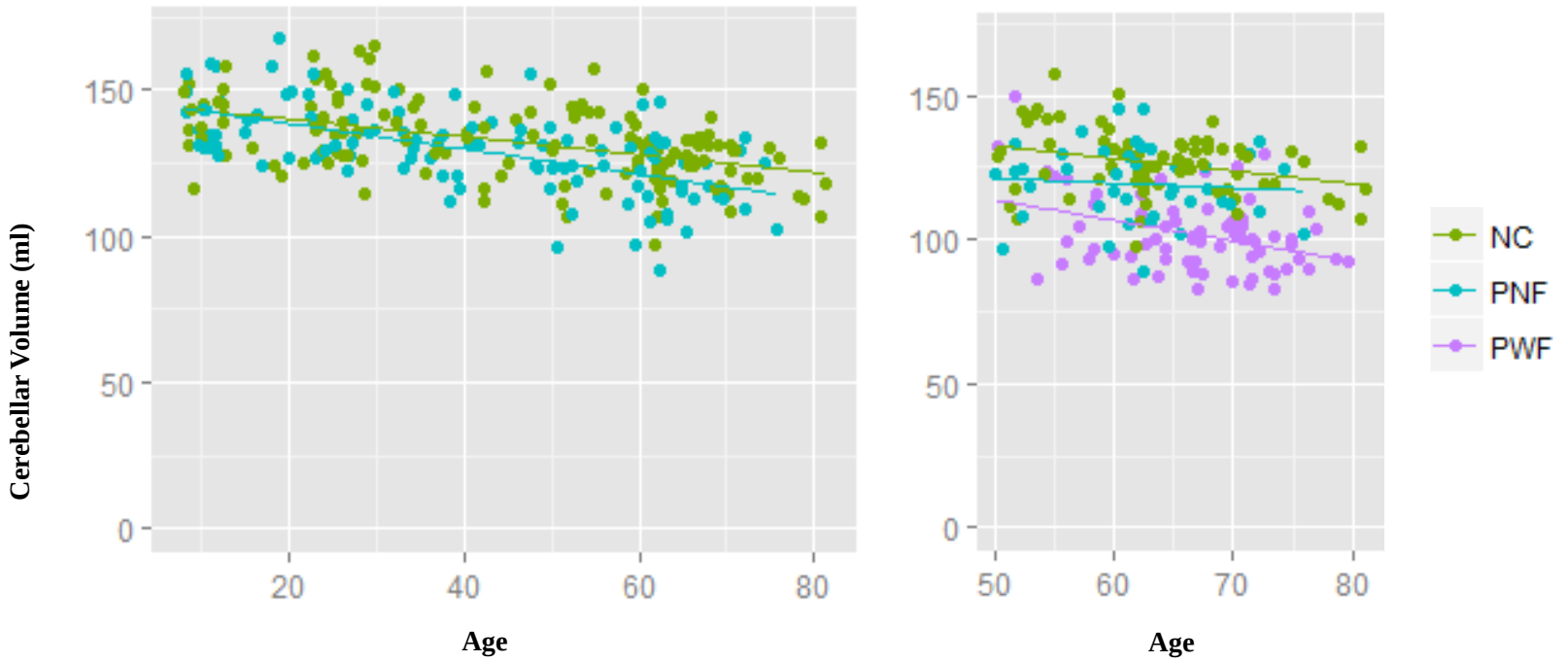
Annual rate of change

NC: –0.07 ± 0.06 ml; PNF: 0.08 ± 0.08 ml

PWF: –0.23 ± 0.05 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 ± 2.53 ml, t = –0.10, p = 0.92
Age x group: –0.14 ± 0.06 ml, t = –2.24, p = 0.026

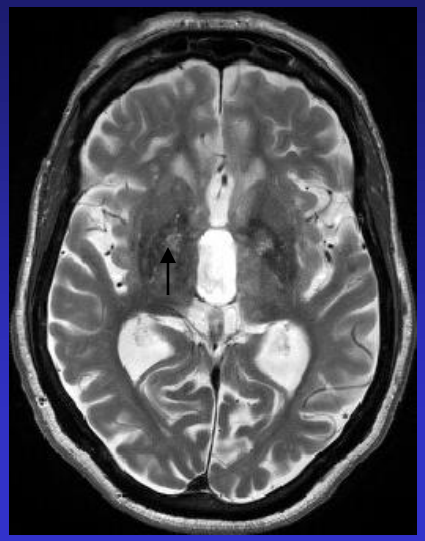
Annual rate of change
NC: –0.36 ± 0.04 ml
PNF: –0.50 ± 0.06 ml

Age of divergence in volume: 6.4 years

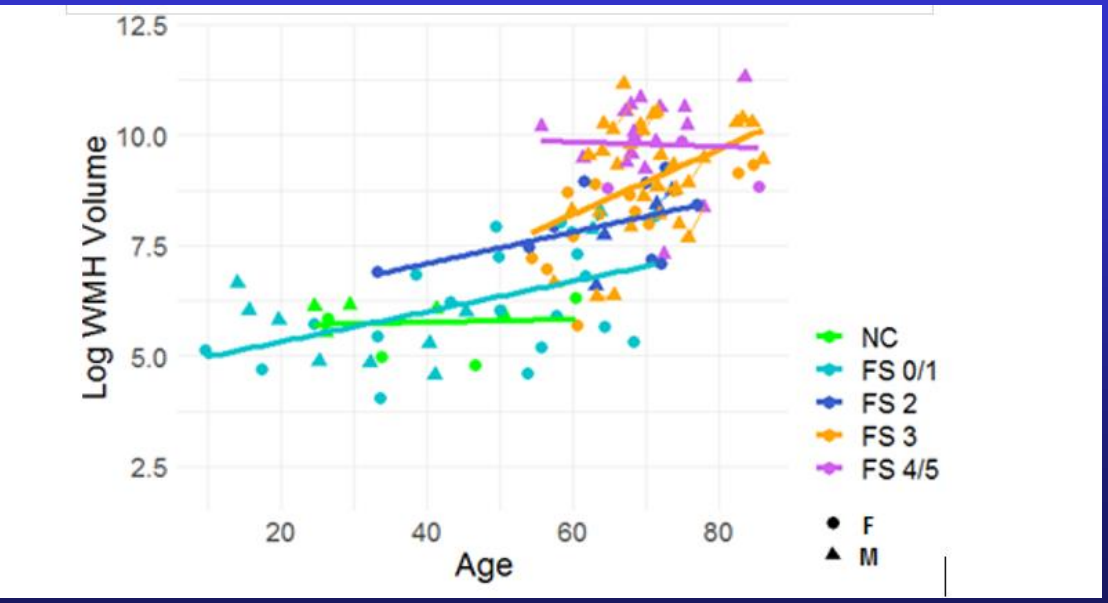
Linear relationship
NC: 22.4 ± 1.86 ml, t = 12.1, p < 0.0001
PNF: 13.0 ± 2.17 ml, t = 5.99, p < 0.0001

Annual rate of change
–0.54 ± 0.11 ml
PWF vs. PNF: difference in volume occurs before age 50

Amazing discoveries by Jun Yi Wang every year (>13 papers/8 yrs)

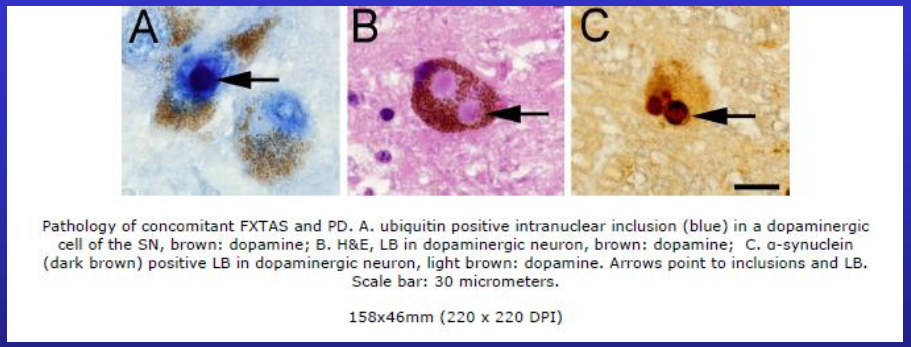


- The brain changes in premutation carriers start in childhood
- The enlarged ventricles in FXTAS distort brain structure
- Hypergyrification and hypogyrification in pres (boys on left)
- The eye of the tiger sign in FXTAS



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 substancia nigra.



Pathology of concomitant FXTAS and PD. A. ubiquitin positive intranuclear inclusion (blue) in a dopaminergic cell of the SN, brown: dopamine; B. H&E, LB in dopaminergic neuron, brown: dopamine; C. α-synuclein (dark brown) positive LB in dopaminergic neuron, light brown: dopamine. Arrows point to inclusions and LB. Scale bar: 30 micrometers.

158x46mm (220 x 220 DPI)

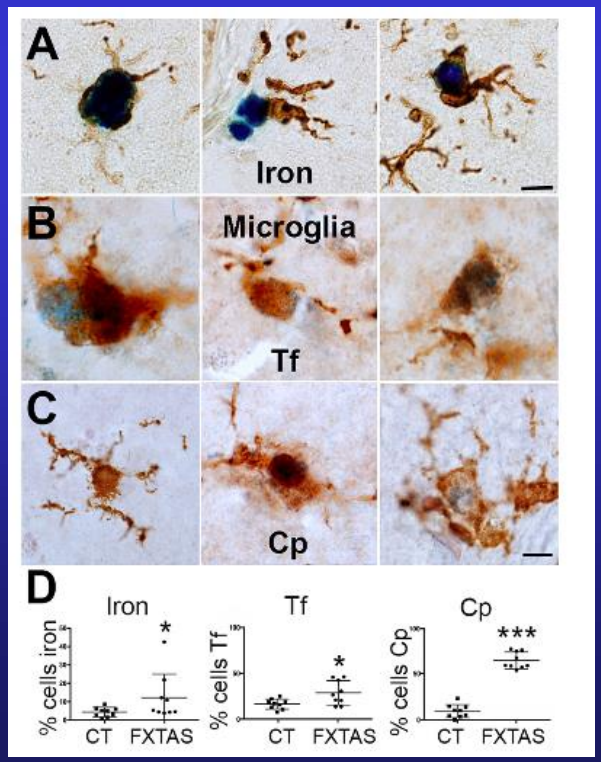
- 2 of 7 with Lewy bodies but 2 more without PD symptoms had Lewy bodies so 10% (4/40) total

Iron deposition within the putamen in FXTAS

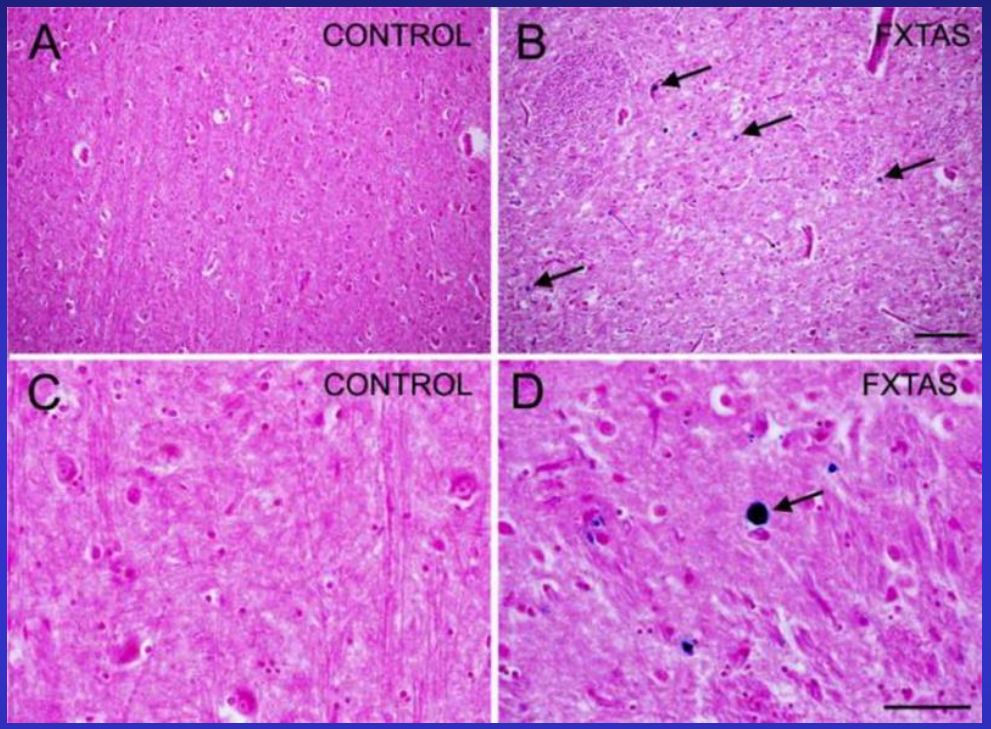
Transport of iron into the brain is altered in FXTAS

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.



Ariza et al. (Veronica Martínez-Cerdeño) 2017



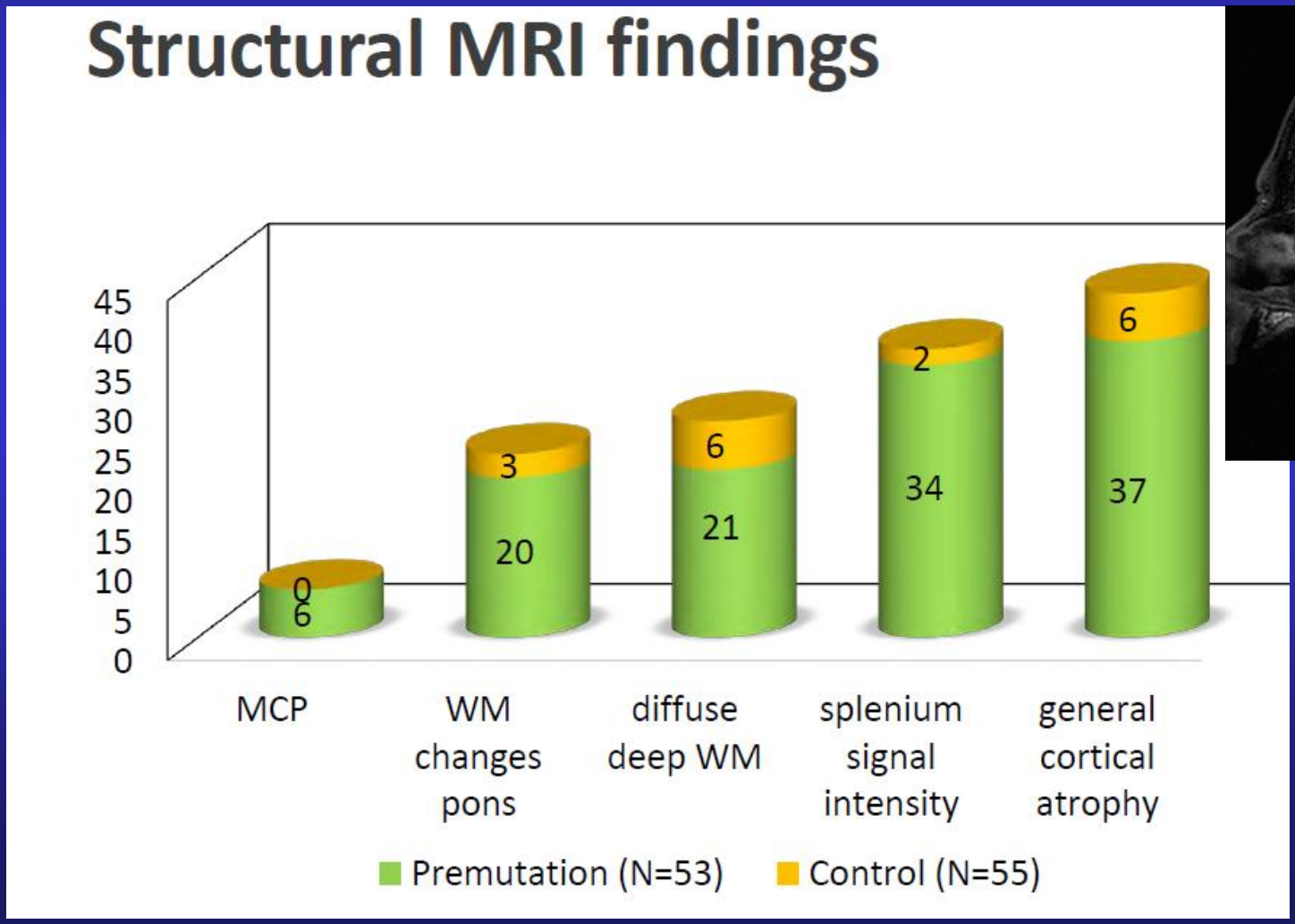
However, increased levels of iron, transferrin, and ceruloplasmin in microglial cells, indicating an attempt by the immune system to remove the excess iron.

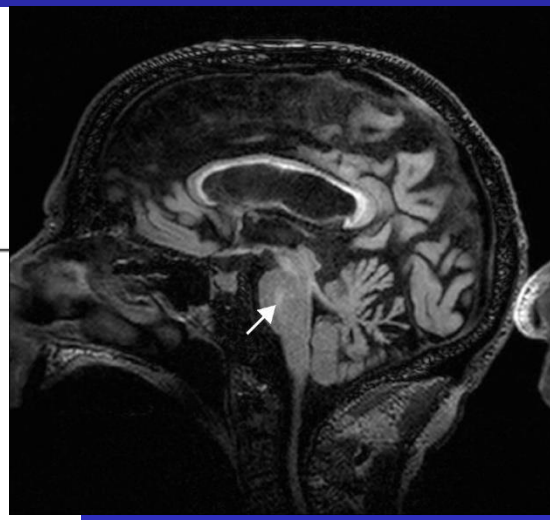
53 female carriers with FXTAS vs 55 controls (Schneider et al 2020)

- 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

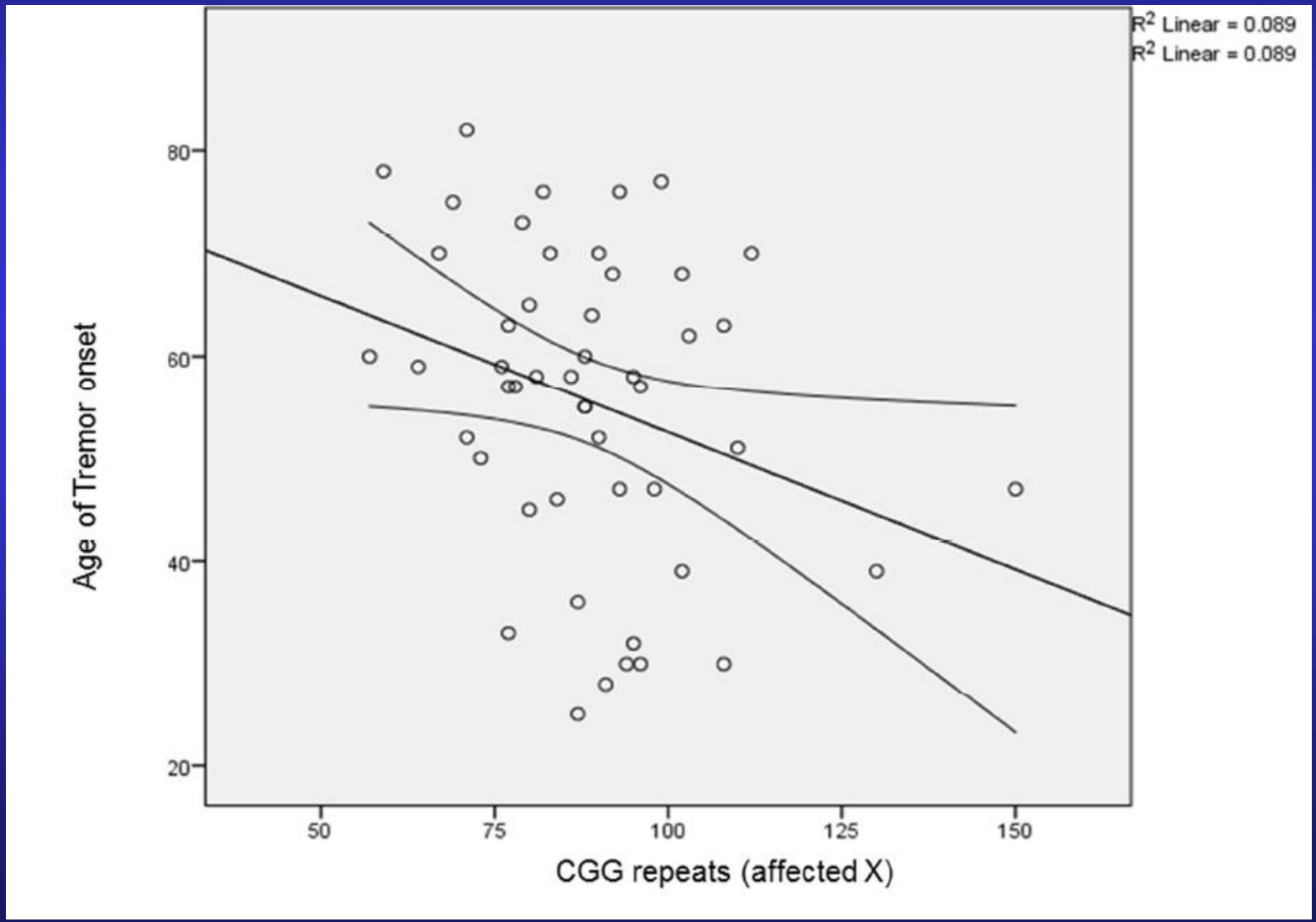
Splenium hyperintensity and cortical atrophy are most common MRI findings in females with FXTAS

(Schneider et al 2020)





Relationship of CGG repeat size and age of tremor onset (Schneider et al 2020)



FXAND: Depression and Anxiety can worsen with age in women with the premutation

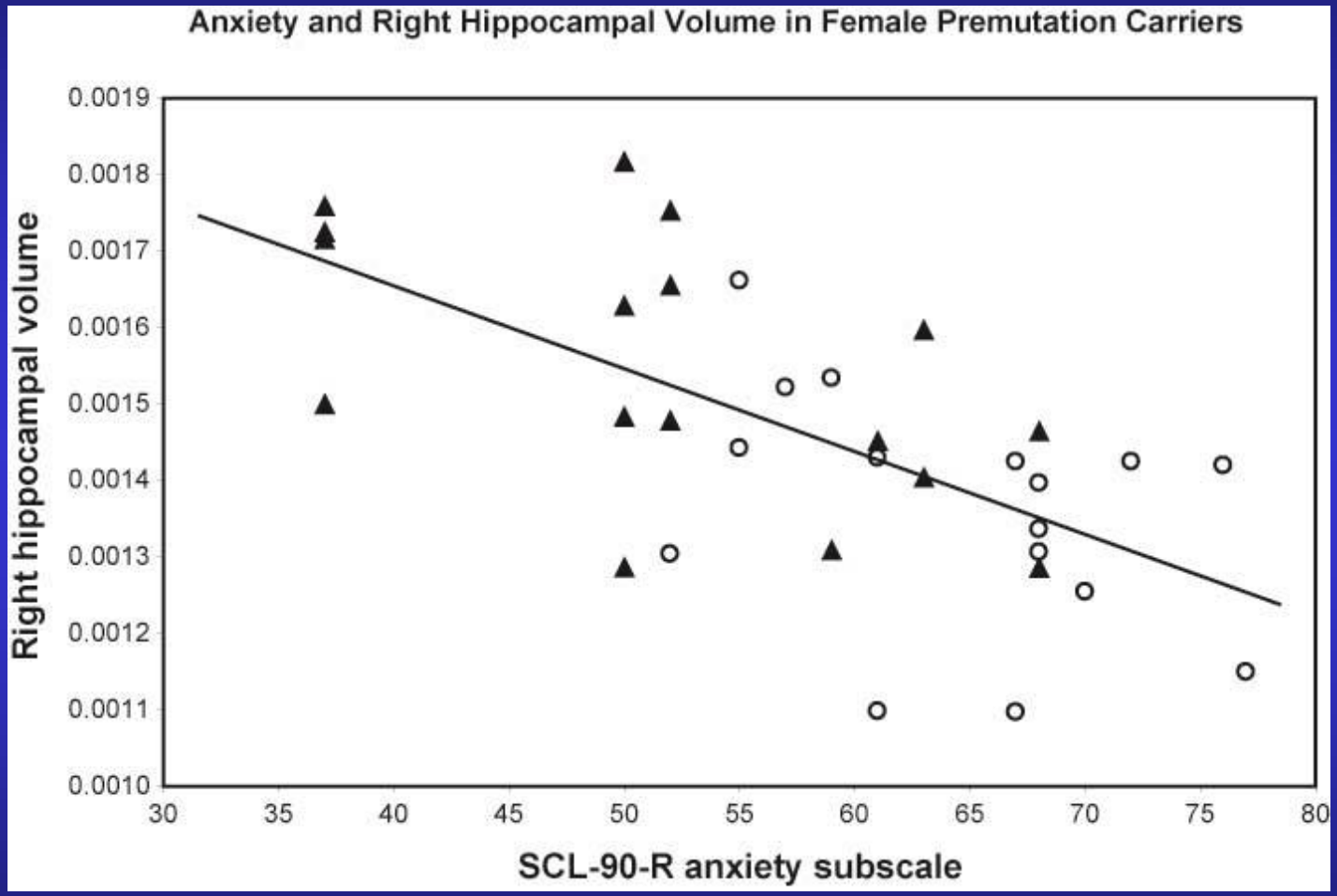
Table 1. Summary of SCID-I Longitudinal Data

Lifetime DSM-IV Disorder	*FMR1* Time 1 [subset of Roberts *et al.* (3)], % (*n*)	*FMR1* Time 2, % (*n*)
Mood Disorders	51.81 (43)	60.24 (50)
Major depressive disorder	45.78 (38)	54.22 (45)
Dysthymia	1.20 (1)	1.20 (1)
Bipolar disorder I or II	4.82 (4)	4.82 (4)
Anxiety Disorders	27.71 (23)	34.94 (29)
Panic disorder with agoraphobia	3.61 (3)	6.02 (5)
Panic disorder without agoraphobia	7.23 (6)	8.43 (7)
Agoraphobia without panic	3.61 (3)	3.61 (3)
Social phobia	8.43 (7)	9.64 (8)
Posttraumatic stress disorder	4.82 (4)	6.02 (5)
Specific phobia	4.82 (4)	7.23 (6)
Generalized anxiety disorder	6.02 (5)	10.84 (9)
Any Mood or Anxiety Disorder	59.04 (49)	66.27 (55)

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

Roberts et al 2016 Biological Psychiatry

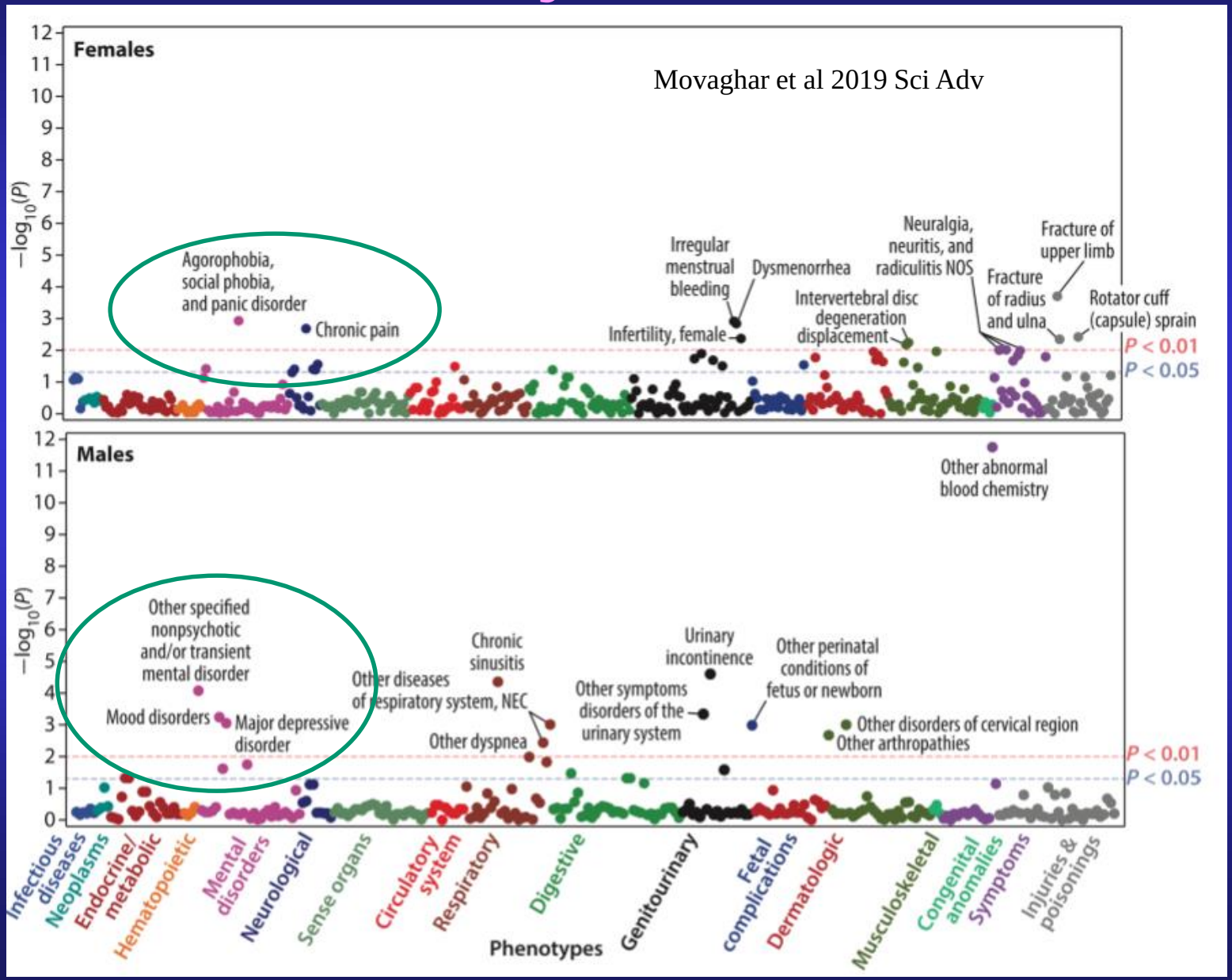
Anxiety and Hippocampal Volumes in Females with the Premutation



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

Adams et al 2009

Marshfield study validates FXAND



Clustering of conditions in 355 pre women

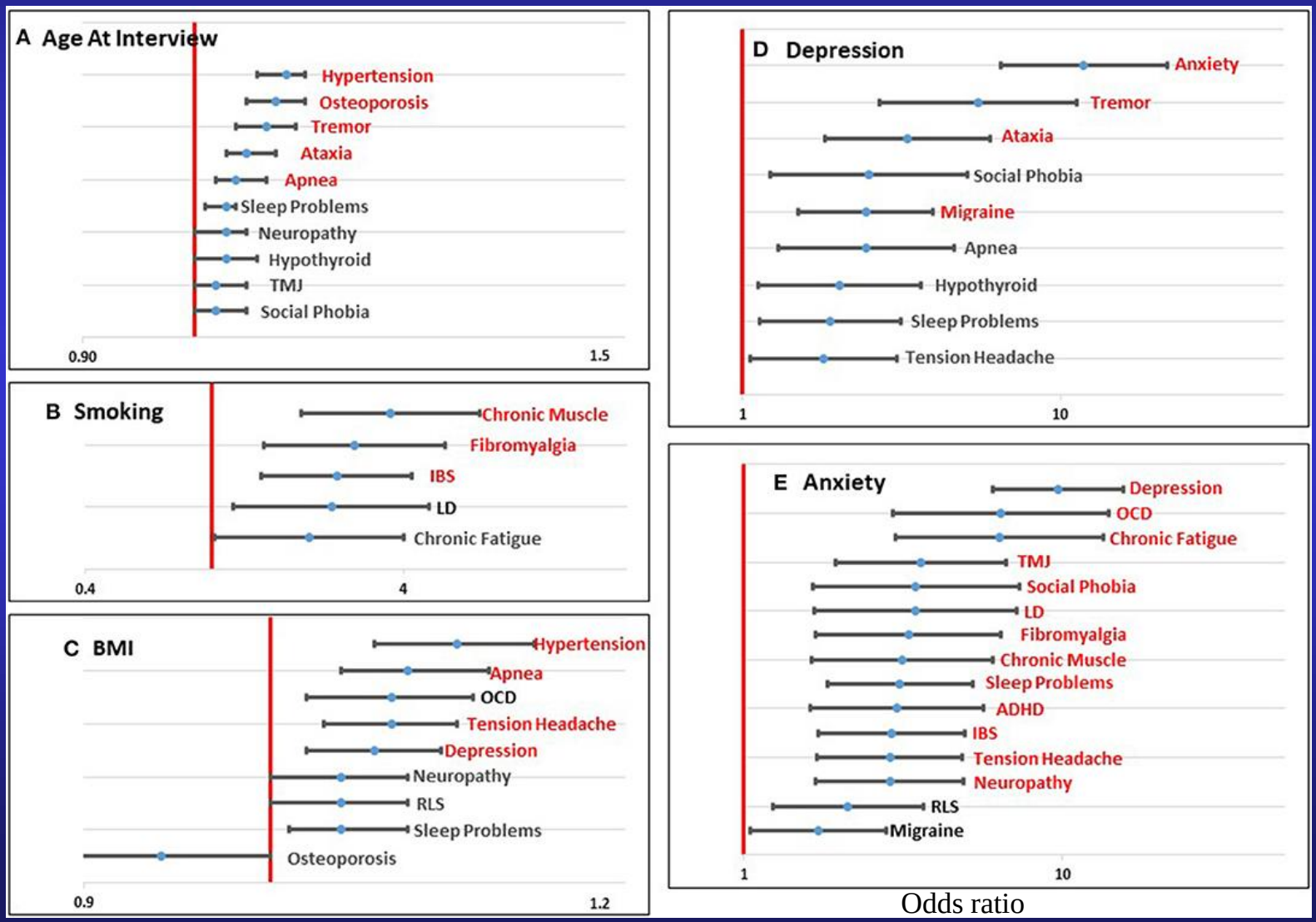
Heat map showing frequencies of reported conditions within each cluster.

	Minimal health problems	Headaches	Sleep problems	Mental health problems	FXPOI			FXTAS symptoms
					Minimal health problems	Mental health problems	Complex profiles	
Anxiety	0.02	0.09	0.43	0.92	0.51	0.89	0.89	0.00
Depression	0.10	0.21	0.67	0.75	0.21	0.86	0.59	0.36
Migraine	0.02	1.00	0.52	0.54	0.01	0.75	0.67	0.36
Tension headache	0.12	0.58	0.38	0.71	0.07	0.61	0.74	0.00
Sleep problems	0.01	0.06	0.76	0.17	0.42	0.61	0.74	0.09
Neuropathy	0.10	0.06	0.29	0.67	0.04	0.20	0.56	0.73
IBS	0.10	0.15	0.19	0.79	0.07	0.02	0.81	0.09
Osteoporosis	0.03	0.09	0.43	0.00	0.46	0.16	0.26	0.36
Hypothyroidism	0.14	0.06	0.43	0.08	0.10	0.30	0.26	0.36
Hypertension	0.17	0.06	0.48	0.04	0.09	0.32	0.11	0.27
Restless leg syndrome	0.11	0.27	0.29	0.13	0.03	0.25	0.26	0.18
Ataxia	0.01	0.06	0.48	0.13	0.09	0.02	0.48	1.00
Sleep apnea	0.07	0.03	0.86	0.08	0.03	0.16	0.22	0.09
Chronic muscle pain	0.05	0.03	0.29	0.08	0.01	0.11	0.78	0.00
Social phobia	0.04	0.00	0.24	0.21	0.00	0.36	0.33	0.09
Fibromyalgia	0.02	0.00	0.33	0.13	0.00	0.16	0.74	0.00
Chronic fatigue syndrome	0.02	0.00	0.19	0.00	0.04	0.18	0.85	0.00
TMJ	0.03	0.00	0.29	0.38	0.13	0.05	0.33	0.00
OCD	0.00	0.03	0.33	0.33	0.04	0.32	0.19	0.00
ADHD	0.06	0.00	0.29	0.00	0.09	0.18	0.37	0.00
LD	0.02	0.18	0.24	0.04	0.09	0.23	0.19	0.00
Tremor	0.00	0.03	0.38	0.13	0.07	0.07	0.22	0.73

Allen et al 2020 Genet Med

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)

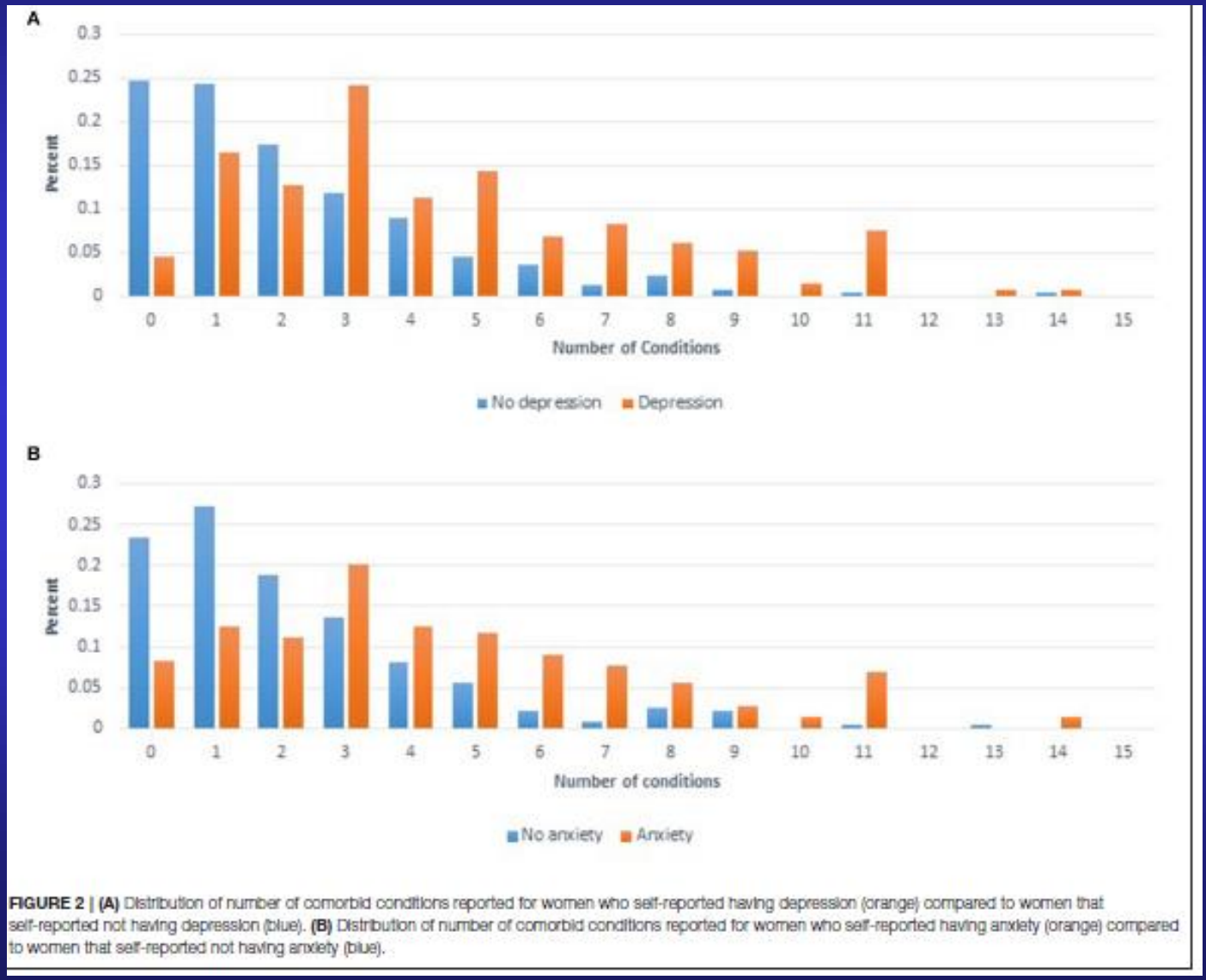
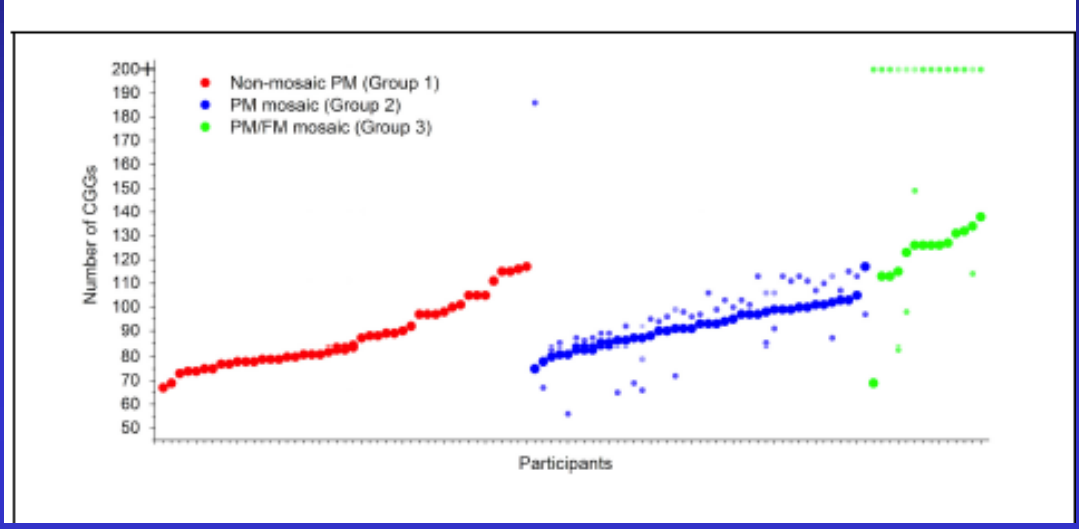


FIGURE 2 | (A) Distribution of number of comorbid conditions reported for women who self-reported having depression (orange) compared to women that self-reported not having depression (blue). **(B)** Distribution of number of comorbid conditions reported for women who self-reported having anxiety (orange) compared to women that self-reported not having anxiety (blue).

Health Profiles in mosaic and non mosaic premutation women

(Mailick et al 2018 Frontiers in Genetics)



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

*$^{+}p < 0.10$, ***$p < 0.001$. [a]For 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)

Variables	Score	Non-mosaic PM (n = 45)	Mosaic PM (n = 41)	Mosaic PM/FM (n = 14)	F-value[a]
1. I felt anxious during the past week (POMS anxiety)	5.83	62.2%	80.5%	28.6%	7.09**
2. I had hot flushes/flashes during menopause	4.44	64.3%	70.3%	16.7%	6.04**
3. I feel worn out (SF-36)	3.89	80.0%	89.7%	85.7%	0.55
4. I am impulsive (BRIEF-A)	3.66	53.3%	53.7%	7.1%	5.74**
5. I expect my health to get worse (SF-36)	3.62	8.9%	27.5%	14.3%	3.19*
6. I start things at the last minute (BRIEF-A)	3.50	80.0%	56.1%	42.9%	5.47**
7. I have trouble finishing tasks (BRIEF-A)	3.42	64.4%	56.1%	14.3%	6.14**
8. I felt uneasy during the past week (POMS Anxiety)	3.24	42.2%	65.9%	35.7%	3.25*
9. I had trouble keeping my mind on what I was doing (CES-D)	3.20	55.6%	63.4%	42.9%	1.10
10. I have been a nervous person (SF-36)	3.11	66.7%	90.0%	64.3%	3.89*
11. My health limits me in walking several blocks (SF-36)	2.79	11.1%	35.0%	7.1%	4.92**
12. My health limits me in walking more than a mile (SF-36)	2.72	20.0%	42.5%	7.1%	4.62*
13. After having a problem, I don't get over it easily (BRIEF-A)	2.63	62.2%	80.5%	42.9%	4.20*
14. I have trouble sitting still (BRIEF-A)	2.54	64.4%	43.9%	35.7%	3.63*
15. I felt nervous during the past week (POMS Anxiety)	2.42	42.2%	61.0%	21.4%	3.97*
16. I talk at the wrong time (BRIEF-A)	2.23	55.6%	51.2%	14.3%	4.37*
17. Lifetime diagnosis of anxiety, depression, or other emotional disorder	2.21	46.7%	43.9%	7.1%	3.94*
18. Lifetime diagnosis of arthritis, rheumatism, osteoporosis, or other bone or joint disease	1.72	37.8%	26.8%	0.0%	3.93*
19. Total number of prescription medications	1.27	2.28	2.07	1.21	0.59
20. I had depression during menopause	1.18	31.0%	27.0%	0.0%	2.57+
21. I had feelings of pain, aches, tingling or cramps during the past week (MDS-UPDRS)	1.01	77.8%	78.0%	50.0%	2.49+

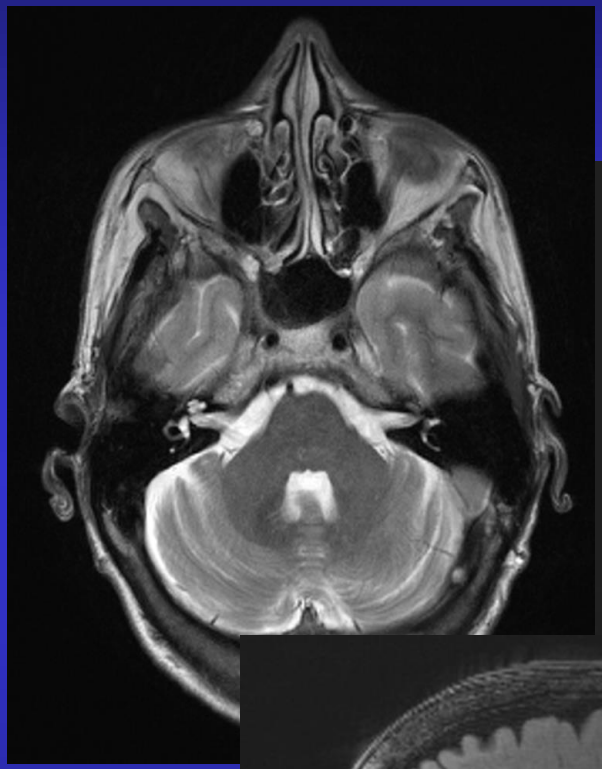
*+$p < 0.10$, *$p < 0.05$, **$p < 0.01$. [a]F-value based on ANCOVA with age as a covariate.*

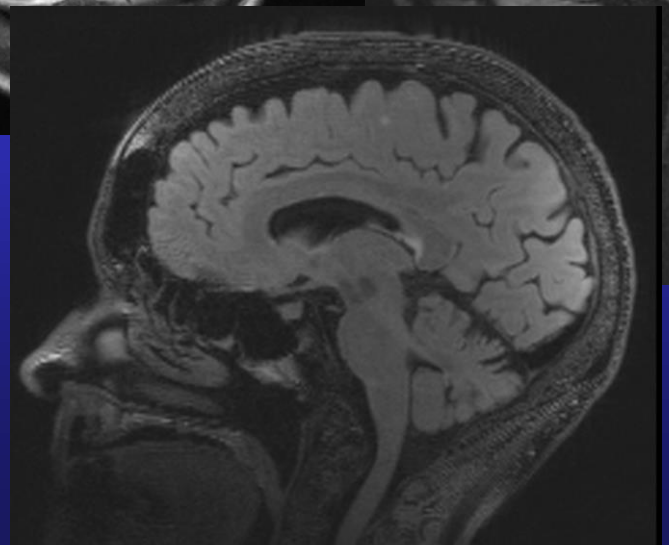
64 yo woman with FXTAS rapid course related to psychiatric and medical problems

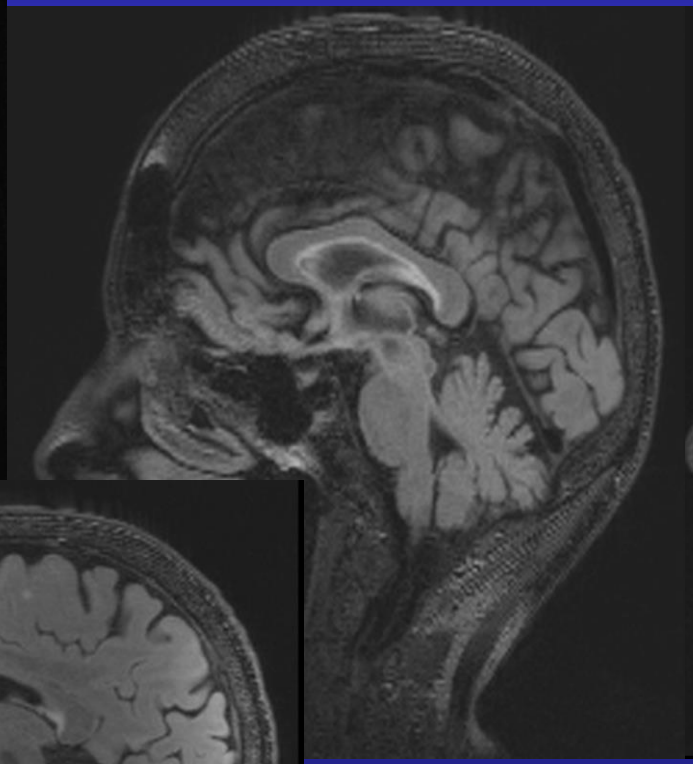
Decline over 7 mo in 64 y woman with FXTAS; 31, 85 repeats

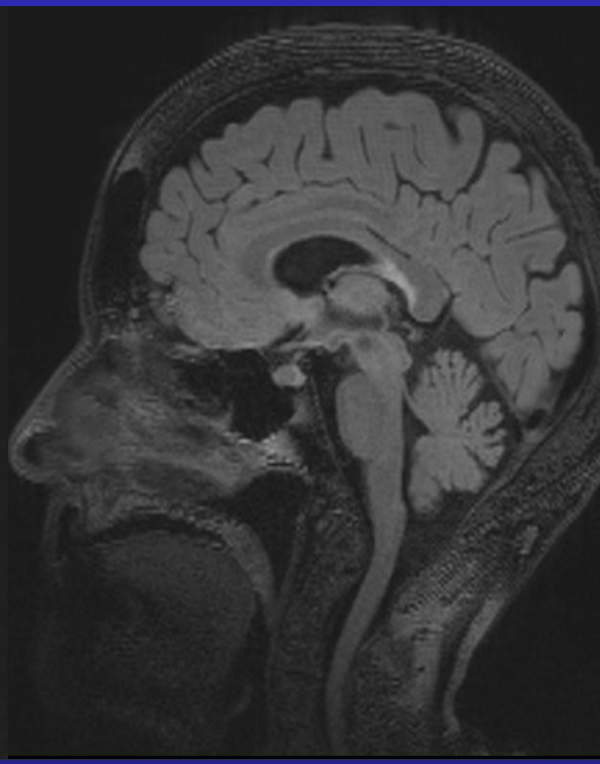
- Well until last November at age 63 when her mother died from FXTAS, she was the caretaker and depressed with mother's death and she was subsequently hospitalized for type 1 diabetes newly diagnosed
- Panic attacks began in January so began fluoxetine and then switched to sertraline
- Neuropathy problems 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



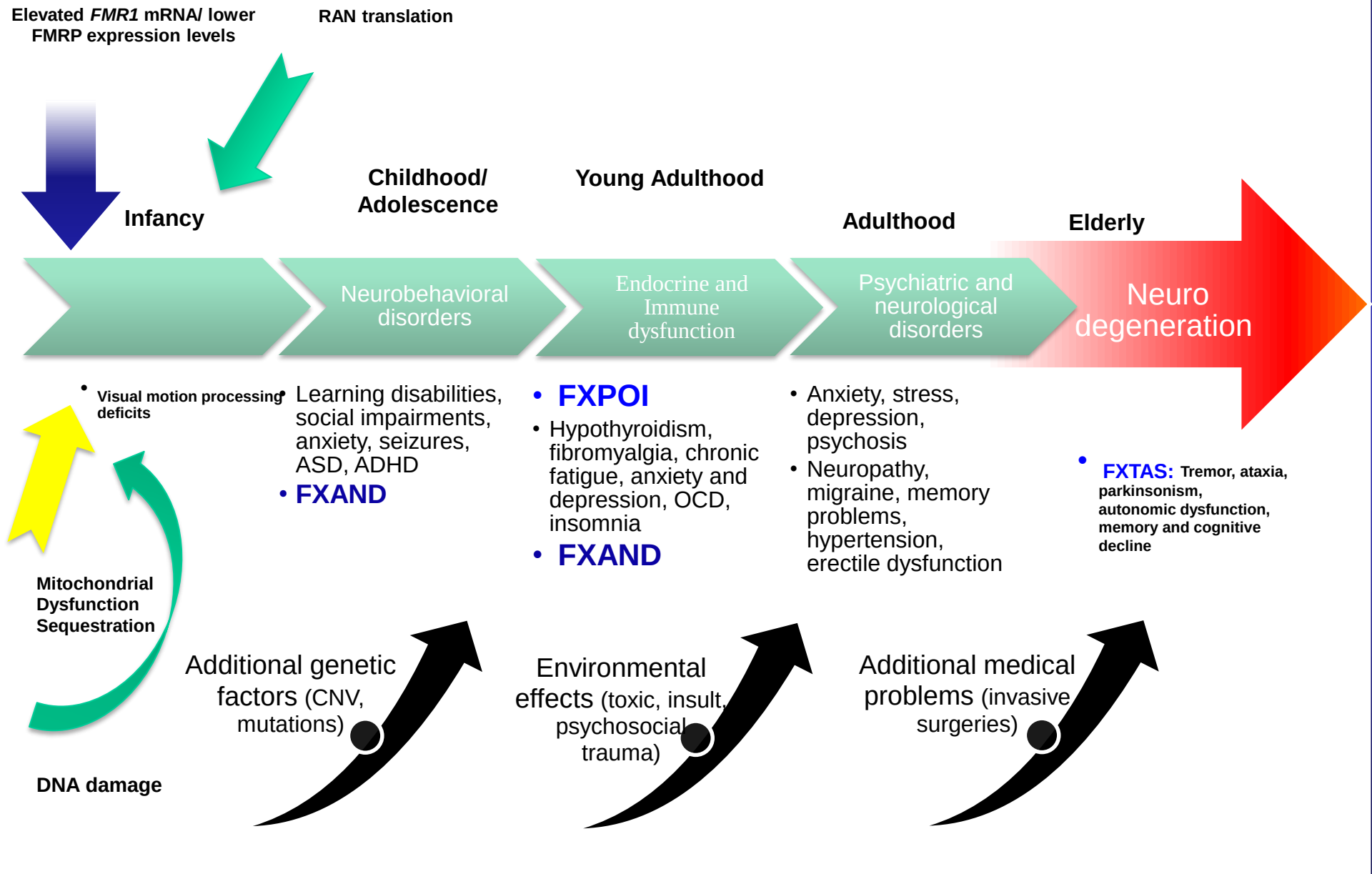






Splenium sign and WMH spots in cerebrum

Premutation involvement across the lifespan



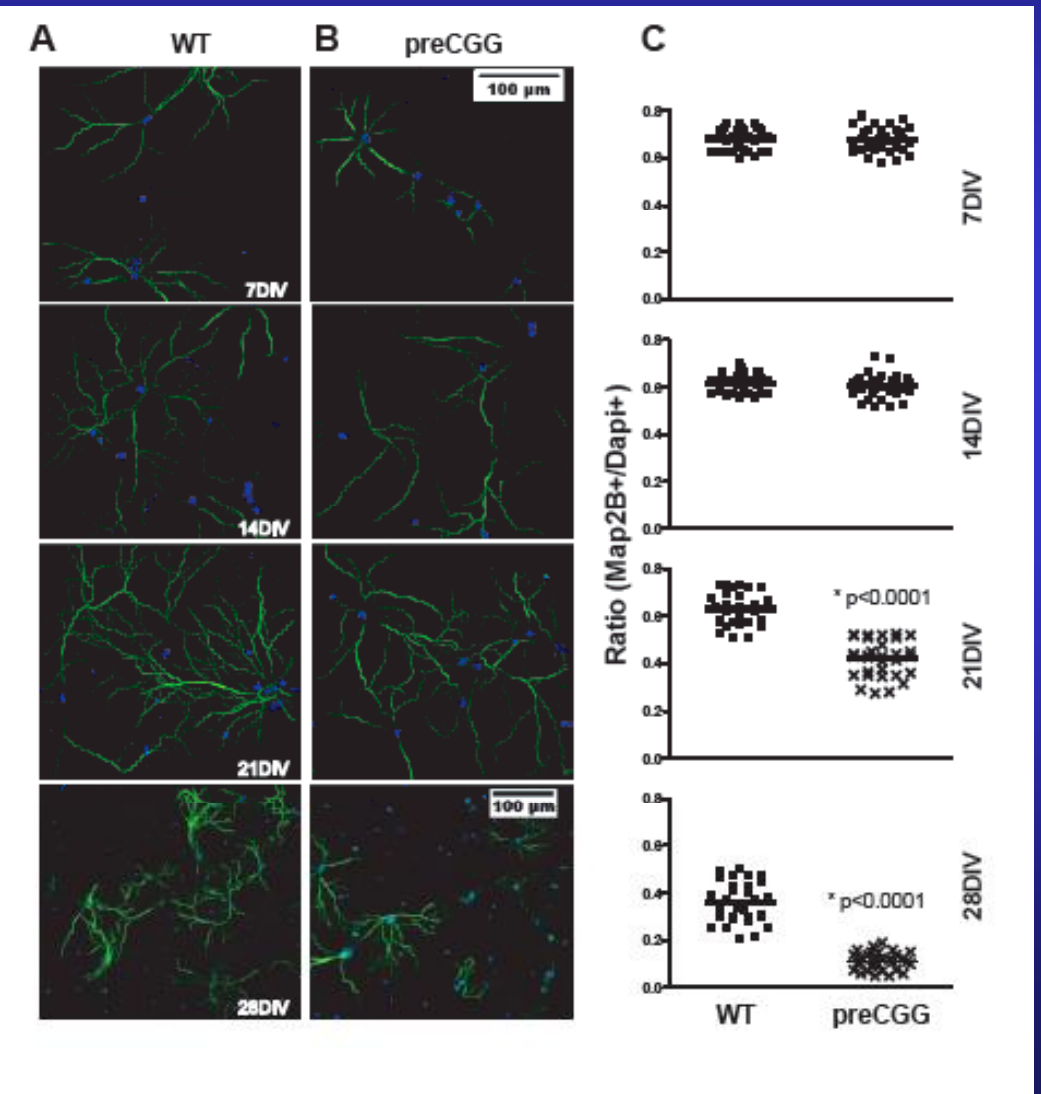
Some get FXTAS and some do not

- 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

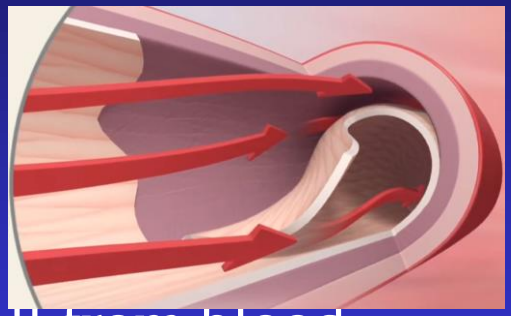
- Low FMRP leads to elastin abnormalities and MMP9 elevation leading to changes in the extracellular matrix and many of the physical features of FXS
  - Prominent and long ears, soft velvet like skin, joint dislocations, hyperextensible finger joints, high-arched palate, flat feet, mitral valve prolapse, 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

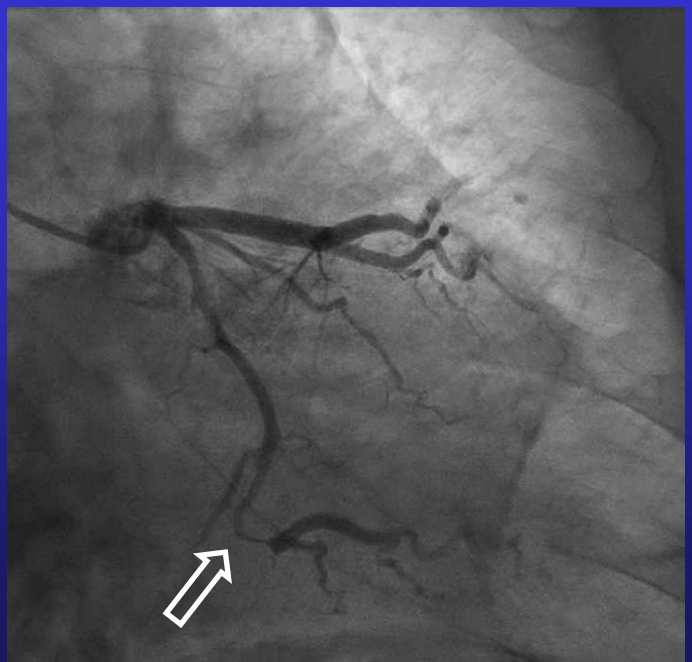


- SCAD is defined by tearing of the arterial wall from blood dissection and no hx of atherosclerotic heart disease

- 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

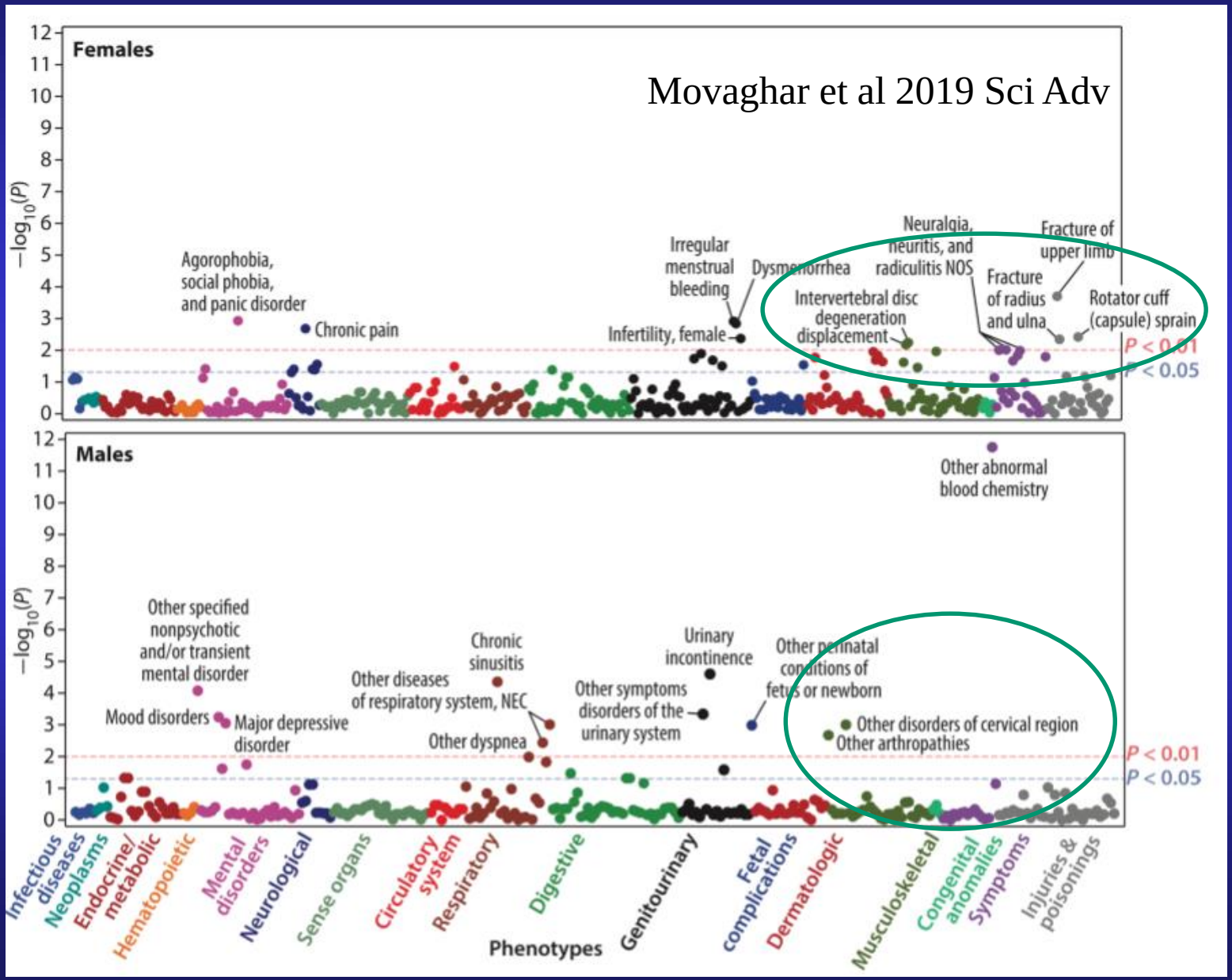
SCAD Case 1: Clinical Background

- 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

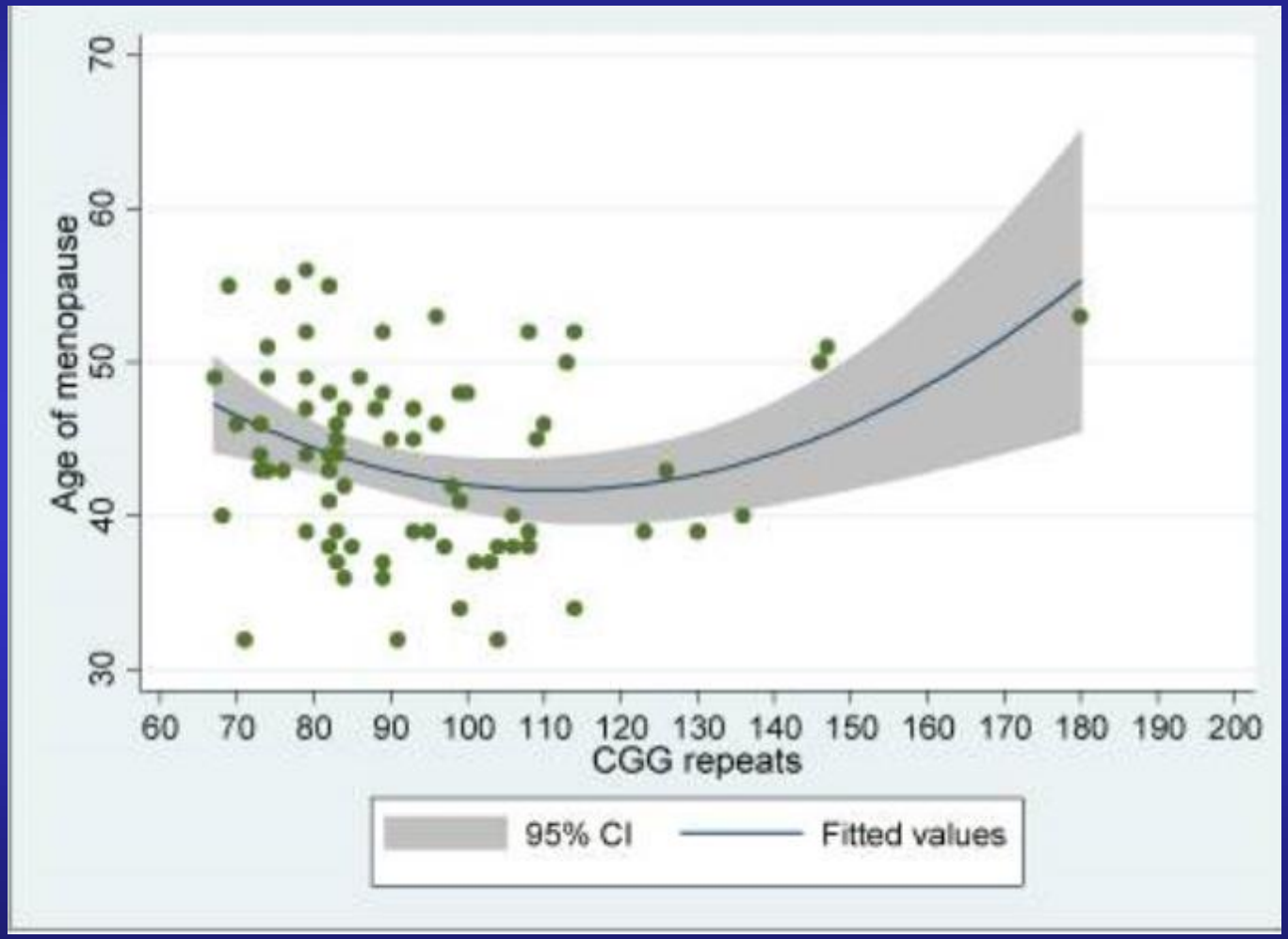


Angiogram demonstrated SCAD at large circumflex artery OM1 branch

Marshfield study validates connective tissue problems



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)

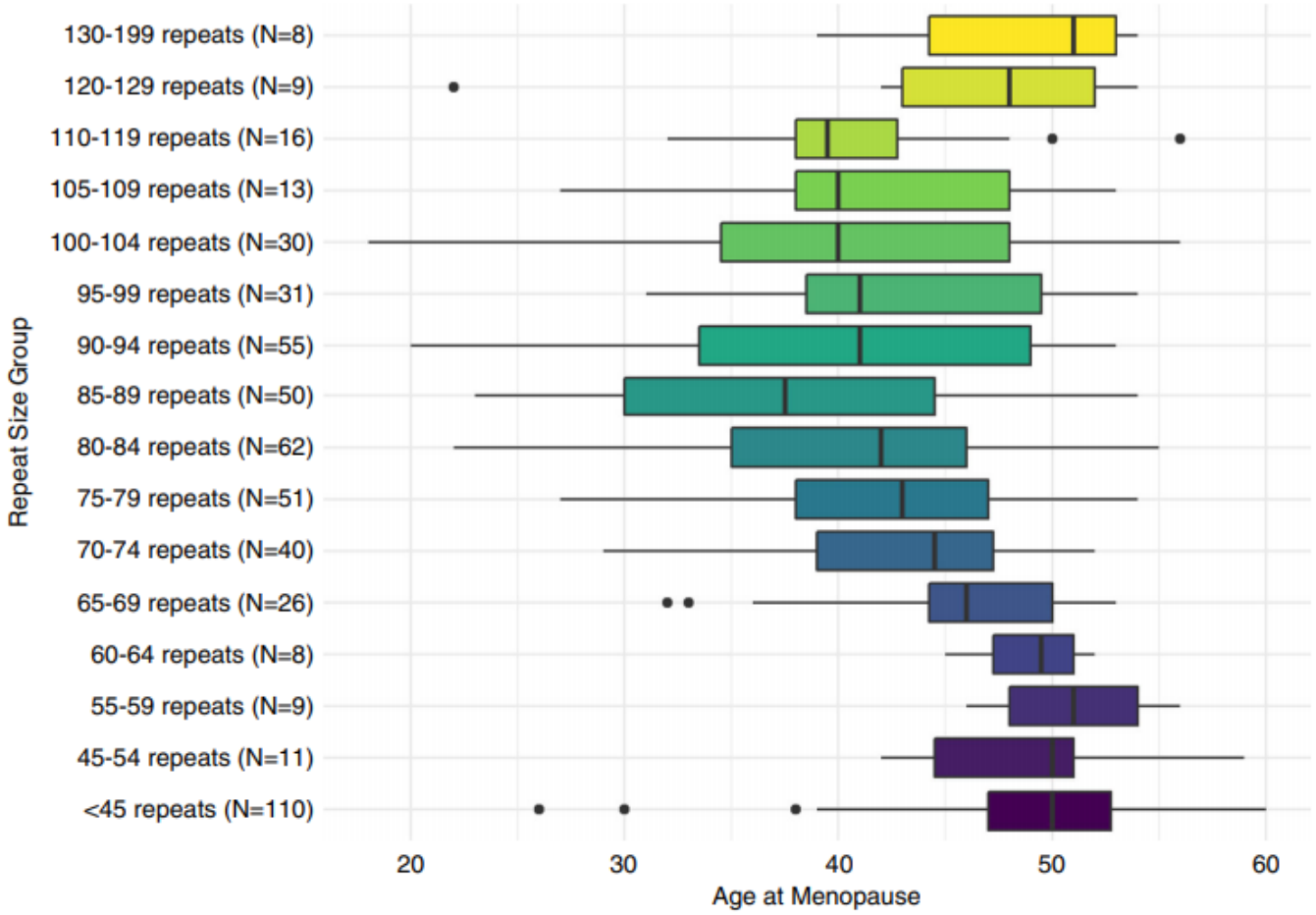


Fig. 1 Box plot of age at menopause distribution by repeat size group. Vertical lines within the box from left to right represent the lower quartile, the median, and the upper quartile, respectively. The horizontal lines represent the 5th and 95th percentiles, and the values beyond these lines, marked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

Risk to have a child with FXS relates to AGG anchors

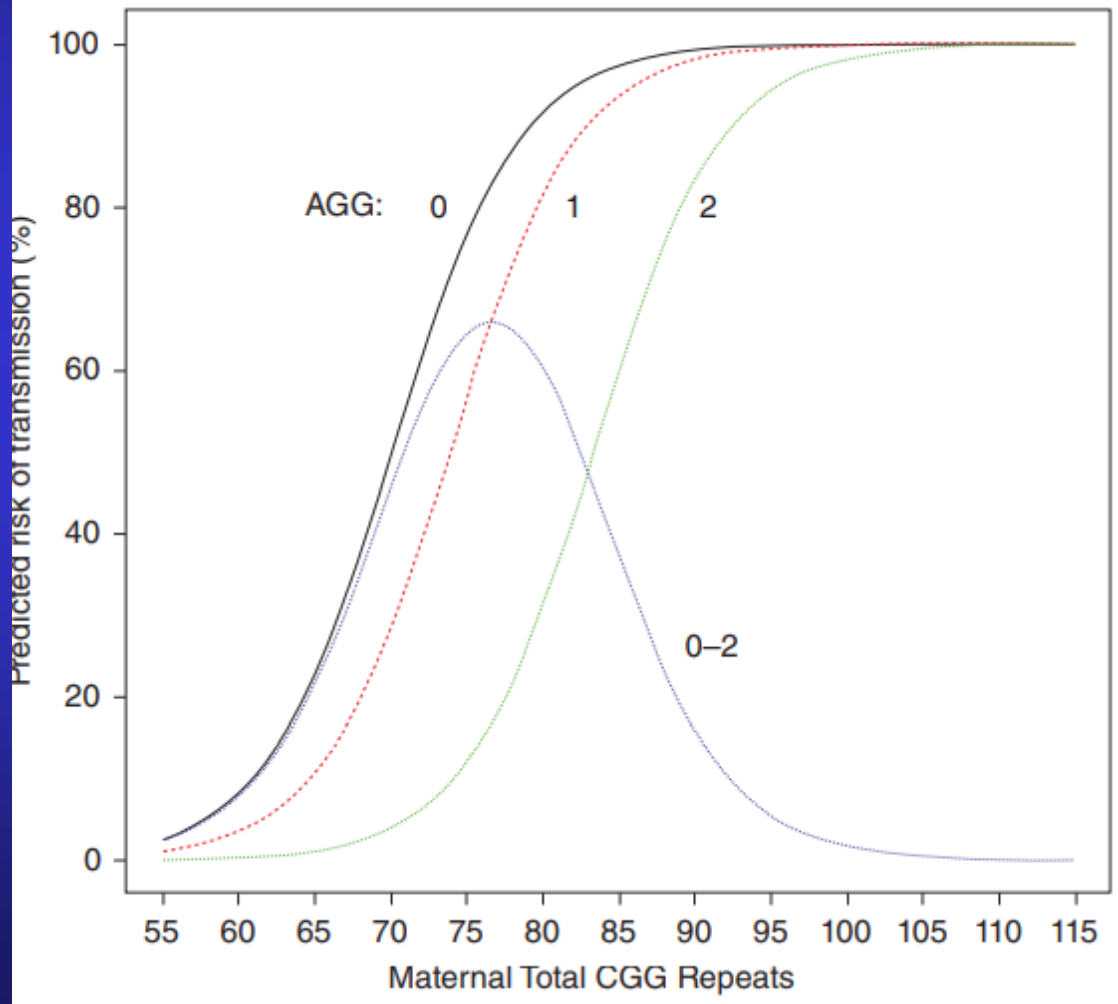
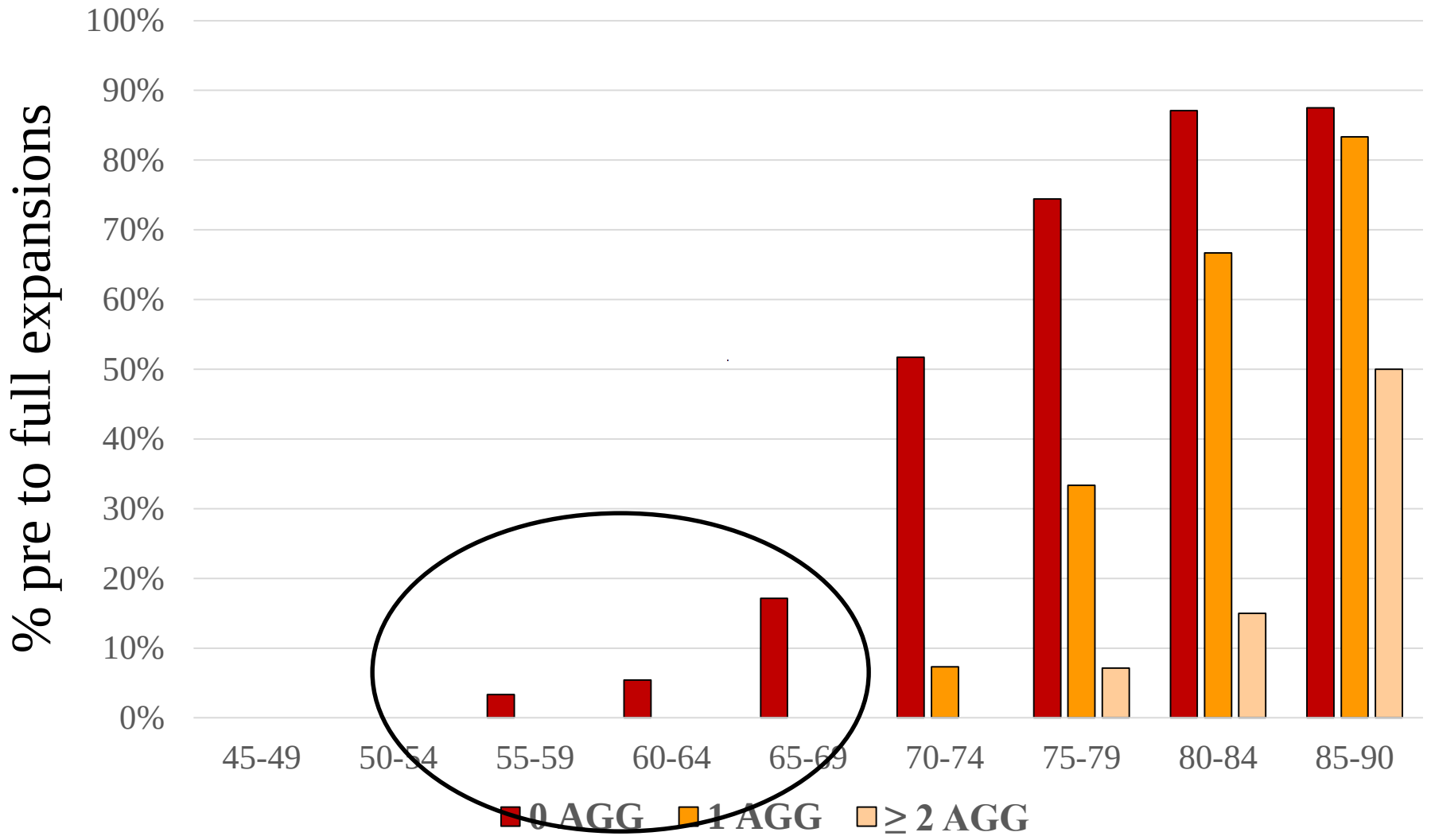


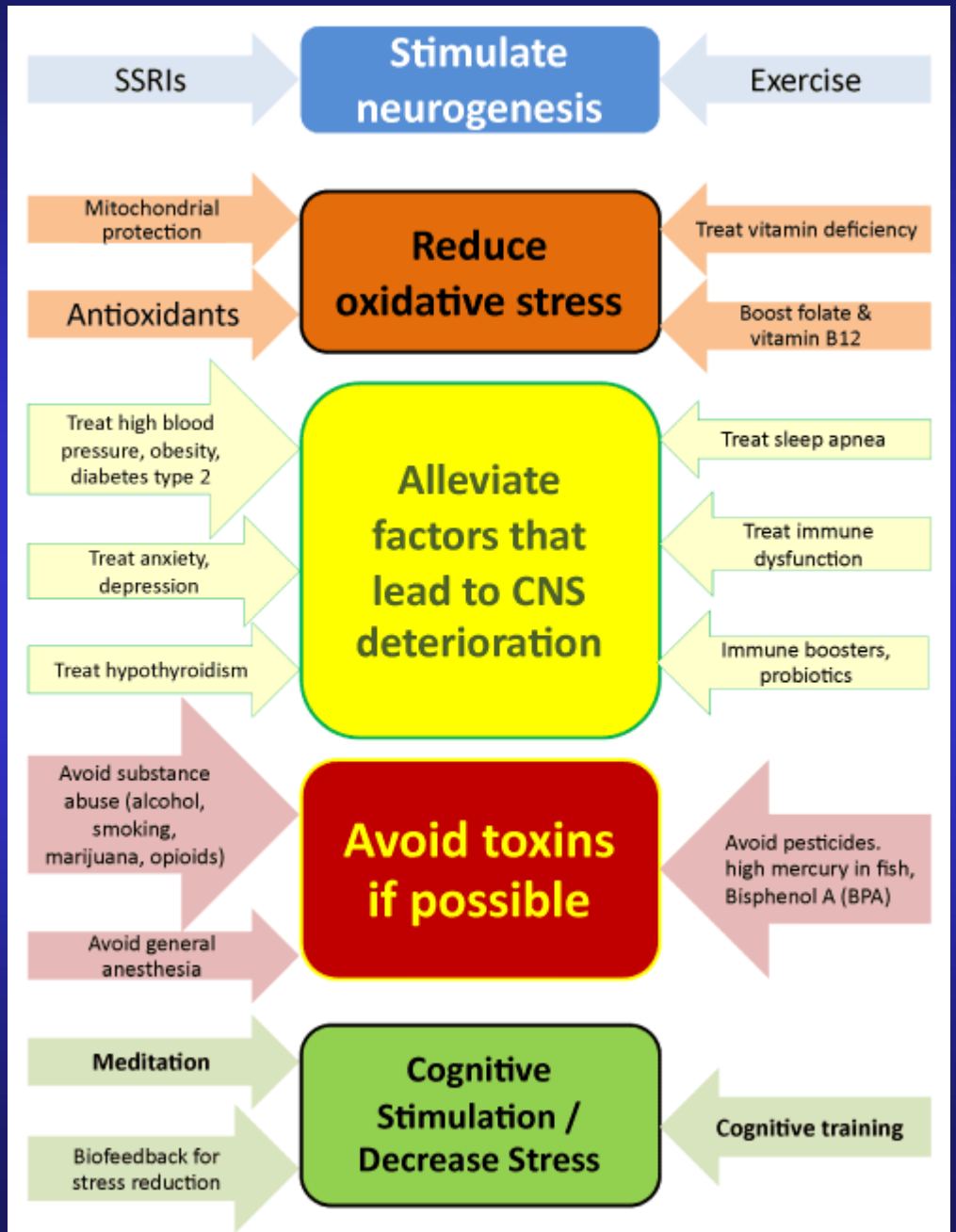
Figure 16.3 Estimated risk for expansion to the full-mutation range of a transmitted, maternal premutation CGG repeat is a sensitive function of the number of AGG interruptions in the maternal allele, decreasing with increasing number of interruptions. The differential risk between zero and two AGG interruptions (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.[157]

Expansion of a premutation to a full mutation depends on mother's repeat size and AGG interruptions



Nolin et al., 2014

Treatments for premutation carriers



Polussa et al 2014 Brain Disorders and Therapy

Symptomatic treatment in FXTAS

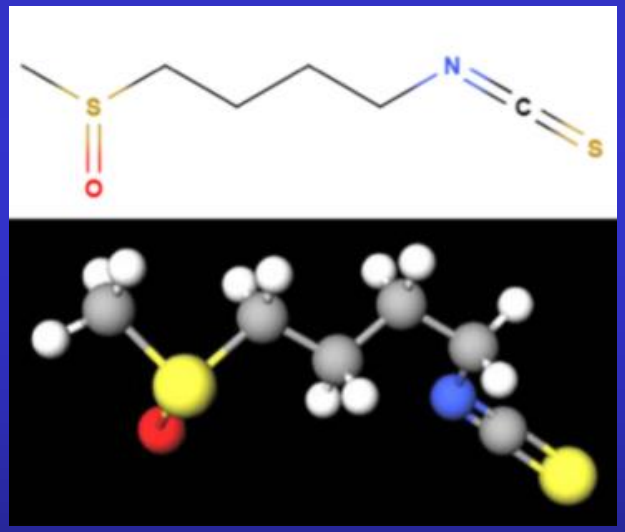
- Tremor: can respond to primidone, beta blocker, anticonvulsant (Keppra) or DBS
- Tremor can be parkinsonian and respond to Sinemet
- Ataxia is difficult to treat could try Amantadine, Riluzole
- Pain: treat with CBD, Gabapentin, or pregabalin or duloxetine (Cymbalta)
- Depression/ Anxiety: treat with 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)

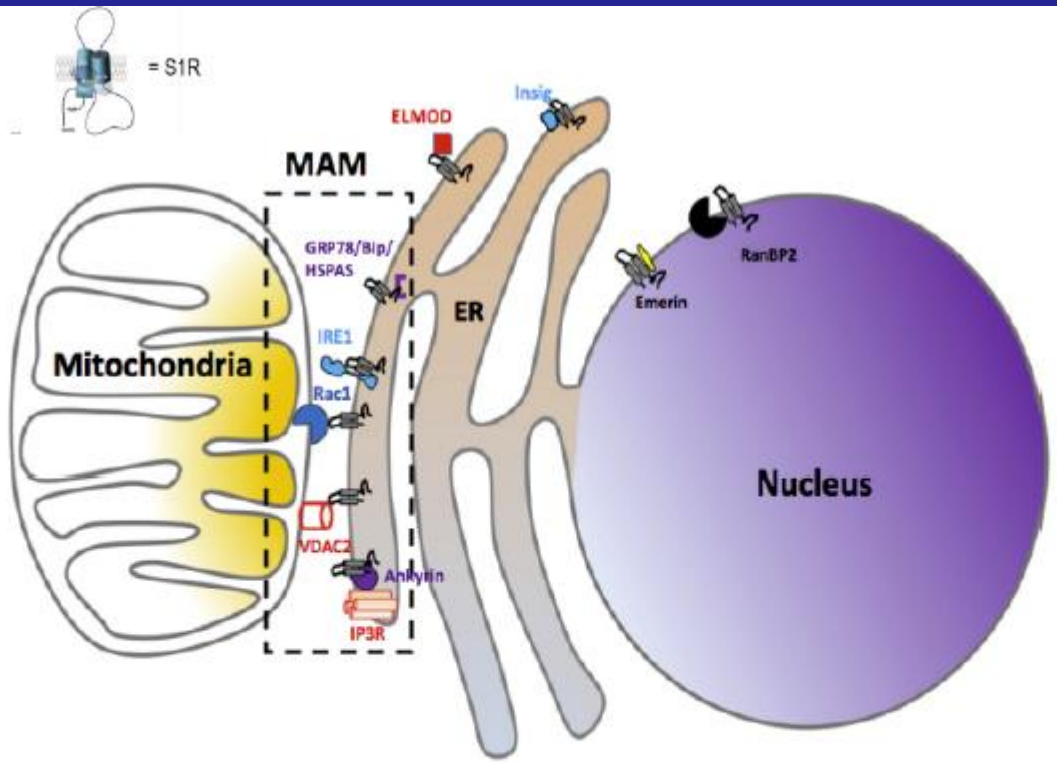
Sulforaphane (SFN) a dietary supplement that turns on the Nrf-2 antioxidant systems in cells

- 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

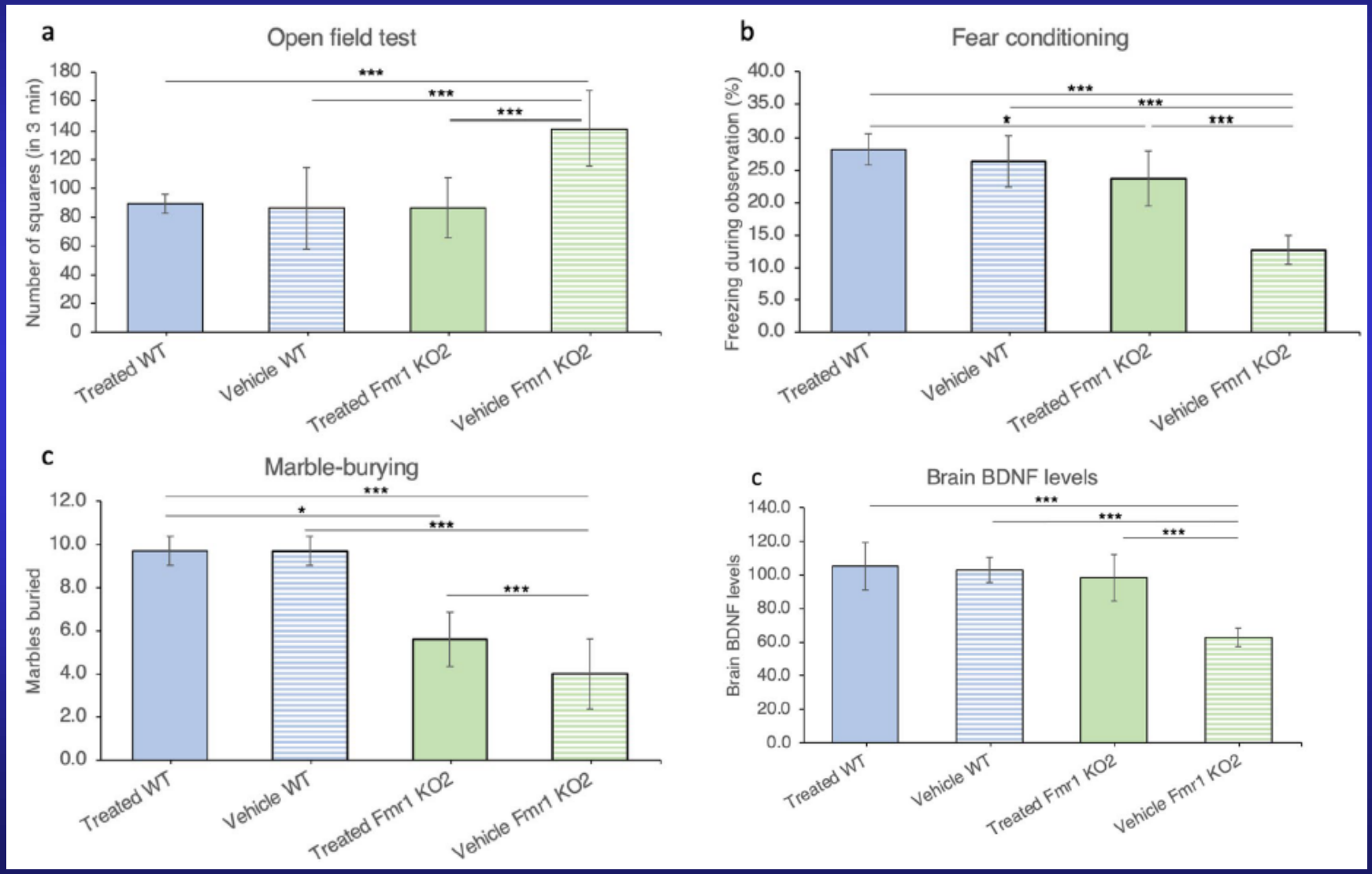


- Reducing mitochondrial dysfunction
- Reducing protein misfolding
- Modulating Ca^{2+}
- Reducing oxidative stress
- Reducing inflammation
- Enabling neuroprotection

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

ACS Chemical Neuroscience

Research Article

Cite This: *ACS Chem. Neurosci.* 2019, 10, 3778–3788

pubs.acs.org/chemneuro

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

International Fragile X Premutation Registry created with the NFXF and the Fragile X Association of Australia to facilitate research

Interested in joining the International Fragile X Premutation Registry?

Join now at fragilex.org/ifxpr

Collaborators

UC Davis School of Medicine

MIND Institute
Len Abbeduto
Kathy Angkustsiri
Mary Jae Leigh
Bibiana Restrepo
David Hessl
Susan Rivera
Andrea Schneider
Maria Jimena Salcedo
Sarah Dufek
Sumra Afzal
Hazel Biag
Yingatrana McLennan
Ellery Santos
Warda Haq
Jasdeep Shergill
Jun Yi Wang
Courtney Clark
Hilary Chason
Norman Brule

Dept. Radiology
James Brunberg

Dept. Neurology
Lin Zhang
John Olichney
Ricardo Maselli
Mike Rogawski

Dept of Psychiatry UCSF
Andreea Seritan

Dept. Biochem & Molec. Medicine
Paul Hagerman Flora Tassone
Glenda Espinal

Department of Rehabilitation
Veronica Martinez-Cerdeno

Colombia: Universidad del Valle: Wilmar Saldarriaga

CES Universidad: Juan Esteban

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

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