Research Advances in Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

Stephen T Nowicki, MD, PhD

Clinical Fellow,
Developmental and Behavioral Pediatrics
M.I.N.D. Institute
University of California, Davis, School of Medicine

Acknowledgements (partial list)

UC Davis School of Medicine

M.I.N.D. Institute

- Randi Hagerman
- Louise Gane
- Sebastien Jacquemont
- Tristan Jardini
- Susan Harris
- Kris Herman
- Faraz Farzin
- Nicole Tartaglia

Dept. Radiology

James Brunberg

Dept. Pathology

Claudia Greco

Dept. Biochem & Molec. Medicine

Paul Hagerman Lab

- Flora Tassone
- Dag Yasui
- Kendra Nannen
- Lexin Li
- Sasha Beilina
- Dolores Garcia-Arocena
- Chris Iwahashi
- Brooke Babineau
- Lisa Becker
- Li-Sheng Chen
- Anna Ludwig
- Chris Brick

University of Colorado Health Sciences Center (Denver)

Dept. Neurology

Maureen Leehey

Deborah Hall

Dept. Pediatrics

Ann Reynolds

Dept. Medicine

James Grigsby

RUSH-Presbyterian-St. Luke's Medical Center (Chicago)

Dept. Pediatrics

Elizabeth Berry-Kravis

Dept. Neurology

Christopher Goetz

Children's Hospital of Orange County

Philip Schwartz

San Diego State University

Dept. Statistics

Richard Levine

Support: NICHD, NINDS, NFXF
UC Davis M.I.N.D. Institute

Fragile X Syndrome

Fragile site

Fragile X Mental Retardation 1 (*FMR1*) Gene

Most common heritable form of mental impairment

- ~ 1 in 260 females and 1 in 800 males carries the mutation
- ~ 20 - 30% of all X-chromosome linked mental retardation
- ~ 1 – 3% of all mental retardation

Most common single gene associated with autism

- ~ 6% of all individuals with autism
- ~ one-third of young children with fragile X syndrome have autism

Fragile X Syndrome

Broad spectrum of involvement

- cognitive deficits (lowered IQ)
- shyness/social anxiety $\rightarrow$ autism
- ADHD
- mild to severe mood instability

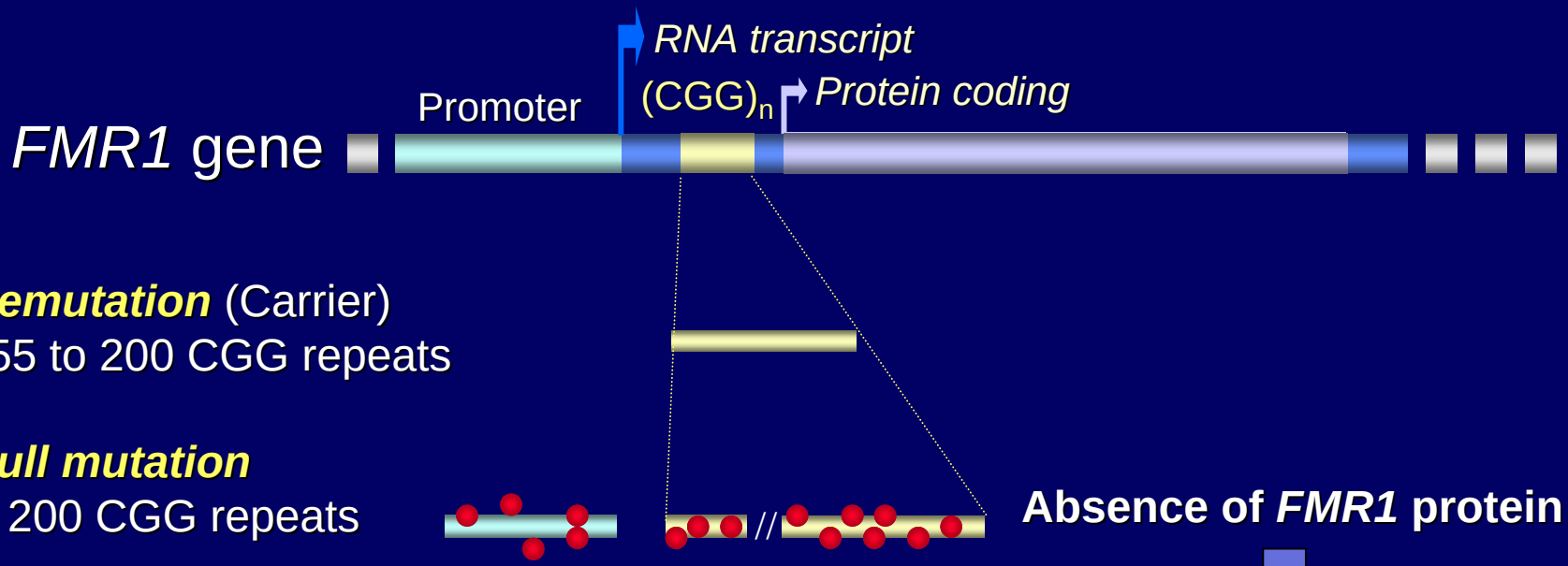
Physical features include

- large/prominent ears
- macroorchidism
- mitral valve prolapse
- joint laxity

Fragile X Syndrome

. . . is caused by a large CGG-repeat expansion in a non-coding portion of the *FMR1* gene

Typical development: n < 55 CGG repeats



Premutation (Carrier)
55 to 200 CGG repeats

Full mutation
> 200 CGG repeats

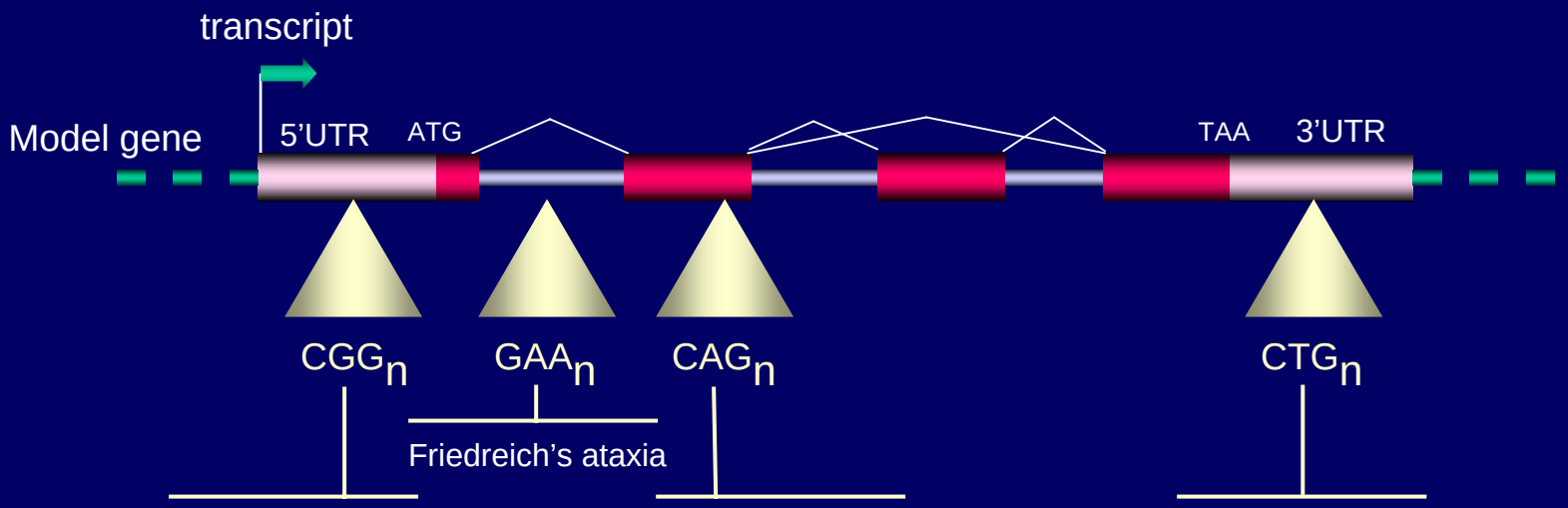
CpG methylation

FMR1 gene silencing (generally)

Absence of *FMR1* protein

fragile X syndrome

Human disease-causing trinucleotide repeat expansions



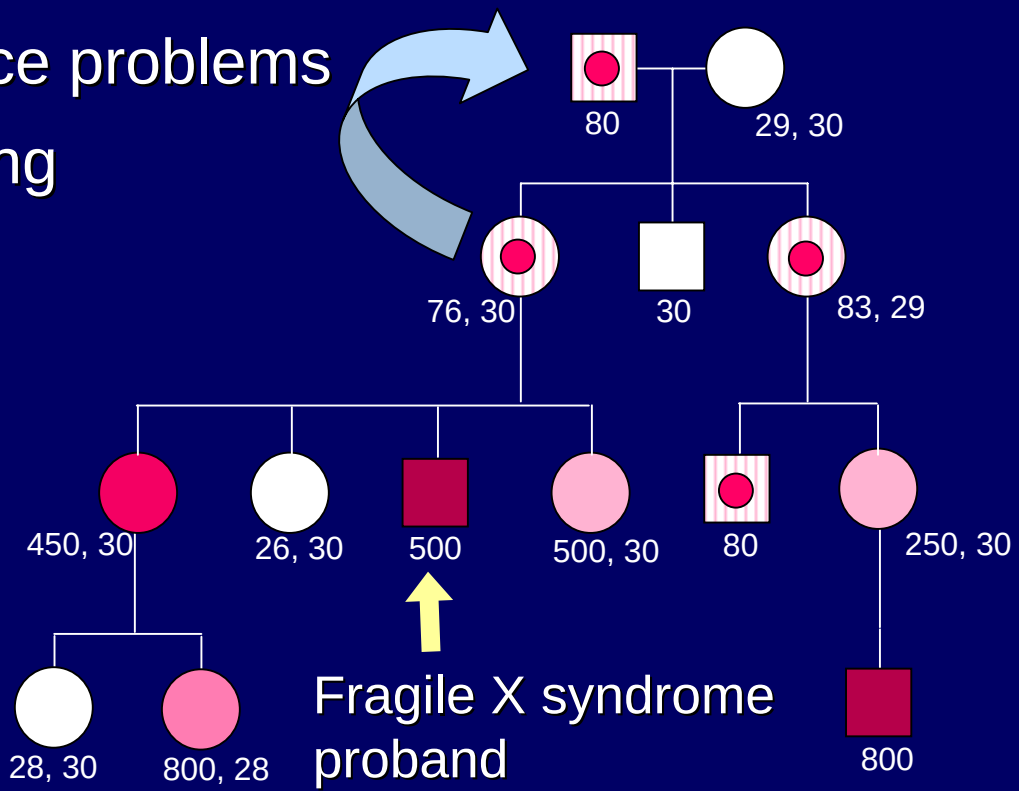
Fragile X syndrome
Fragile XE syndrome
Fragile site 11B
Jacobson syndrome (chromosomal deletion disorder)

Huntington's disease
Dentatorubral-pallidoduysian atrophy (DRPLA)
Spinal and bulbar muscular atrophy (SBMA)
Kennedy's disease
Spinocerebellar atrophy – (SCA-1)
SCA-2
SCA-3 (Machado-Joseph disease)
SCA-6
SCA-7
SCA-17 (TBP)

Myotonic dystrophy (DM1)

Mothers of fragile X children complained of specific problems with their own (carrier) fathers

- Frequent falls/ balance problems
- Difficulty writing, eating
- Memory loss



Case 1 (DR)
63 grandfather 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
  - VIQ-93, PIQ-73, FSIQ-83

Clinical features of Fragile X-associated tremor/ataxia syndrome

Intention tremor

Gait ataxia

Peripheral neuropathy

Cognitive decline

Autonomic dysfunction

Anxiety, mood instability

Parkinsonism

Hagerman et al (2001) Neurology

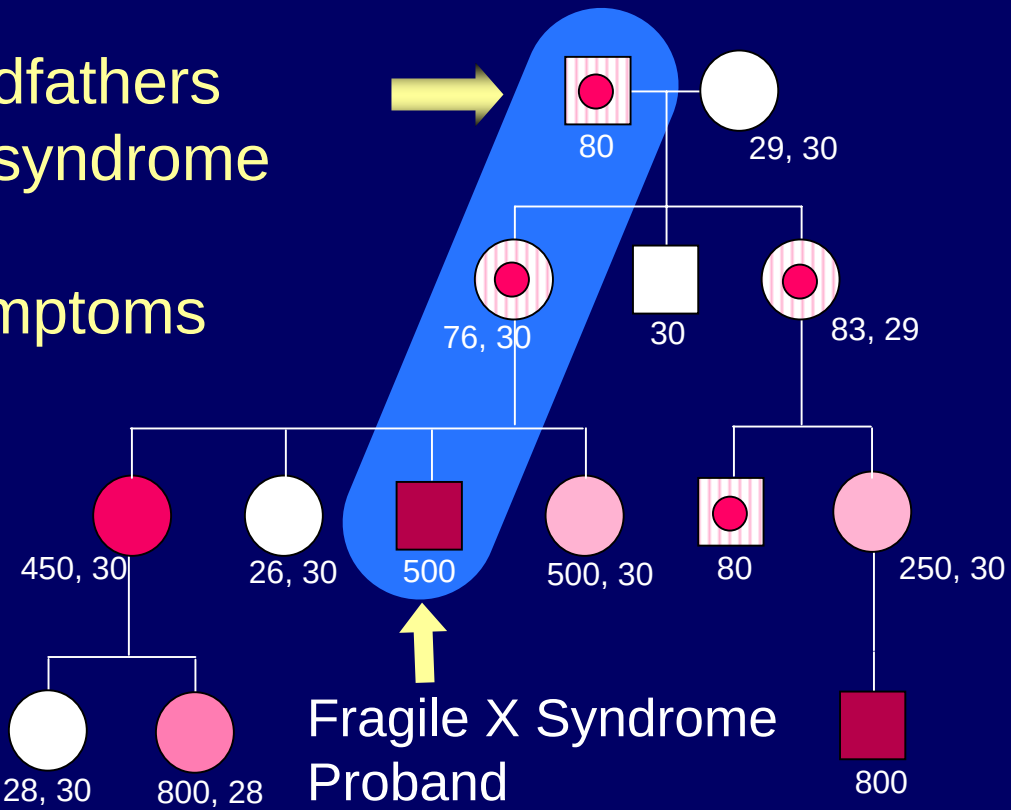
First 5 cases described – all males over 50 yo

Fragile X-associated tremor/ataxia syndrome (FXTAS)

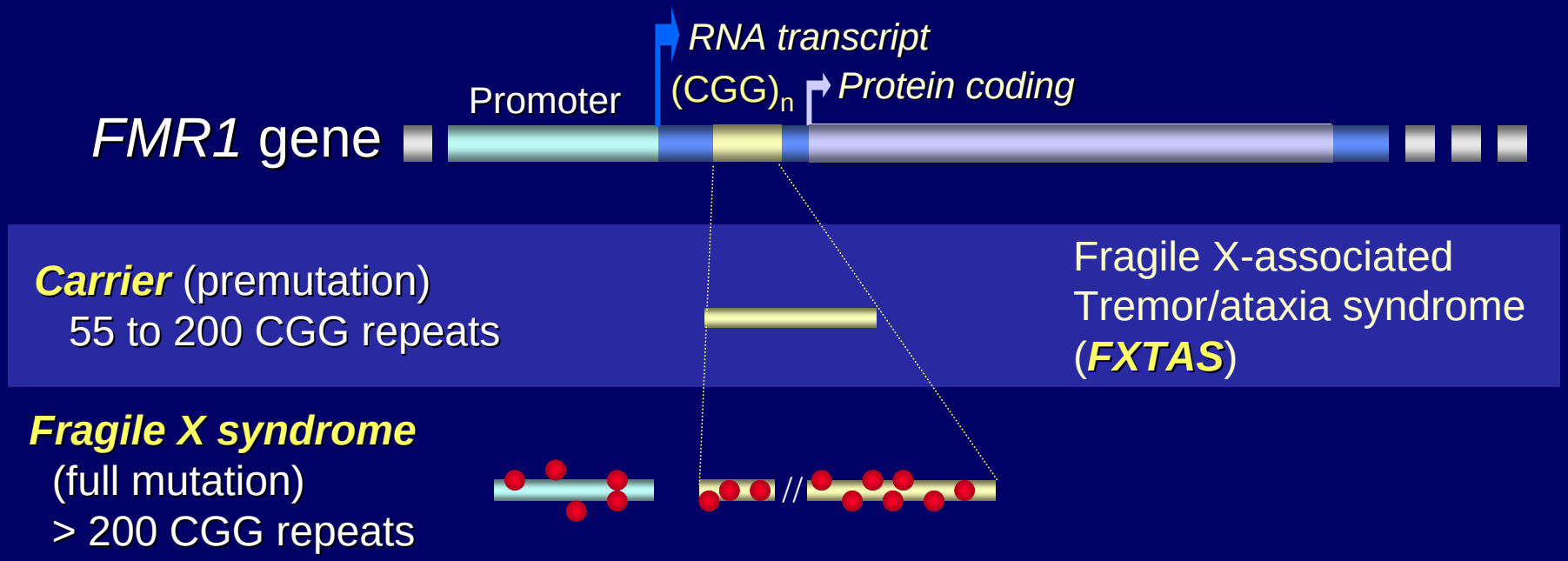
Appears to affect (premutation) carriers exclusively

Usually identified in grandfathers of children with fragile X syndrome

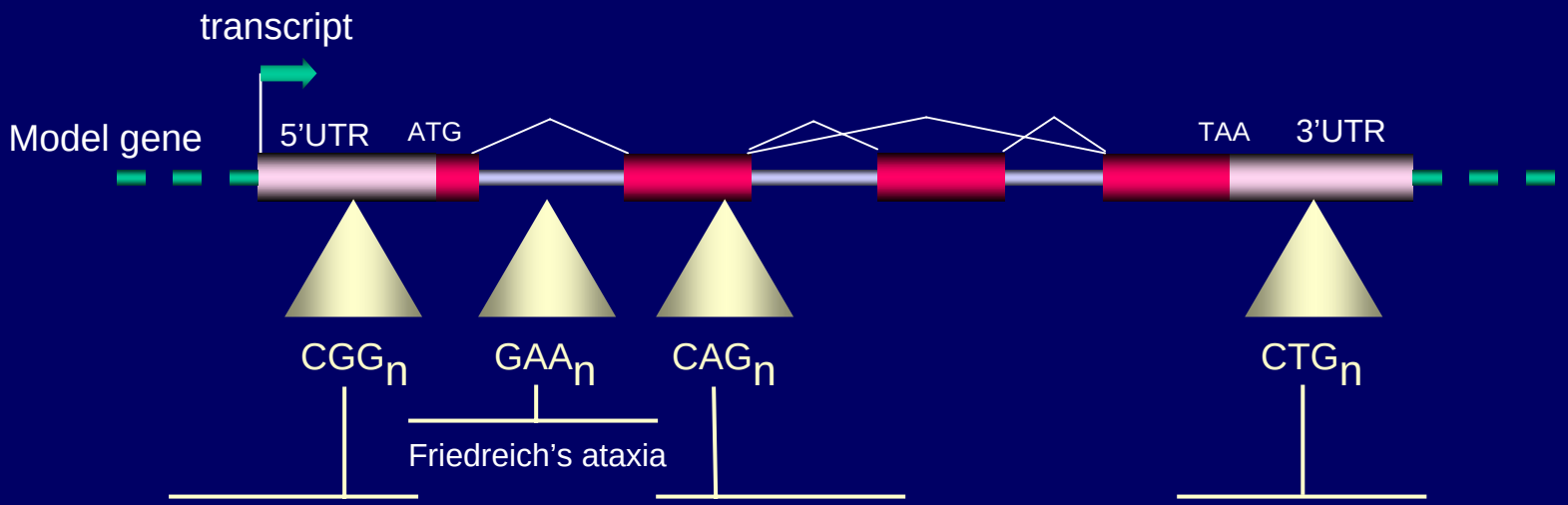
Onset of neurological symptoms nearly always over 50 yr



A distinct neurological phenotype among carriers of premutation alleles



Human disease-causing trinucleotide repeat expansions



FXTAS
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SCA-7
SCA-17 (TBP)

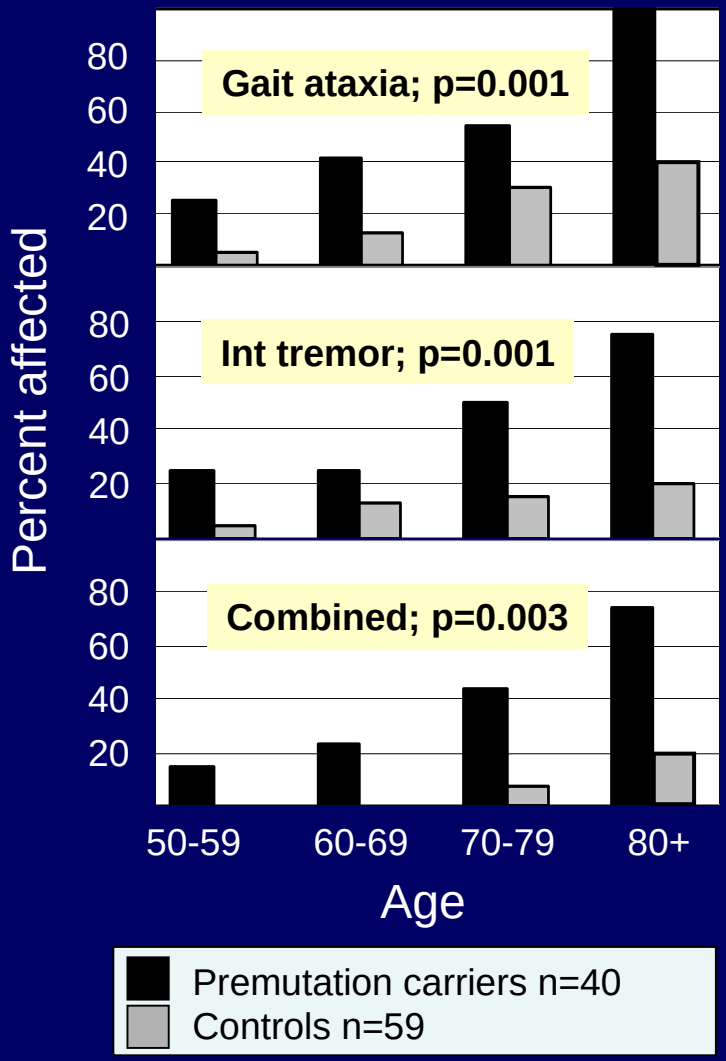
Myotonic dystrophy (DM1)

Penetrance of FXTAS: a California family study

Jacquemont et al (2004) JAMA 29:460

- 123 families with FXS in the Northern and Southern Fragile X Associations
- 192 individuals >50yr (premutation carriers and controls) penetrance in male carriers:

Penetrance of tremor/ataxia in male carriers of premutation alleles



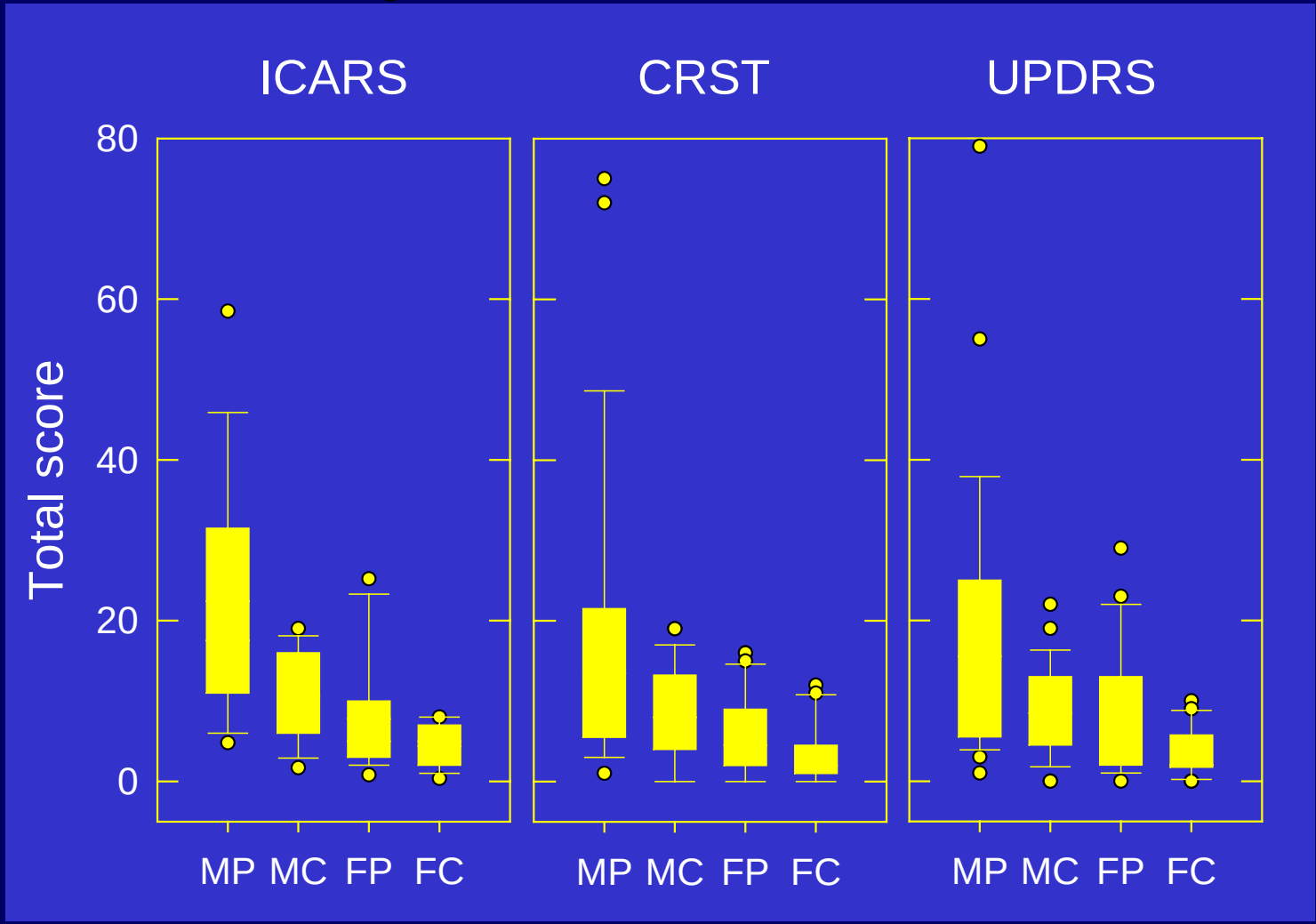
Jacquemont et al (2004) JAMA

- At least *one-third* of male premutation carriers (>50 y) have combined tremor and ataxia
  ~ 1/3,000 males in general population with lifetime risk of FXTAS
- Penetrance increases with age
- Penetrance far lower in females
  differences seen on the neurological exam may represent subclinical features

Neurological Exam

- Motor portion of the Unified Parkinson's Disease Rating Scale (UPDRS)
- The International Cooperative Ataxia Rating Scale (ICARS)
- Clinical Rating Scale for Tremors (CRST)

ICARS, CRST, and UPDRS total scores, by sex and carrier status



Clinical presentation of 87 premutation males

	FXTAS n=64 (51-89)	No FXTAS n=23 (51-80)
Tremor: intention	88%	9%
Tremor: resting	42	0
Gait ataxia	91	0
Falling	48	9
Walking aid	52	0
Neuropathy	48	22
Incontinence	34	0
Lower limb weak	34	9
MCP in 27/46	59	0

Jacquemont et al 2004; AJMR

Females with FXTAS

	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
Age	67y	57	85	62	74
FSIQ	126	99	100	111	87
VIQ	130	103	104	110	88
PIQ	116	94	94	111	86
Tremor onset	42y	30	82	52	71
Ataxia onset	59y	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	0.50	0.21
mRNA level	3.2x nl	4.6	1.4	2.5	2.6
MRI	+ MCP	- MCP	pacemk	pacemk	- MCP

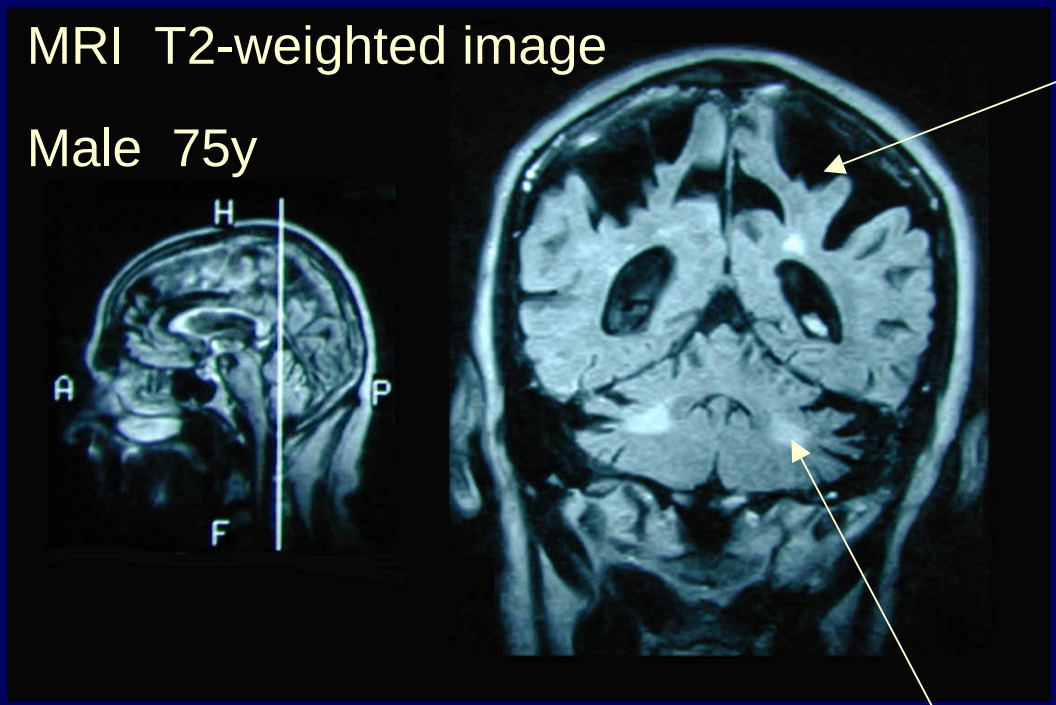
FXTAS in female carriers

Case 1

Written consent for
videotaping and display

FXTAS: Imaging correlates

Brunberg et al (2002) AJNR

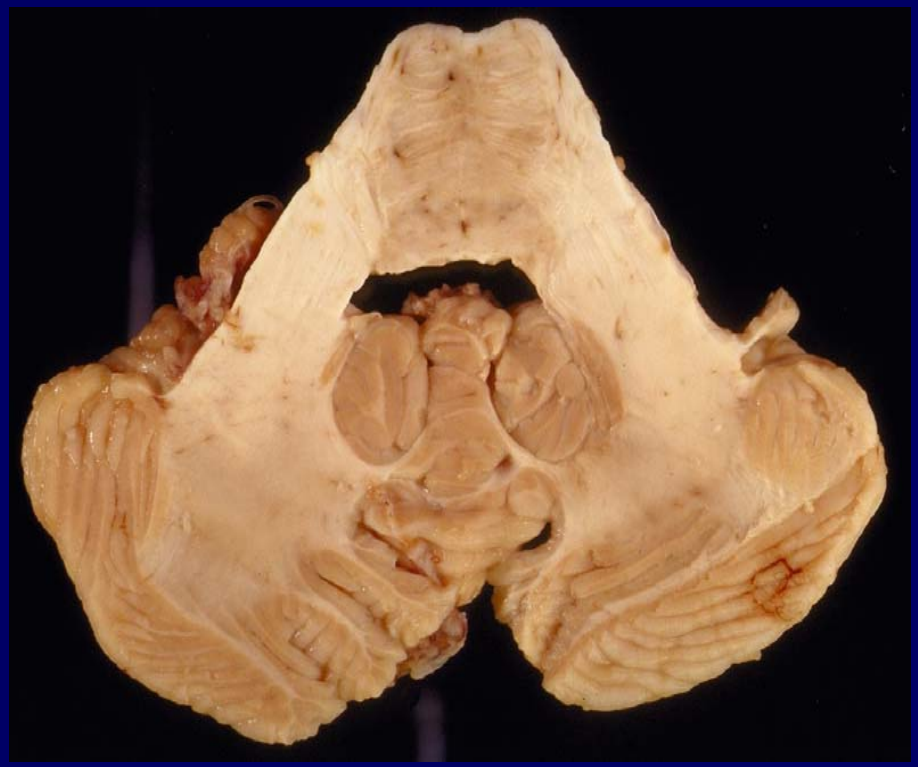


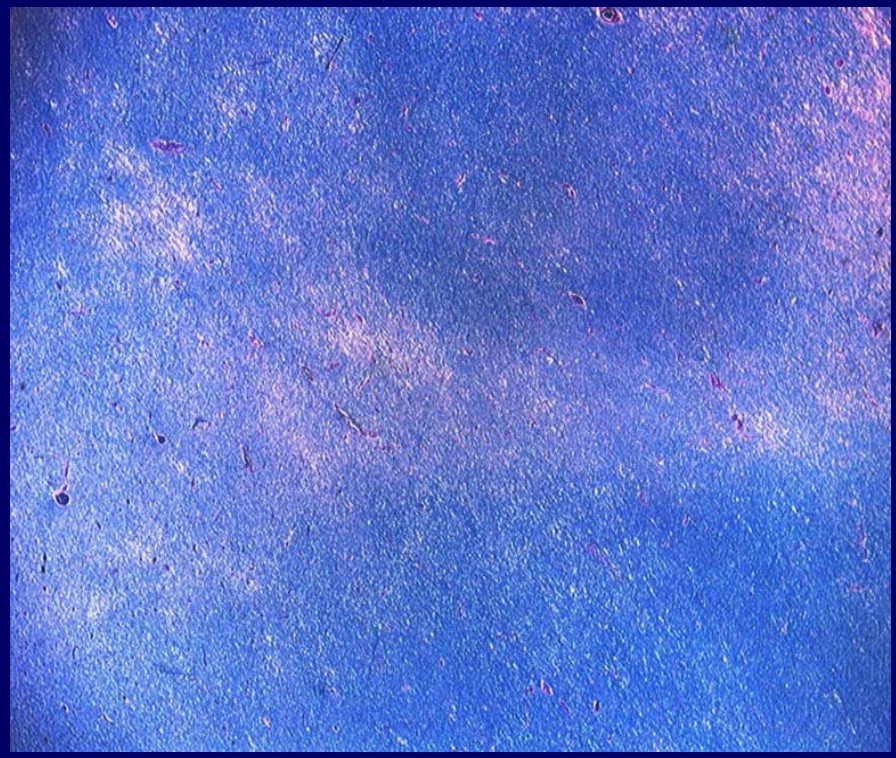
Severe cortical atrophy

Hyperintensities of the deep cerebellar white matter and middle cerebellar peduncle

"MCP" sign

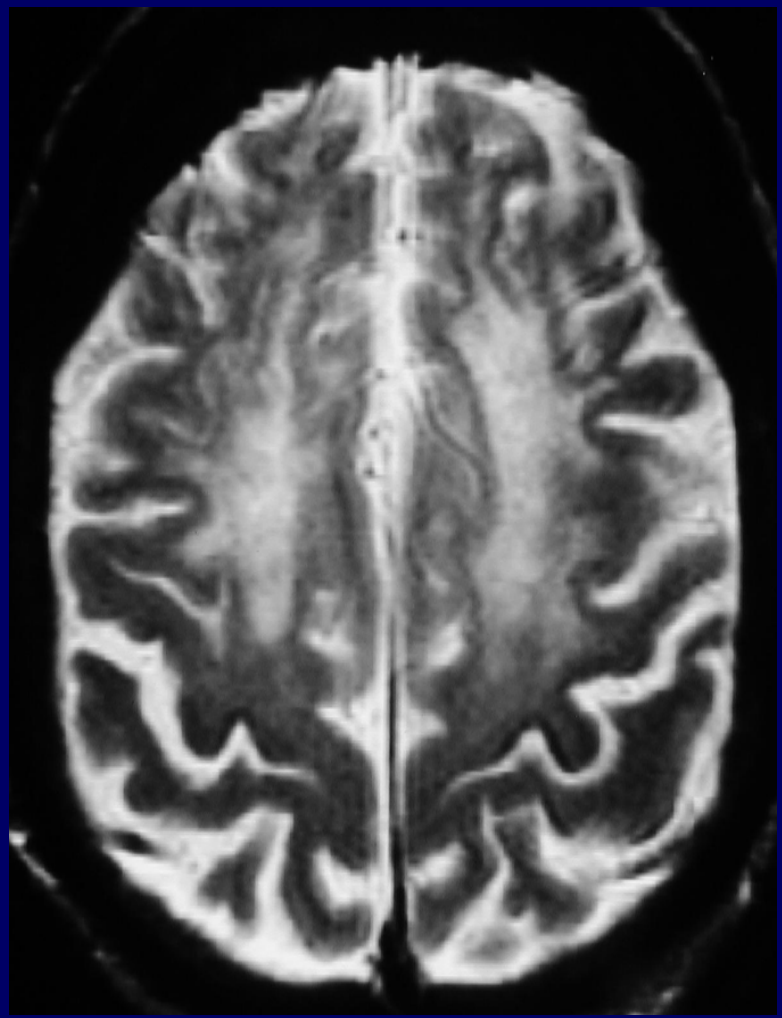
Middle cerebellar peduncle white matter involvement

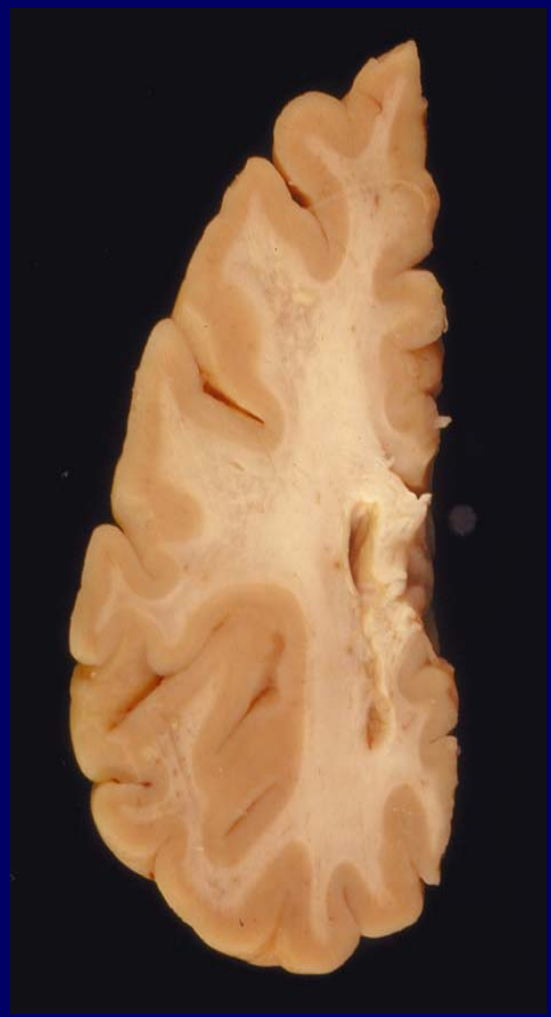




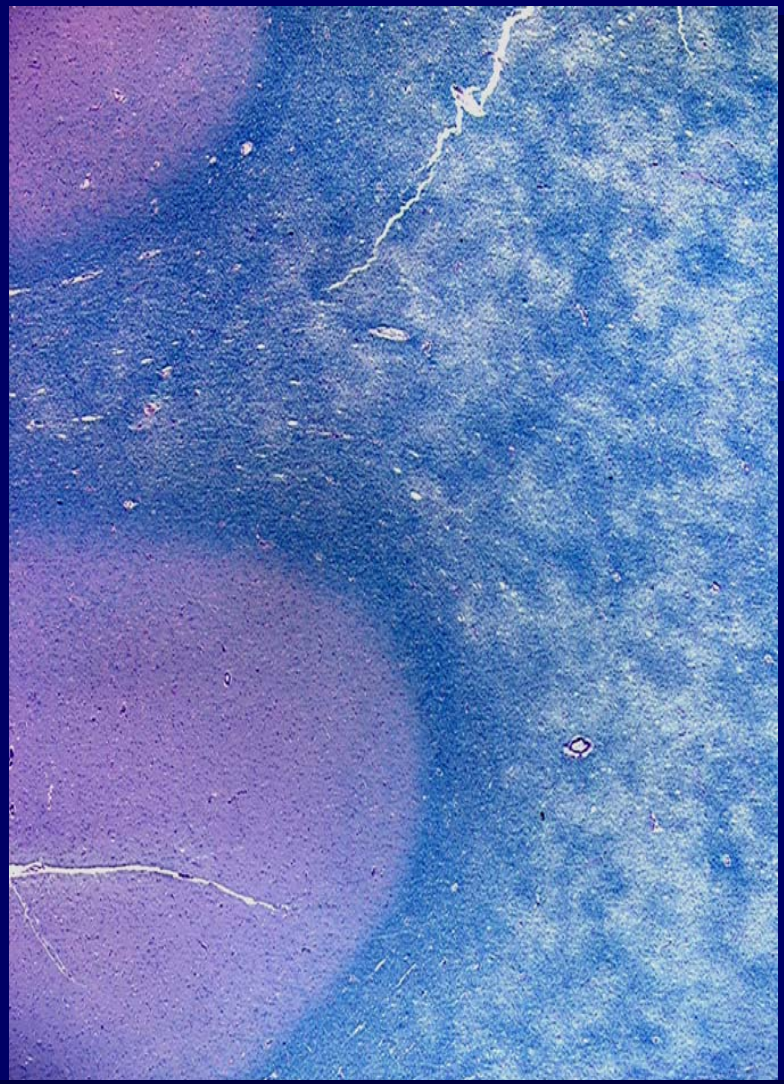
Patchy Pallor
LFB/PAS
Myelin stain

Subcortical white matter involvement

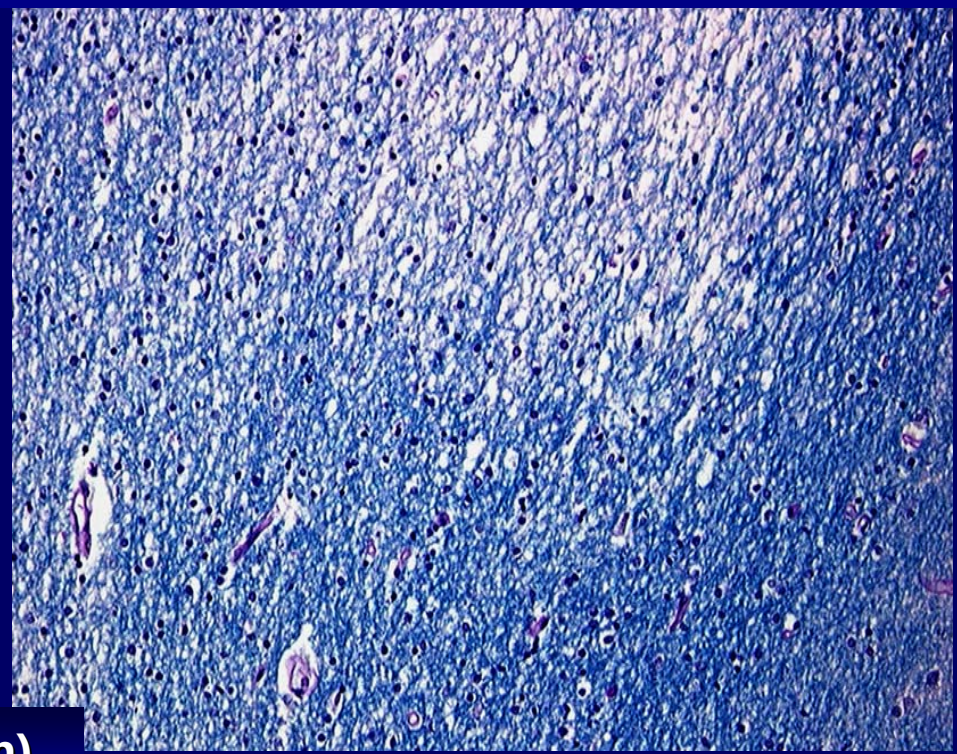




Frontal white matter

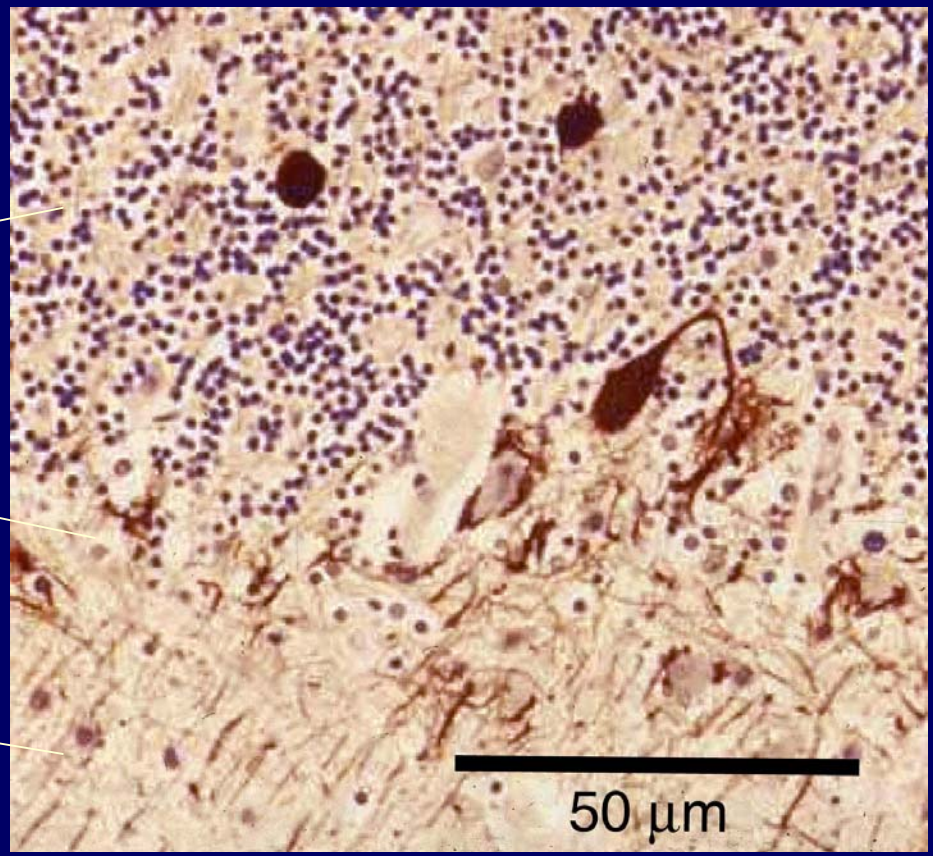


Patchy myelin pallor
Arcuate fibers involved
Glial cells decreased
Normal vessels
No inflammatory cells



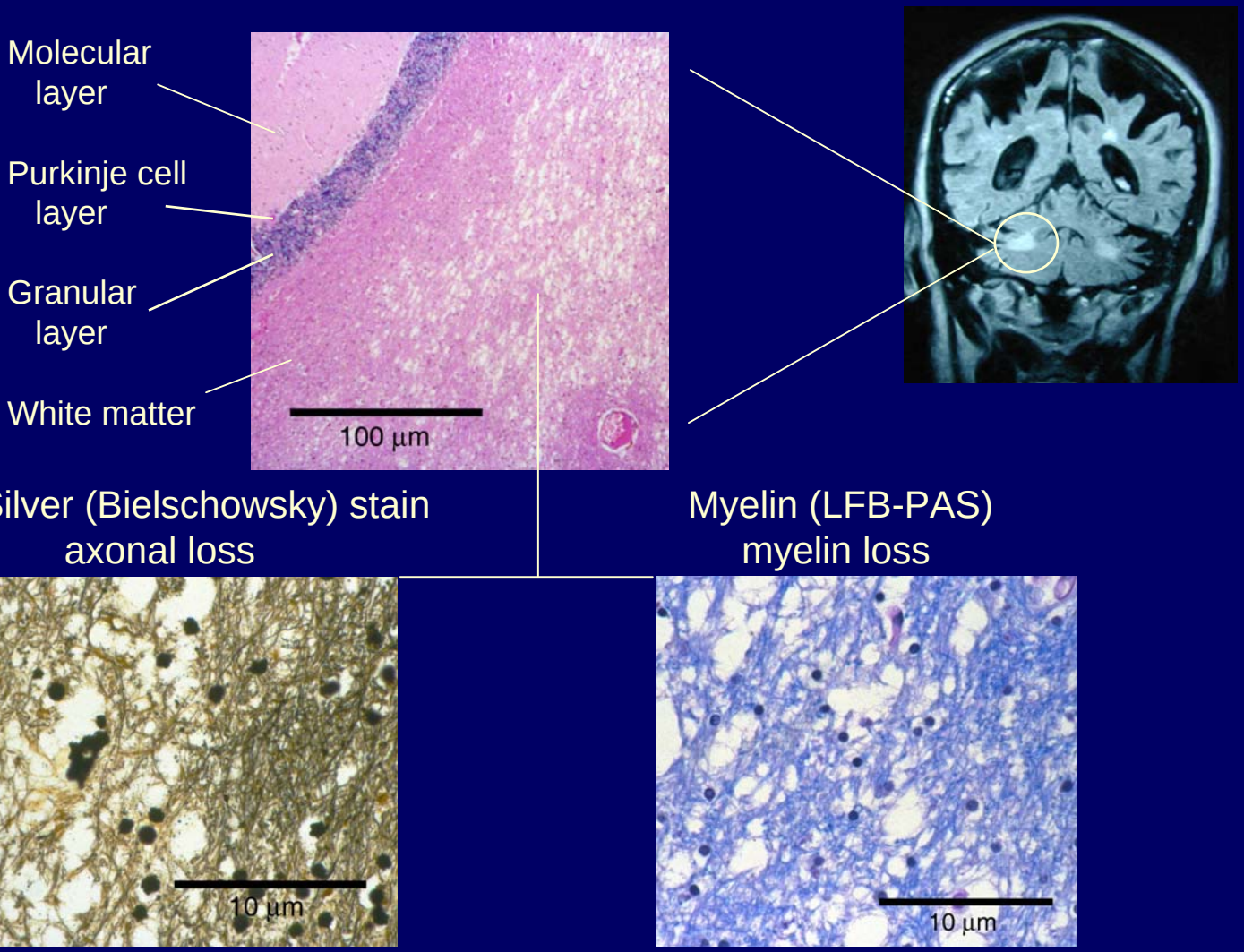
Luxol fast blue – PAS (Myelin stain)

Scattered (cerebellar) axonal torpedoes, Purkinje cell dropout, Bergmann gliosis



Neurofilament Abs

Spongioform changes of deep cerebellar white matter

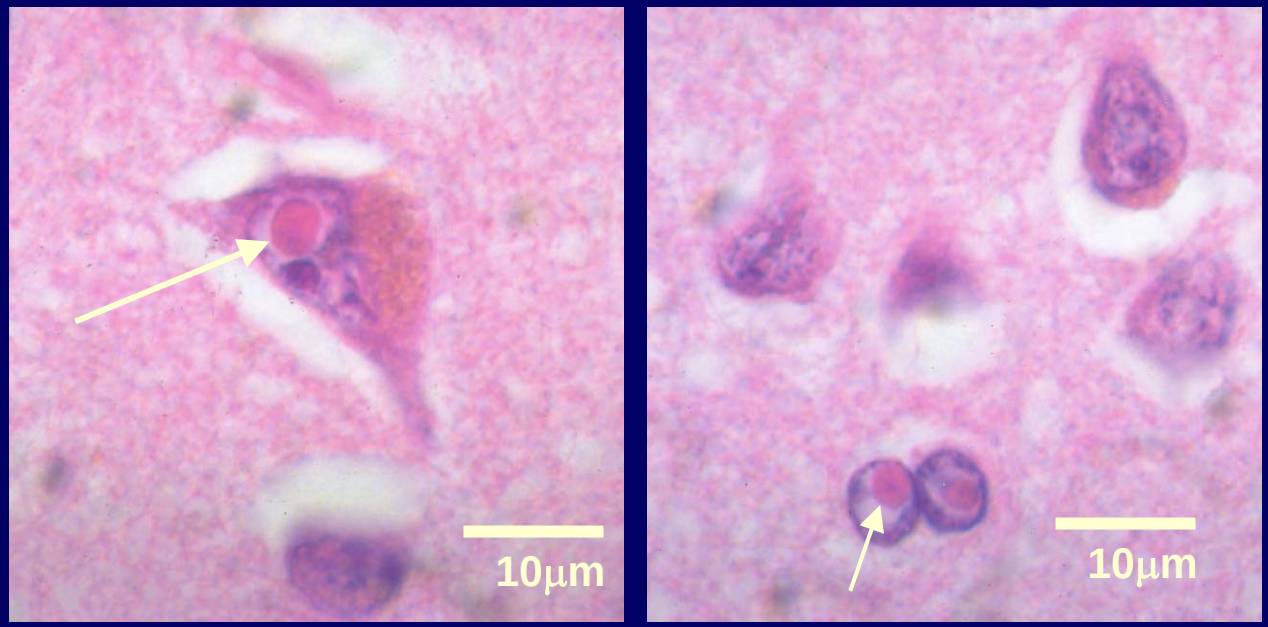


FXTAS is an inclusion disorder

Greco et al (2002) Brain

Cortical neuron

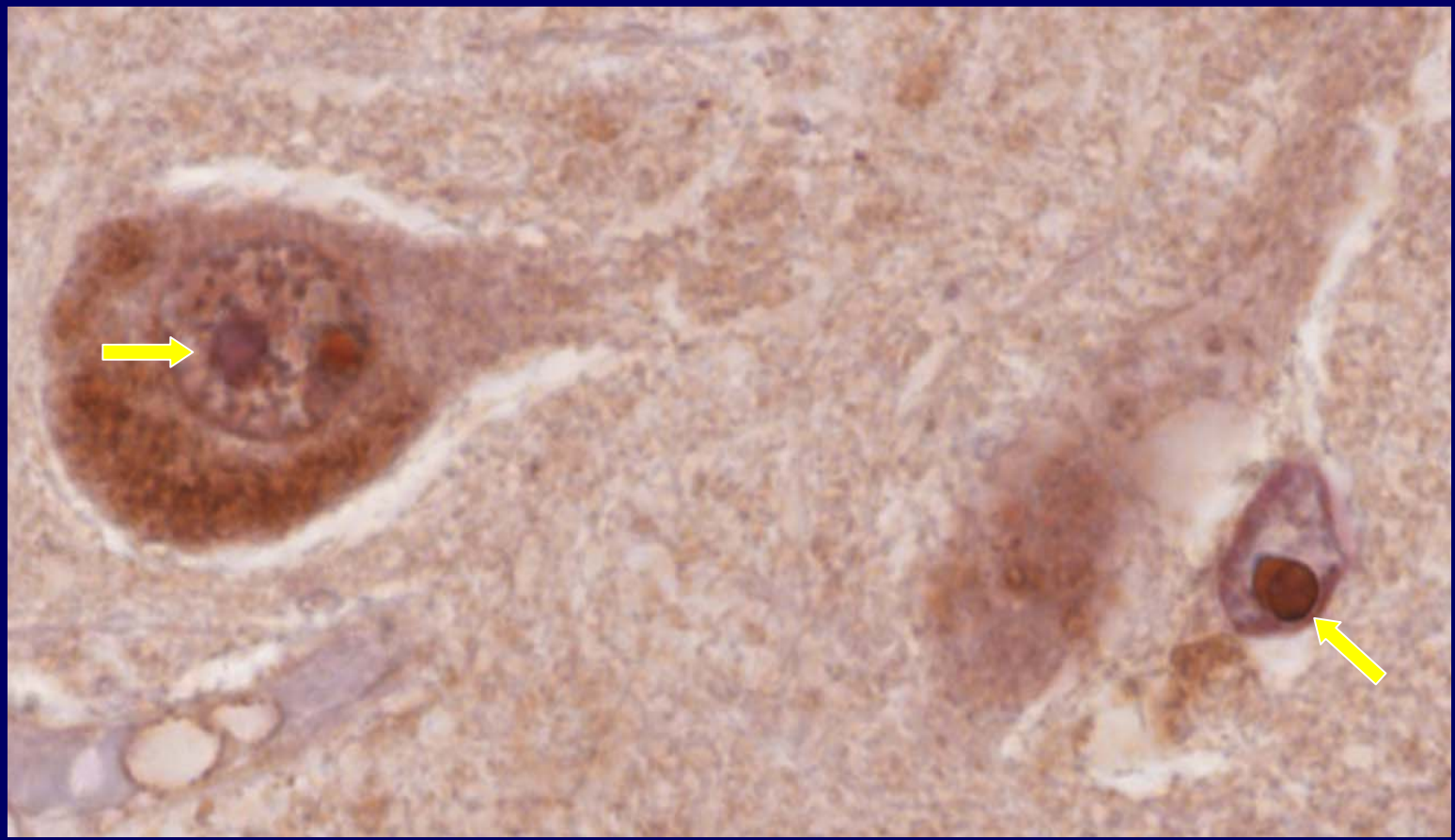
Astroglia



H & E

All brains (12/12) from individuals with FXTAS examined thus far – 11 males and 1 female – possess inclusions

Neuronal and astrocytic inclusions - also in females with FXTAS



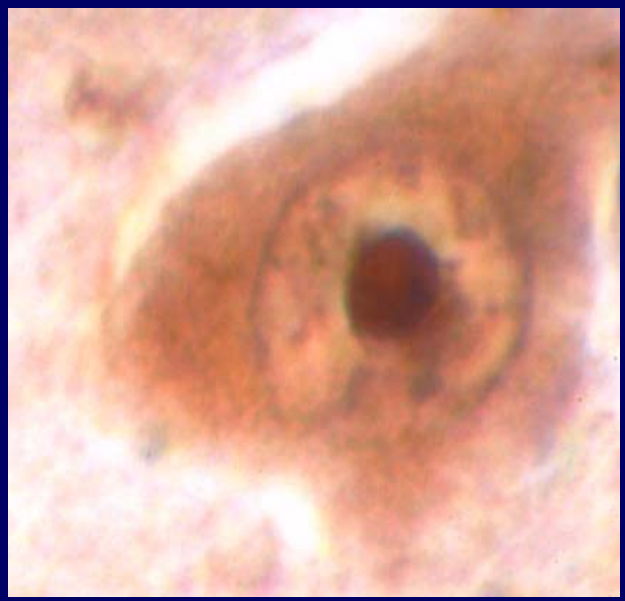
Case 3

Intranuclear inclusions

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

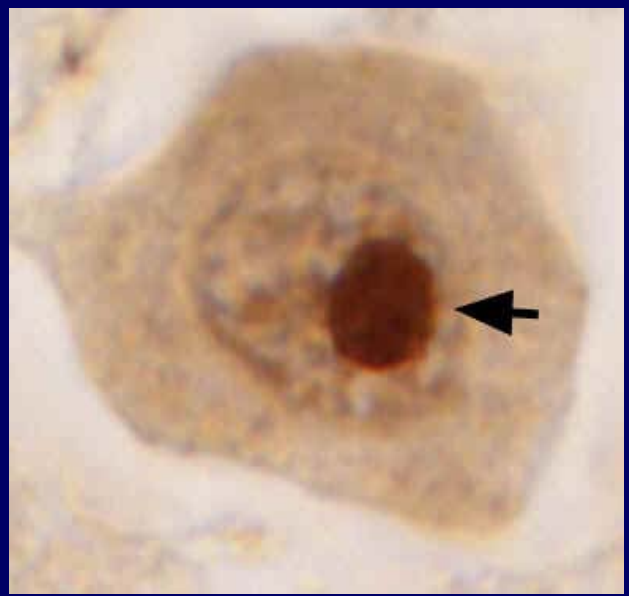
FXTAS inclusions are similar to those in CAG-repeat disorders

FXTAS: CGG repeat expansion
NO abnormal protein



Greco *et al*., Brain (2002)

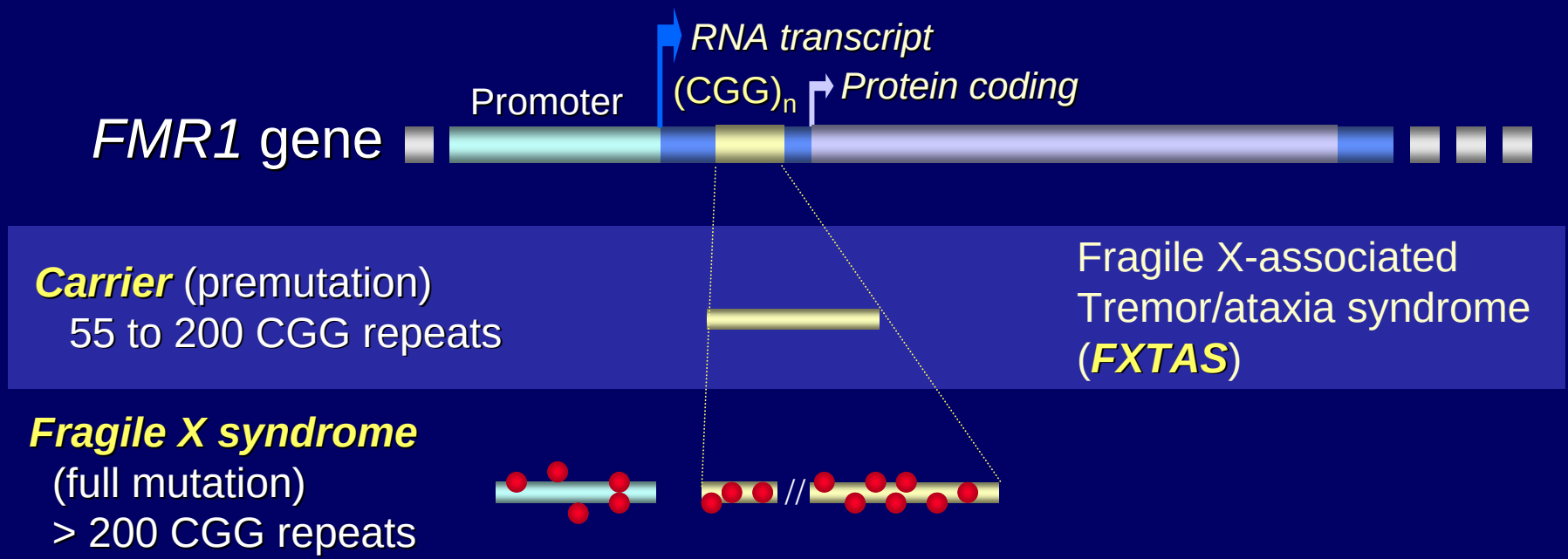
SCA3: CAG repeat expansion –
Abnormal (polyQ)protein



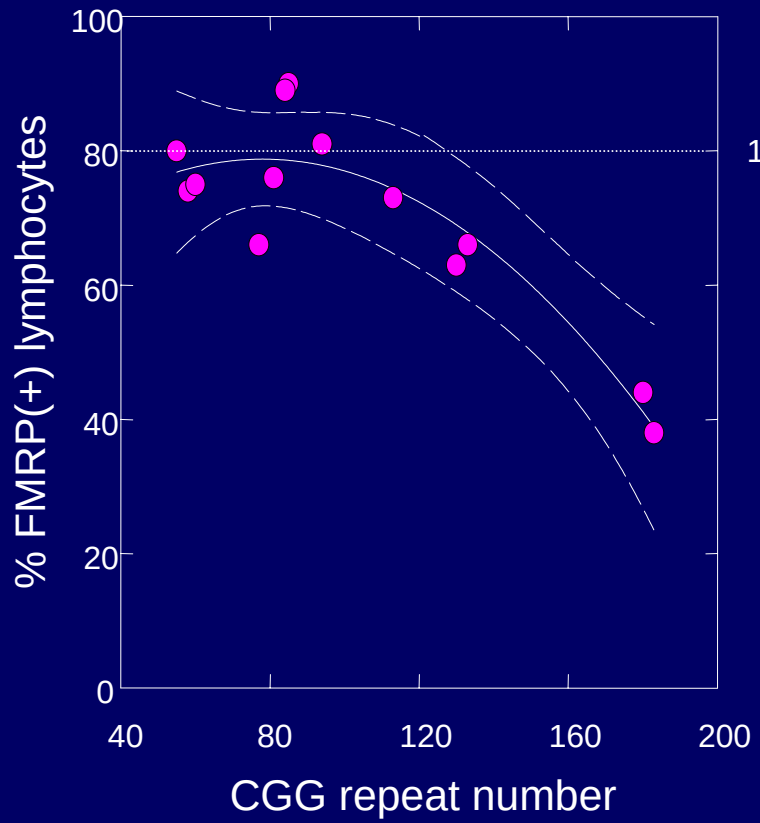
Anti-Ubiquitin

Chai *et al*., Hum Mol Genet (1999)

What is the molecular basis of FXTAS?



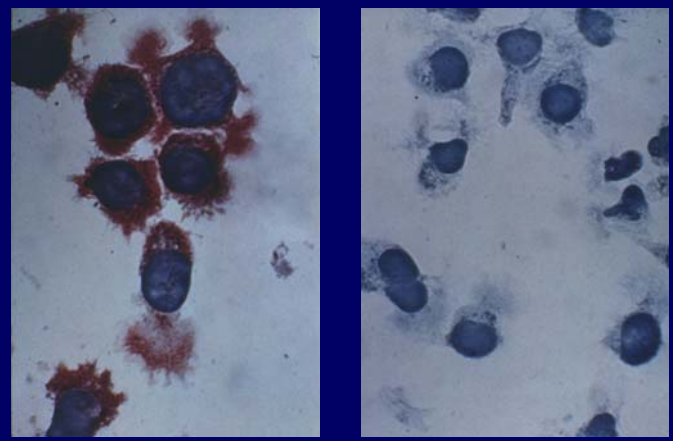
FMRP levels are reduced for CGG expansions in the upper premutation range



1 SD below mean of ~89%

Typical FMRP determination

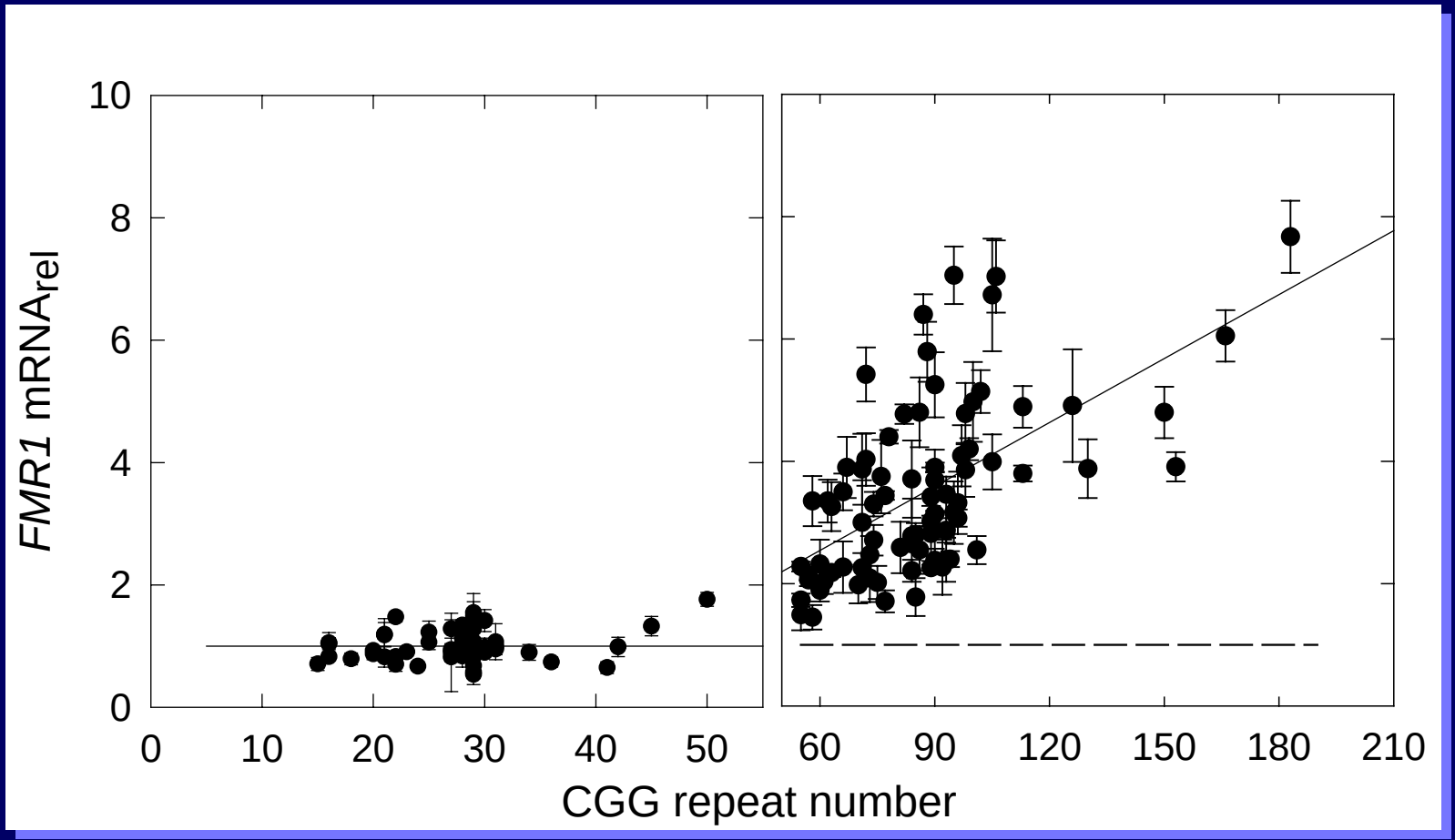
- 200 lymphocytes
- positive or negative call
- SEM ~4% of the mean



Willemsen et al., 1995, 1997

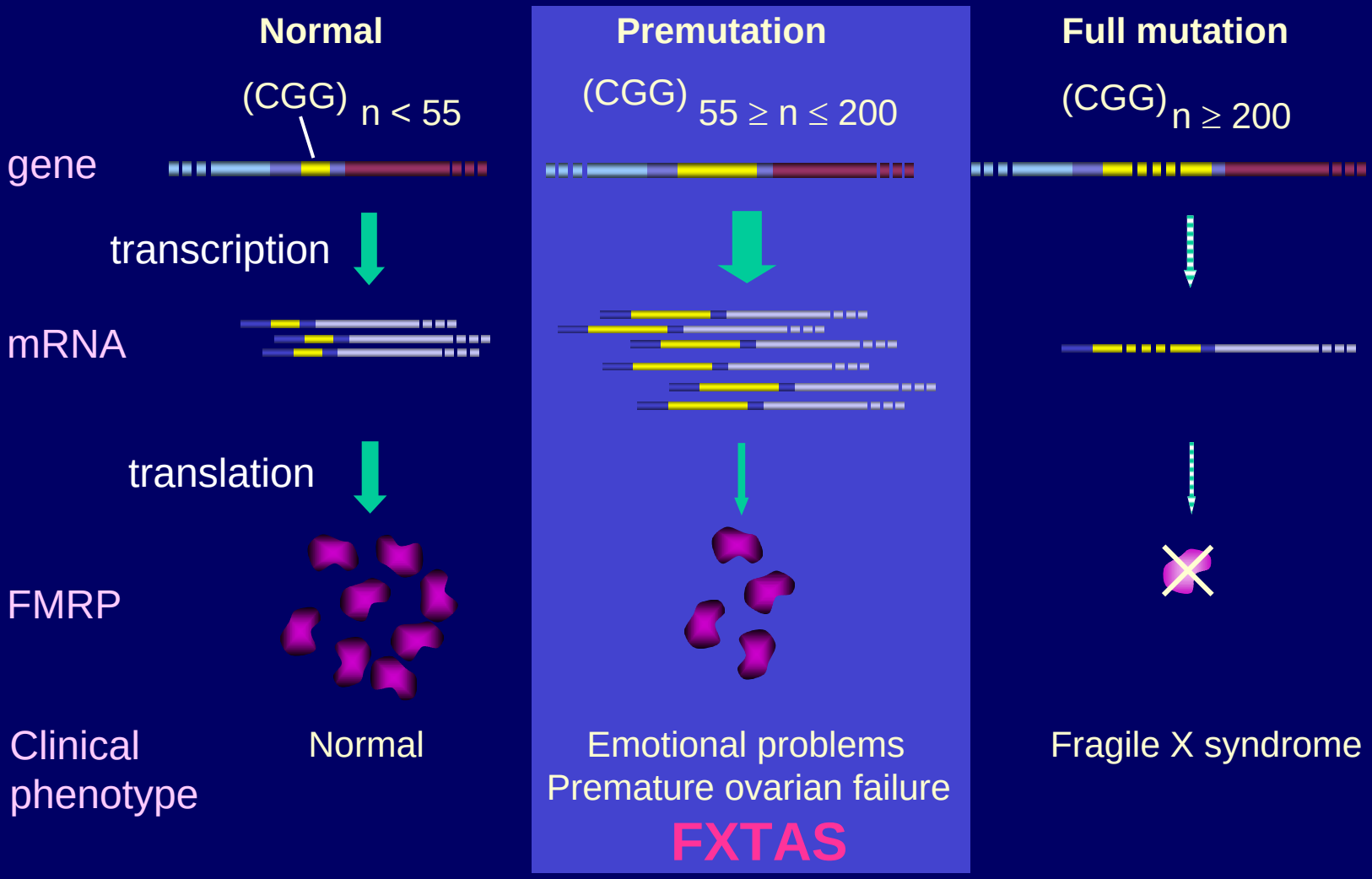
Why ? … the FMR1 gene is unmethylated in this range

FMR1 mRNA is *elevated* in the premutation range



Tassone et al (2000) AJHG and unpublished

Expression of the *FMR1* gene



RNA “toxic gain-of-function” model for FXTAS

Hagerman et al (2001) Neurology
Greco et al (2002) Brain

- FXTAS is not due to absence of protein
  - Premutation carriers have near normal levels of FMRP

- Not due to expansion of CGG repeat, *per se* (as DNA)
  - Males with full mutation alleles apparently do not develop FXTAS

- Likely due to presence of abnormal *FMR1* mRNA in carriers of premutation alleles

expanded CGG repeat in 5’ leader
elevated levels of *FMR1* mRNA

This RNA model predicts (rare) cases of FXTAS in full mutation range

A Precedent for an RNA gain-of-function disorder:

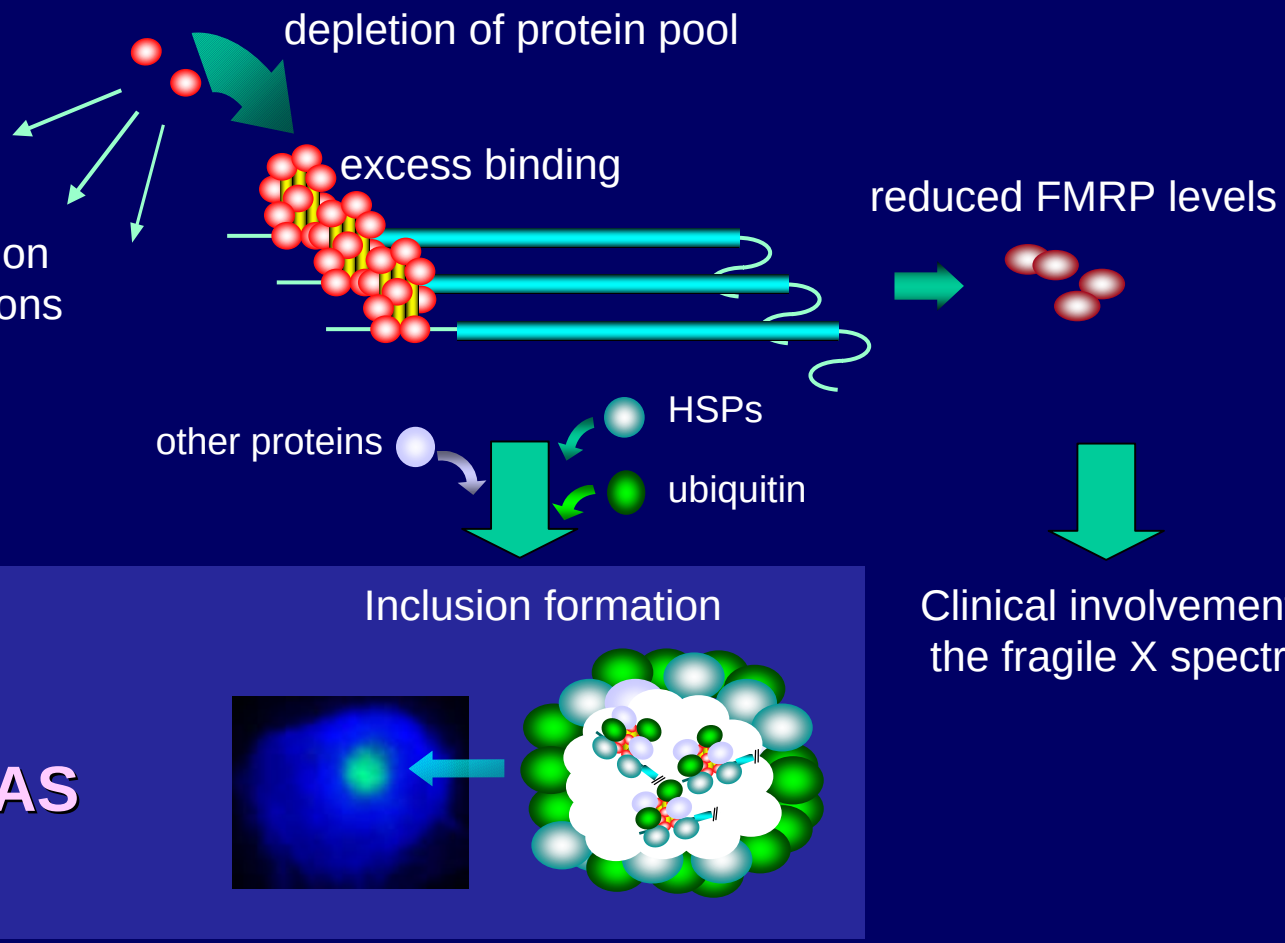
Myotonic dystrophy

- Two genes of unrelated function, *DMPK* (19q) and *ZNF9* (3q), give rise to myotonic dystrophy (DM1 and DM2, respectively)
- No point mutations/deletions in either gene cause DM
- Both loci possess ***non-coding*** repeats, CUG and CCUG, that are expanded in the disease state
- Both repeat-containing RNAs accumulate in nuclear foci
- Expression of $(CUG)_{250}$ in transgenic mice leads to both myotonia and histopathological features of DM1 muscle.

RNA gain-of-function model for FXTAS

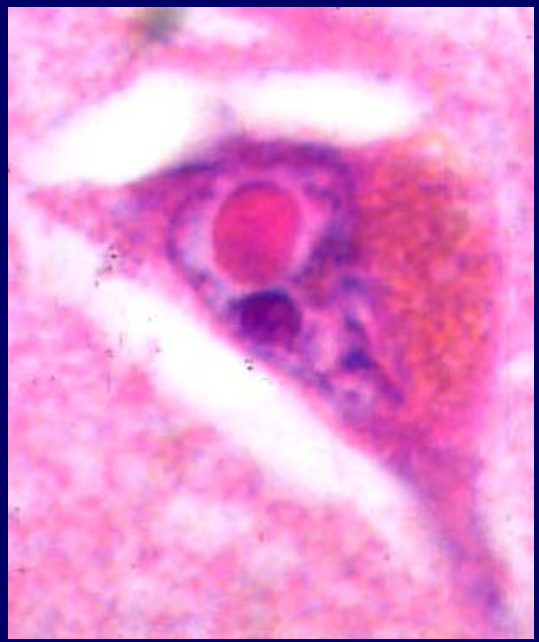
Premutation allele > 54 CGG repeats

Hypothetical:
DM-type model

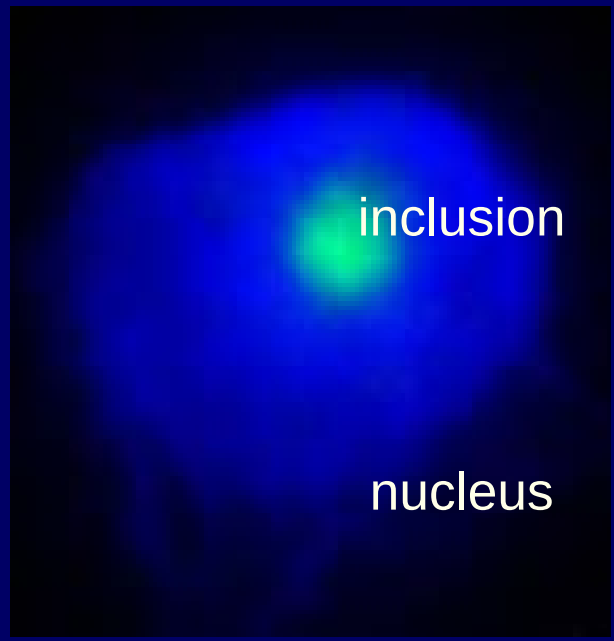


The inclusions may reveal the origin of FXTAS

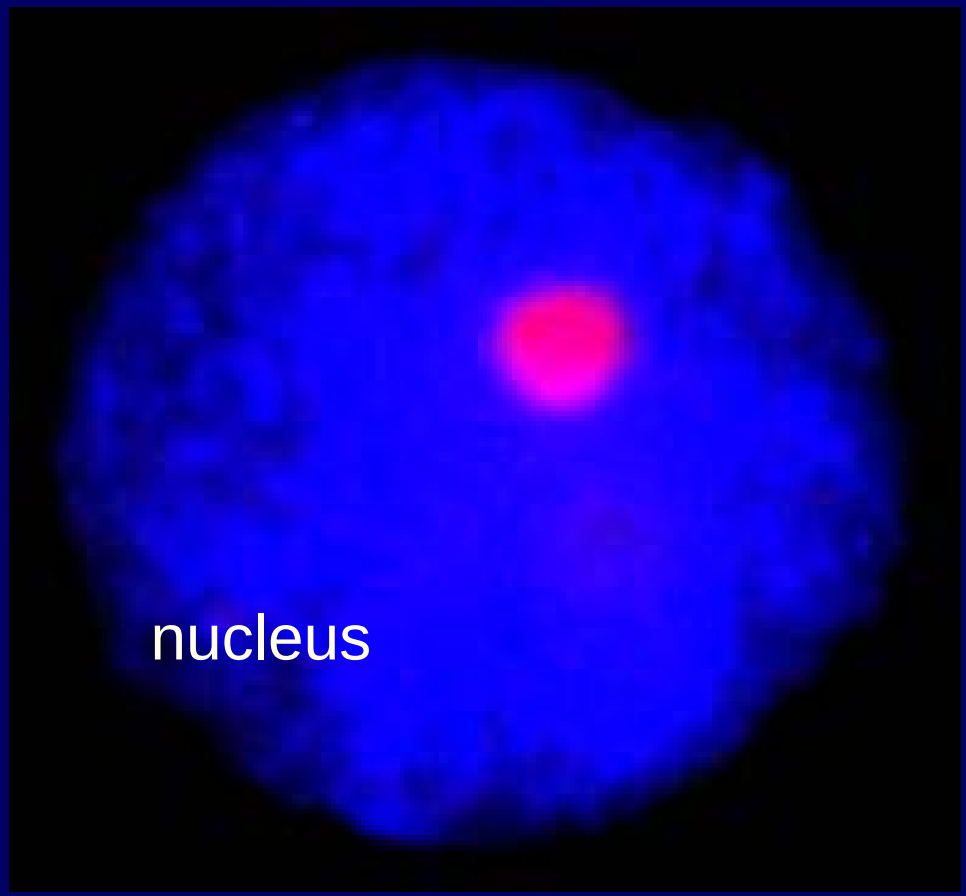
Iwahashi et al.



Isolate the nucleus



Do the inclusions contain the mRNA from the *FMR1* gene?



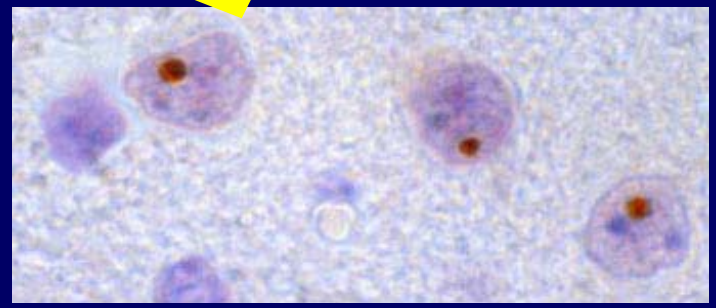
Tassone et al.

Yes

Fluorescent staining of the FXTAS inclusions with a probe that is specific for the fragile X mRNA

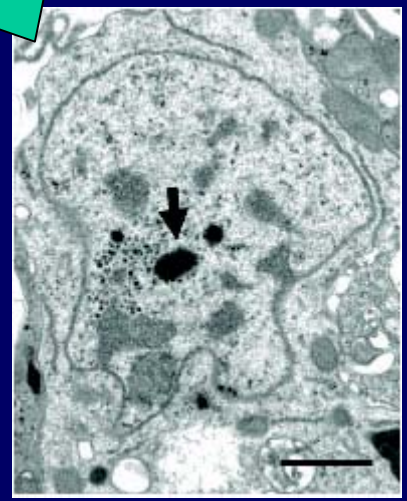
The CGG repeat – *as RNA* – stimulates formation of inclusions

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



Willemsen

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



Warren

RNA “gain-of-function” model for FXTAS

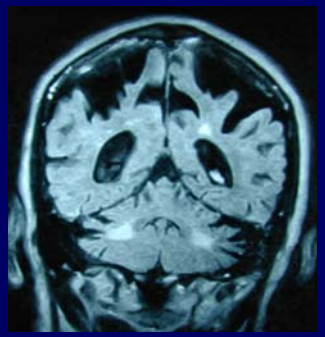
Evidence strong for an RNA gain-of-function model, but what mechanism?

– How is the mRNA involved?

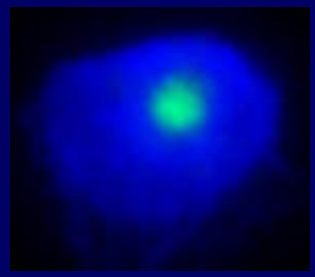
– What are the protein players?

What proteins are in the inclusions?

Isolation of inclusions

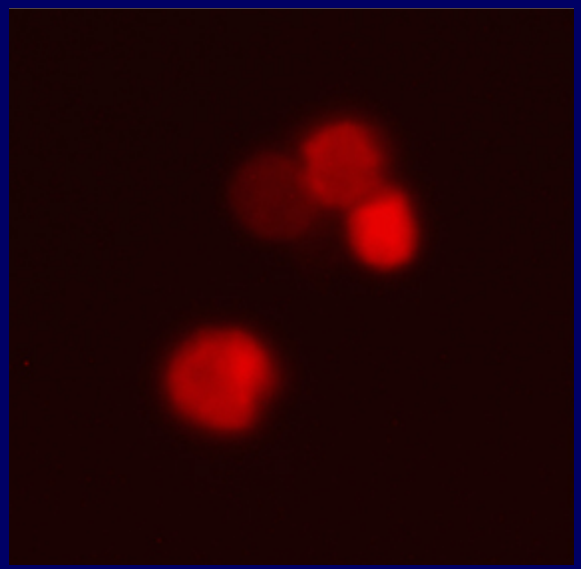


Isolate nuclei from
frozen cortical tissue

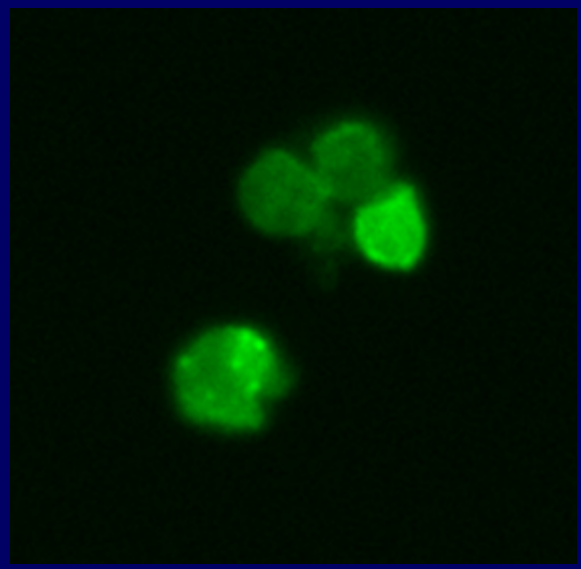


Disruption of nuclei and preparative flow sorting to yield purified inclusions (~10^6 inclusions /gram brain tissue)

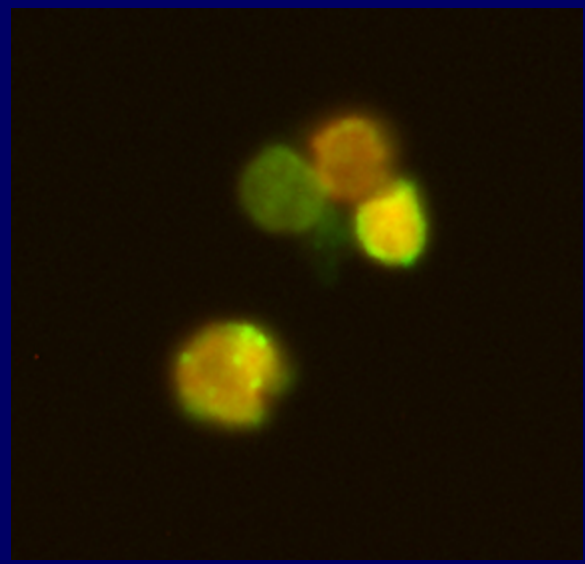
αB-crystallin



ubiquitin

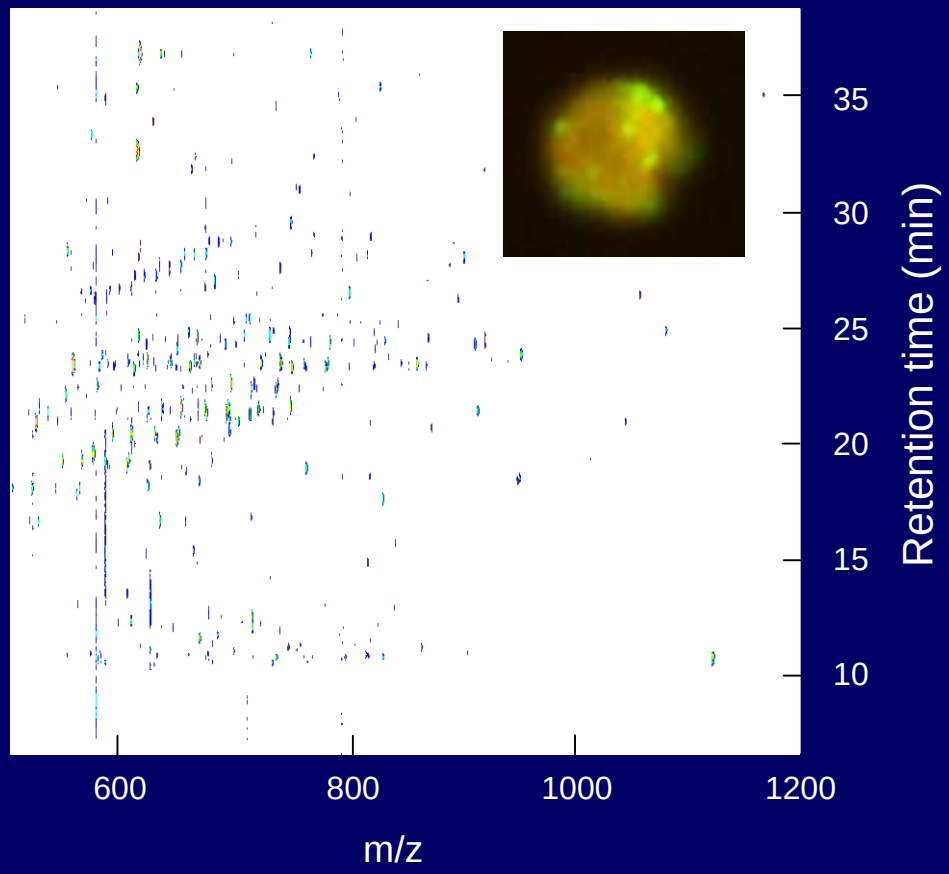


merge

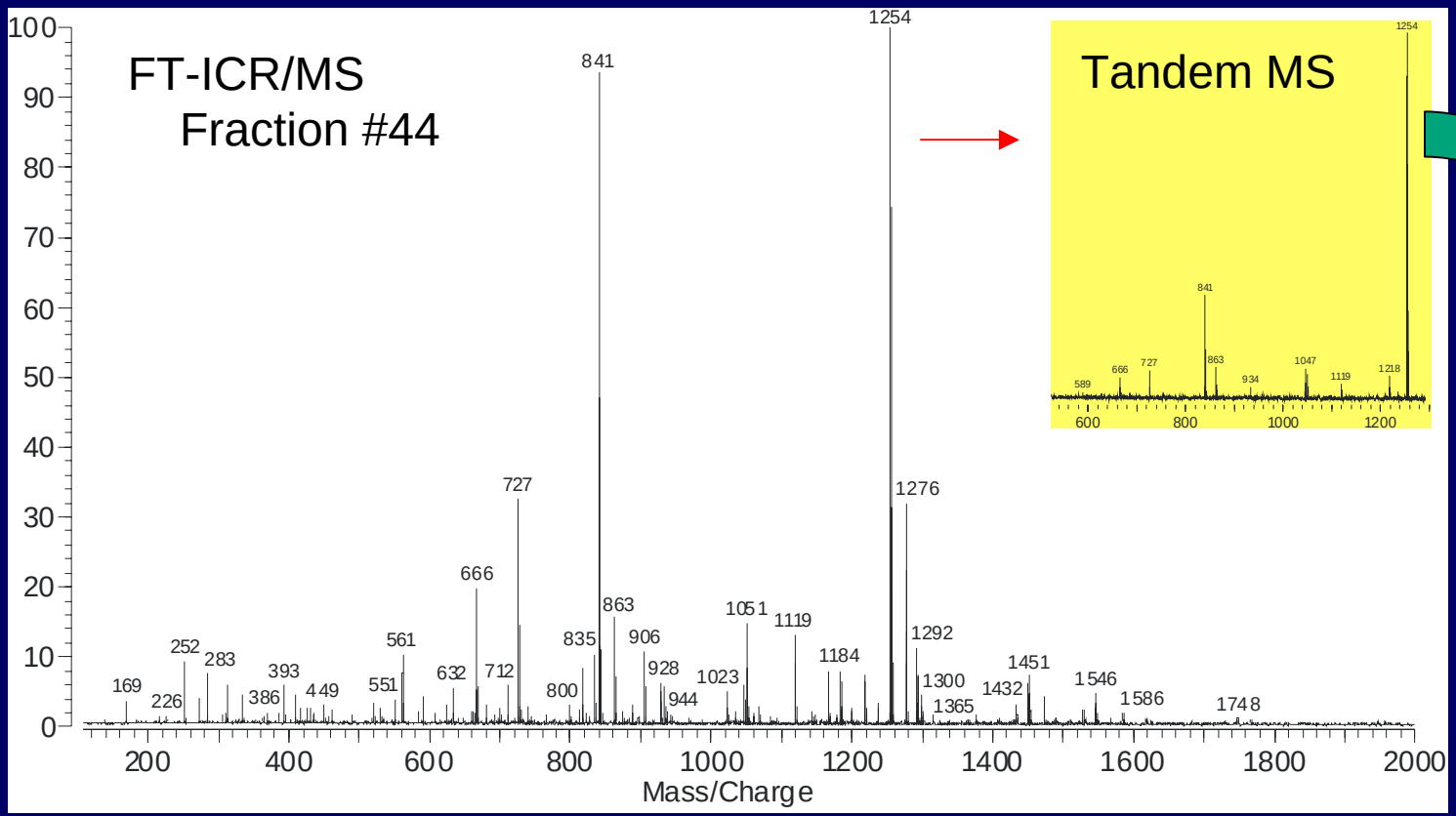


Fourier transform ion cyclotron resonance MS (FT-ICR-MS)

- Ultra high mass resolution <1ppm
- Wide Dynamic Range >100kDa



FT-ICR-MS, continued



Candidate proteins ← SVIPDGPAAQDGK

For example:

gi2947232 (membrane associated guanylate kinase 2)

Identification of inclusion proteins

Proteasomal subunits

HSP70

αB crystallin

- possible phosphorylated forms
- possible ubiquitinated forms

14-3-3ζ isoform

4-6 additional major protein species

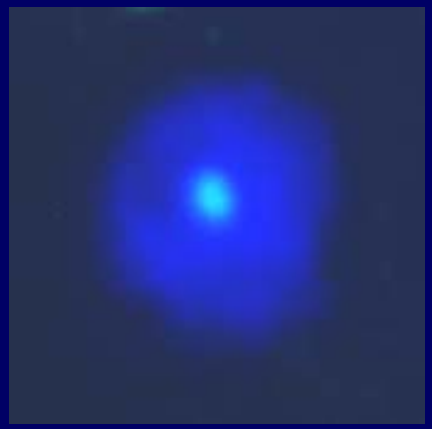
– identifications not complete

In progress

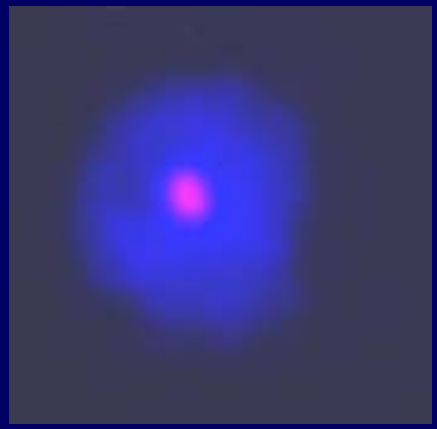
Identifications of interactions

e.g. ubiquitinated species (mono- ? poly- ?)
14-3-3 targets?
etc.

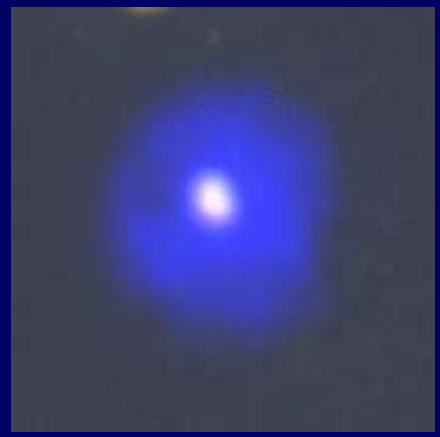
Another puzzle – myelin basic protein (MBP) appears to be in the inclusions



Ubiquitin

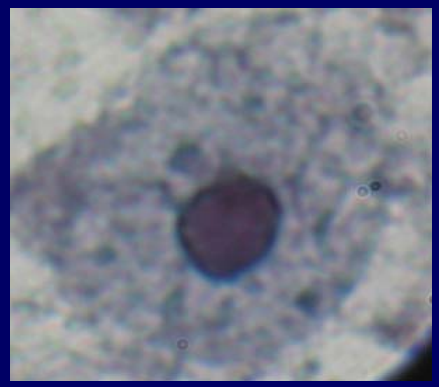


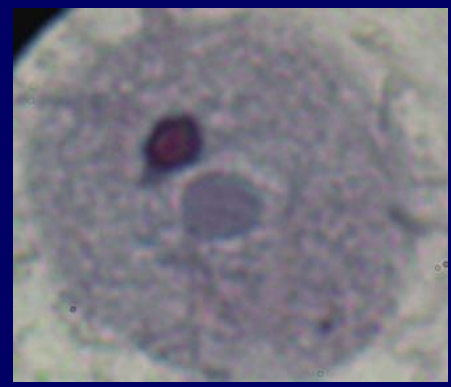
MBP



merge

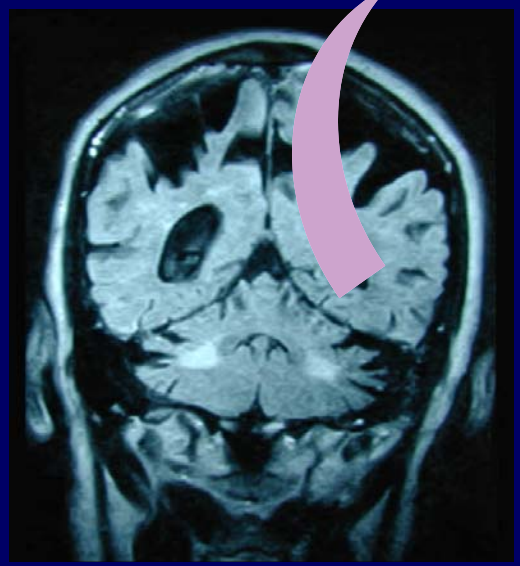
Non-fluorescent
Anti-MBP staining

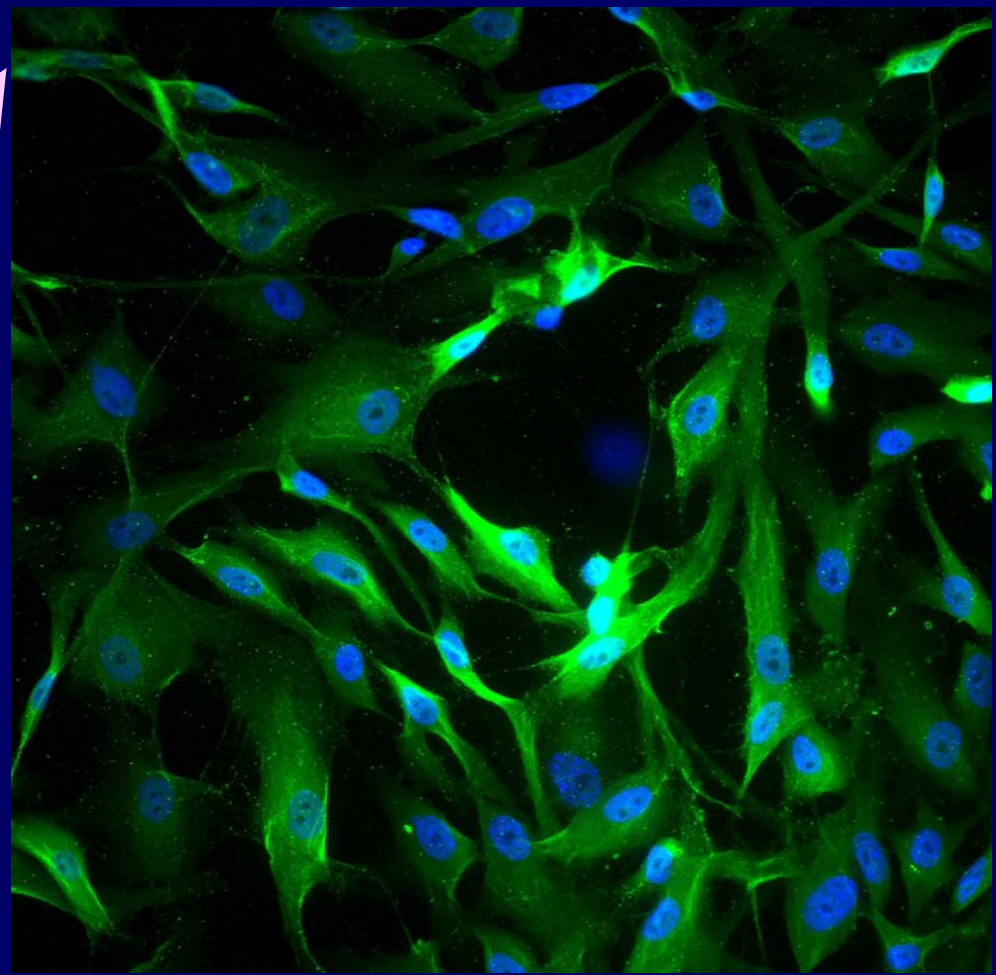




Research directions for FXTAS

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





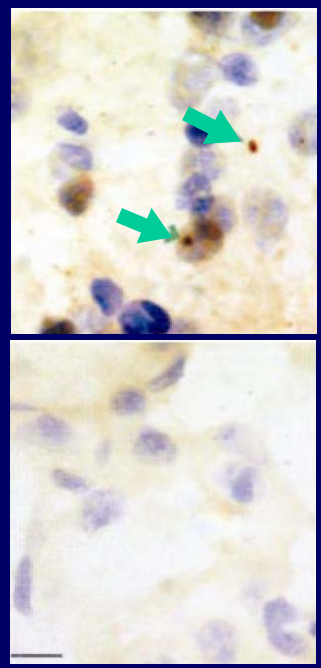
Schwartz and
Tassone, in preparation

Research directions for FXTAS

Scientists at Columbia University created mice with Huntington disease, another trinucleotide (CAG) repeat disorder

Huntington gene could be turned off in response to a chemical signal

Mice whose abnormal gene was turned off at 18 wk experienced nearly full recovery by 34 weeks



Clear neuropathology: inclusions, loss of brain mass

Reversal of brain pathology, recovery of normal behavior

Yamamoto et al.

Where to go from here?

Further research to understand the cause of FXTAS

- *identify targets for treatment – delay or block onset*

FXTAS research furthers our understanding of fragile X syndrome

- *it's the other face of the same gene*
- *are there restrictions to higher expression?*
- *can FXTAS help to explain the silencing in fragile X?*

The fragile X gene is a portal to a broader understanding of the causes of both neurodevelopmental and neurodegenerative disorders

Diagnostic criteria for FXTAS

Diagnostic Criteria for FXTAS (Mandatory Criterion: *FMR1* Allele Size of 55–200 CGG Repeats)

1. Definite:
   - A. one clinical major criterion (clinical major criteria: intention tremor and gait ataxia; clinical minor criterion: parkinsonism) *and*
   - B. one radiological major criterion (radiological major criterion: symmetric white-matter lesions involving the middle cerebellar peduncles; radiological minor criteria: white-matter lesions in cerebral white matter, moderate-to-severe generalized atrophy) *or*
   - C. presence of inclusions (the presence of intranuclear—neuronal and astrocytic—inclusions has been added as an additional criterion for FXTAS, on the basis of examination of postmortem brain tissue).
2. Probable:
   - A. two clinical major criteria *or*
   - B. one radiological major criterion *and*
   - C. one clinical minor criterion.
3. Possible:
   - A. one clinical major criterion *and*
   - B. one radiological minor criterion.

Acknowledgements (partial list)

UC Davis School of Medicine

M.I.N.D. Institute

- Randi Hagerman
- Louise Gane

Sebastien Jacquemont
Tristan Jardini
Susan Harris
Kris Herman
Faraz Farzin
Nicole Tartaglia

Dept. Radiology

James Brunberg

Dept. Pathology

Claudia Greco

Dept. Biochem & Molec. Medicine

Paul Hagerman Lab
Flora Tassone
Dag Yasui
Kendra Nannen
Lexin Li
Sasha Beilina
Dolores Garcia-Arocena

Chris Iwahashi
Brooke Babineau
Lisa Becker
Li-Sheng Chen
Anna Ludwig
Chris Brick

University of Colorado Health Sciences Center (Denver)

Dept. Neurology

Maureen Leehey
Deborah Hall

Dept. Pediatrics

Ann Reynolds

Dept. Medicine

James Grigsby

RUSH-Presbyterian-St. Luke's Medical Center (Chicago)

Dept. Pediatrics

Elizabeth Berry-Kravis

Dept. Neurology

Christopher Goetz

Children's Hospital of Orange County

Philip Schwartz

San Diego State University

Dept. Statistics

Richard Levine

Support: NICHD, NINDS, NFXF
UC Davis M.I.N.D. Institute