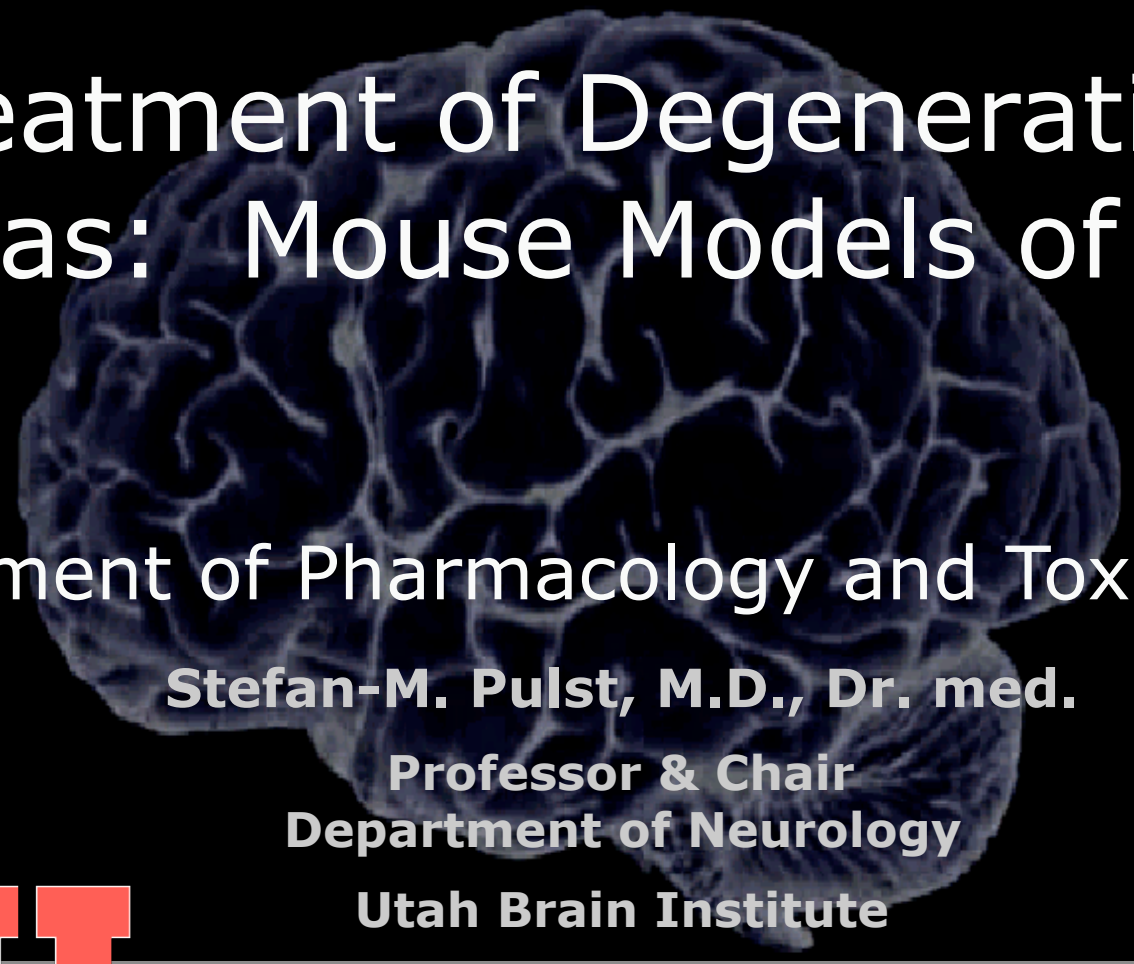
Treatment of Degenerative Ataxias: Mouse Models of SCA2



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Relevant Financial Relationship(s):

Athena Neuroscience: Consultant & Speakers' Bureau

Apopharma: DSMB

Off Label Usage: None

1-10-11

Overview

- Genes

  SCA2:

  - Polyglutamine (polyQ) disease
  - Genetic modifiers

- Models
  - SCA2 transgenic model
  - Modifiers in Mice

-

- Treatments
  - HT Coumpound screen
  - Antisense
  - IP3R signaling & dantrolene in the SCA2 mouse model

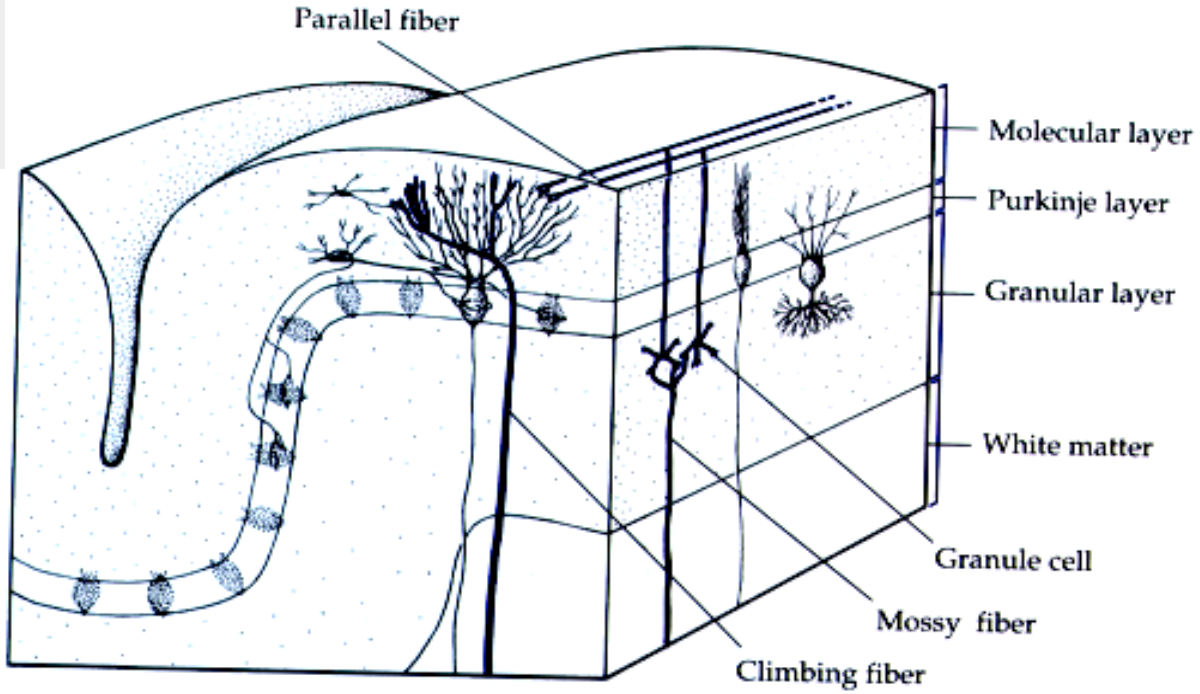
What are SCAs?

- Neurodegenerative Disorders
- Affect primarily cerebellum
- Often Purkinje cells
- Other neurologic systems as well
- Autosomal dominant

SCA Symptoms/Signs

- Cerebellar
  - Gait
  - Appendicular Ataxia (Precision/overshoot, rhythm)
  - Speech
  - Eye movements (nystagmus, overshoot)
- Other
  - Slow saccades
  - Parkinsonism
  - Spasticity
  - Neuropathy

Cerebellum



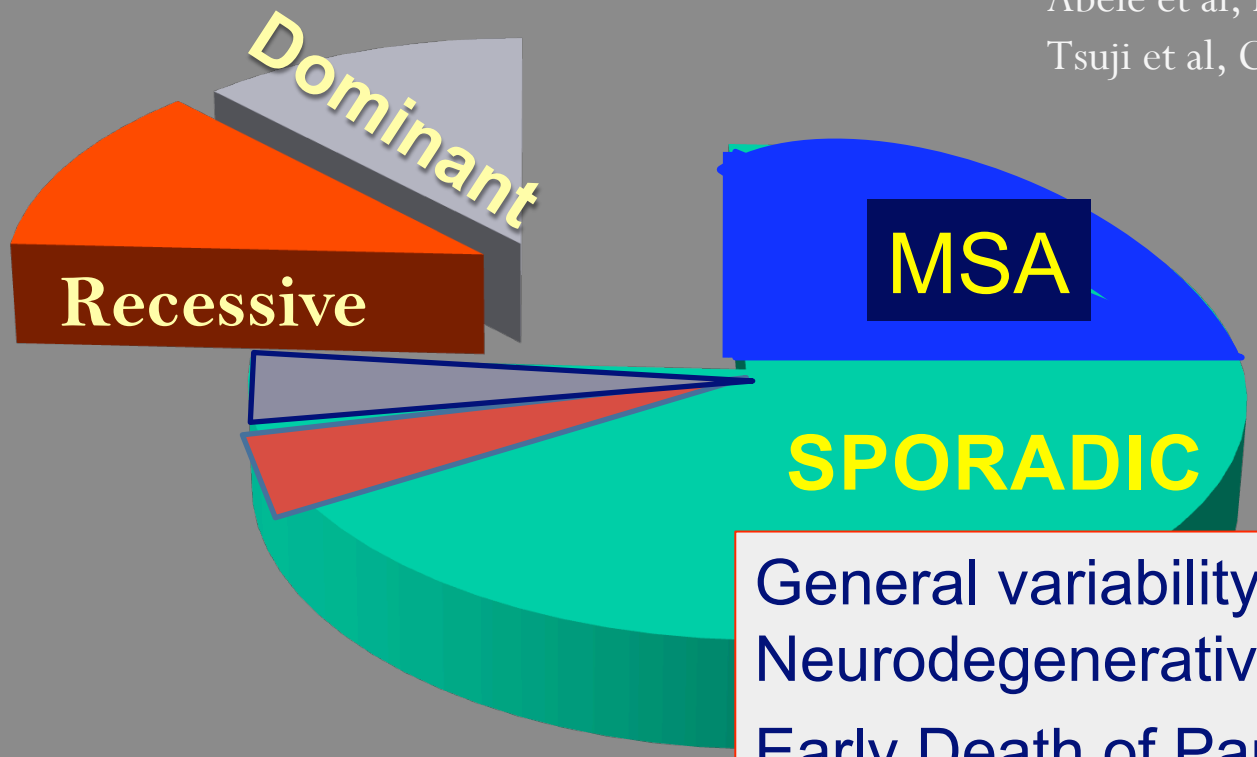
- Cerebellar surface = 1 cerebral hemisphere
- 10^{11} neurons
  (haploid genome : $3x10^{9}$ basepairs)
- 15 - 30 million Purkinje cells
  each with >200,000 synapses

Degenerative Ataxia with and without Family History

Prevalence rate: 18.6/100,000

Abele et al, Brain 2002

Tsuji et al, Cerebellum, 2009



General variability of AO in Neurodegenerative Dx

Early Death of Parents

Small Families & Adoption

Anticipation

Presence of Mendelian Mutations in Neurodegenerative Diseases

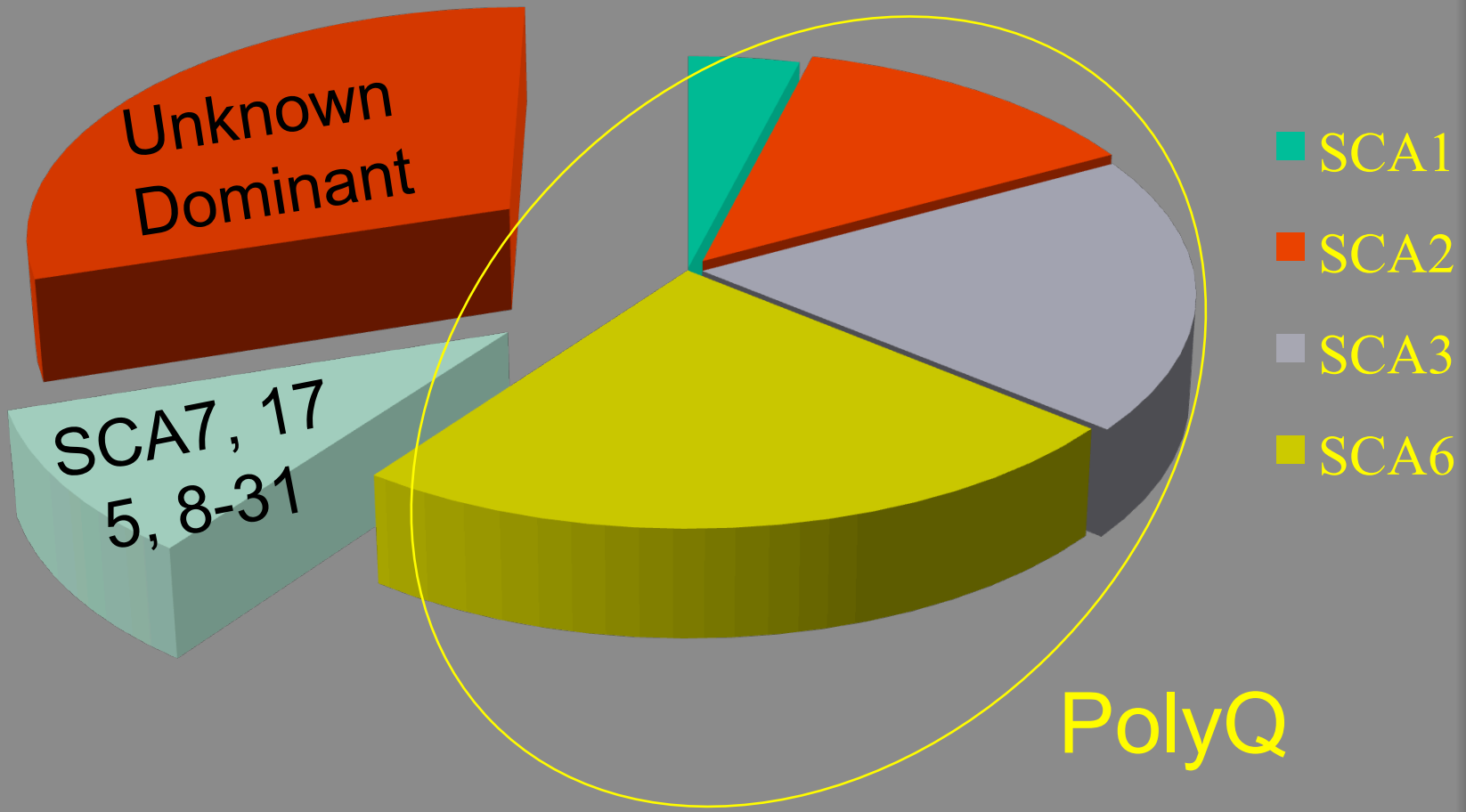
- Alzheimer: 2-3%
- Parkinson disease: 5%
- ALS: 10%

- Degenerative Ataxias: 25%

Genes

"We think it has something to do with your genome."

Genetic Architecture of SCAs

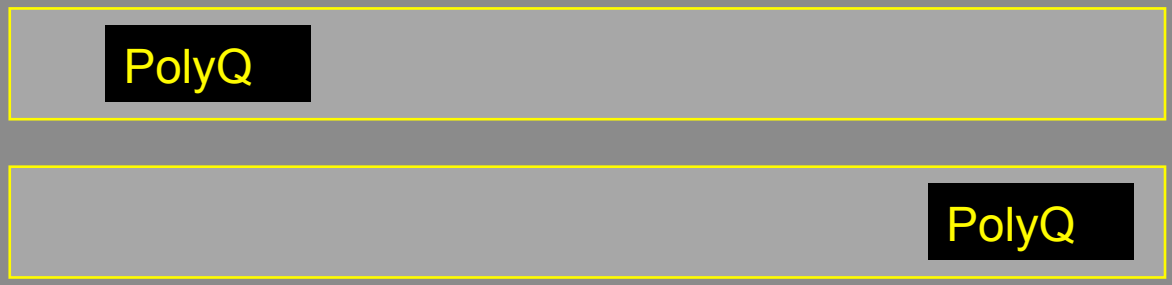


Non-polyQ Ataxias

- EA2 — CACNA1a
- SCA5 — beta3-spectrin
- SCA10 — toxic RNA
- SCA11 — Kinase (TTBK2)
- SCA13 — Voltage- gated K^+ - channel
- SCA14 — Kinase (PKCγ)
- SCA15,16 — Ca^{++} release (ITPR1 LoF)
- SCA20 — Dup 11q (260kb)
- SCA23 — Prodynorphin
- SCA27 — FGF14 LoF
- SCA28 — mitochondrial AAA protease
- SCA31, 35 — toxic RNA

Dominant SCAs

Coding CAG repeat expansions:
SCA1, 2, 3, 6, 7, 17



Repeat is variable in normals

Pathological repeat length different

Reduced penetrance alleles

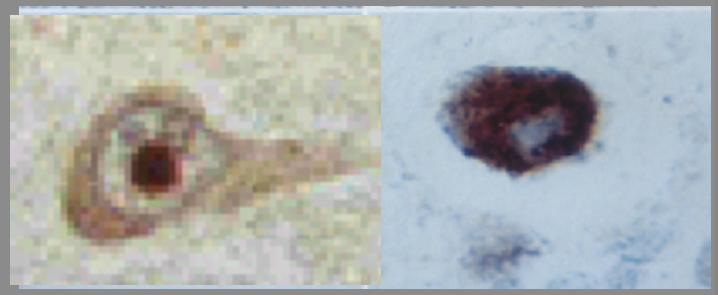
No homologies other than polyQ-tract

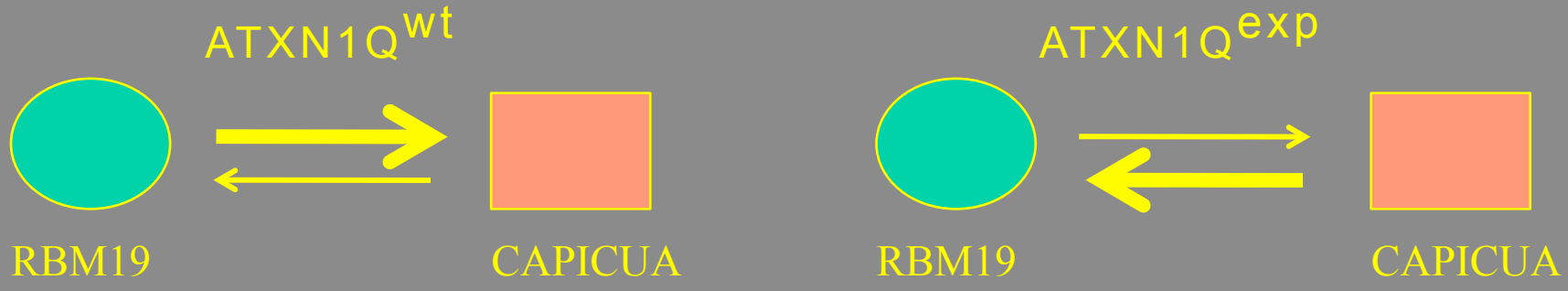
SCA6: Ca^{++} channel (CACNA1A)

SCA17: transcription factor (TBP)

Poly-Q Pathogenesis

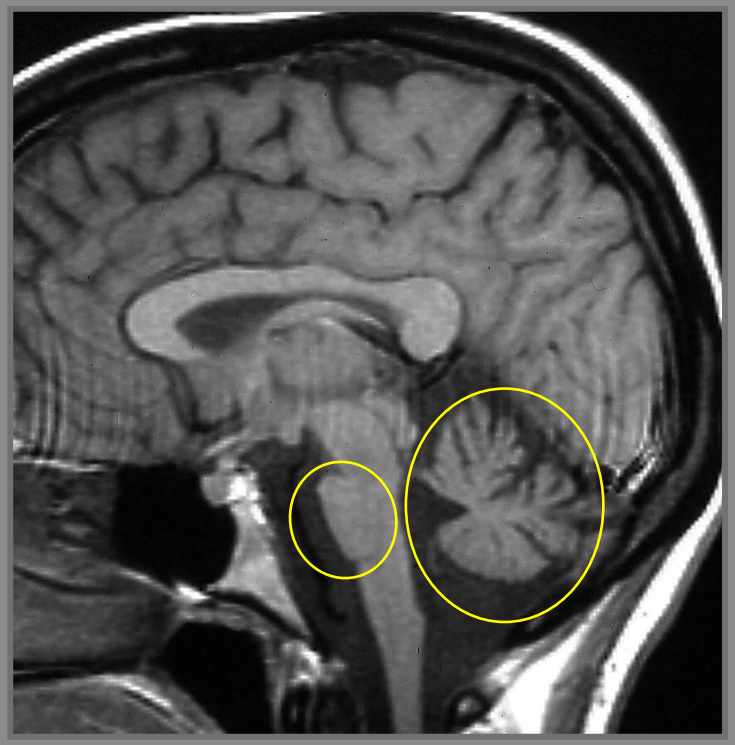
- Gain of Toxic Function
  - Aggregation of misfolded proteins
  - Misfolded toxic oligomeres
- Gain of Normal Function
- Allele-specific Gain/Loss of Normal Function



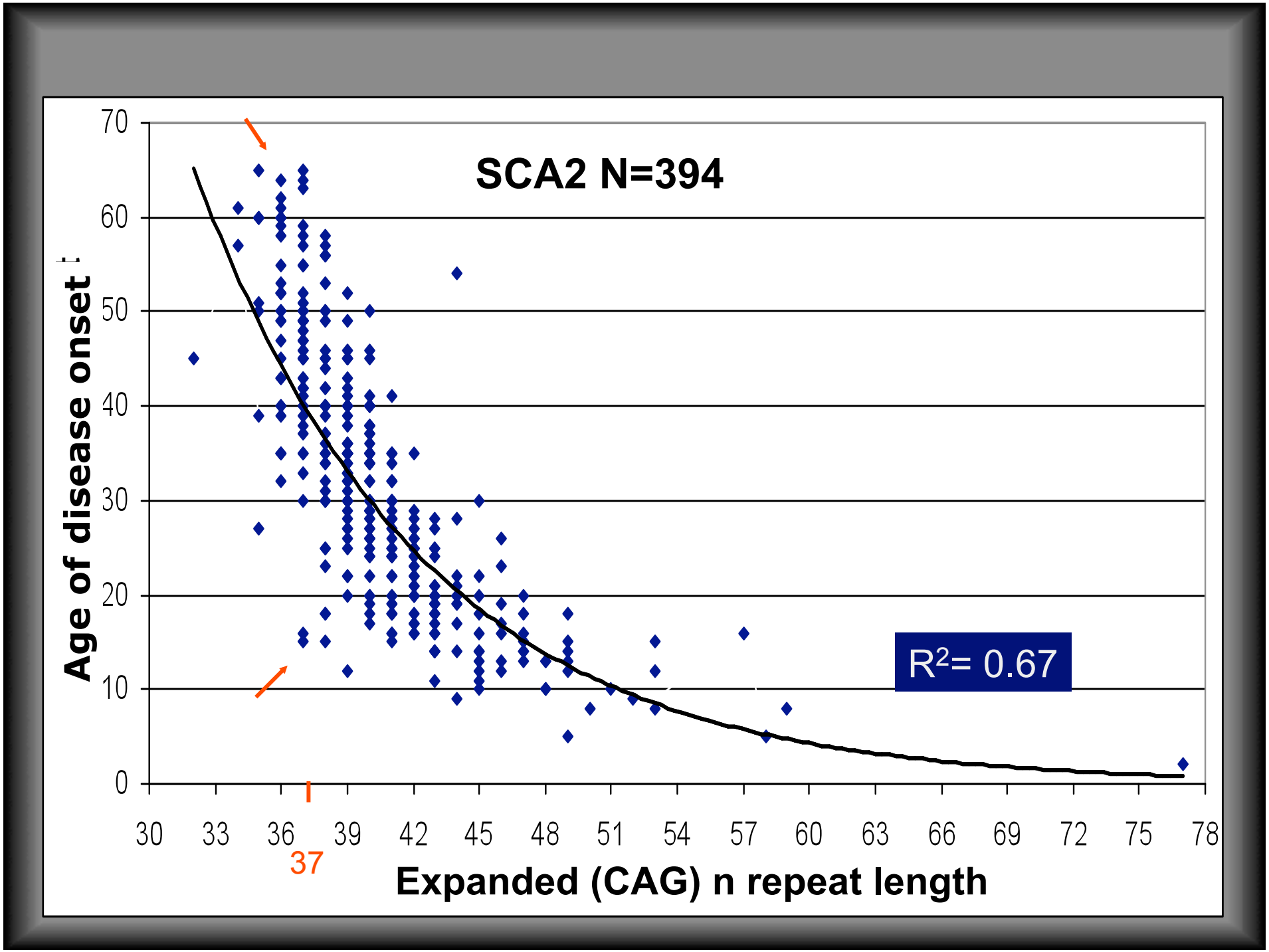


SCA2: Phenotype & Gene

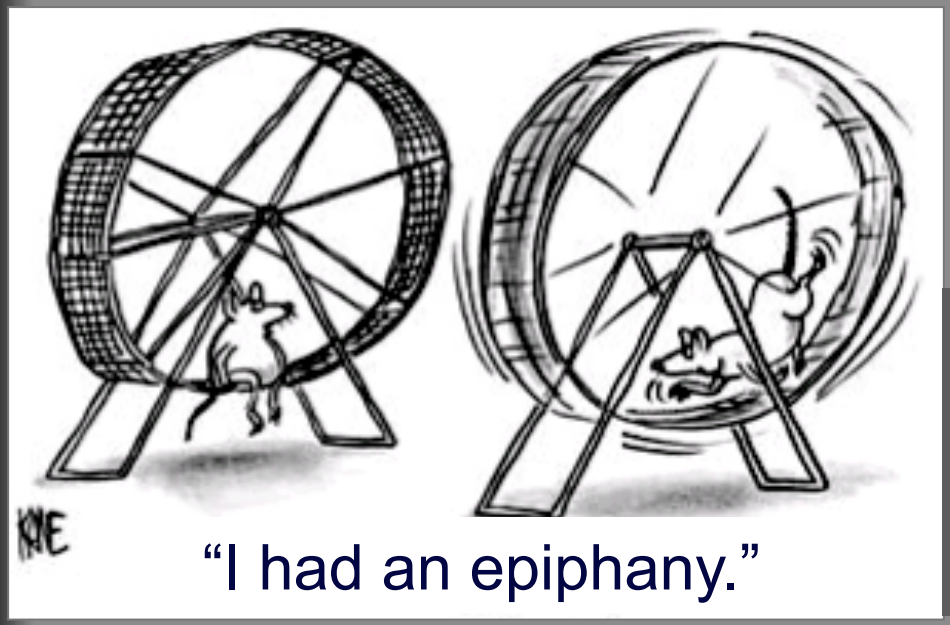
Ataxia
Slow saccades
Neuropathy
Parkinsonian features
Dystonia & Spasticity
ALS-like



Pulst et al Nature Genet 1996


SCA2 N=394
Age of disease onset
70
60
50
40
30
20
10
0
30
33
36
39
42
45
48
51
54
57
60
63
66
69
72
75
78
37
Expanded (CAG) n repeat length
R2= 0.67

Models



"I had an epiphany."

Why the Mouse ?

- Cells do not have a Cerebellum
- Cerebellar Circuits very similar in Mouse and Human.

- **Treatment trials in rodents**
  - Cost & Safety
  - Precise timing of disease onset and treatment.
  - Easier Differentiation between symptomatic and disease-modifying effects.

Animal Models

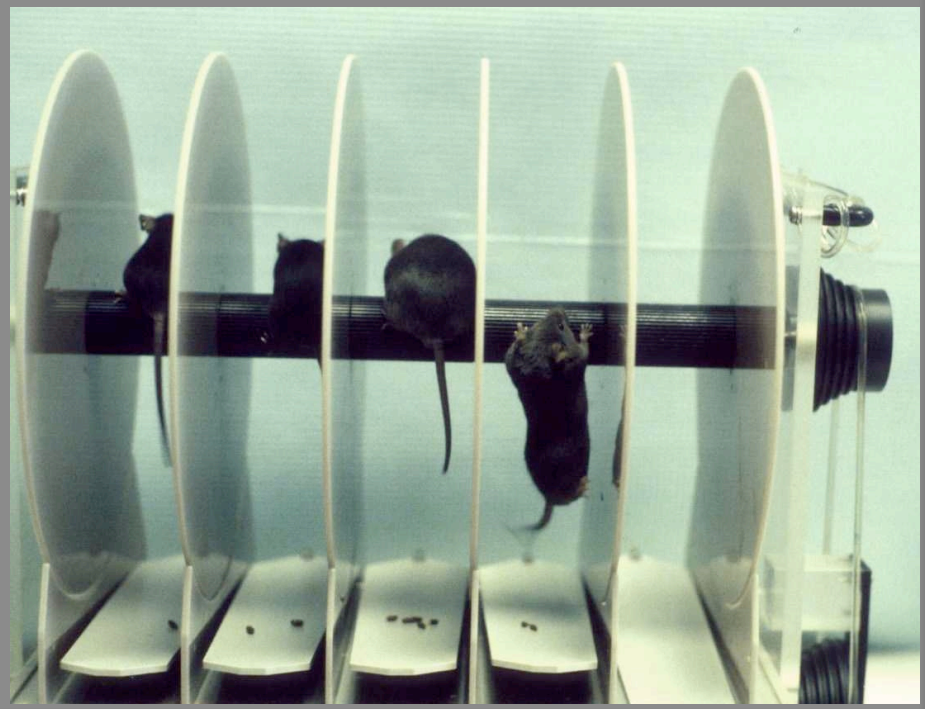
A model is a model is a model.

- Transgenic with cDNA:
  - Pcp2
  - PrP
  - endogenous
- BAC transgenics
- Conditional transgenics
- Knock-in:

  Usually very long CAG repeats required

Outcomes: Moutaxia

- **Morphologic**
  - Calbindin staining
  - Molecular layer thickness
  - PC number
- **Biochemical**
- **Functional**
  - Rotarod
  - Beam
  - Gait Analysis

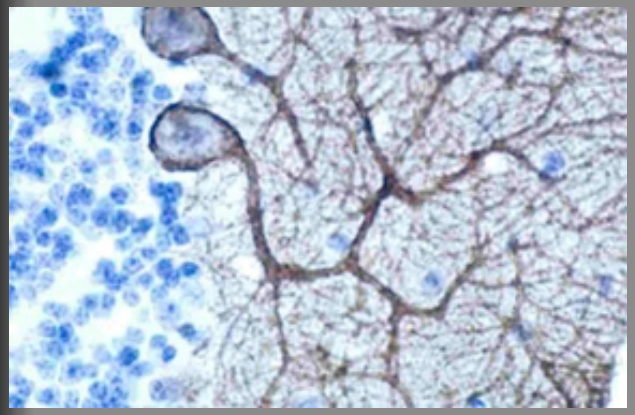


The tg-Pcp2-ATXN2[Q58] mouse

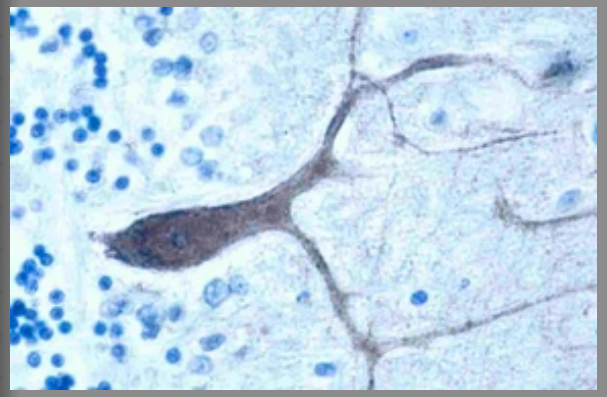
Promoter: Purkinje cell specific PcP2

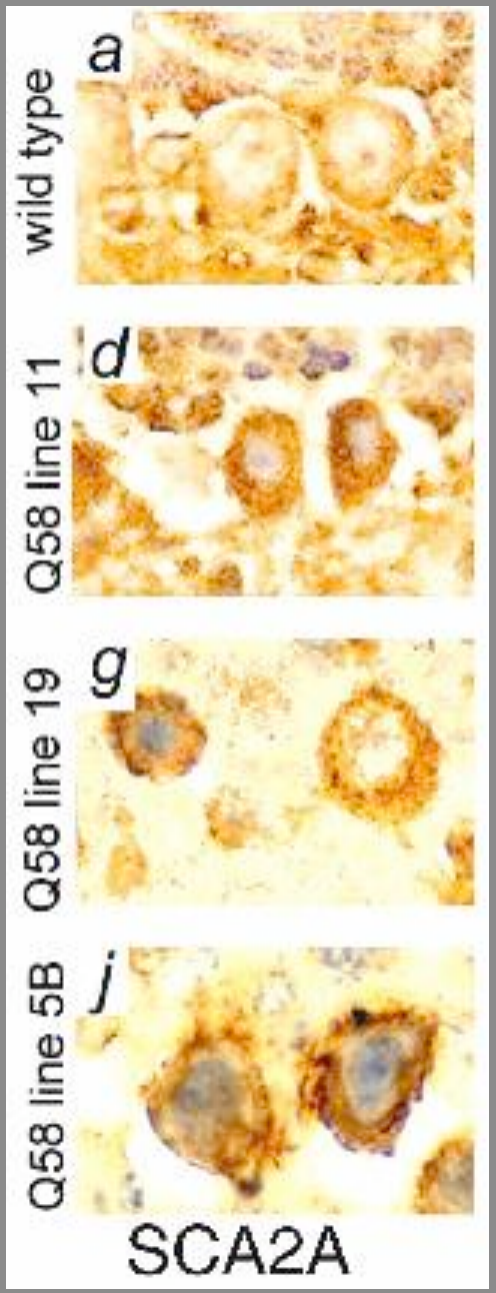
Huynh et al Nat Genet 2000

Human control

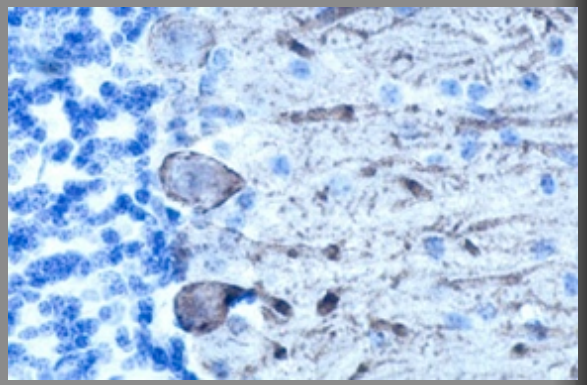


Wildtype Mouse

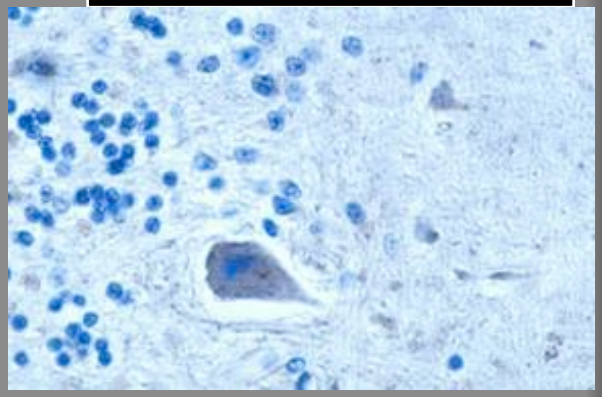




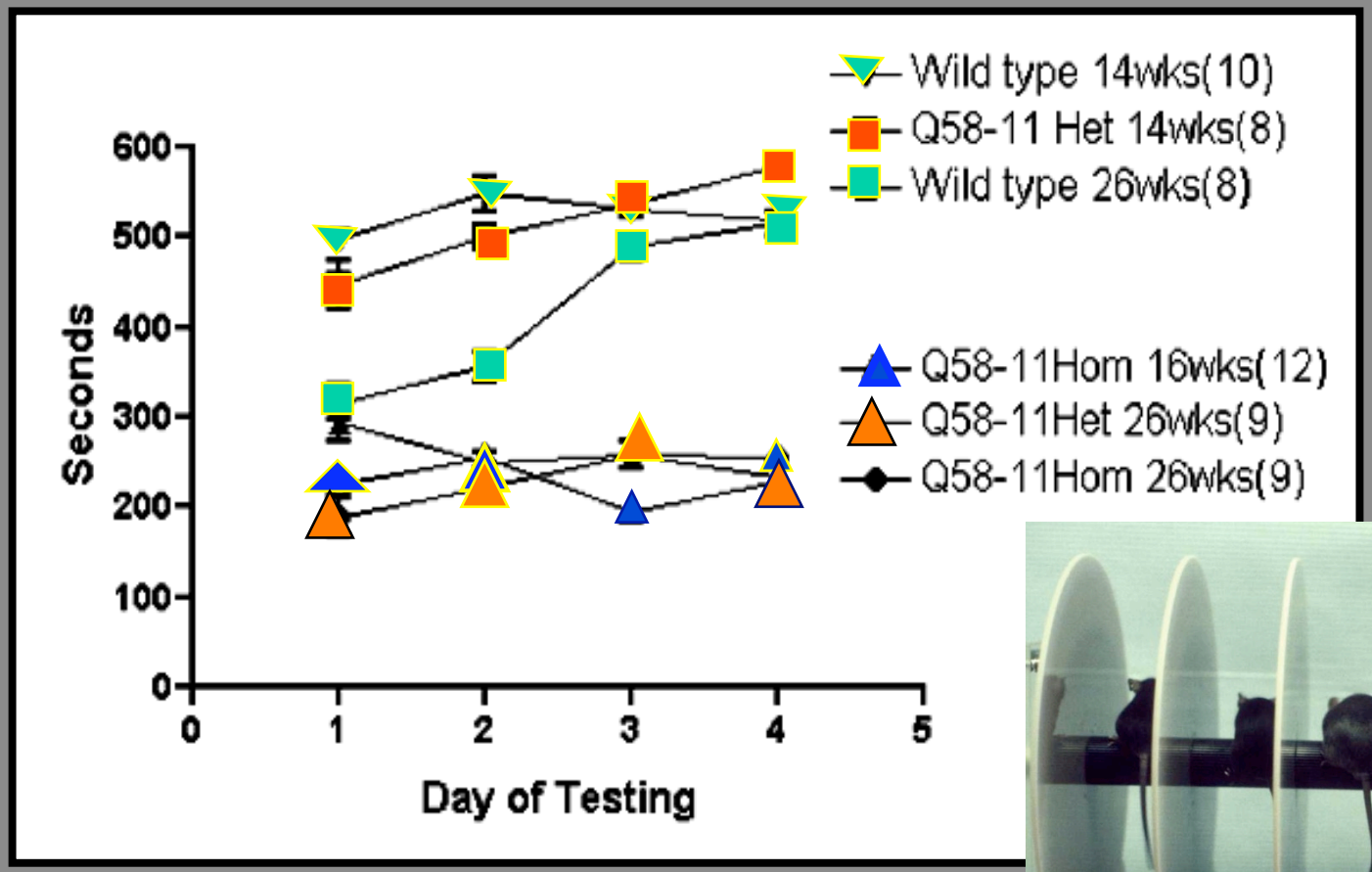
SCA2 patient

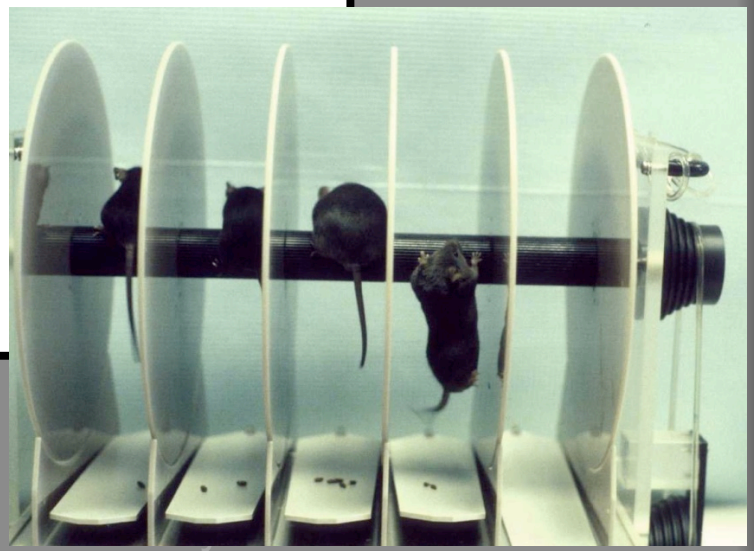


Ataxin-2$_{[Q58]}$ mouse



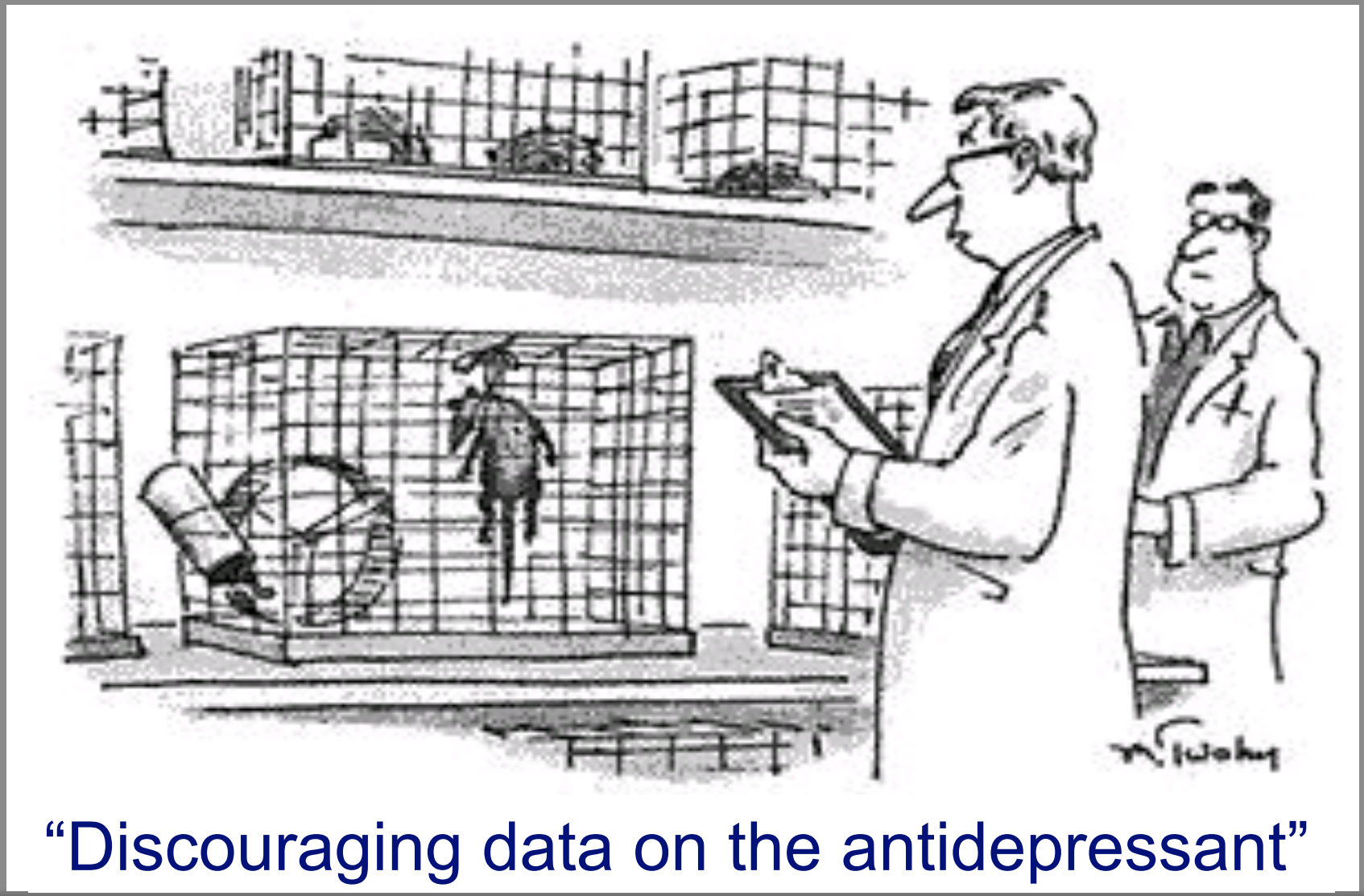
Functional Analysis





Huynh et al Nat Genet 2000

Treatments



"Discouraging data on the antidepressant"

Treatment Strategies for SCAs

- SCA-type specific
  - siRNA knockdown (SCA1, 3)
  - Modified Antisense (SCA2 in progress)
  - Small molecules (SCA2 in progress)
- Directed at potentially shared mechanisms
  - Correcting deranged gene expression: SCA1 Lithium
  - Correcting abnormal PC firing: riluzole
  - Glutamate-stimulated Ca-release: SCA2 & SCA3, Dantrolene

Targeted therapy for SCA2

Reduction of ataxin-2 dose is therapeutic for SCA2.

- SCA2 phenotype is worse in patients homozygous for the disease allele.
- SCA2 phenotype is worse in homozygous vs heterozygous *ATXN2* transgenic mice.
- *ATXN2* knockout mice are obese but have no neurodegeneration, while SCA2 patients are lean.
- SCA1 & SCA3 mouse phenotypes are reversible.
- *ATXN1* shRNA injection improves *ATXN1* mouse phenotype.

Compound Screening

NIH Chemical Genomics Center (NCGC)

NCGC	Primary assay:	qHTS, 400K compounds on ATXN2-luc inhibition
NCGC	Secondary assay 1:	Recombinant FF Luc counter-screen
NCGC	Secondary assay 2:	SH-SY5Y toxicity test
UTAH	Secondary Assay 3:	ATXN2-lac repressor / lac operator luc (pos. readout)
UTAH	Secondary Assay 4:	CMV-luc
UTAH	Tertiary assay 1:	qPCR for endogenous *ATXN2*
UTAH	Tertiary assay 2:	Western blots for ataxin-2

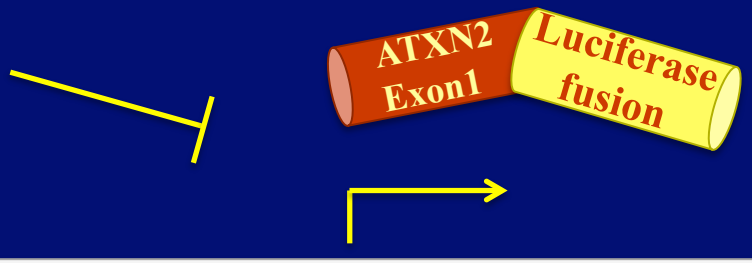
SCA2 mouse

Knockdown of *Atxn2 in vivo*

Motor phenotype testing

NCGC Compound Screen

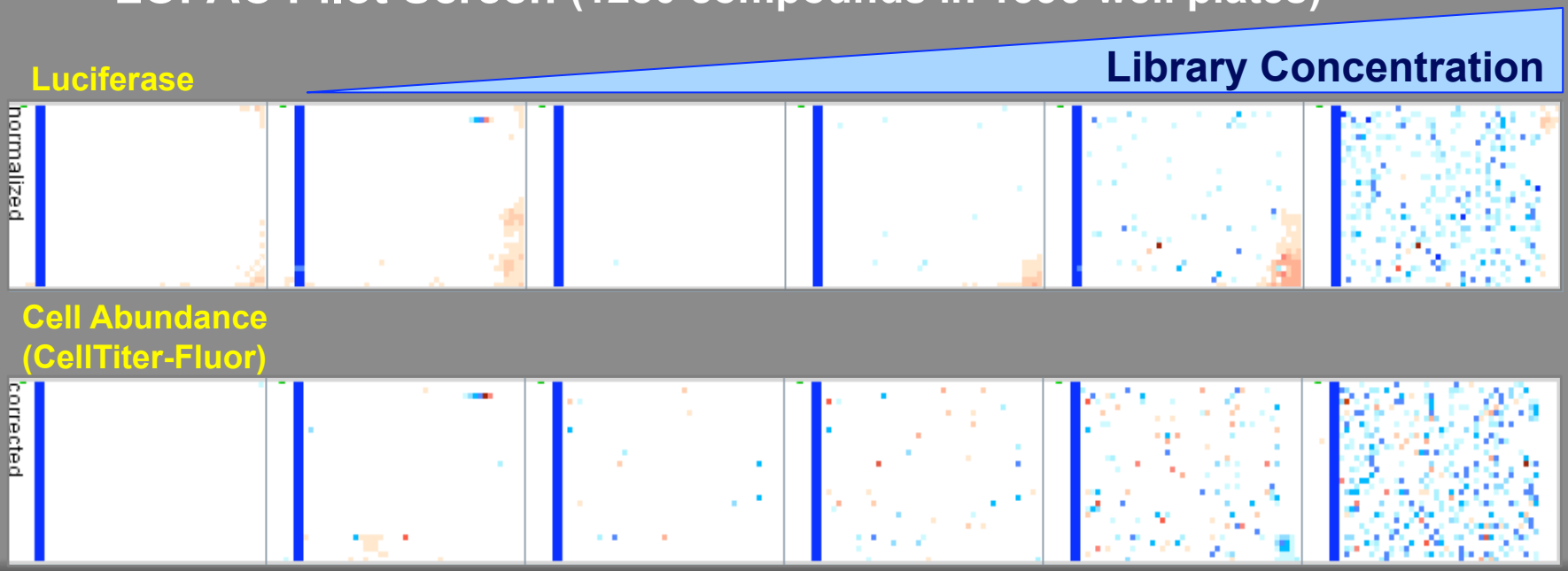
Assay:
Screen 400,000 compounds for inhibition of *ATXN2-luc* expression

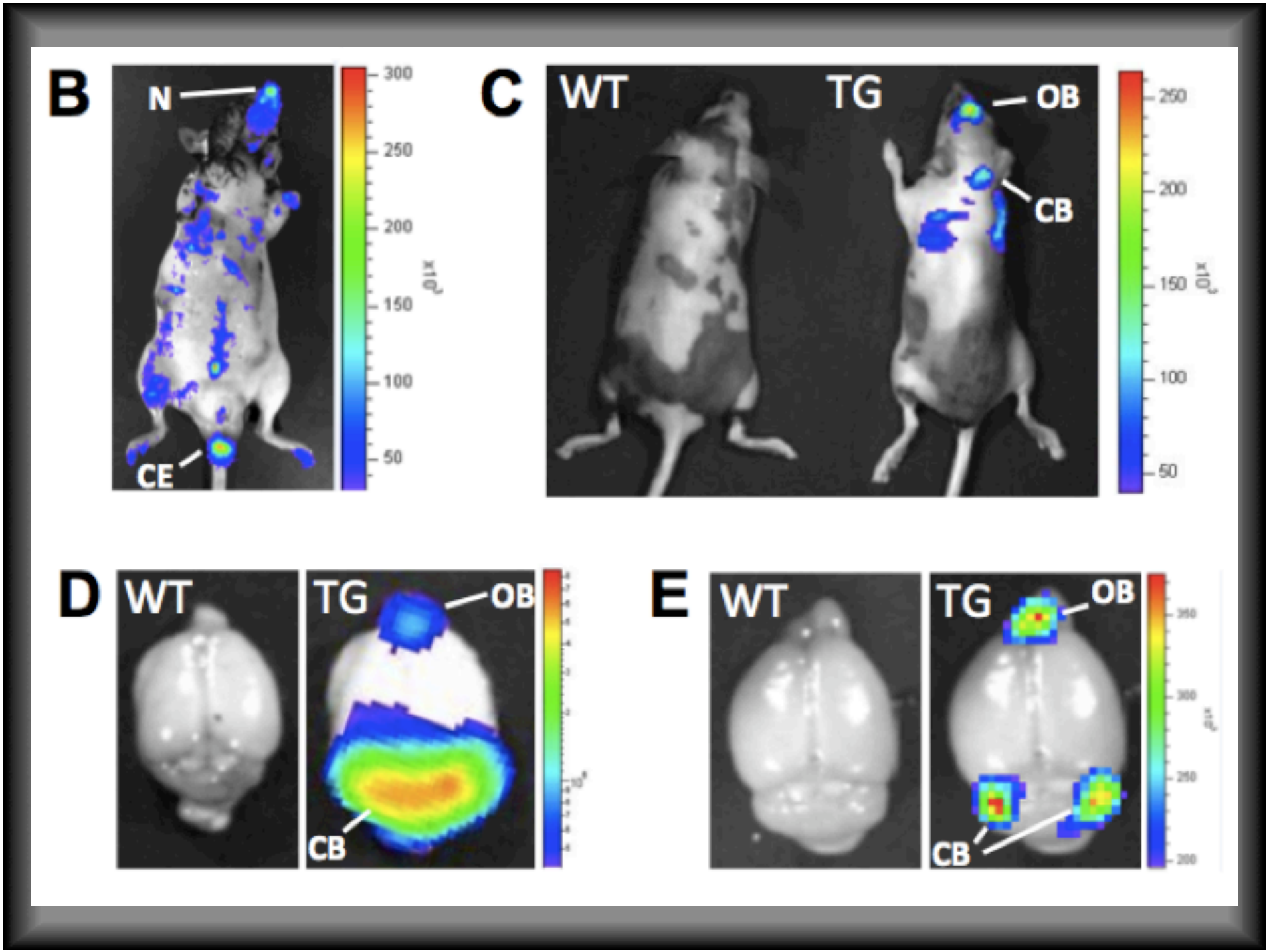


Readout:
Luciferase Assays with integrated assay for determining compound toxicity

1000 bp *ATXN2* US---Luciferase---*ATXN2* 3'-UTR

LOPAC Pilot Screen (1280 compounds in 1536-well plates)




B
N
CE
300
250
200
150
100
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C
WT
TG
OB
CB
250
200
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100
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D
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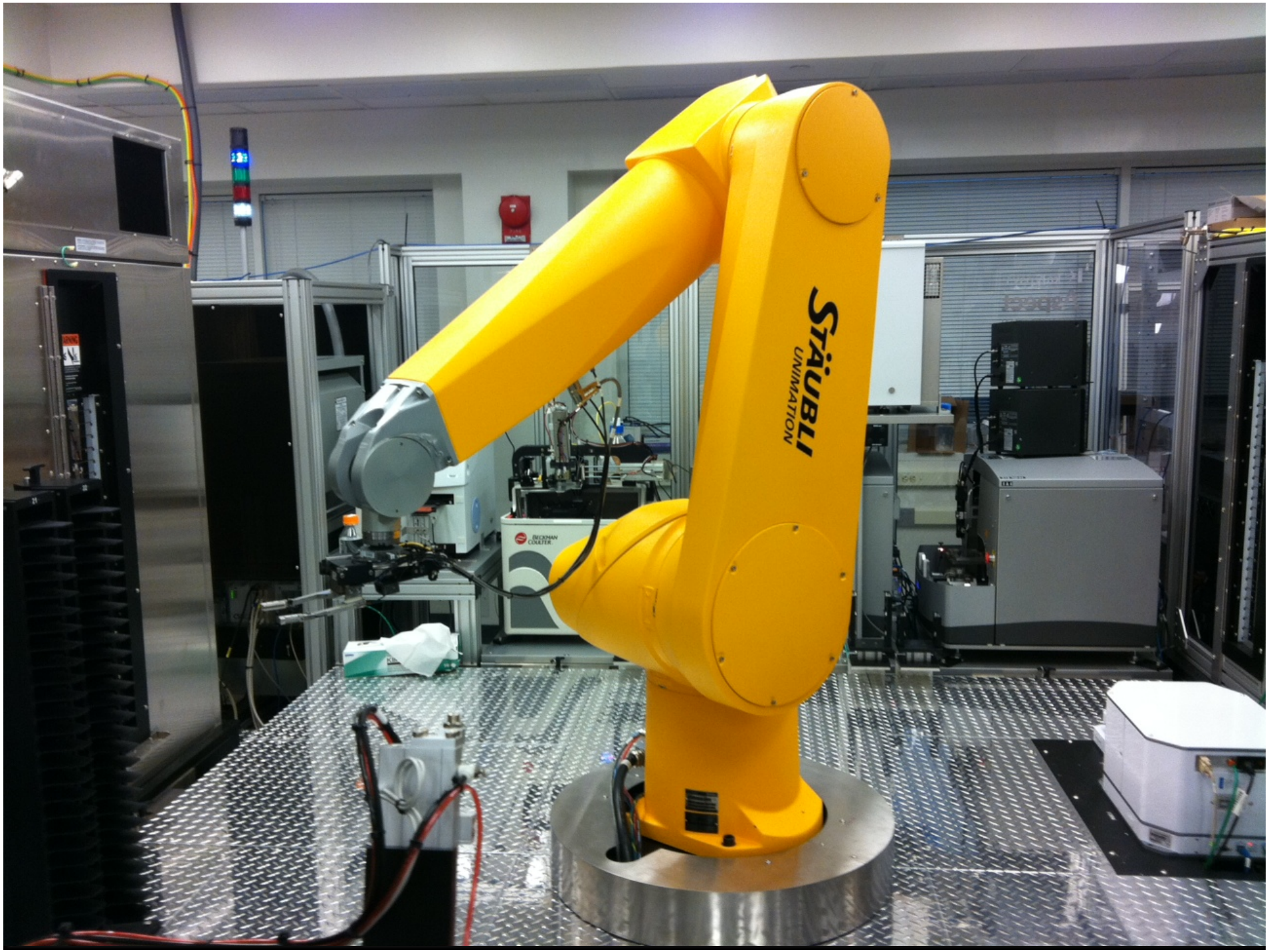
TG
OB
CB
E
WT
TG
OB
CB


400,000 compounds in 1536 well plates


STÄUBLI
UNIMATION
BECKMAN COULTER

ATXN2 Antisense Screen

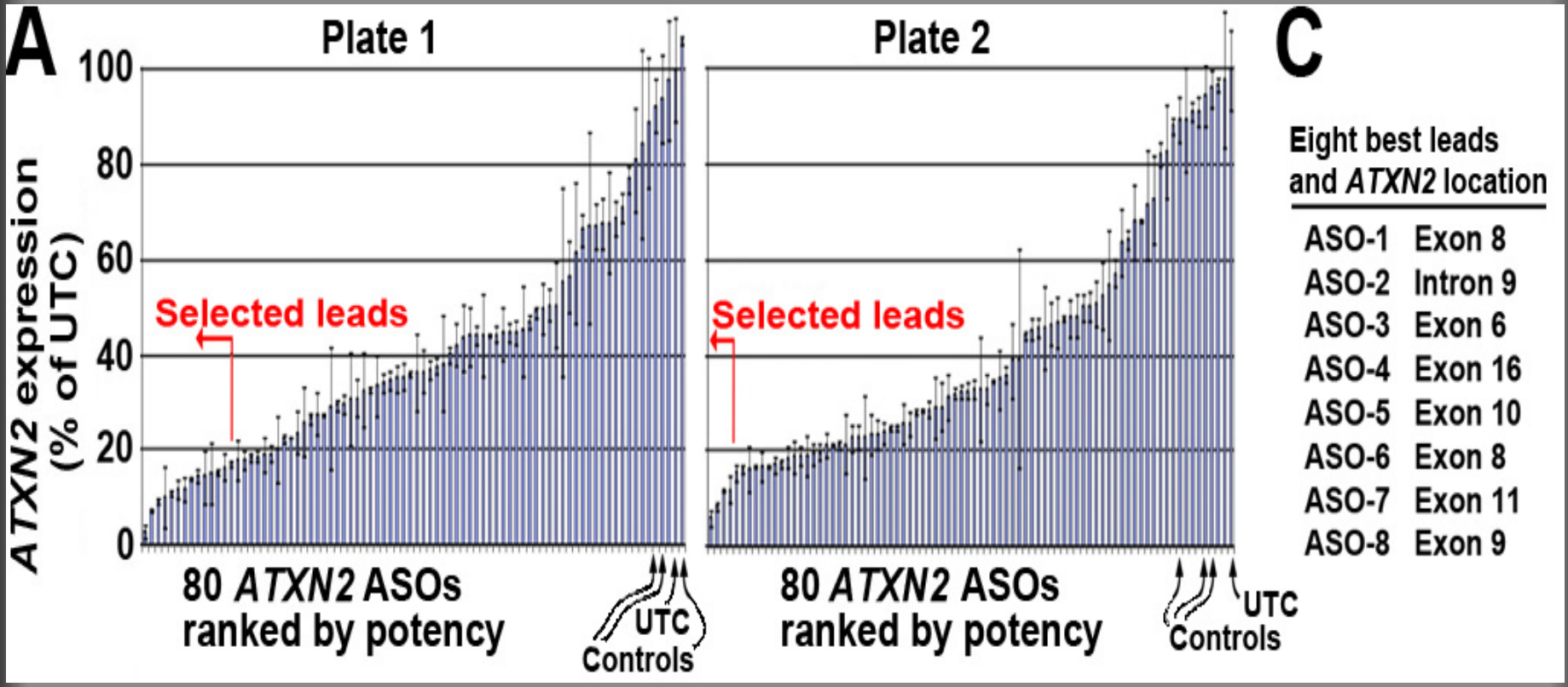
ISIS Pharmaceuticals

160 ASOs were designed by ISIS that are predicted to only target *ATXN2* intronic and exonic sites.

These were screened in 96 well qPCR assays for knockdown of human *ATXN2*.

Eight leads were selected after retesting dosewise.

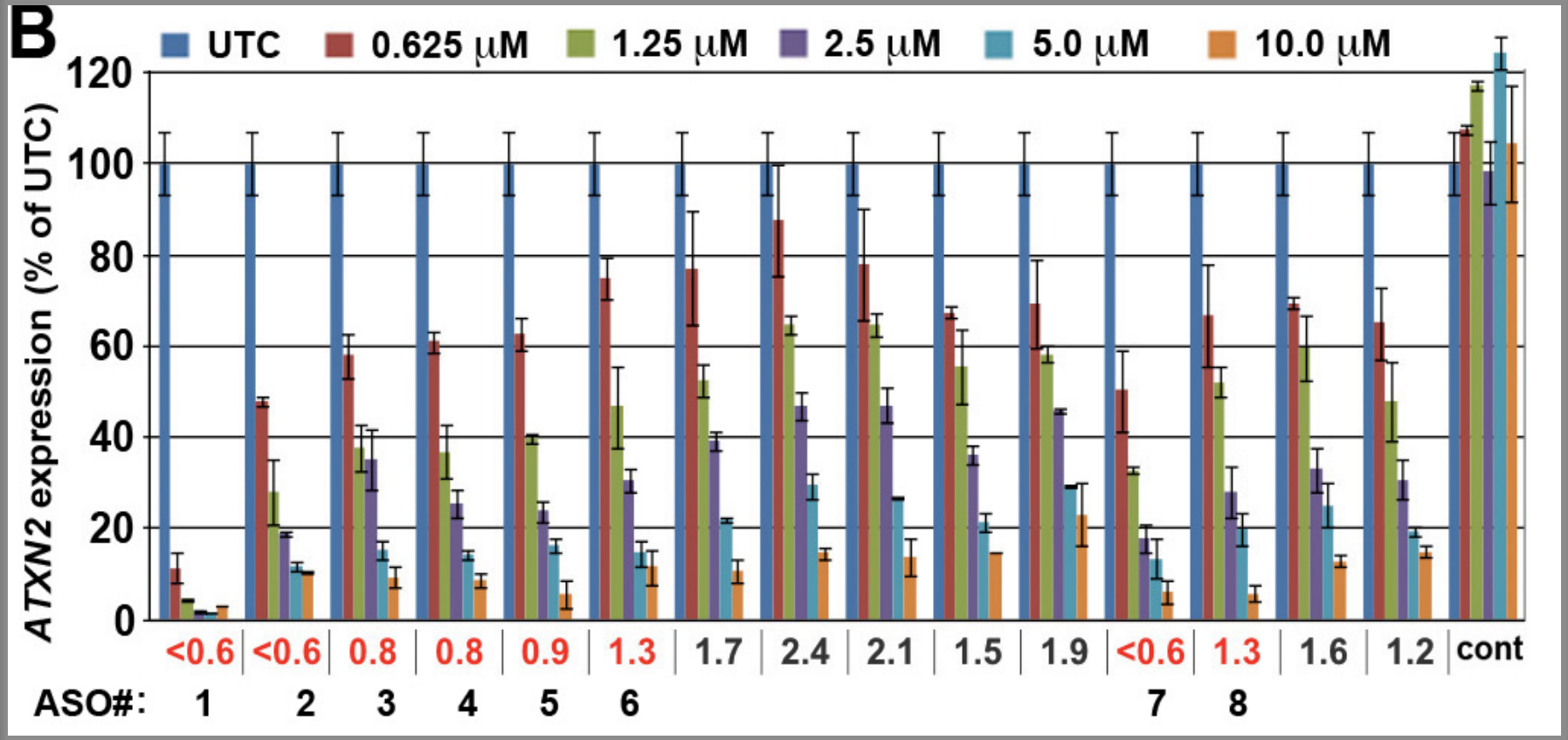
ATXN2 Antisense Primary Screen



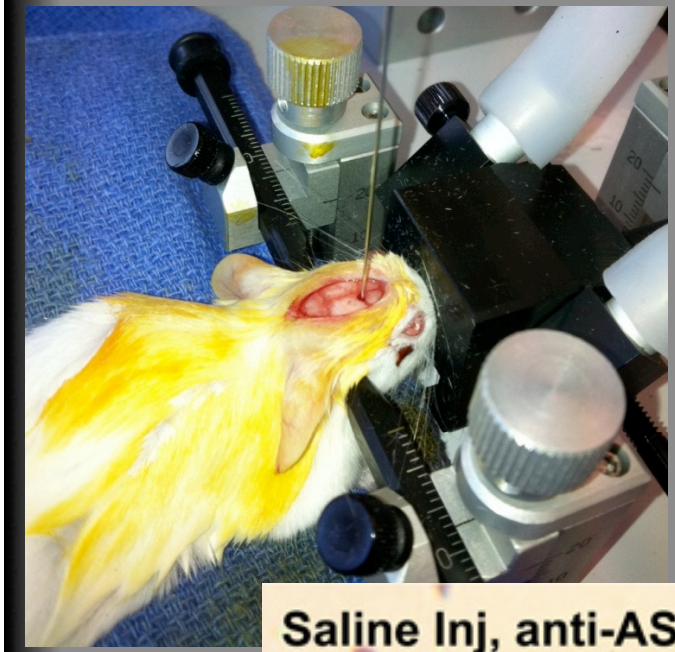
HEPG2 cells

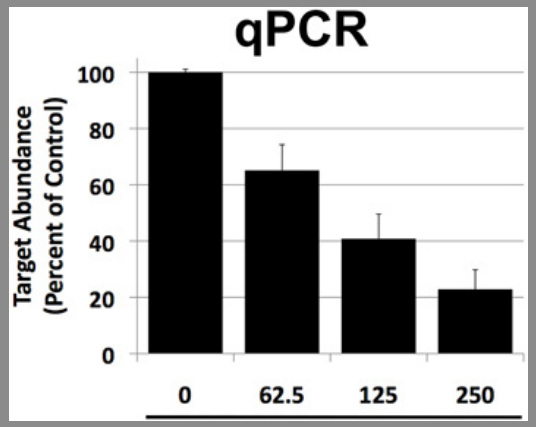
ATXN2 Antisense Secondary Screen

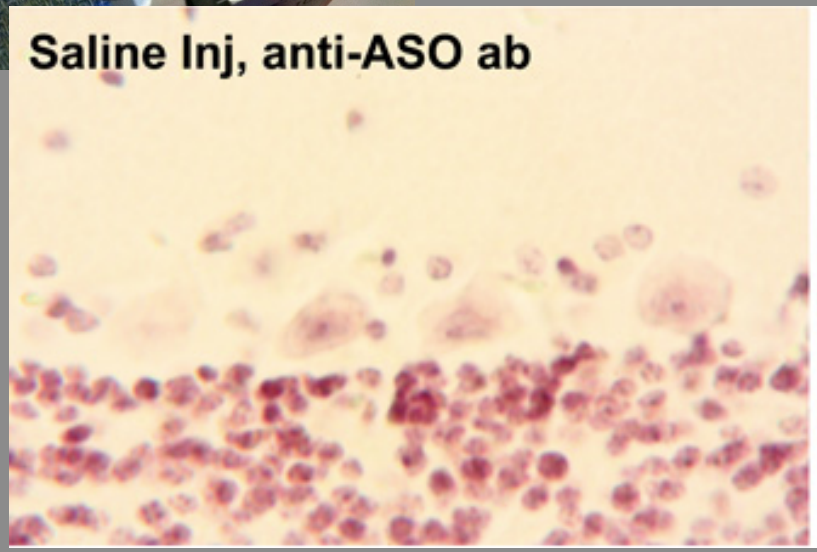
Eight leads were selected after retesting dosewise.
All hit *ATXN2* exons except for one located in an intron.

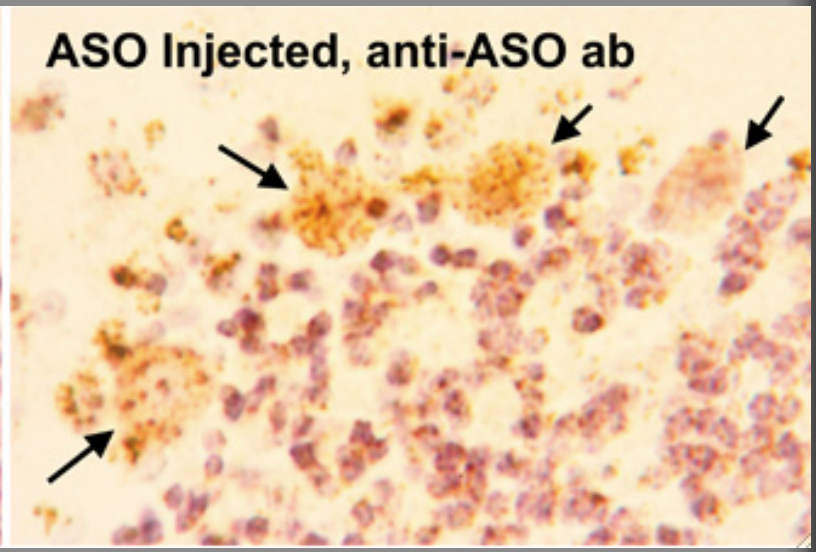


Malat1 Antisense ODN pilot tests

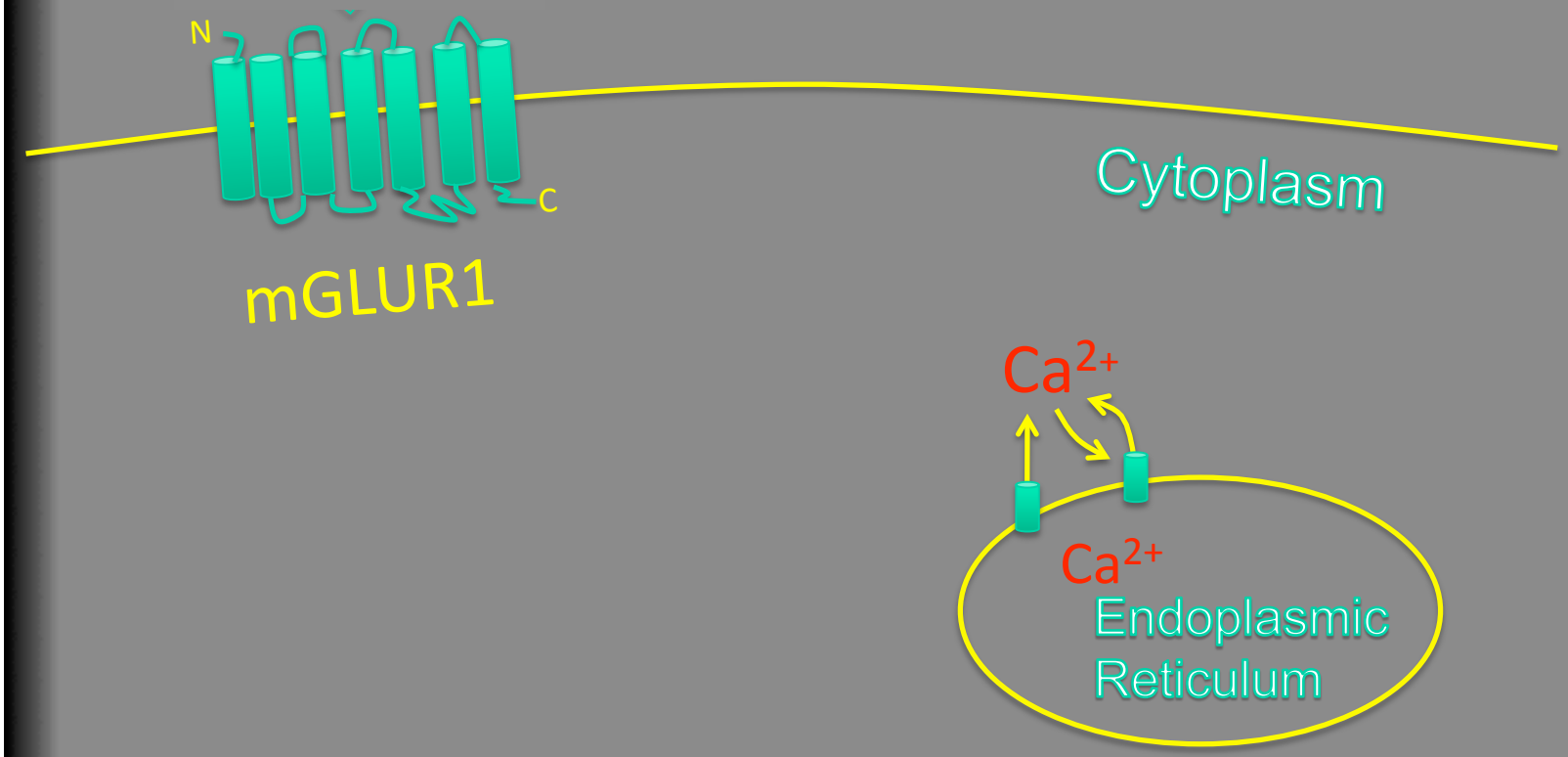








Mutant ATAXN2 and Ca^{2+} Release



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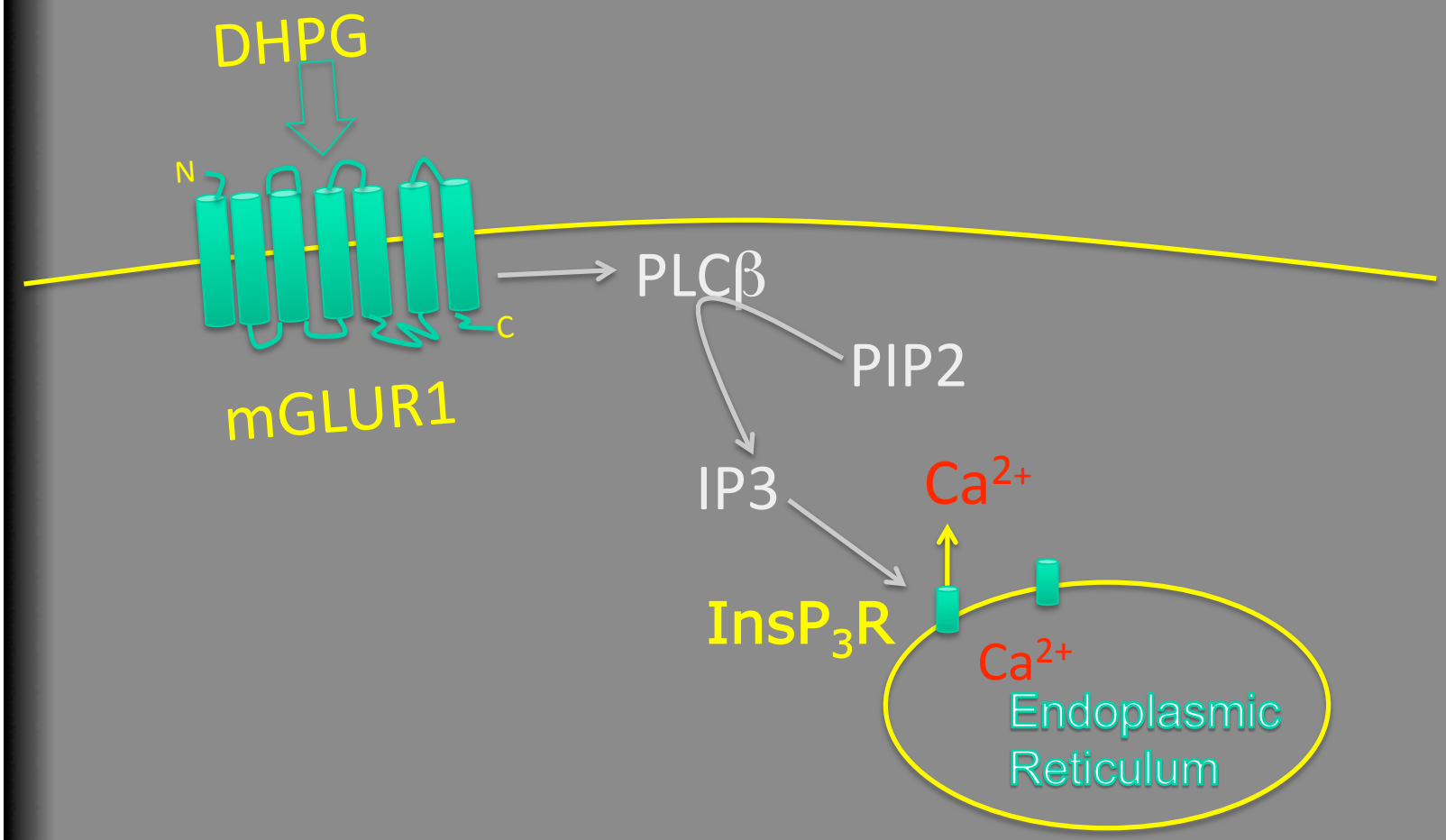
Mutant ATAXN2 and Ca^{2+} Release



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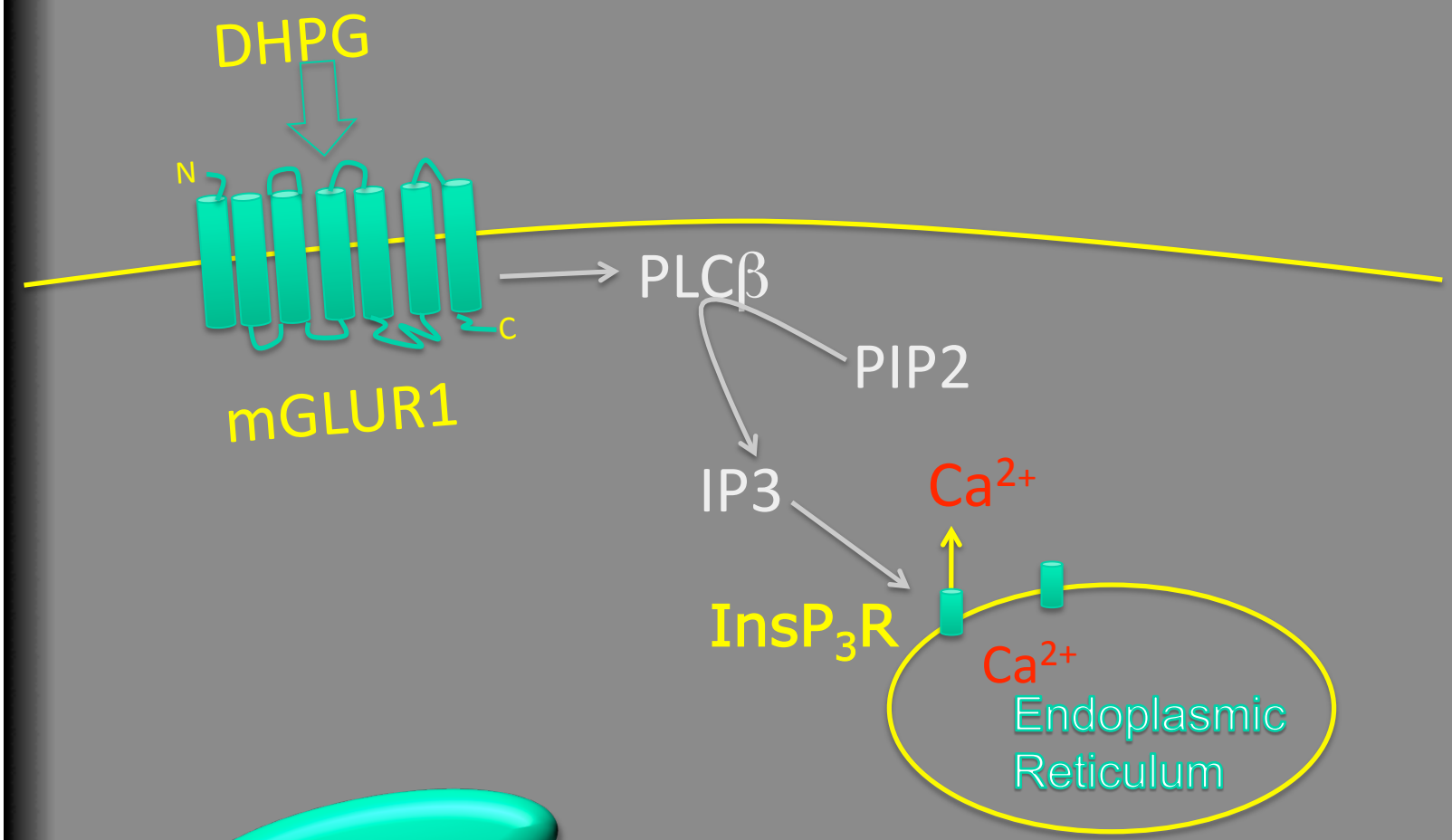
Mutant ATAXN2 and Ca^{2+} Release



Mutant Ataxin-2 binds $InsP_3R$ more strongly to mediate Ca^{2+} exit from ER .

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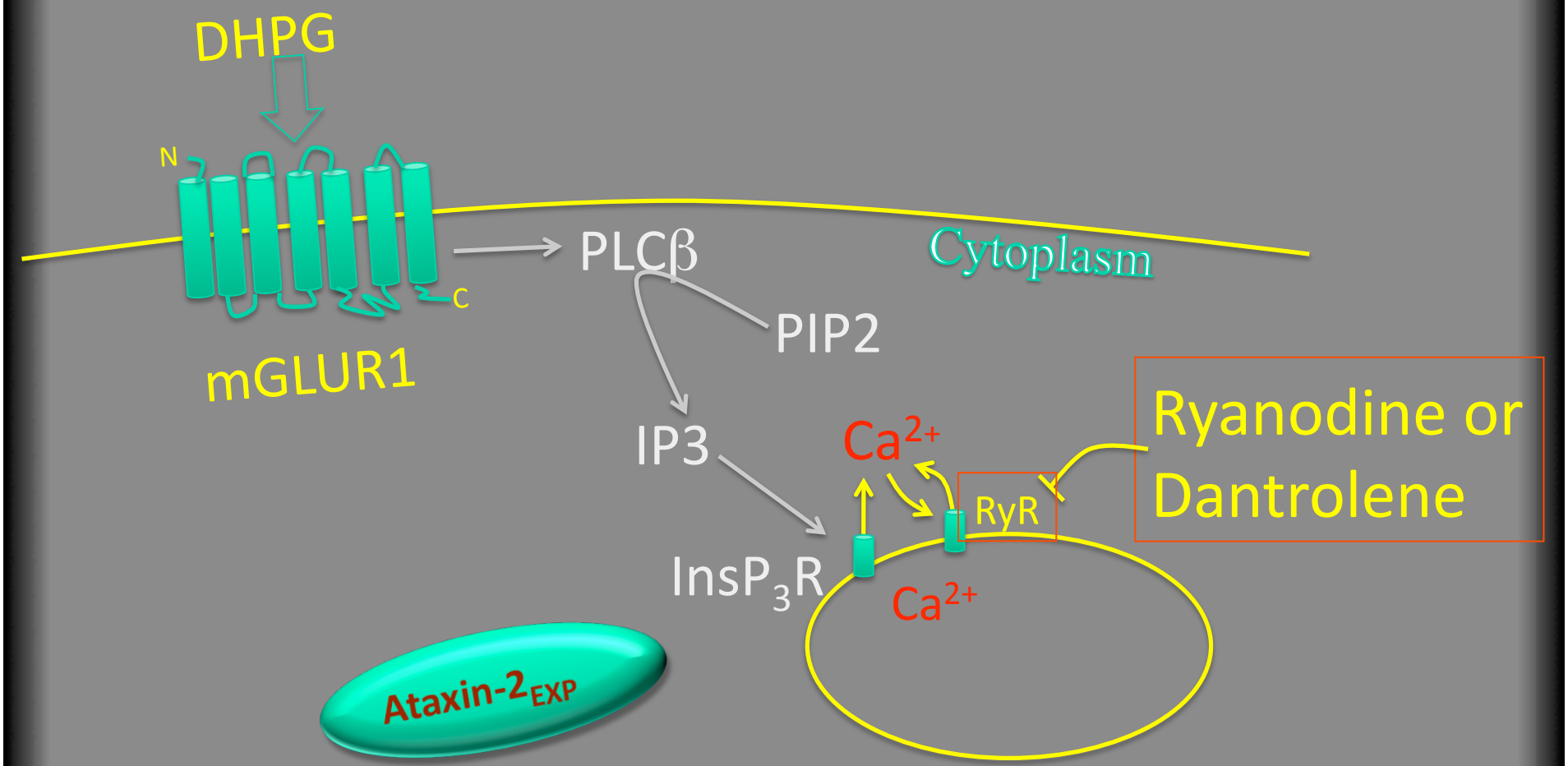
Mutant ATAXN2 and Ca^{2+} Release



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Ataxin-2 action on Ca^{2+} movement in vitro.
(cultured primary Purkinje cells from *ATXN2* transgenic mice)

Differential interaction for wt and mutant ATXN2 with InsP3R1

Exaggerated responses in 58Q PCs to DHPG stimulated Ca^{++} release

Enhanced Ca signals in 58Q PCs cause Glutamate induced cell death

Dantrolene recovery of cellular phenotype in 58Q PCs in vitro

Does dantrolene have an effect *in vivo* ?

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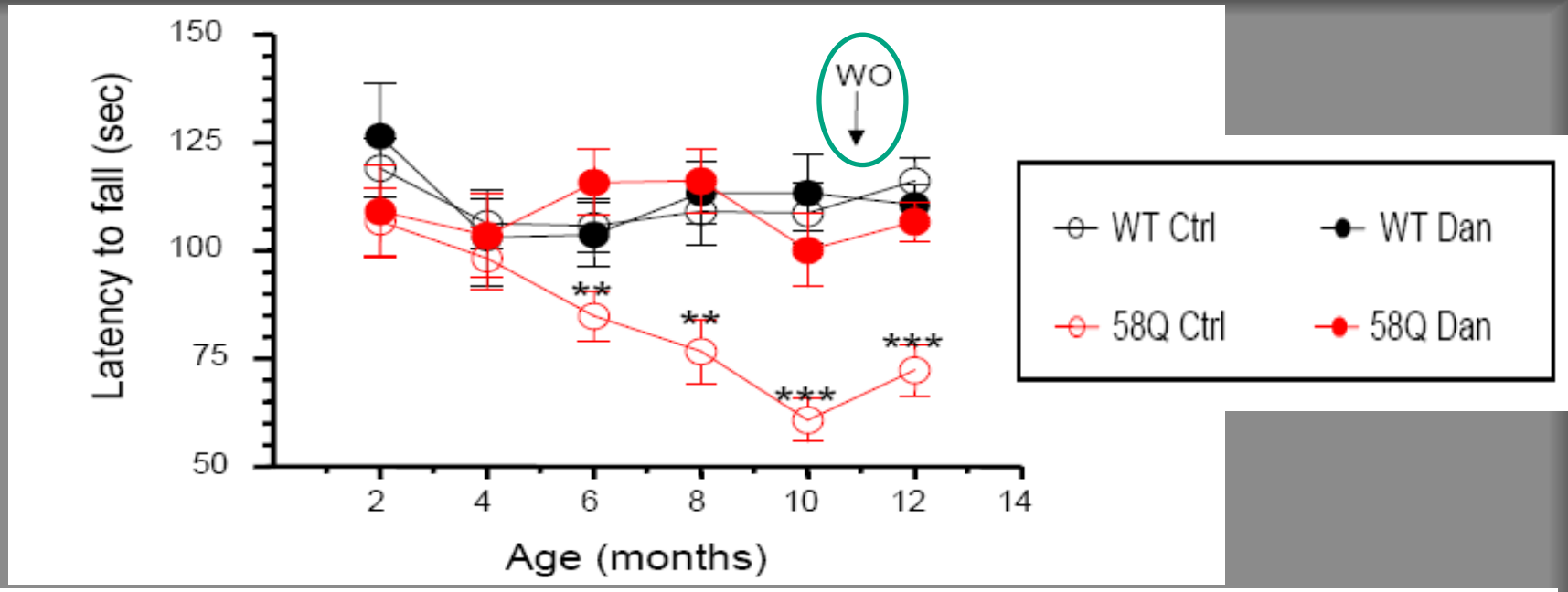
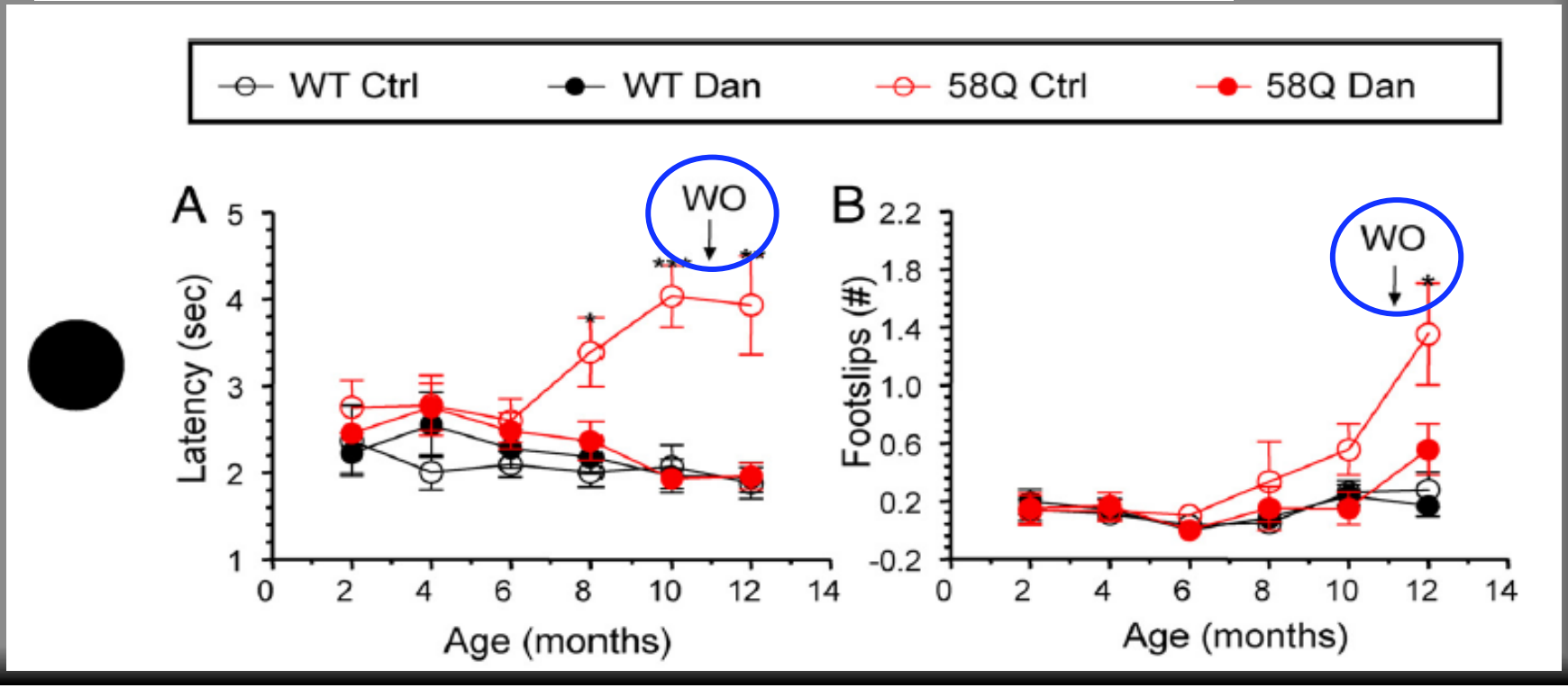
- Design
  - 4 groups
  - Wt +/- Dantrolene & Q58 +/- Dantrolene
- Dantrolene Feeding
  - Age- and weight-matched females
  - 5mg/kg body weight PO twice per week
  - Controls PBS only
  - Washout phase beginning at 11 months
- Motor coordination assessment
  - Beam walk @ 17mm R, 10mmR, 5mm Sq
  - Rotarod: 3 days training, test day with 3 trials


Latency to fall (sec)
150
125
100
75
50
2
4
6
8
10
12
14
Age (months)
WO
**
**
***
***
WT Ctrl
WT Dan
58Q Ctrl
58Q Dan


WT Ctrl
WT Dan
58Q Ctrl
58Q Dan
A
Latency (sec)
5
4
3
2
1
0
2
4
6
8
10
12
14
Age (months)
WO
***
**
*
B
Footslips (#)
2.2
1.8
1.4
1.0
0.6
0.2
-0.2
0
2
4
6
8
10
12
14
Age (months)
WO
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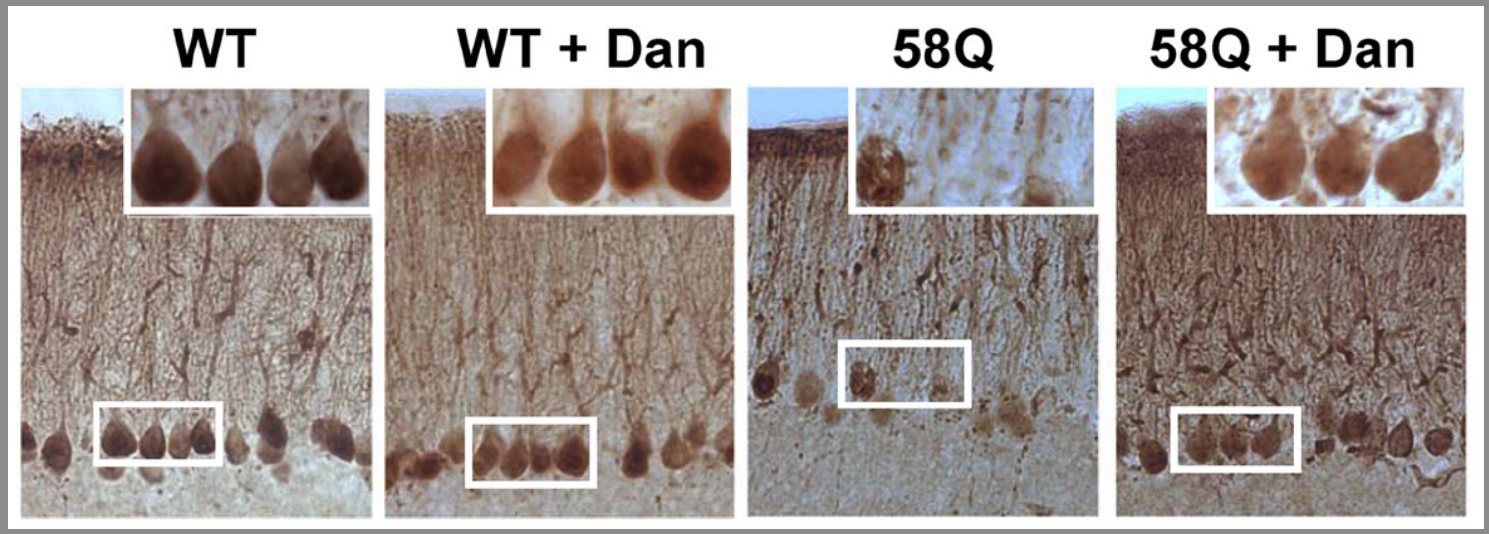

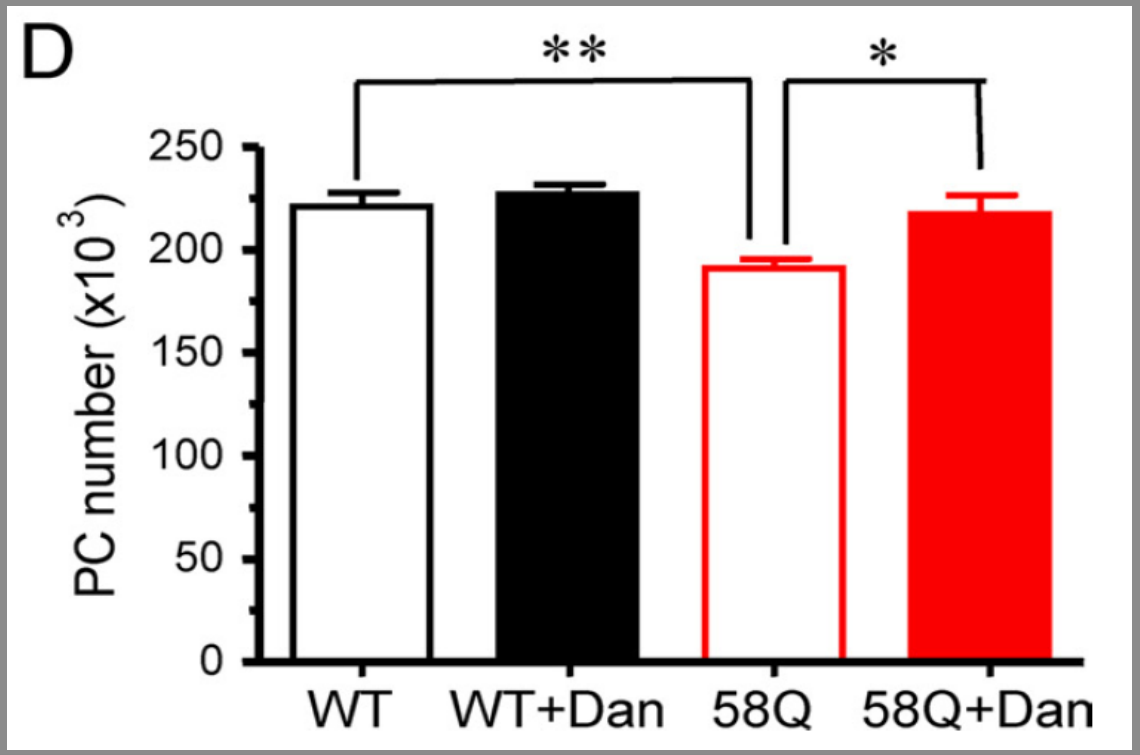

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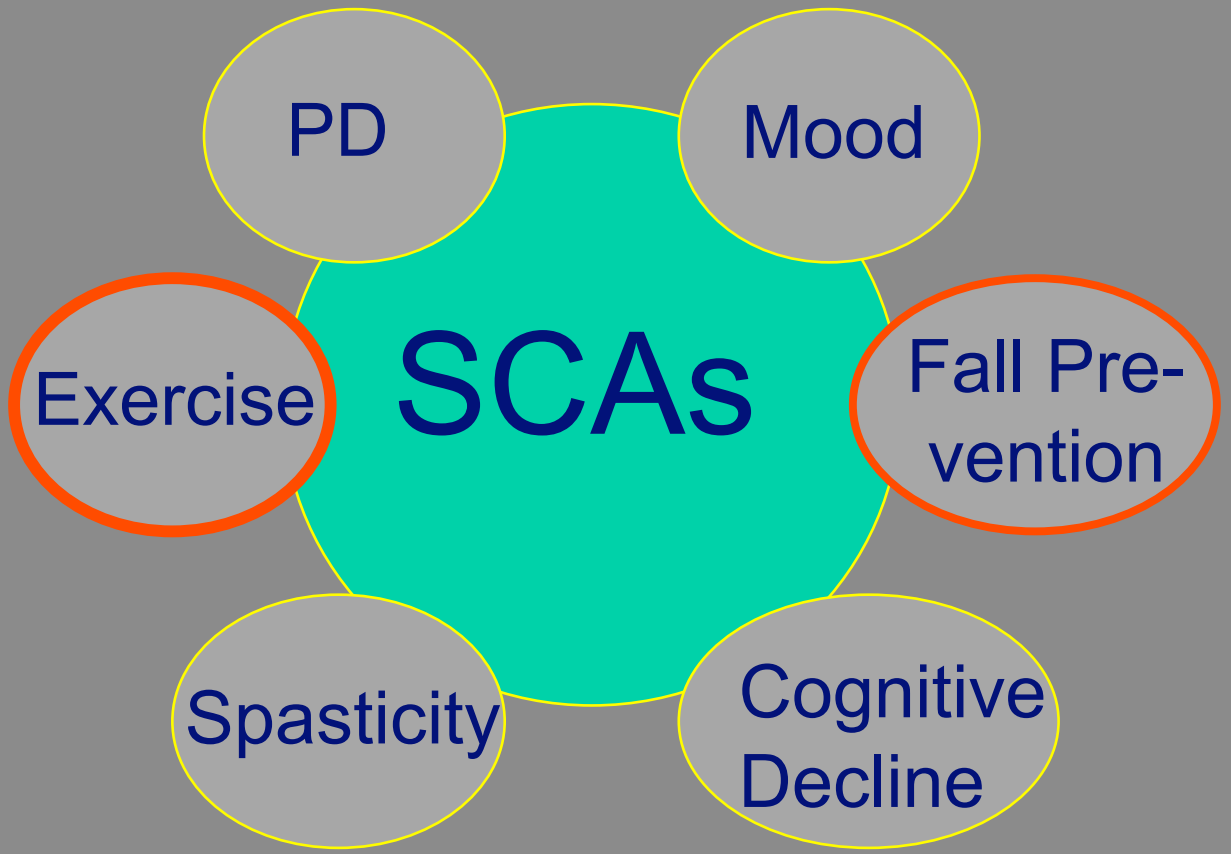
Dantrolene in SCA2:

- Pros
  - Effects on motor function and PC number
  - Also successful in SCA3 BAC transgenic mouse model
  - Dantrolene already in human use
- Further Studies
  - Treatment at or after symptom onset in mouse models
  - Different dosages
  - Other SCA2 mouse/rat models
  - Replication in other laboratories

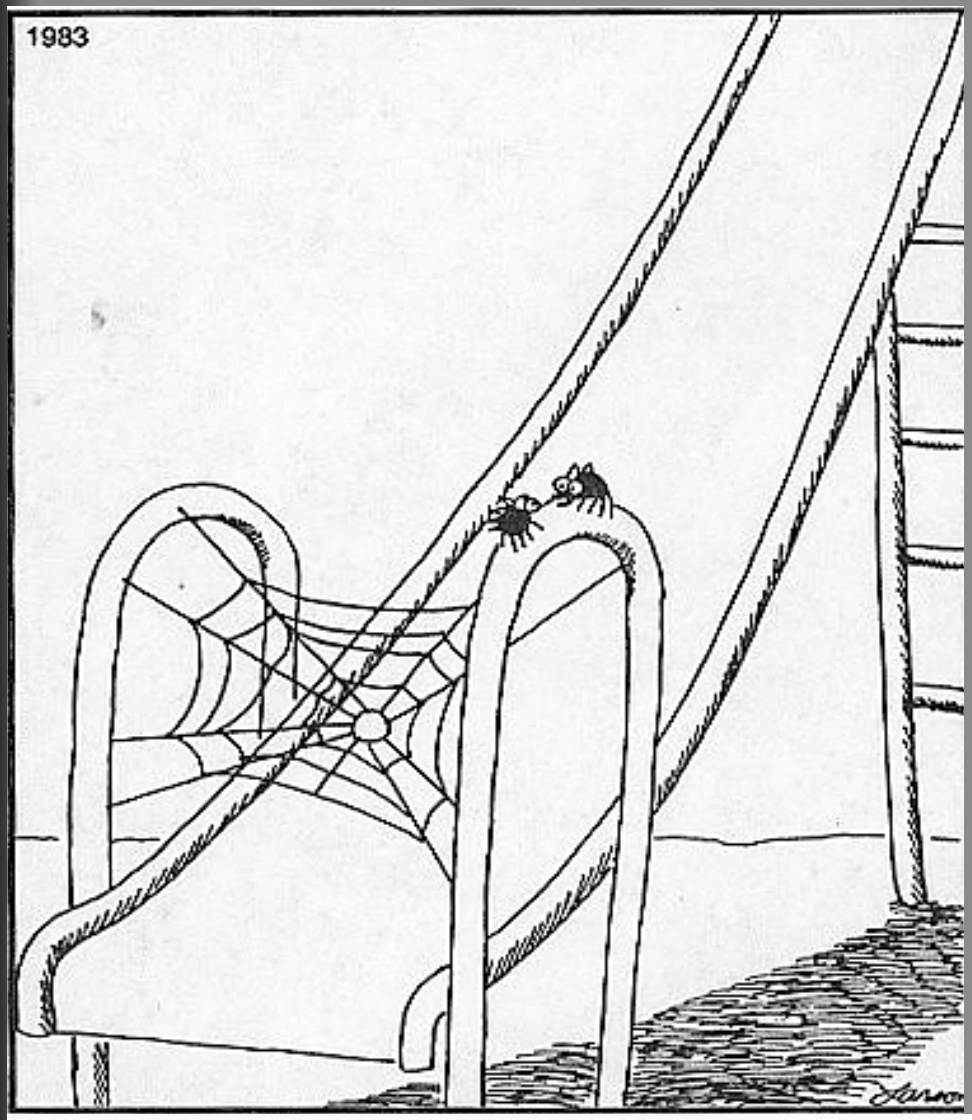
Symptomatic Rx



Summary

- Genes
  - >30 dominant SCA genes/loci identified
  - SCA2 → polyQ disease
- Models
  - Transgenics
  - Ca signaling
- Treatments
  - Compound screen (NCGC)
  - Antisense (ISIS)
  - Dantrolene in mice

Collaborators



If we pull this off, we'll eat like kings.

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