Fragile X: Lessons From Aging

Pacific Northwest Neurological Society 3-5-05

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Fragile X Syndrome

Fragile site

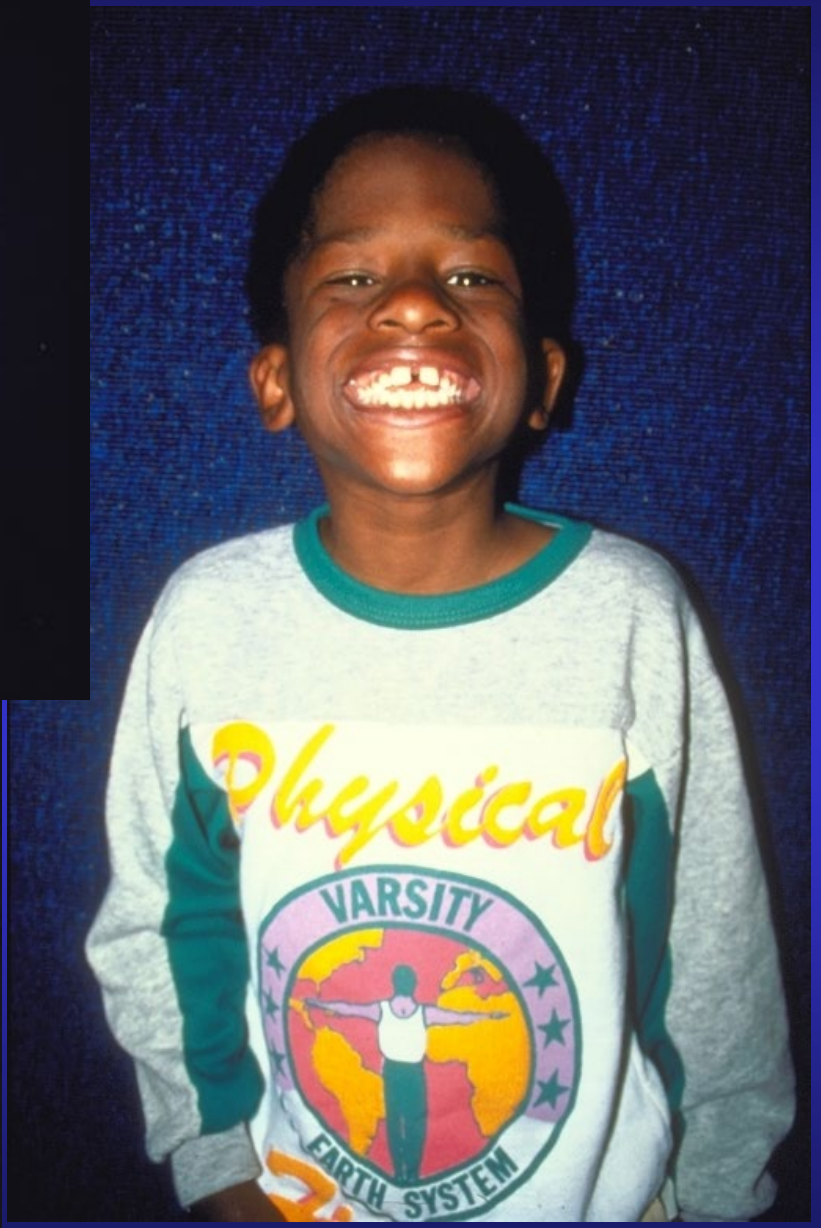
Leading heritable form of mental retardation

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

Leading (known) single gene associated with autism

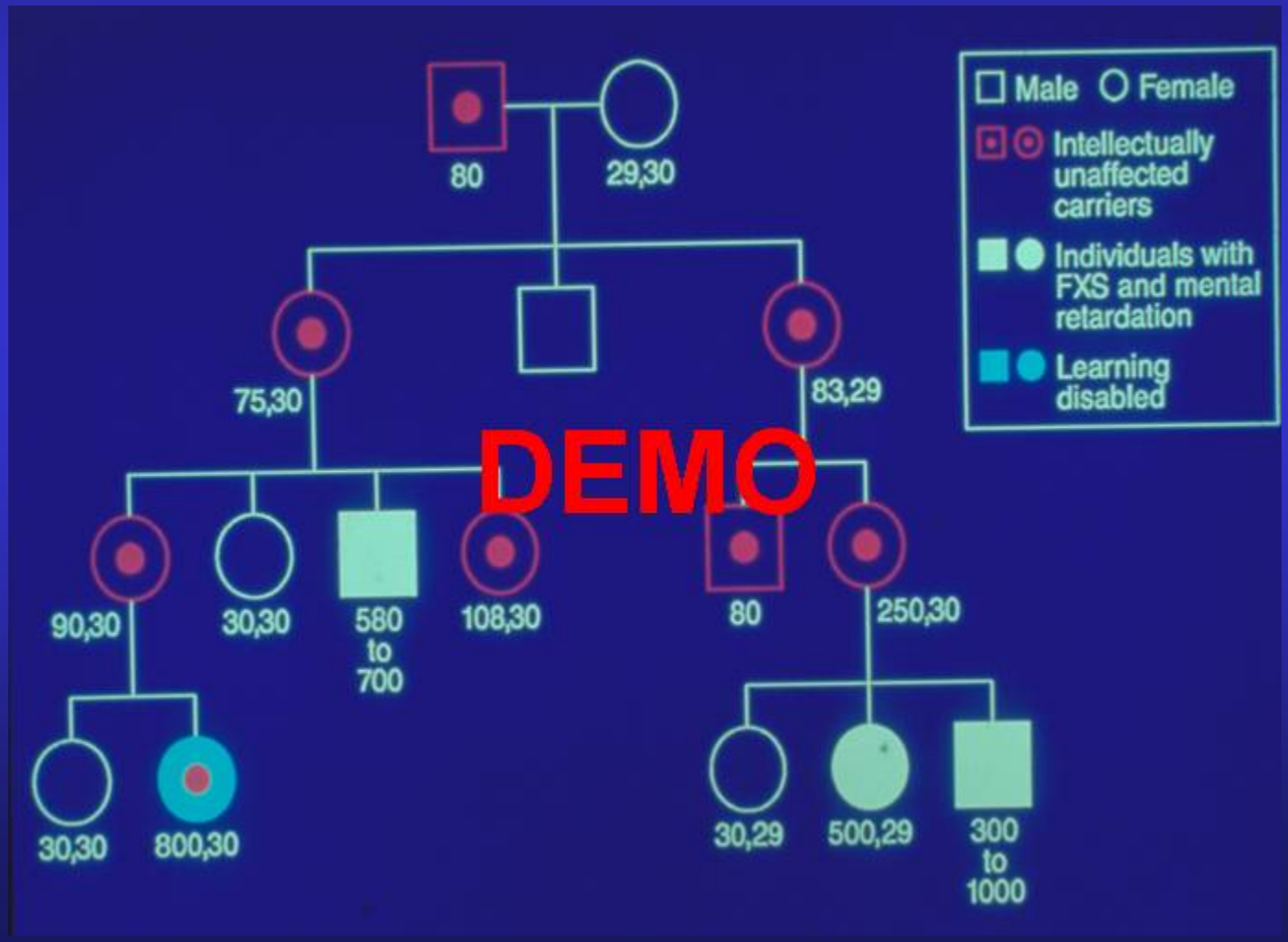
- 3-6% of all children with autism
- Approximately 30% of young children with fragile X syndrome have autism


DEMO


Physical
VARSITY
EARTH SYSTEM


DEMO

Normal: 5 to 44 CGG repeats
Gray Zone: 45 to 54
Premutation: 55 to 200
Full mutation: >200



Handbiting 60%

Handflapping 80%

Poor eye Contact 90%

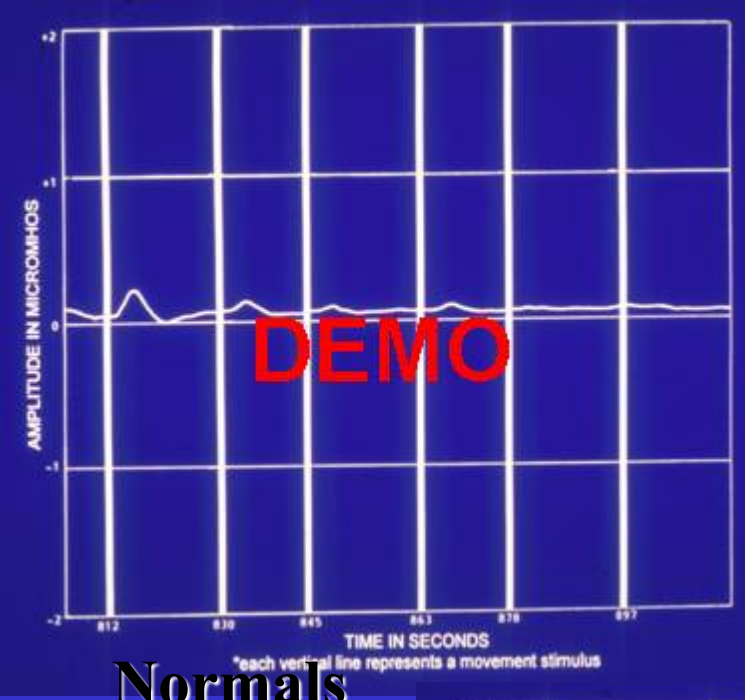
Tactile 80% defensiveness

Unusual sensory responses to stimuli

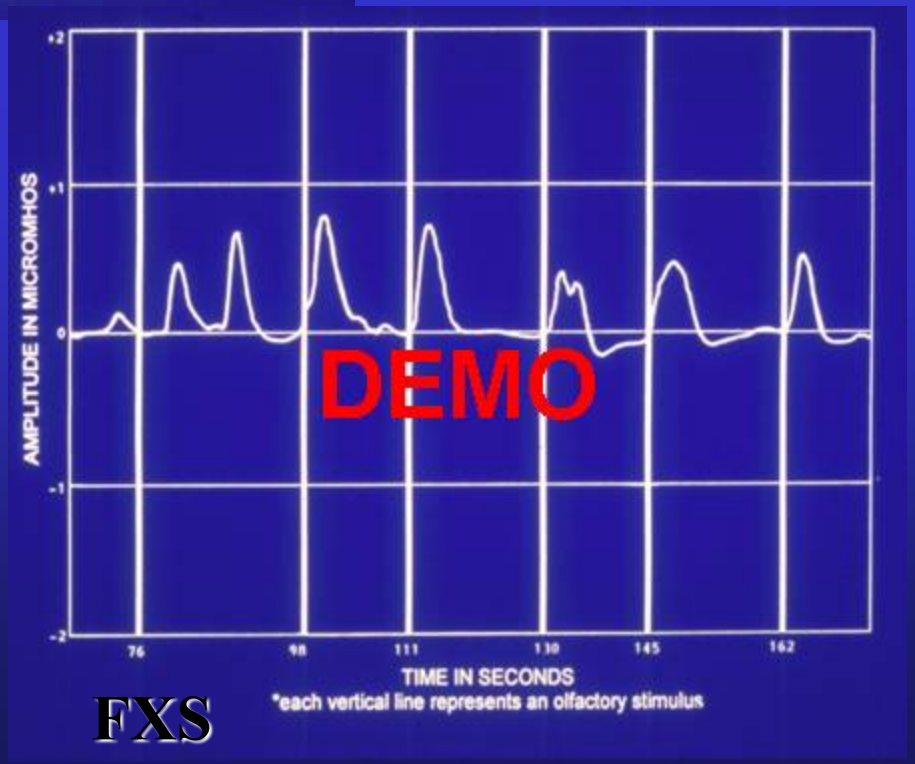
Perseverative speech or behavior in almost all-routines

One brother with autism and disinterested in people and the other without autism but both have the sensory hyperarousal and autistic features

Enhanced electrodermal responses to sensory stimuli correlate inversely with FMRP levels (Miller et al 1999)



Normals



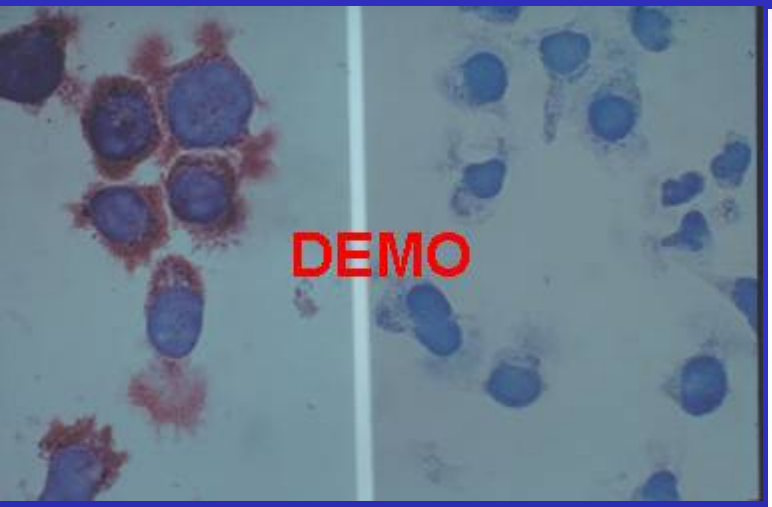
FXS

Emotional & Neurocognitive Features

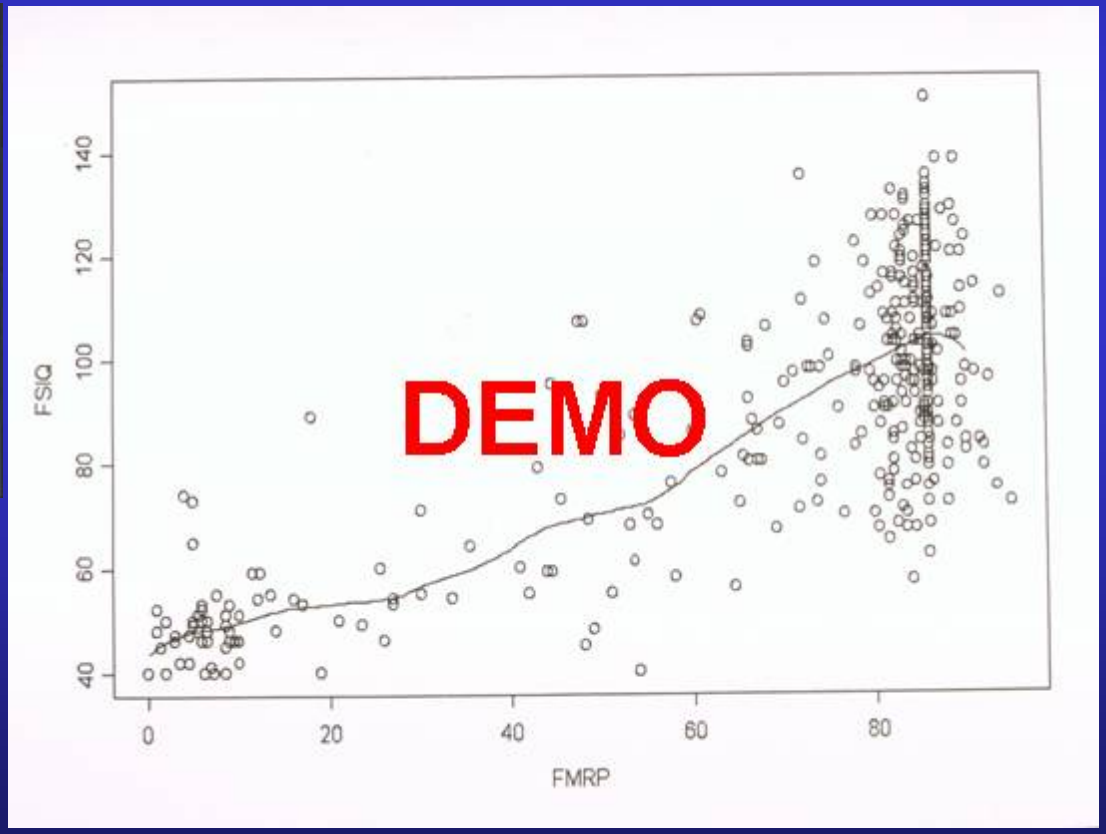
- Hyperactivity, impulsivity and/or short attention span
- Executive function deficits: problems with organization, shifting set, planning, inhibition tangential speech, schizotypal features, ADHD, perseveration
- Overreactivity to stimuli: enhanced electrodermal response to stimuli; enhanced cortisol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

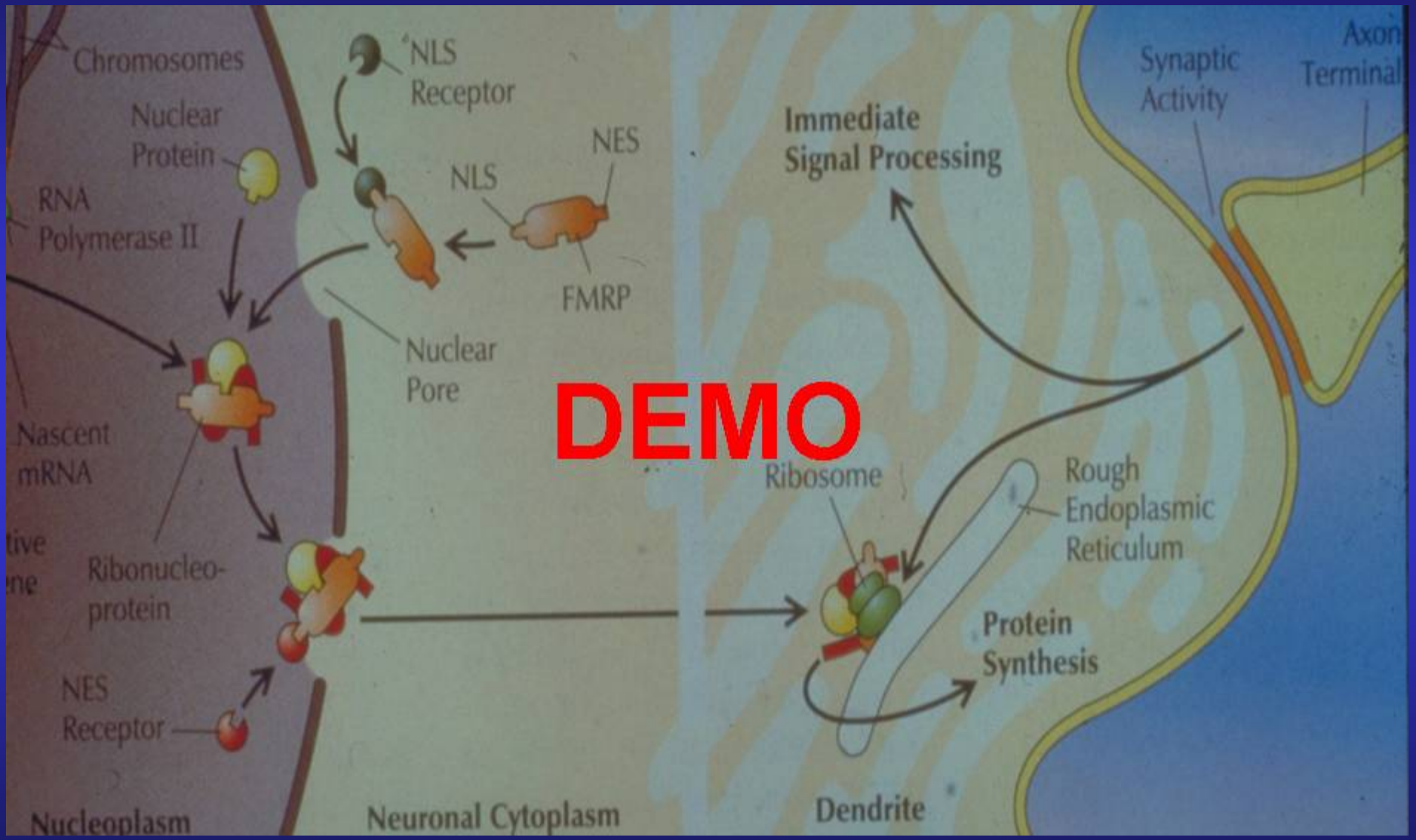
FMRP and IQ

Variations in FMRP levels correlate with IQ and neurocognitive involvement in FXS (Tassone et al 1999; Kaufmann et al 1999; Loesch et al 2002, 2003)



Anti-FMRP antibody stains lymphocytes + for FMRP (red)





Warren et al 1997

Genomics approach to the FXS Phenotype

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

Classification of Glutamate Receptors

Metabotropic (mGluRs)

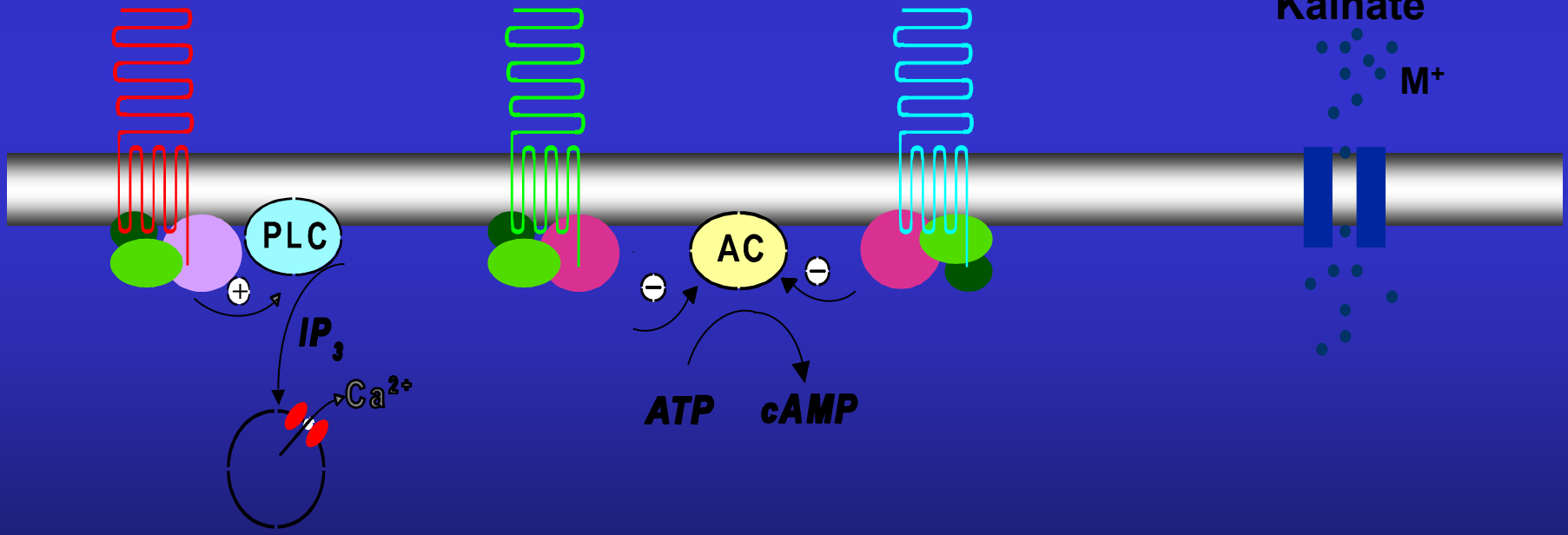
Ionotropic (iGluRs)

Group I
mGluR1,-5

Group II
mGluR2,-3

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

NMDA,
AMPA,
Kainate

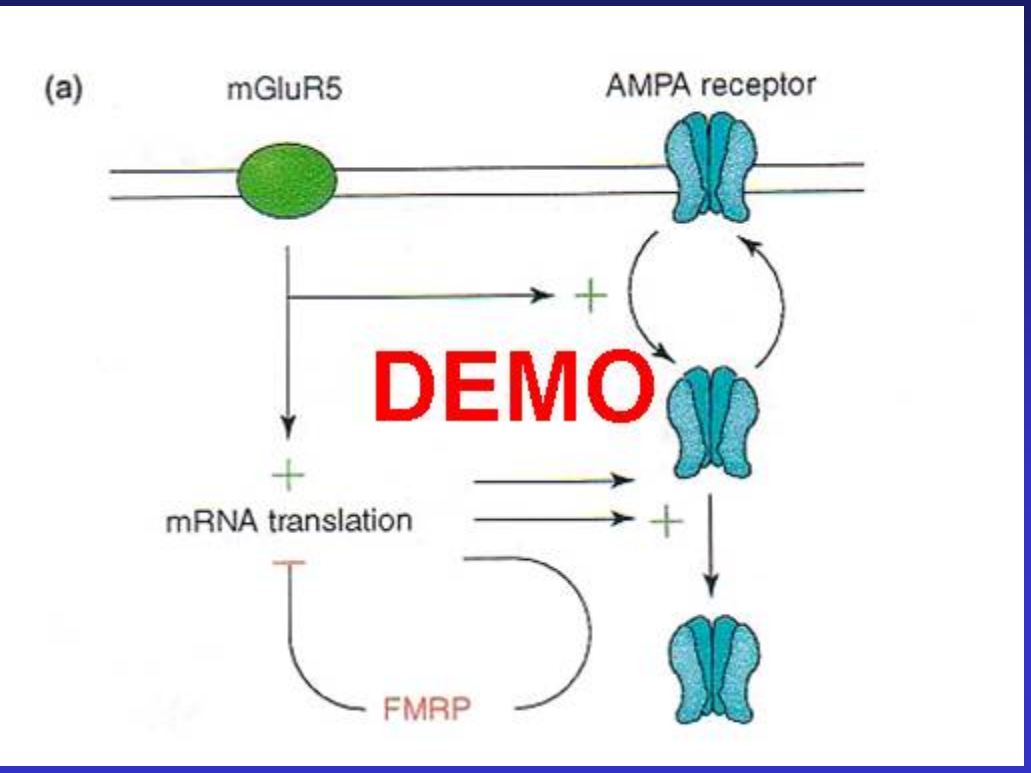


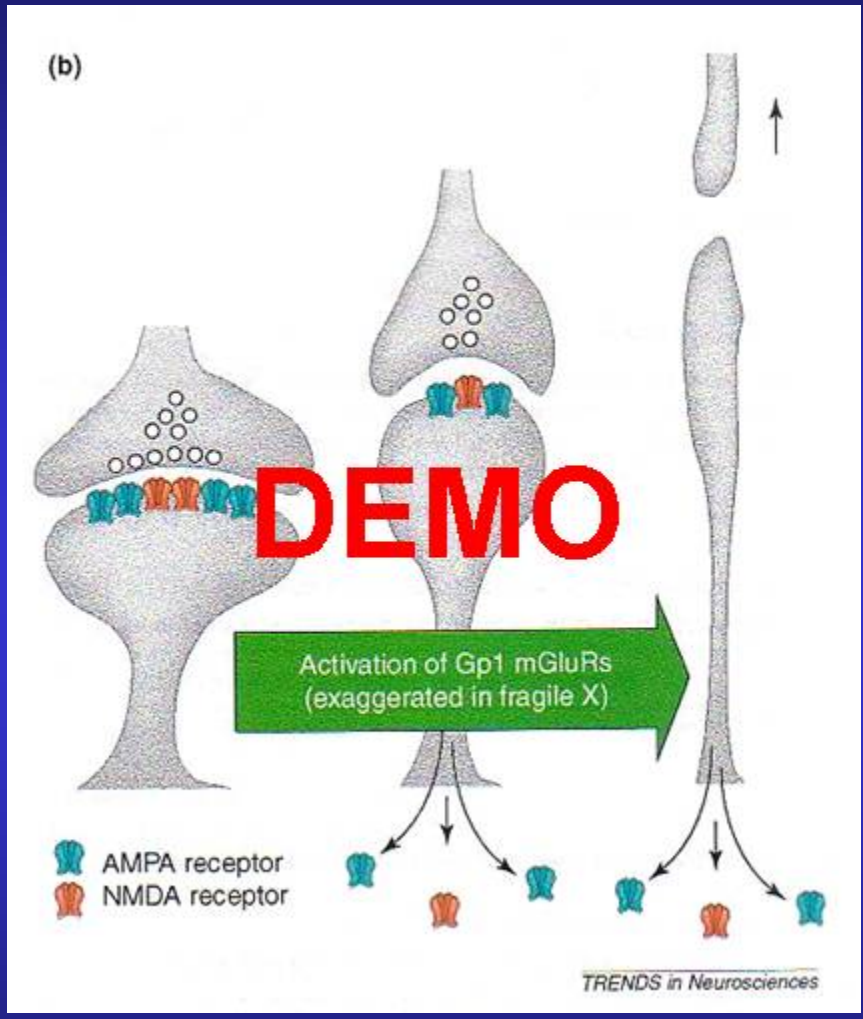
Neurobiology of FXS

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

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

» Huber et al 2002; Snyder et al 2001; Willemsen et al 2004





mGluR5 stimulation leads to LTD; FMRP puts the breaks on this.

Bear et al 2004

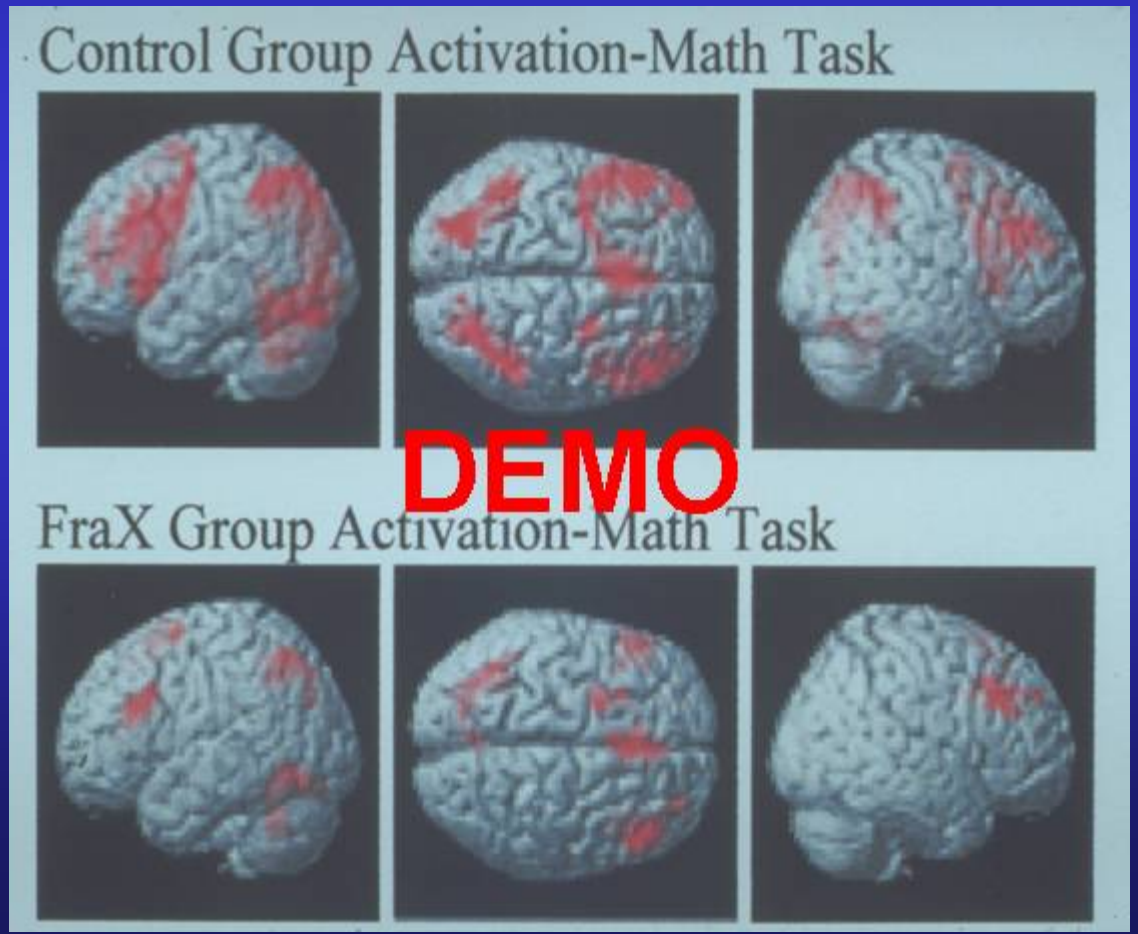
So in FXS there is dramatically increased LTD

Functional Studies: Decreased activation in tasks of
executive function (Menon et al 2000)
visuospatial processing (Kwon et al 2001)
math tasks (Rivera et al 2002)

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

These deficits correlate
inversely with FMRP

The more FMRP,
the better the activation

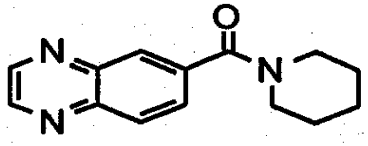


Specific Psychopharmacological Interventions

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

Chemical Name of CX516: **1-(quinoxalin-6-ylcarbonyl) piperidine**

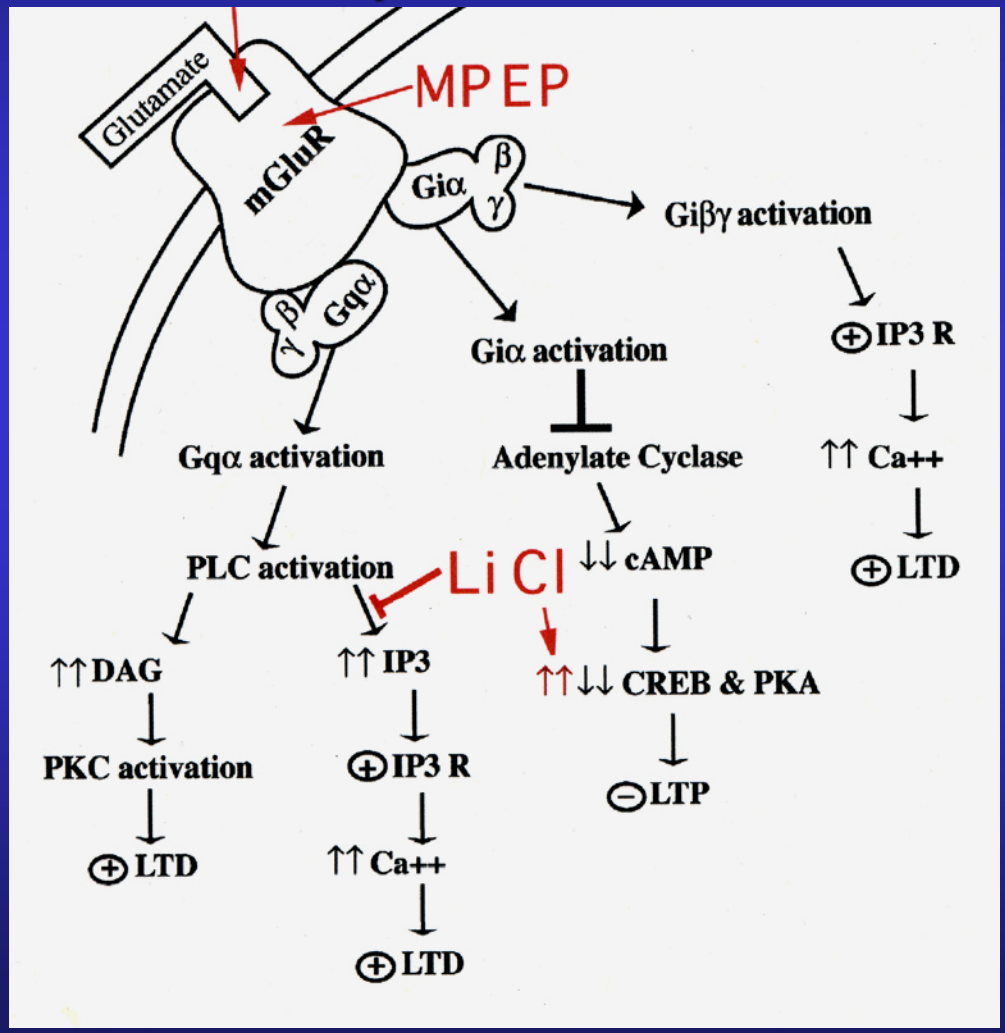
Structure:



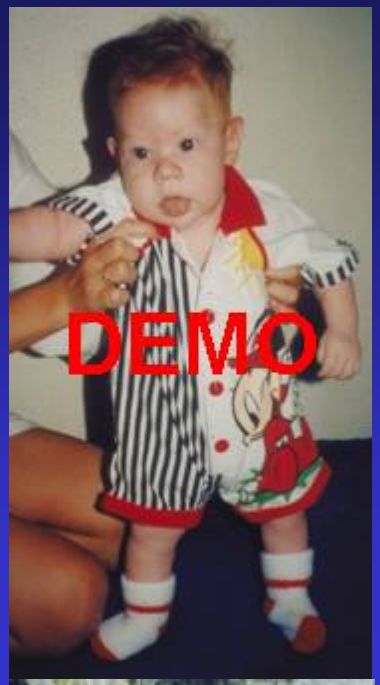
- mGluR5 antagonists: MPEP studies are helpful for seizures in KO mice and there is some enhancement of memory and cognition (Bauchwitz et al 2004)

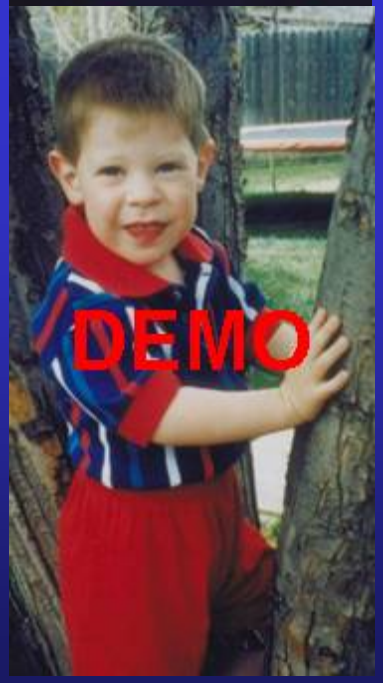
Lithium down regulates the mGluR5 pathway by blocking IP3 (inositol triphosphate) turnover. Controlled trials are taking place in the KO mouse and the fly.

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

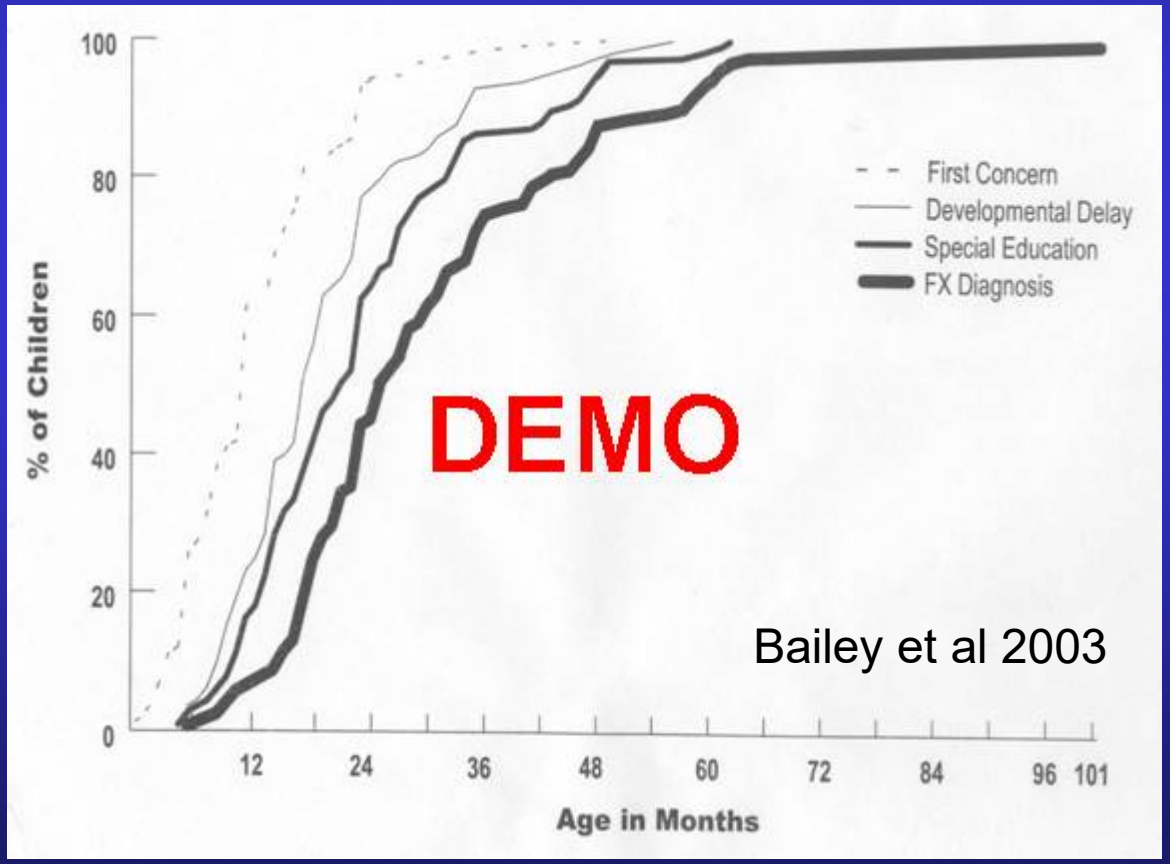


Mc Bride et al 2004



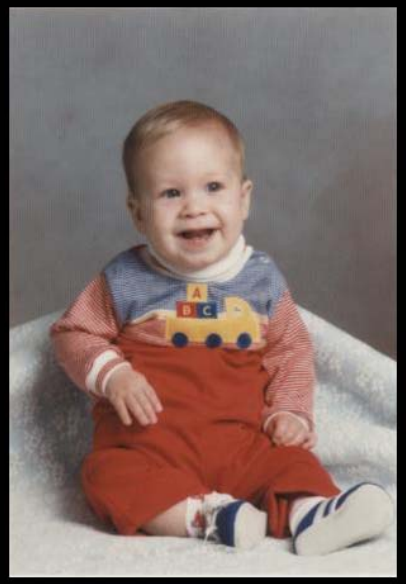


Time delay between first concerns and diagnosis of fragile X syndrome



Newborn Screening with blood spots analyzed by PCR


Sun's
San Jose, CA

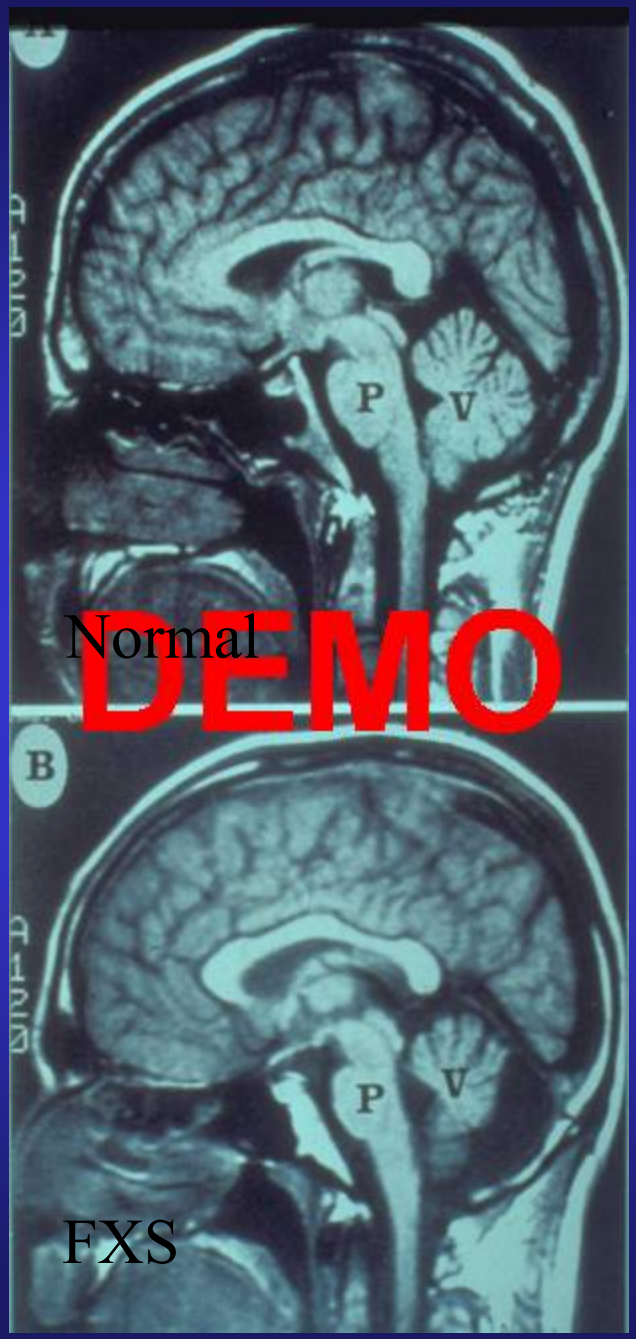



DEMO

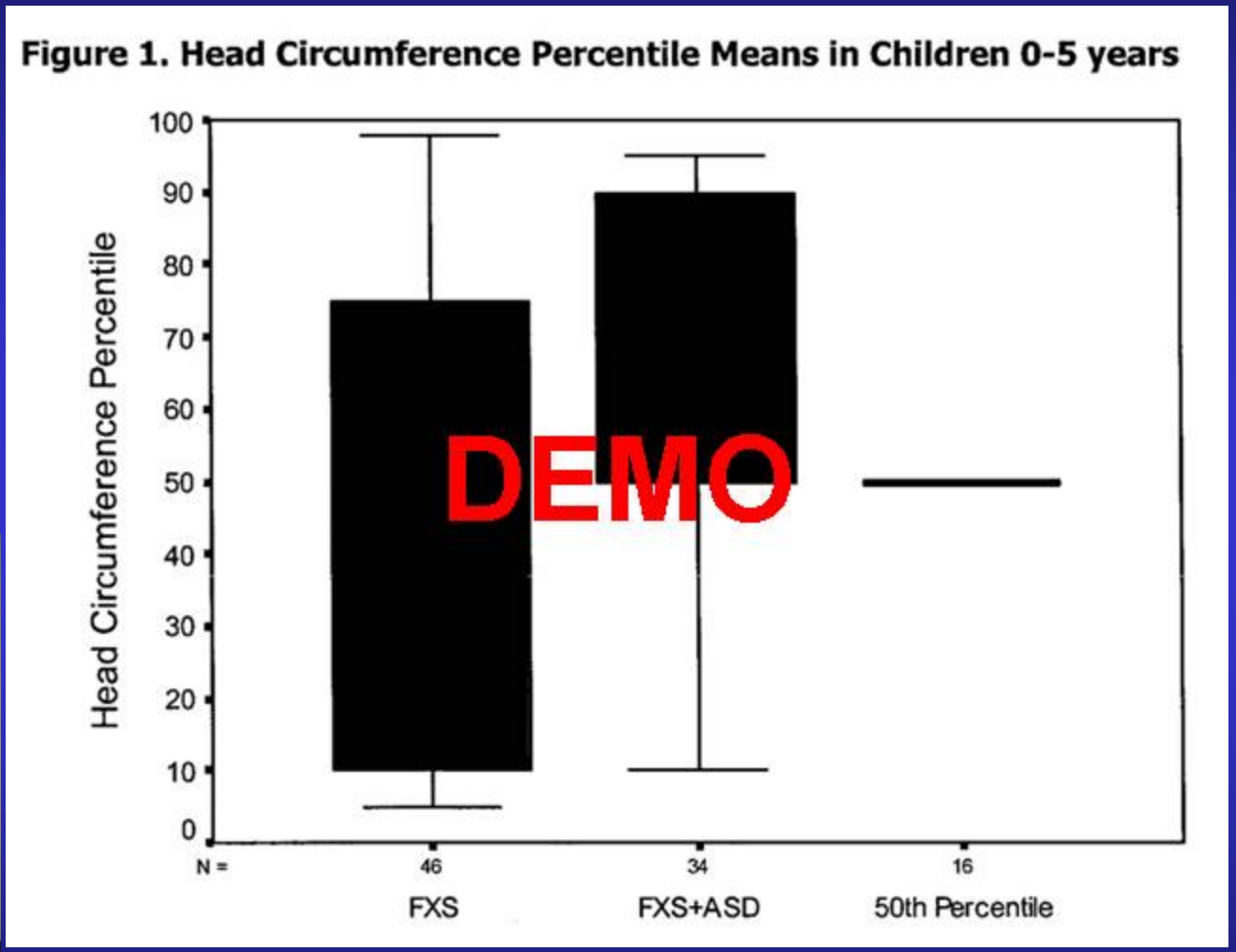
Neuroimaging findings in FXS:

Enlargement of
- **overall brain**
- **caudate**
- **hippocampus**
- **ventricles**

Smaller:
- **posterior cerebellar vermis**
- **Superior temporal gyrus**



Study of 80 children with FXS with and without ASD



Jenkins et al

Fragile X and Autism

- Approximately 3 to 6% of children with autism have fragile X syndrome (Brown et al. 1986, Hagerman 1996, Bailey et al. 1993)
- Approximately 25 to 33% of children with FXS have autism (Baumgardner et al. 1995, Hagerman et al. 1986, Reiss & Freund 1992, Cohen 1995, Turk & Graham 1997, Bailey et al. 1998; Rogers et al 2001)
- Autistic-like features are seen in the majority of patients with FXS
- Boys with FXS and autism have a lower IQ than those with FXS or those with autism (Bailey et al. 1998, 1999). Autism does not correlate with FMRP.

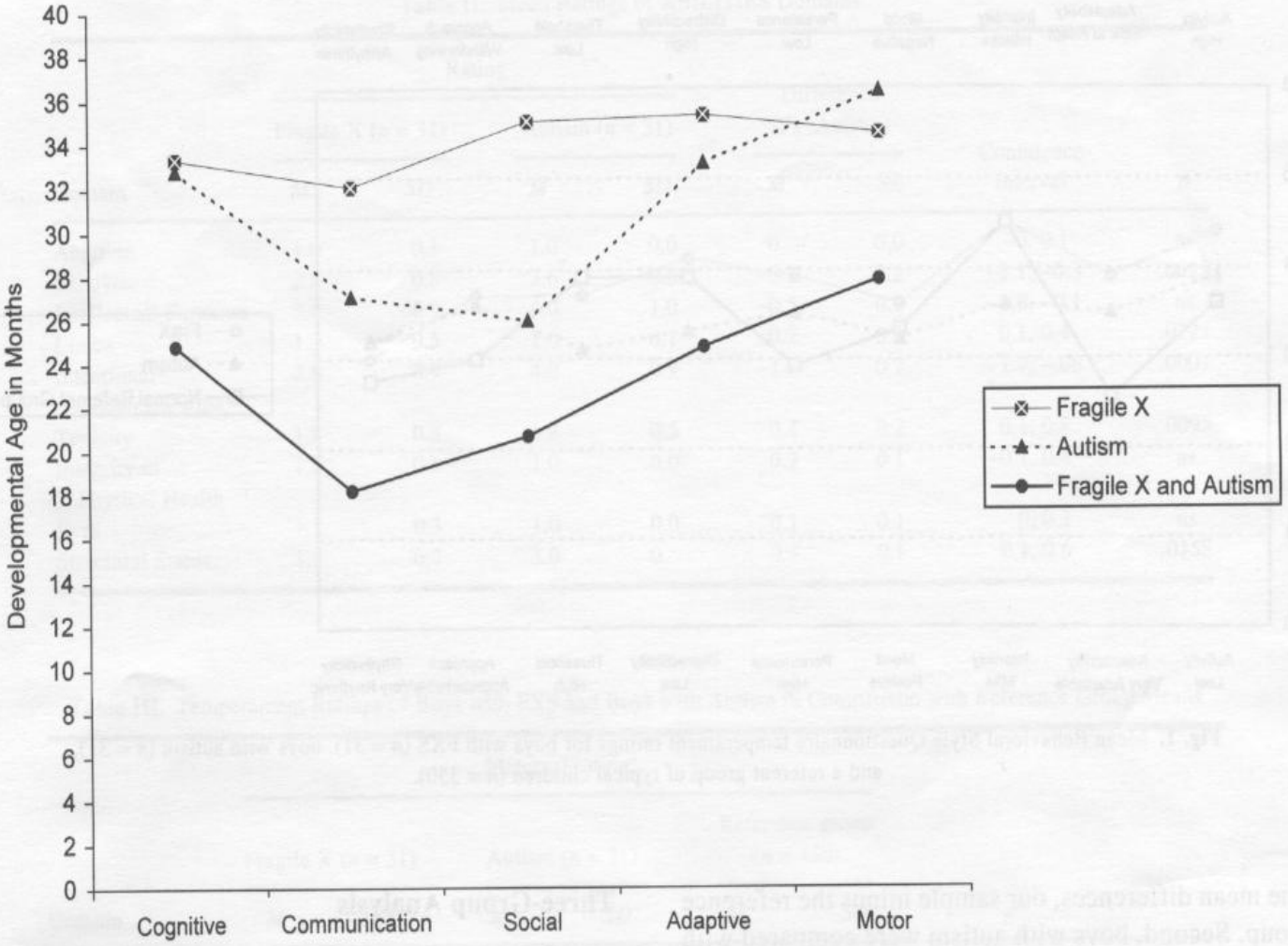


Fig. 2. Mean Battelle Developmental Inventory developmental age scores for boys with FXS ($n = 13$), boys with autism ($n = 13$), and boys with FXS and autism ($n = 13$).

Bailey et al 2000, J Aut Dev Disord 30:49

Studies of young children with FXS with and without autism compared to autism and DD controls

- Philofsky et al 2004 and Rogers et al 2003: FXS+autism have lower cognitive scores on the Mullens and lowered expressive language than autism or FXS alone. Receptive language and imitation skills are a strength in FXS alone.
- Rogers et al 2003: Children with FXS and children with autism had more sensory sxs on SSP than children with DD or MA matched typicals. Children with FXS had lowest scores on low energy/muscle weakness scale

Autism Evaluation of Boys with FXS

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

- Of 69 boys with FXS assessed using the ADI-R, ADOS-G and DSM IV clinical criteria:
  - 29% (n=20) met classification criteria for Autism
  - 16% (n=11) met classification criteria for PDDNOS
  - 55% (n=38) did not meet criteria for either autism or PDDNOS
- Correlations between ADOS scores and FMRP, CGG repeats or FMR1-mRNA were not significant

Autism and Fragile X

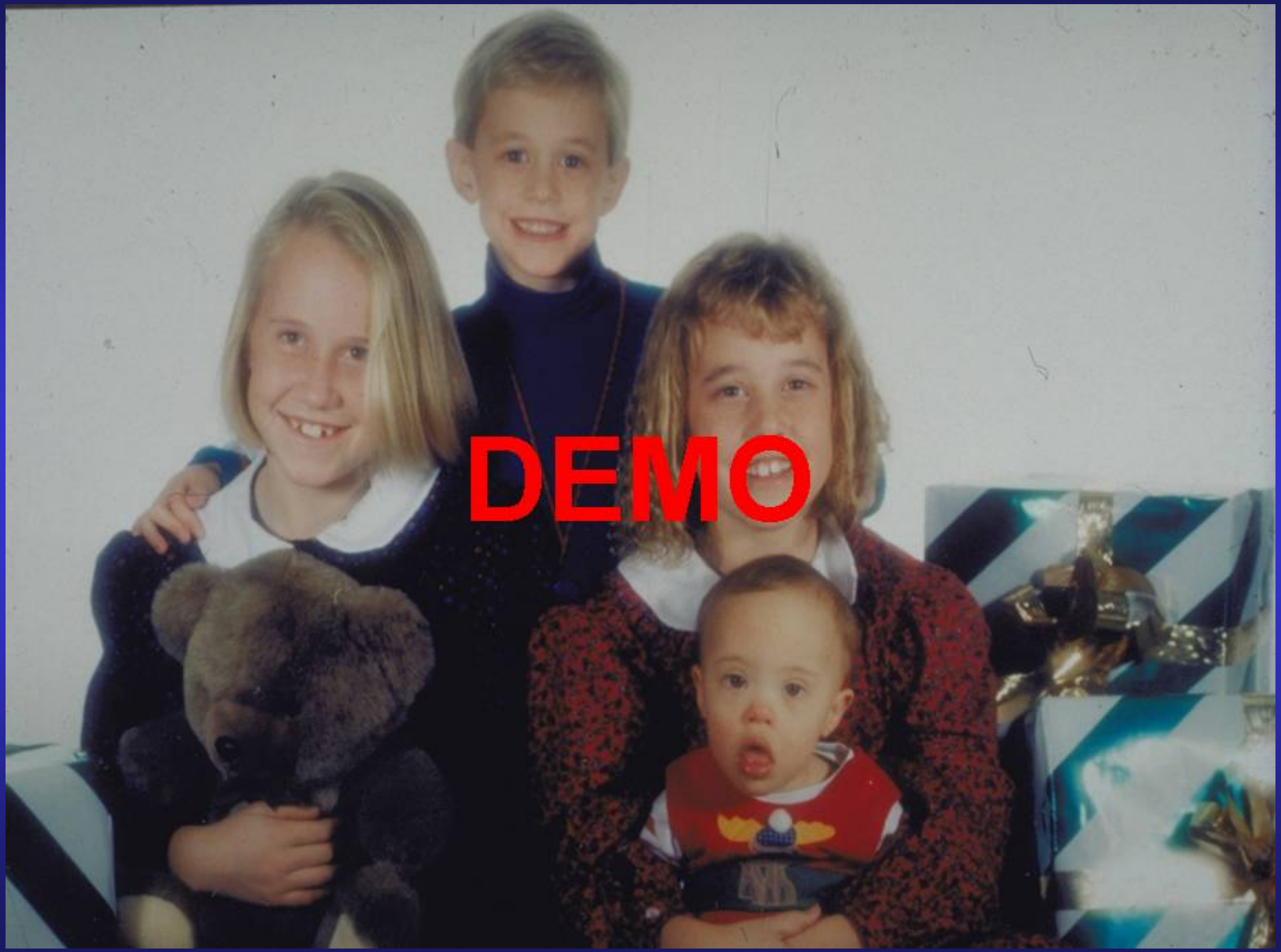
may be caused by genes that are epistatic with FMRP

Autism with no interest in social interactions

Autism with limited interest in social interactions but anxiety interferes with interactions

Obvious Second Hits Leading to Autism

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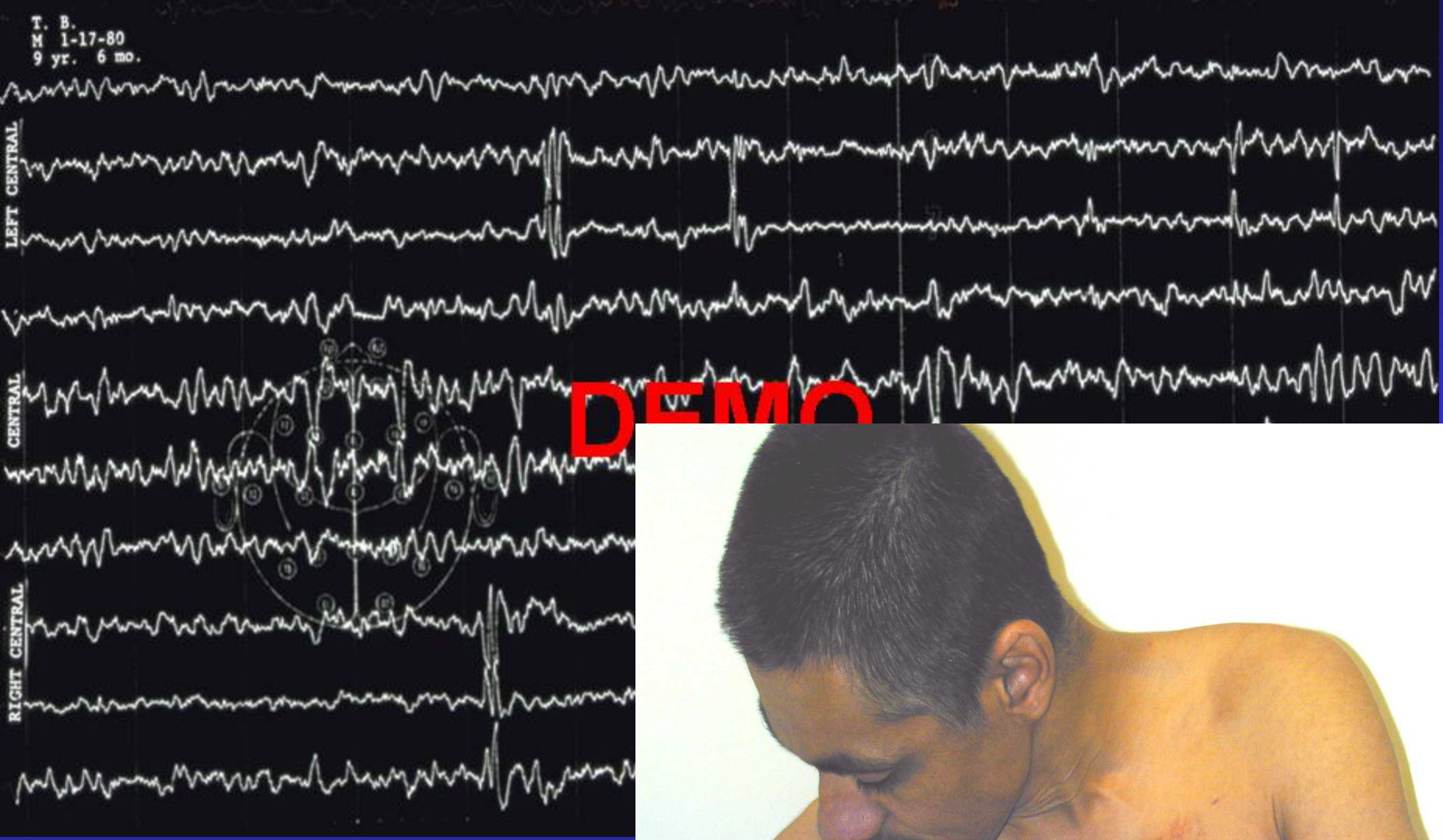
History of
birth trama
with subsequent

- CP
- Seizures
- Severe MR
- Strabismus
- Autism



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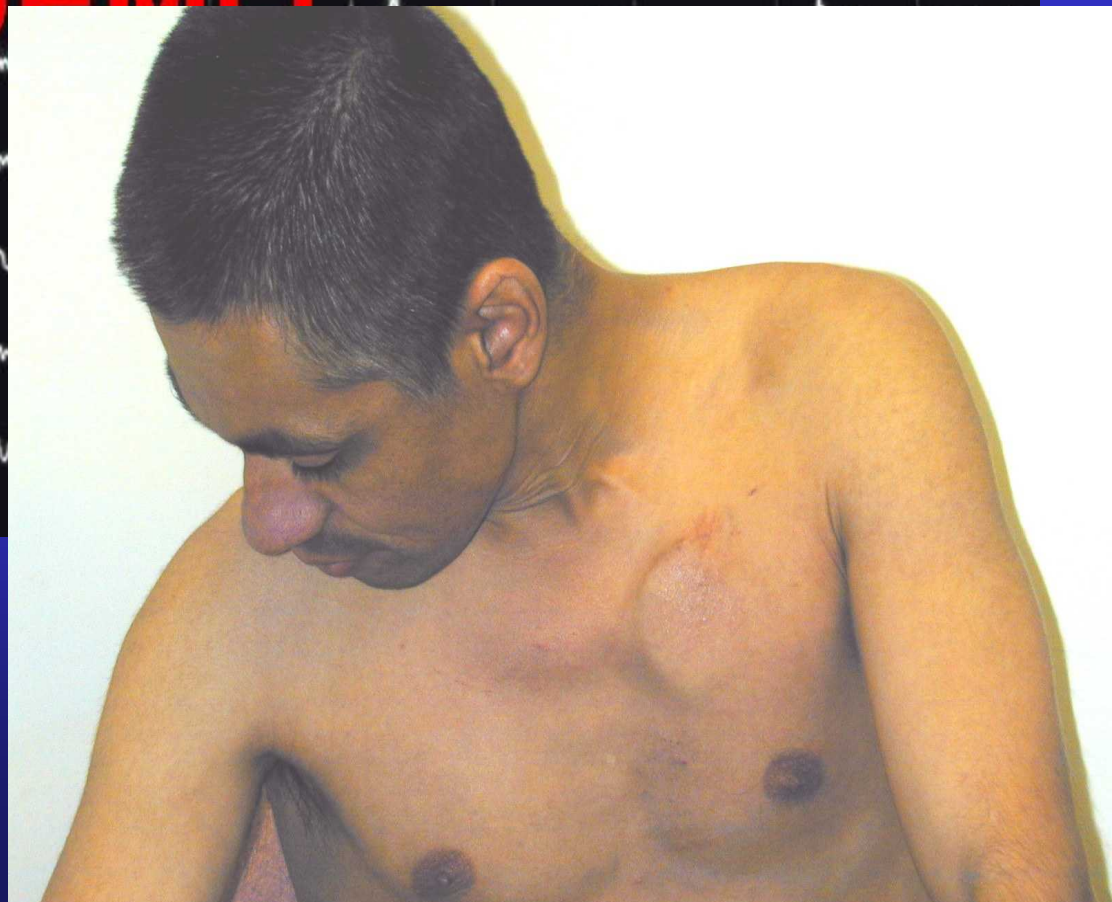
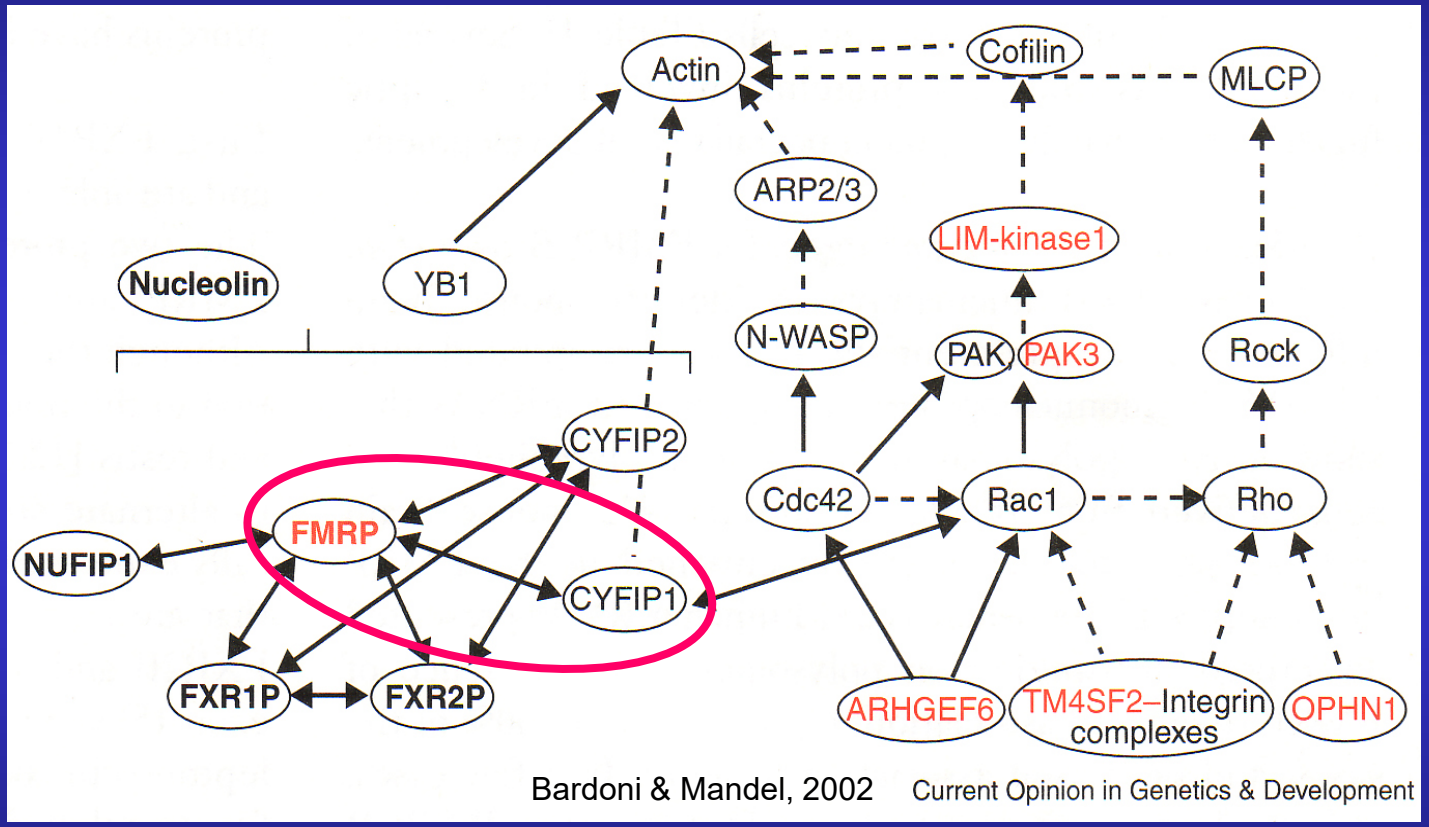
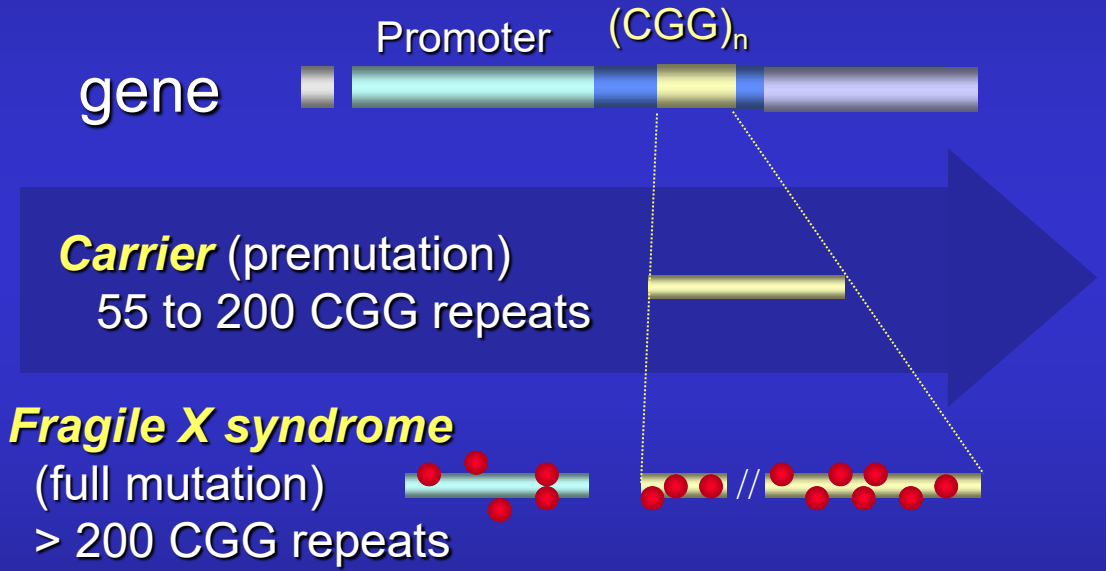


The Prader-Willi Phenotype of Fragile X Syndrome

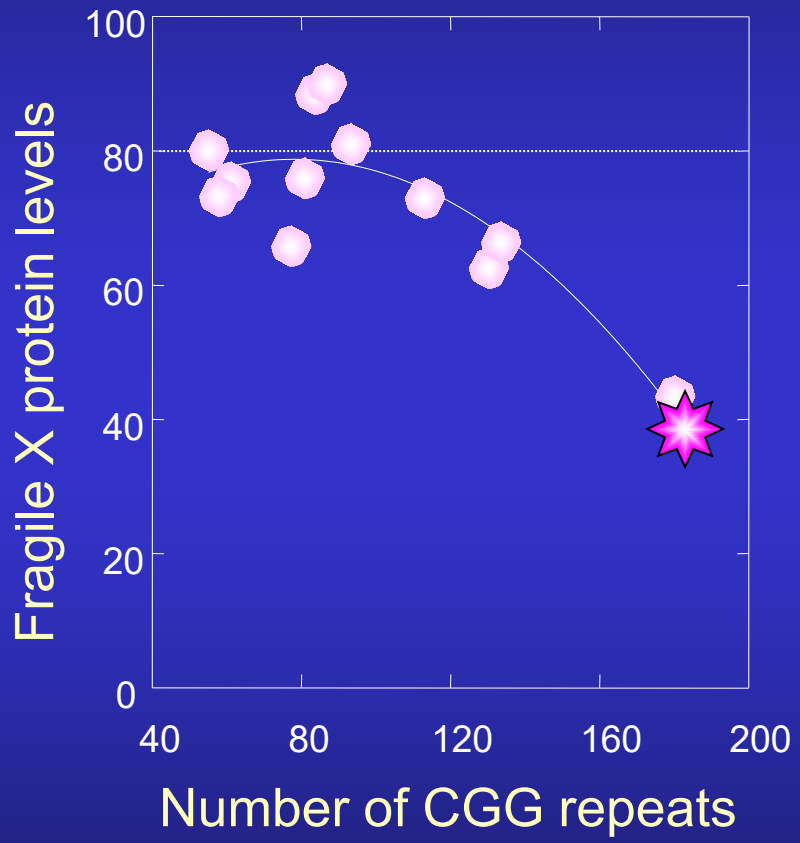


Autism is also seen in some premutation carriers



Aziz et al 2003, Tassone et al 2000
Beth Goodlin-Jones et al 2004;

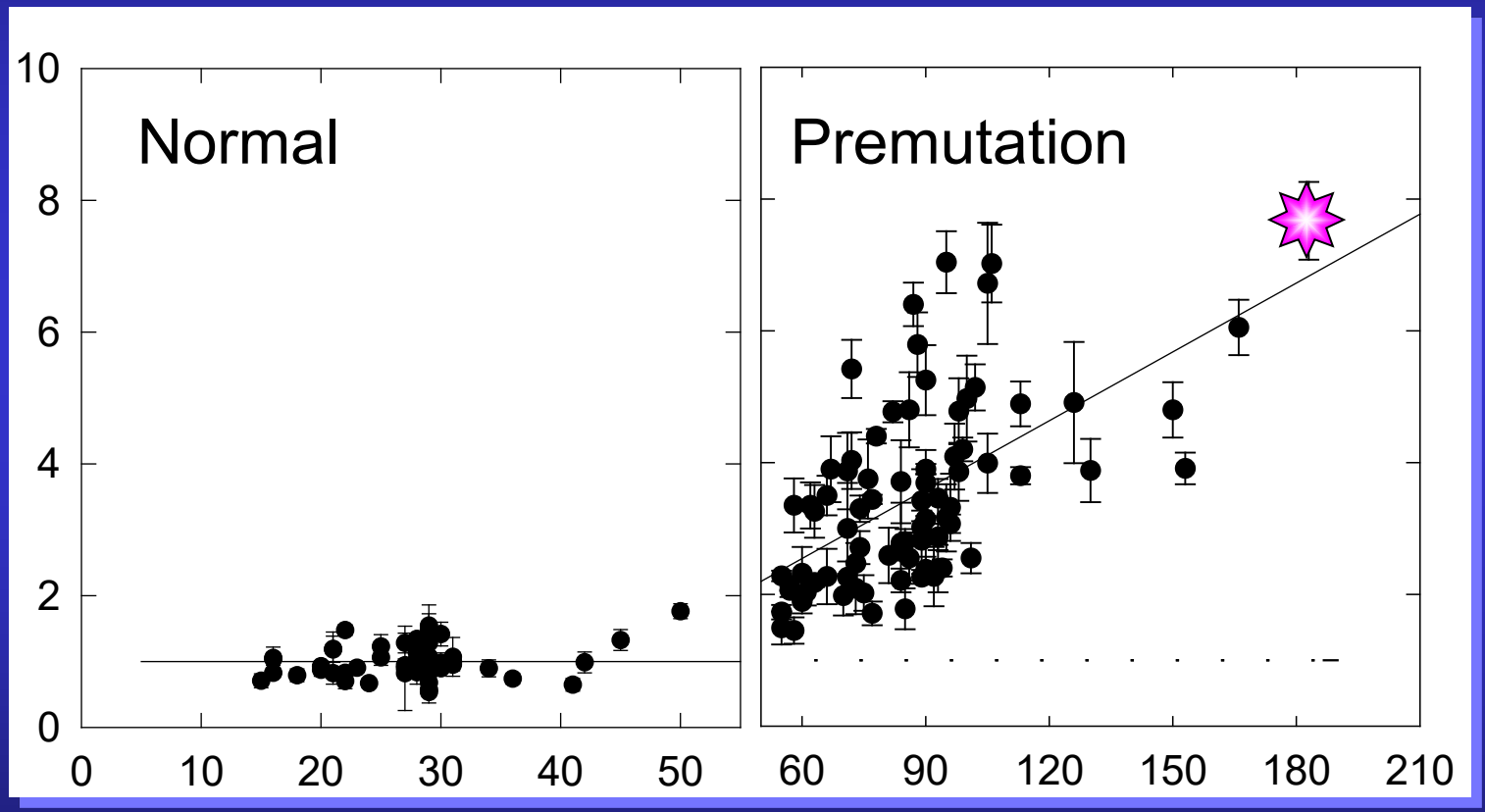
Fragile X protein levels decrease in upper premutation range



Tassone et al 2000.

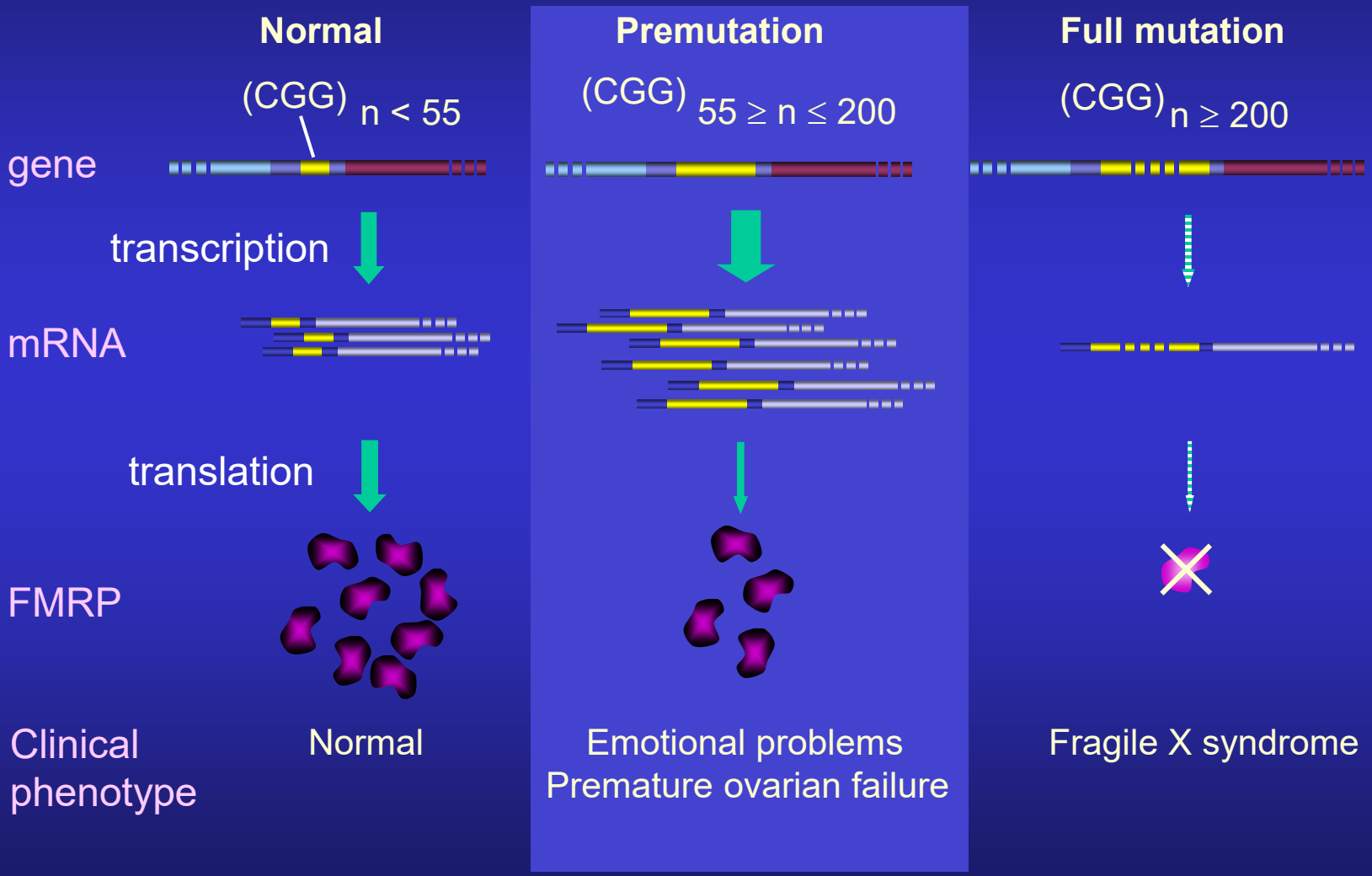
Why? the FMR1 gene is unmethylated in this range

FMR1 mRNA (gene activity) is *elevated* in the premutation range



Tassone et al 2000 AJHG.

Expression of the *FMR1* gene



Study of probands vs non probands with premutation

- ADHD (CGI≥15 and DSM-IV)
  - 73% (11/15) of probands*
  - 50% (6/12) of nonprobands
  - 12% (2/17) of controls*
    p<.01
- ASD (SCQ≥15)
  - 67% (10/15) of probands*
  - 8% (1/12) of nonprobands
  - None of controls*
    p<.05
- ASD (DSM-IV)
  - 73% (11/15) of probands*
    - 33% (5/15) Full autism
    - 40% (6/15) PDDNOS
  - 17% (2/12) of nonprobands
    - 8% (1/12) Full autism
    - 8% (1/12) PDDNOS
  - None of controls*
    p<.05
- Medication (Stimulants, SSRIs and/or atypical antidepressants)
  - 93% (14/15) of probands*
  - 17% (2/12) of nonprobands
  - 6% (1/17) of controls*
    p<.01

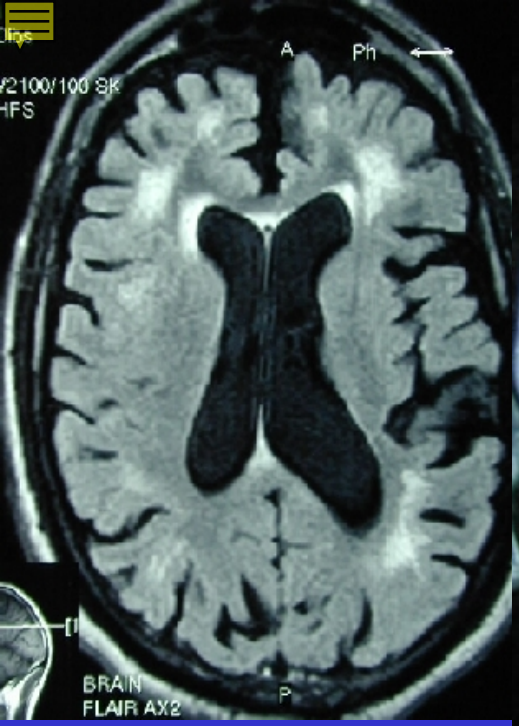
Two brothers with the *FMR1* premutation ages 6 and 7. Boy on right presented as proband with autism and ADHD and his brother has anxiety and ADHD.

Case 1: DR. 63 GF with 89 CGG repeats

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

Fragile X–associated Tremor/Ataxia Syndrome -FXTAS

- Intention tremor that is progressive
- Ataxia and/or frequent falling
- Parkinsonian features: masked facies, intermittent resting tremor, increased tone or response to L-dopa
- Cognitive deficits: memory problems & executive function deficits – decrease in PIQ first
- Psychological features: anxiety, mood liability, outbursts or reclusive behavior
- Peripheral neuropathy: decreased sensation in lower extremities
- MRI global brain atrophy
- MRI – deep cerebellar white matter hyperintensities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Ph
BRAIN
FLAIR AX2
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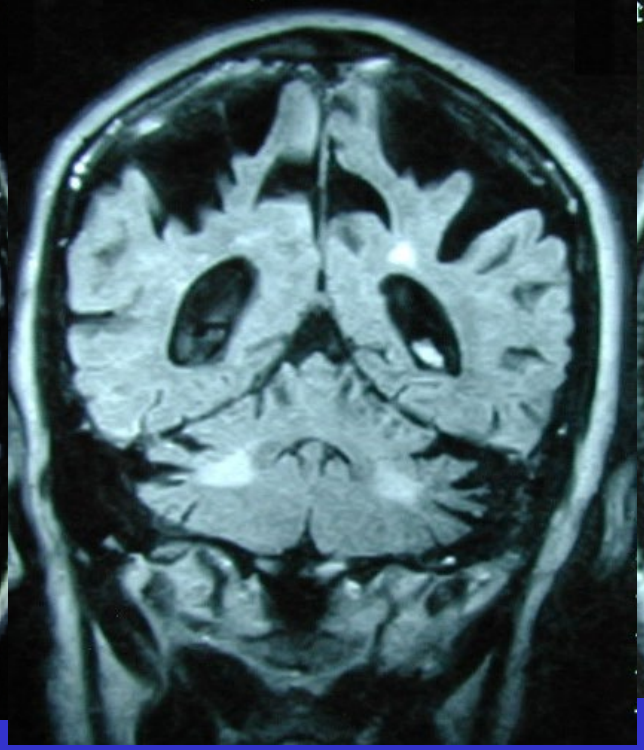
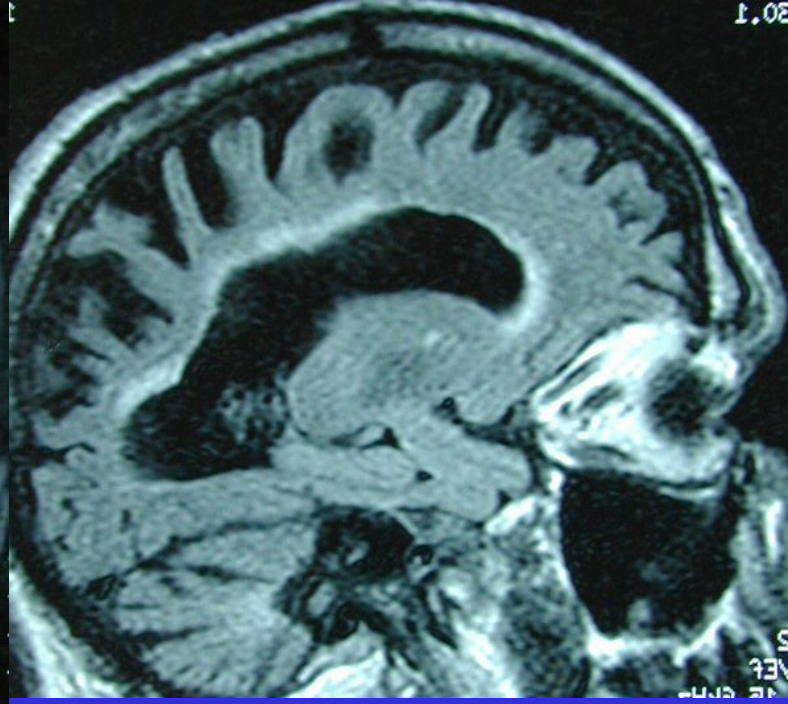
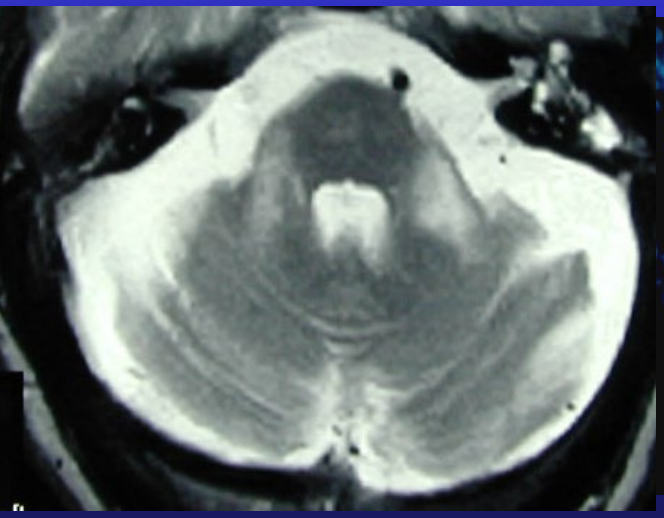
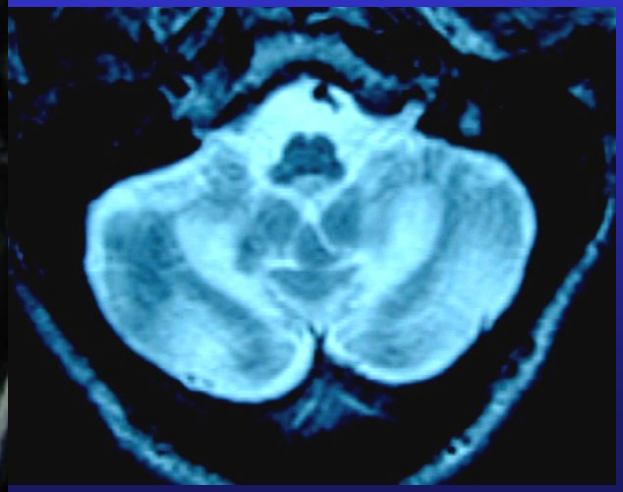
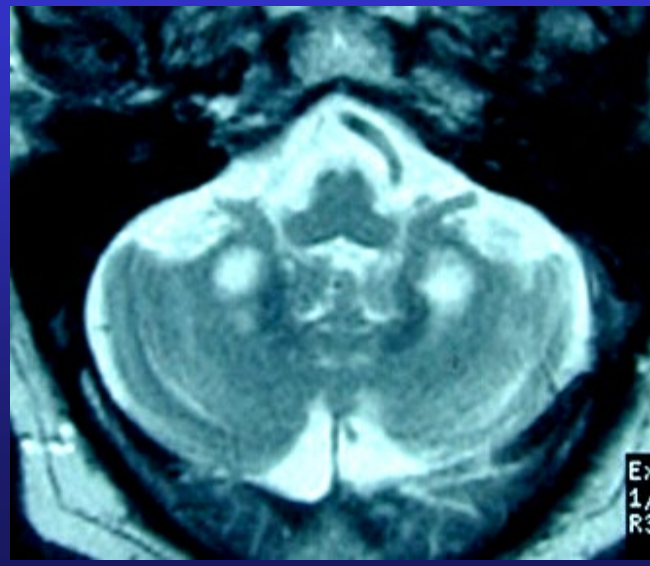










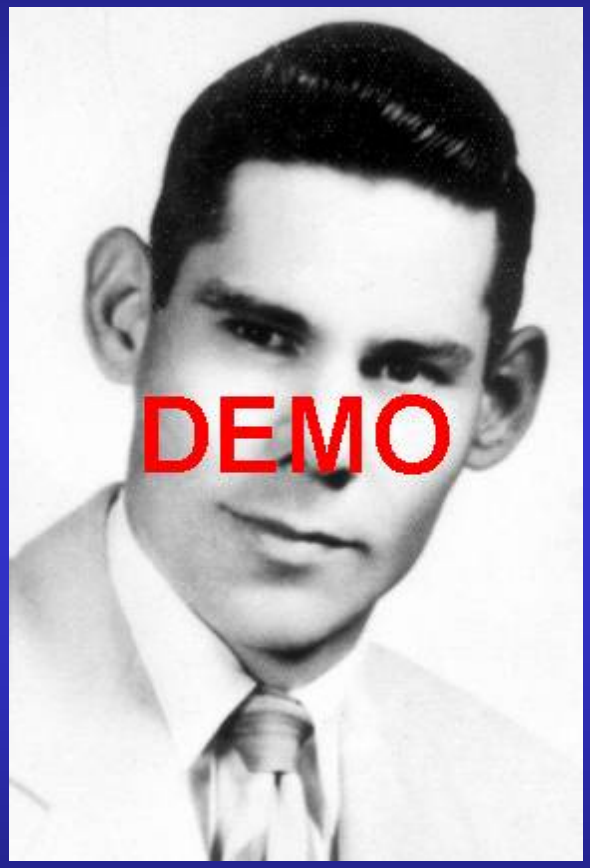
Diagnostic Criteria

Inclusion criteria: CGG repeat 55-200		
MRI	***Major***	Middle cerebellar peduncles lesions
	Minor	Cerebral white matter hyperintensity
	Minor	Moderate to severe generalized atrophy
Clinical	***Major***	Intentional Tremor
	Major	Gait Ataxia
	Minor	Parkinsonism
	Minor	Short term memory deficits
	Minor	Executive function deficits

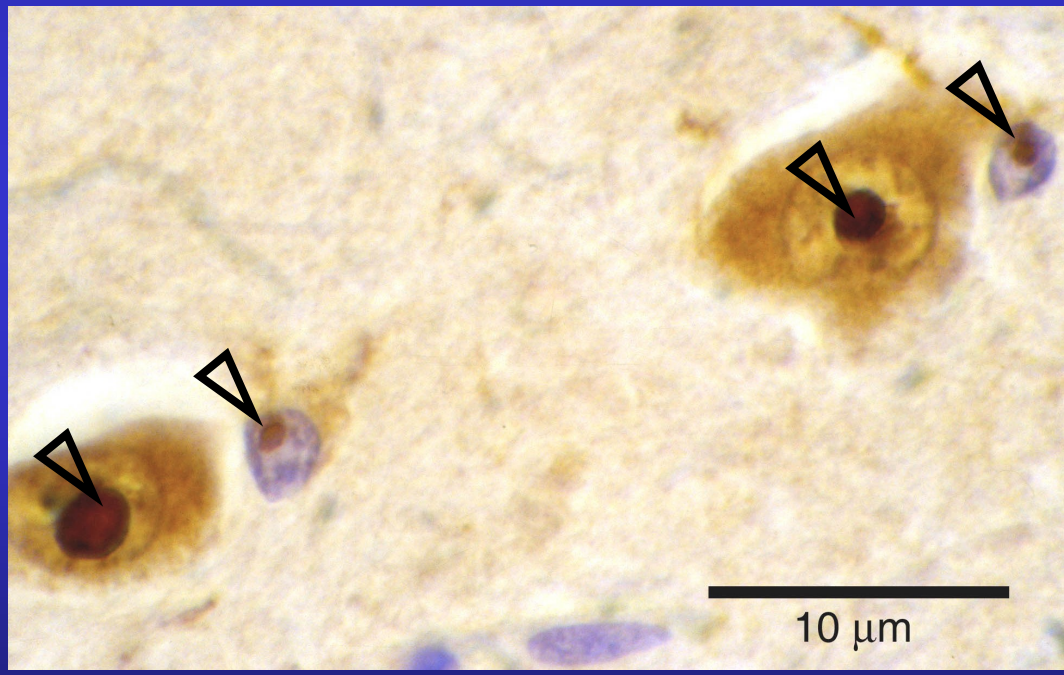
Diagnostic Categories CGG repeat 55-200		
Definite	Probable	Possible
1 MRI ***Major*** + 1 Clinical ***Major***	1 MRI ***Major*** +1clin minor or 2 clin ***Major***	1 MRI Minor + 1 Clinical ***Major***

Intranuclear inclusions

Neurons – Astrocytes in humans



Greco et al 2002 Brain



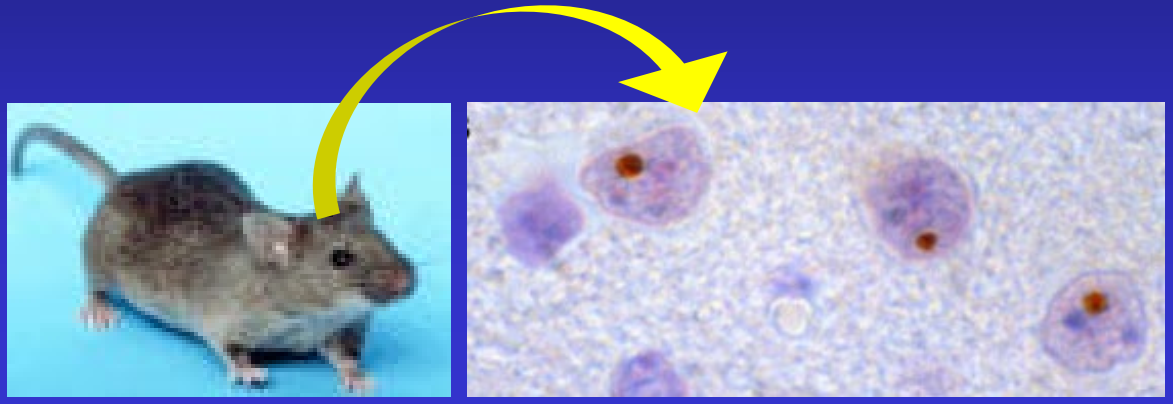
Anti-ubiquitin antibody

Intranuclear inclusions

region	case1 %	case 2 %	case1 %	asce 2 %
• Frontal cortex	6	3	45	15
• Temporal cotex	4	2	44	11
• Putamen	4	4	45	7
• Globus Palidus	4	1	42	13
• **Hippocampus**	**38**	**43**	-	-
• Dentate nucleus	3	3	49	17
	Neurons		**Astrocytes**	

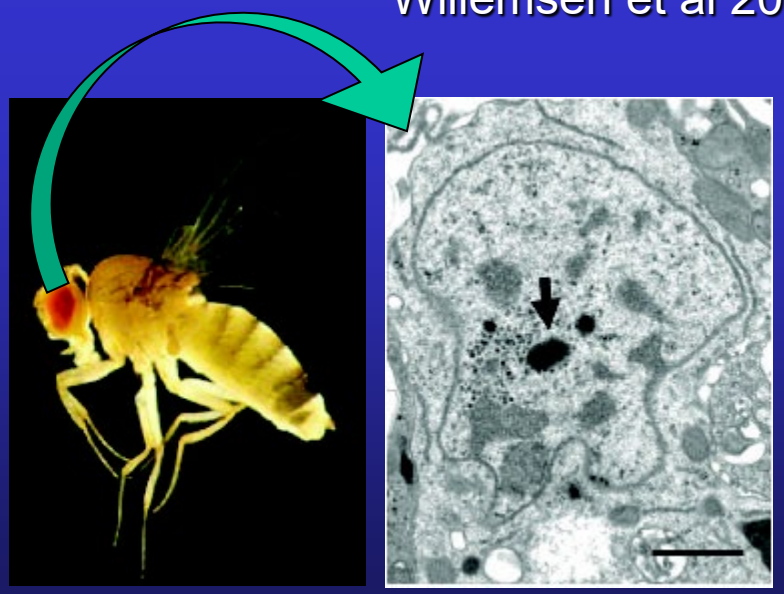
The CGG repeat – *as RNA* – stimulates formation of inclusions

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



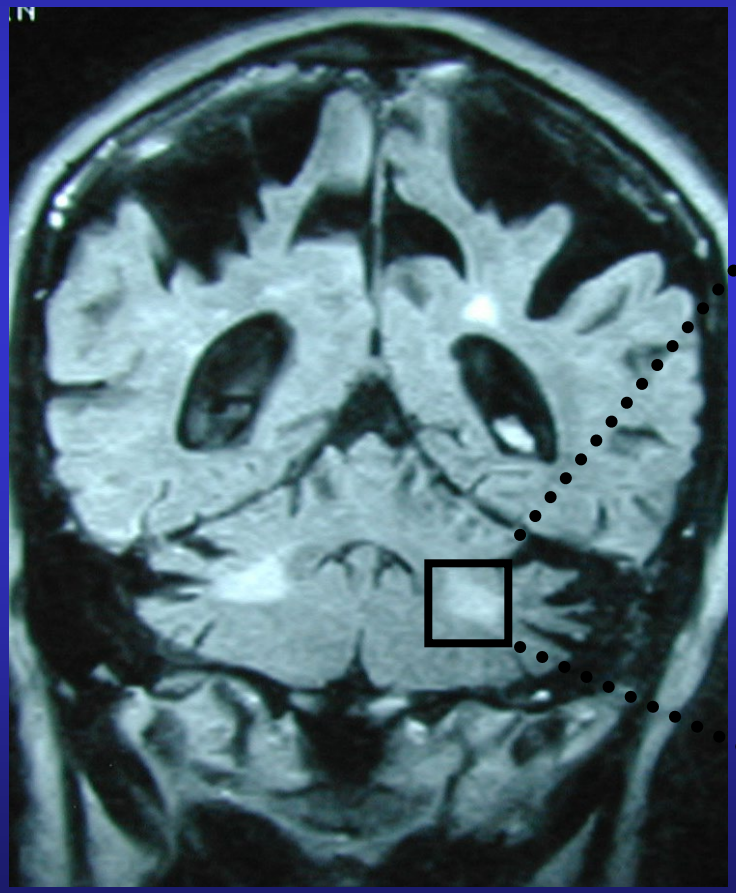
Willemsen et al 2003

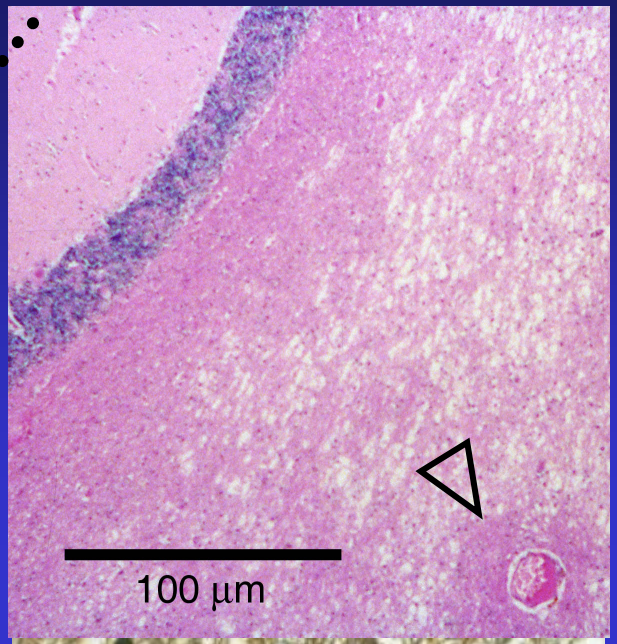
Fly with ~90 CGG repeats placed in an unrelated reporter gene (Jin et al., 2003)

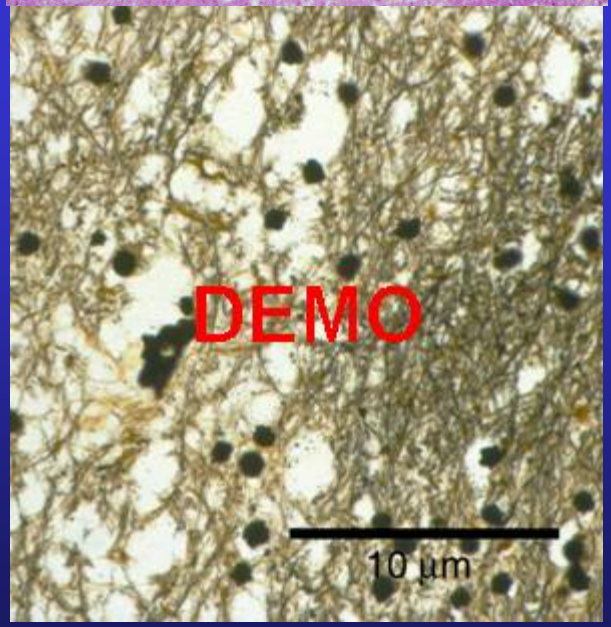


Jin et al 2003

White matter Spongiosis







Neuropsychiatric Phenotype

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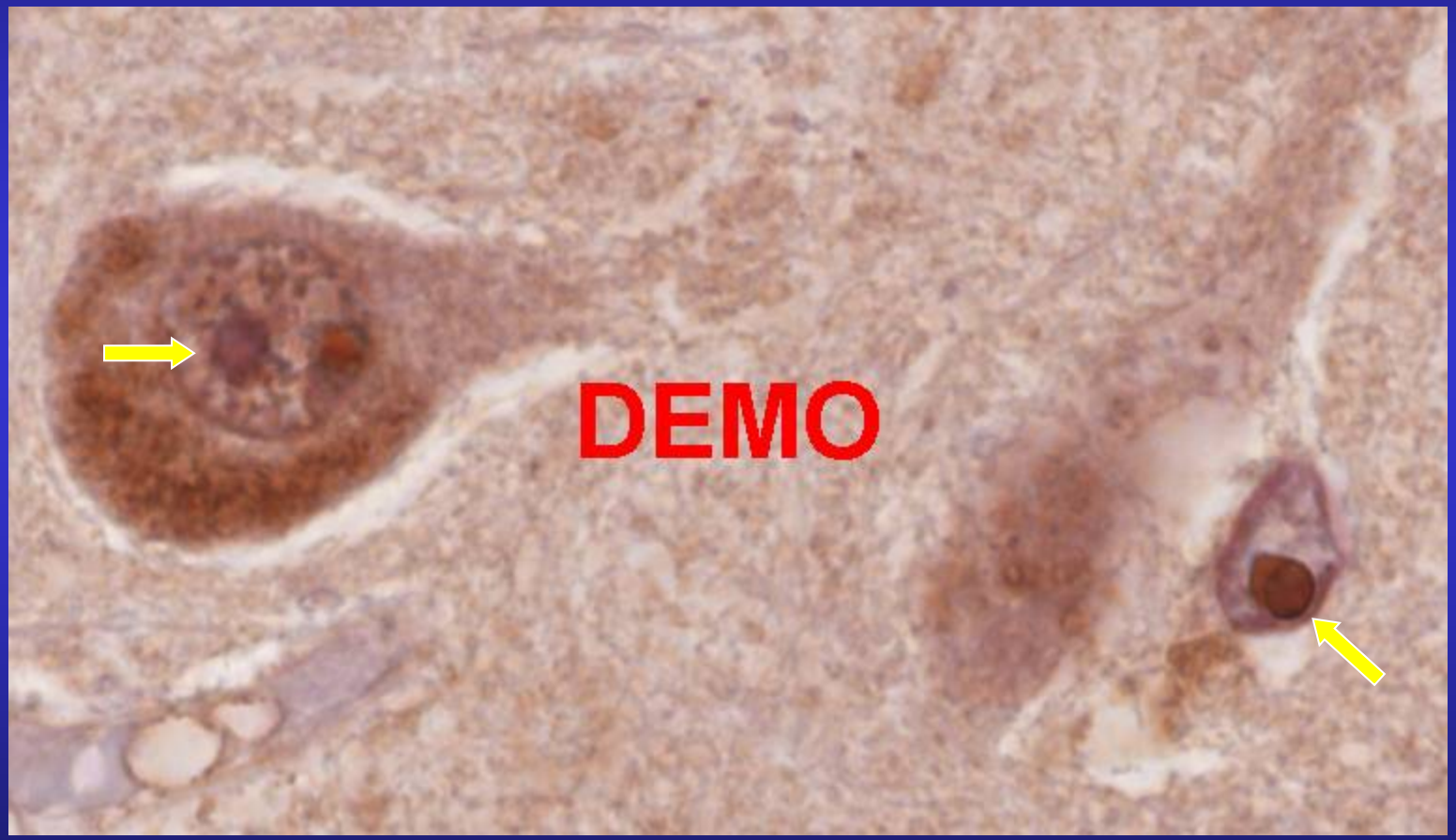
California Family Study of the prevalence of FXTAS

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

Females with FXTAS

	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
Age	67	57	85	62	74
FSIQ	126	99	100	111	87
VIQ	130	103	104	110	88
PIQ	116	94	94	111	86
Age of onset tremor	42	30	82	52	71
Age of onset ataxia	59	37	79	60	71
CGG repeat	18, 90	29, 93	29, 87	18, 90	30,78
FMRP level*	89	96	80	70	90
Activation ratio**	0.51	0.35	0.53	0.5	0.21
mRNA level	3.25 $\pm$ 0.55	4.6 $\pm$ 0.29	1.40 $\pm$ 0.07	2.52 $\pm$ 0.27	2.6 $\pm$ 0.04
MRI	+ MCP sign	No MCP sign	pacemaker	pacemaker	No MCP sign
FXTAS diagnosis	Definite	Probable	Definite	Probable	Probable

Neuronal and astrocytic inclusions - also in females with FXTAS



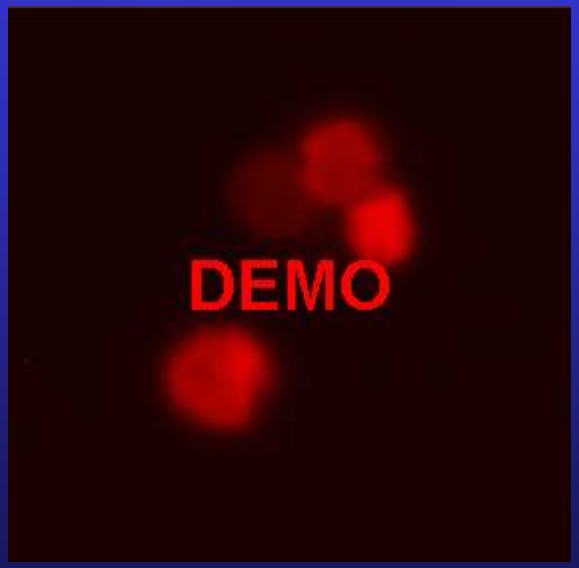
Case 3

Isolation of inclusions

Isolate nuclei from frozen cortical tissue

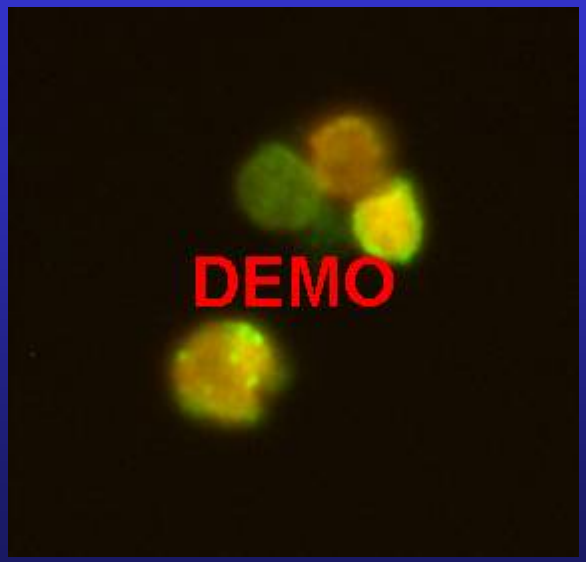
Disruption of nuclei and preparative flow sorting to yield purified inclusions (~10^6 inclusions /gram brain tissue)

αB-crystallin



ubiquitin

merge



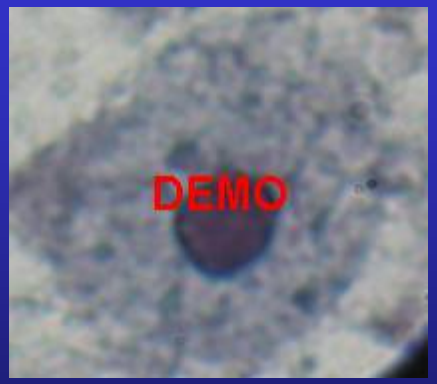
Another puzzle:
Myelin Basic Protein appears to be in the inclusions

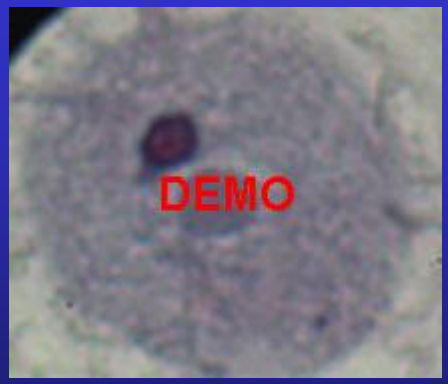
Ubiquitin

MBP

merge

Non-fluorescent
Anti-MBP staining





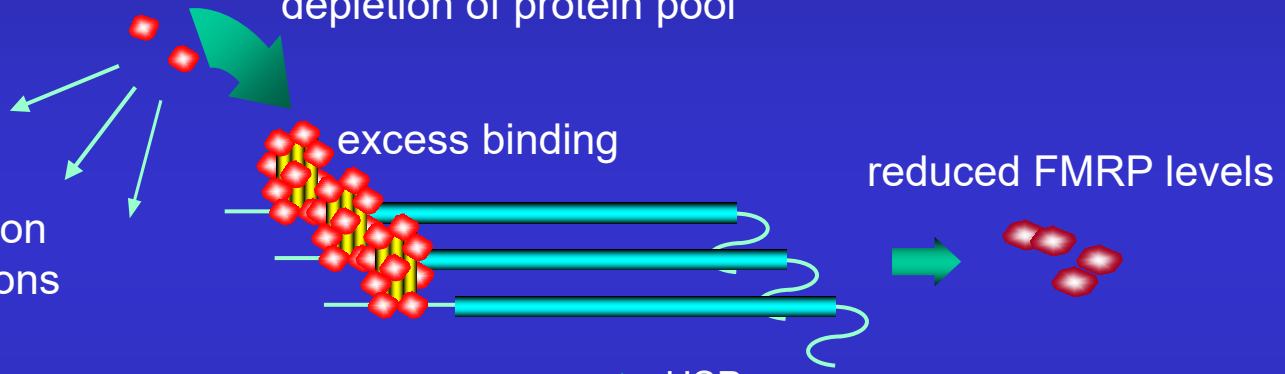
4 sisters (50 to 36 years)
with the premutation
the oldest two have FXTAS
and the younger ones have
intermittent tremor and ataxia
all have anxiety and mood
problems

One sister with FXTAS
also has autoimmune problems
including a lupus like rash,
joint pain and muscle pain;
others have presented with MS sxs

RNA gain-of-function model for FXTAS

Premutation allele > 54 CGG repeats

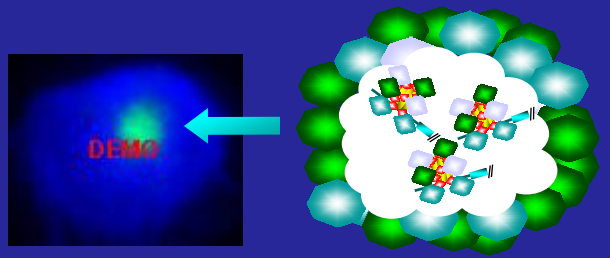
Hypothetical:
DM-type model



Neurological dysfunction

FXTAS

Inclusion formation

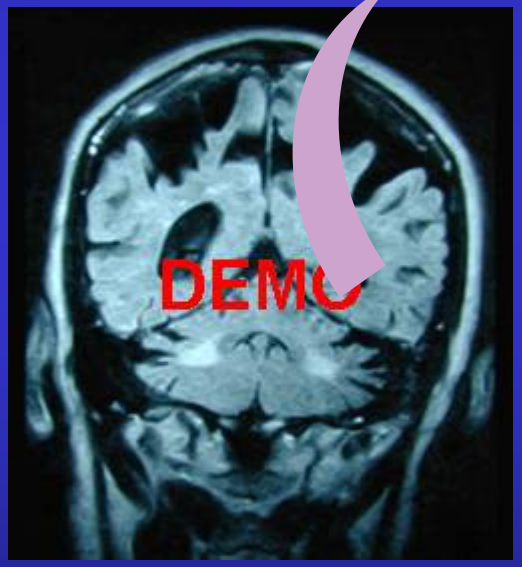


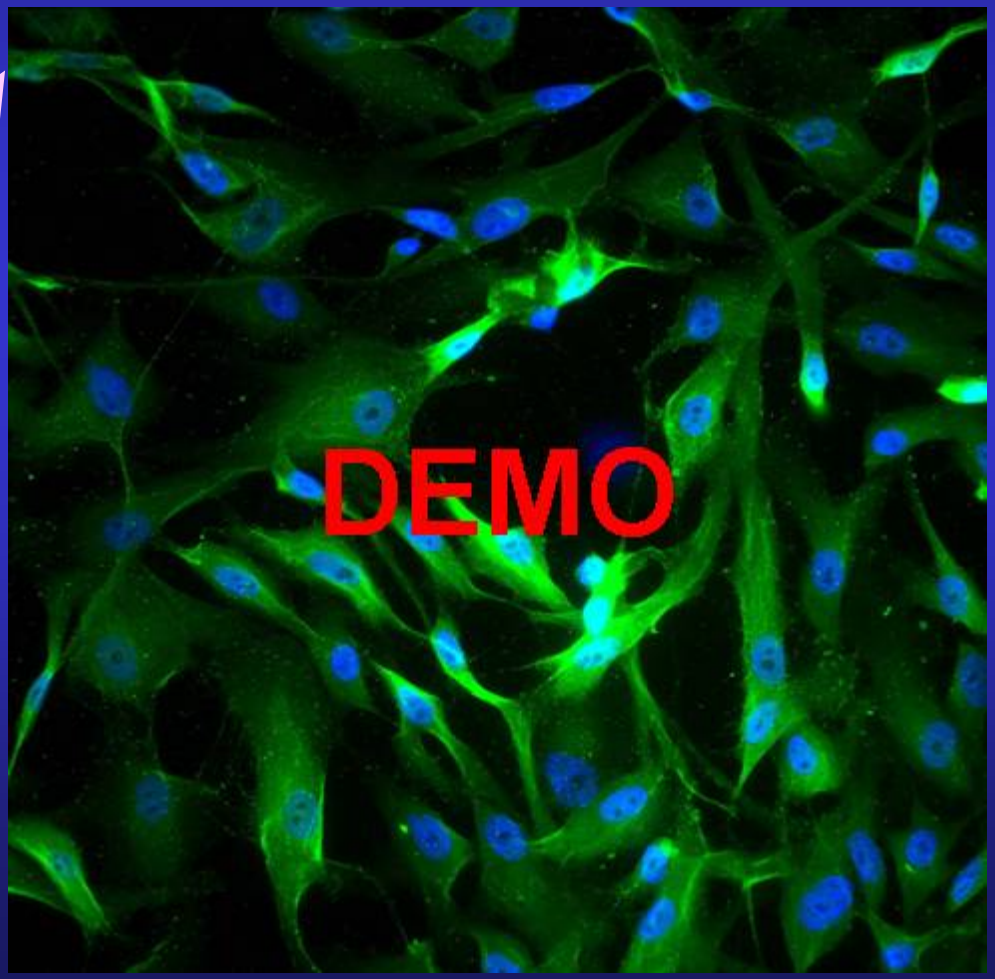
Clinical involvement on the fragile X spectrum

Charcot Marie Tooth and FXTAS

Research directions for FXTAS

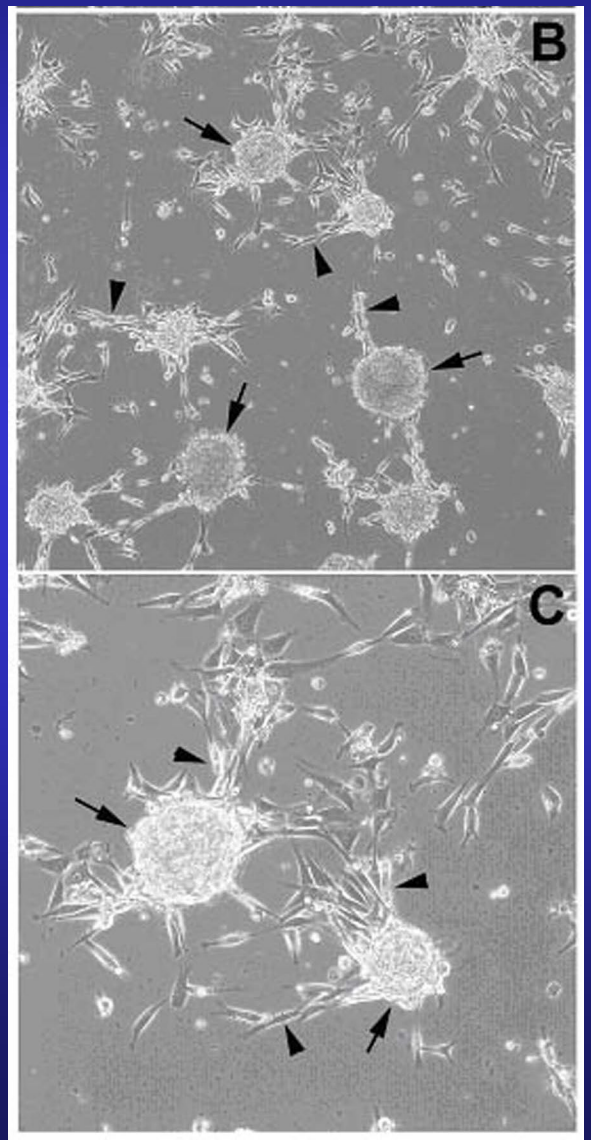
Successful growth of neural stem cells from the brains of adults who have died with FXTAS or with fragile X syndrome

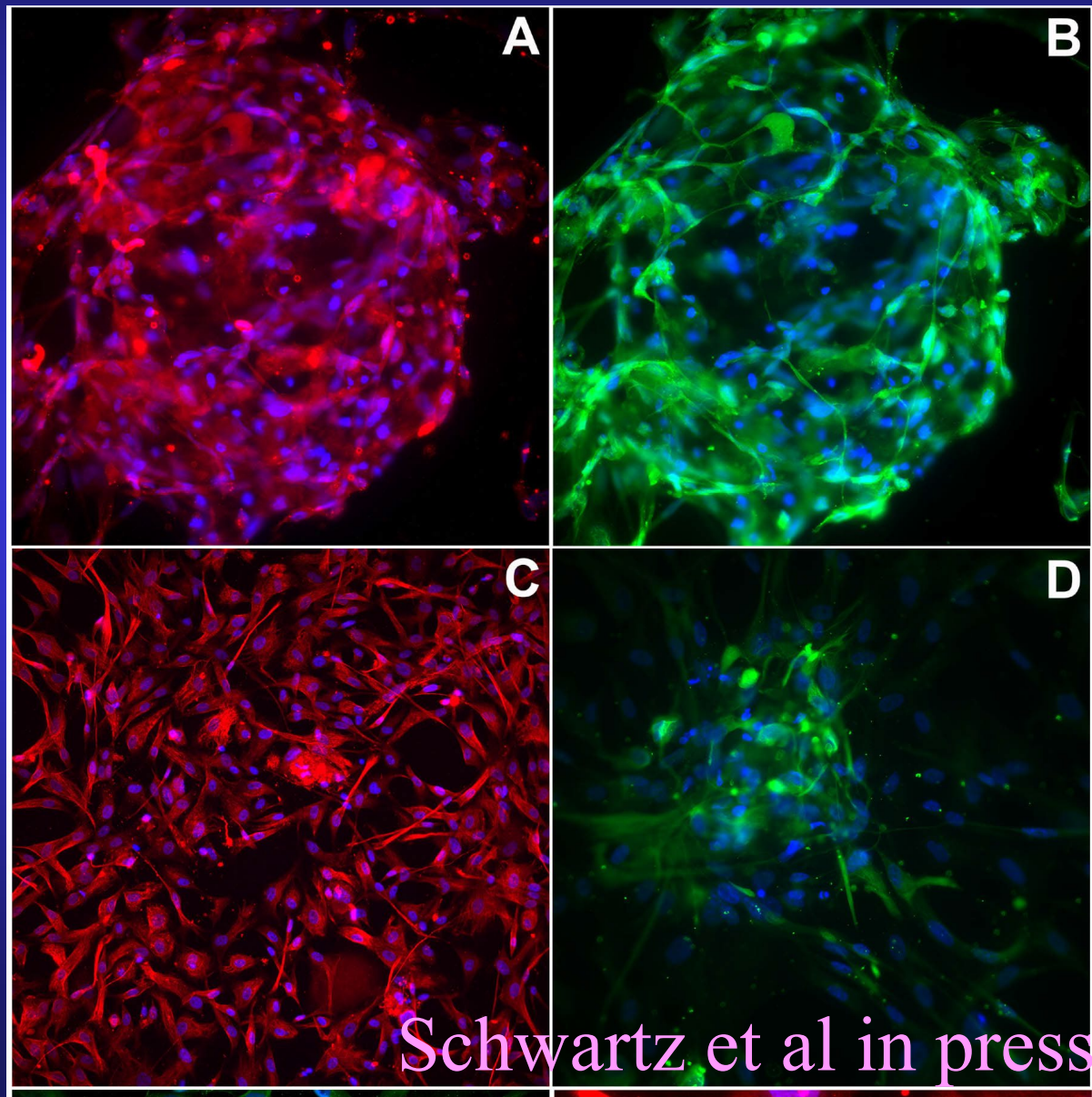




Schwartz et al 2004

Neural stem cells from a boy with FXS





Schwartz et al in press

The National Fragile X Foundation has Lesson Plans on line

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FXS and FXTAS

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