The spinocerebellar ataxias: wobbling towards translation.

Neurology Grand Rounds

University of Michigan

THE UNIVERSITY OF UTAH

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Apopharma: DSMB

Off Label Usage: None

What are SCAs?

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

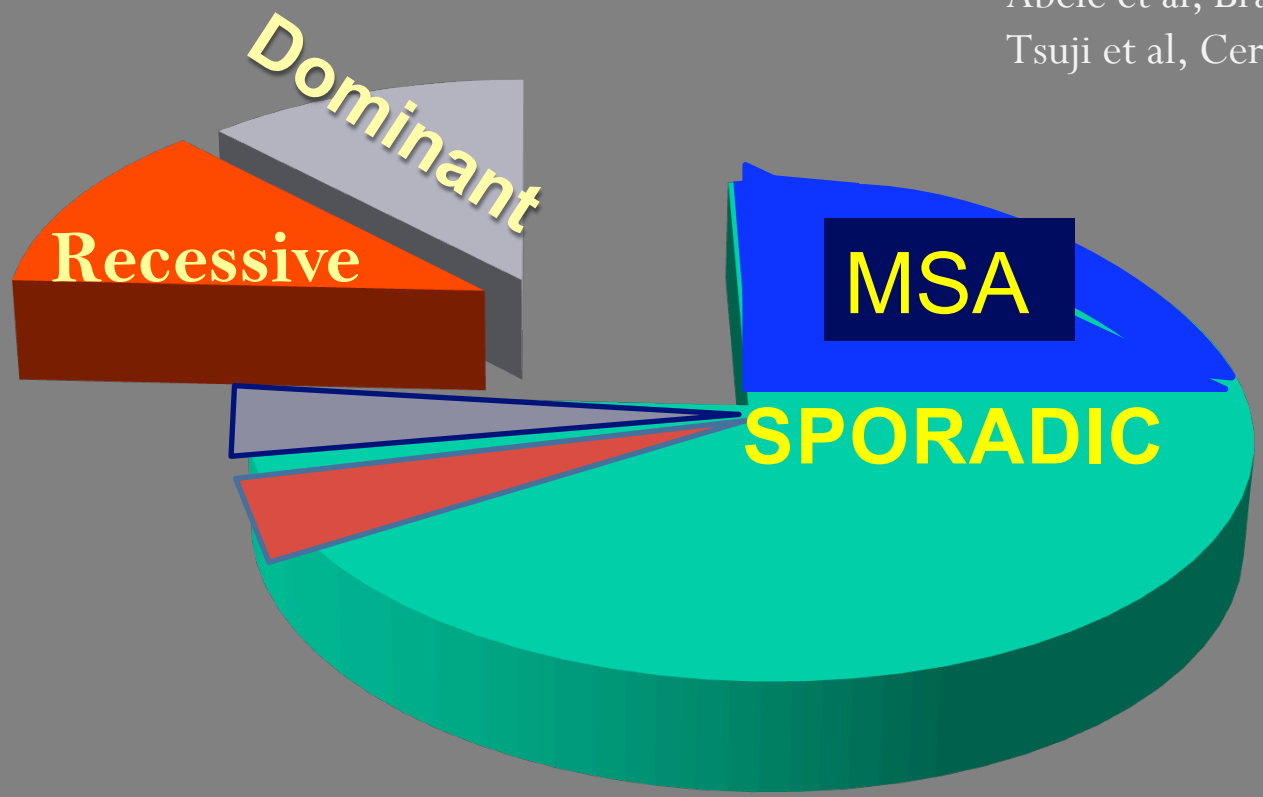
SCA Symptoms/Signs

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

Degenerative Ataxia with and without Family History

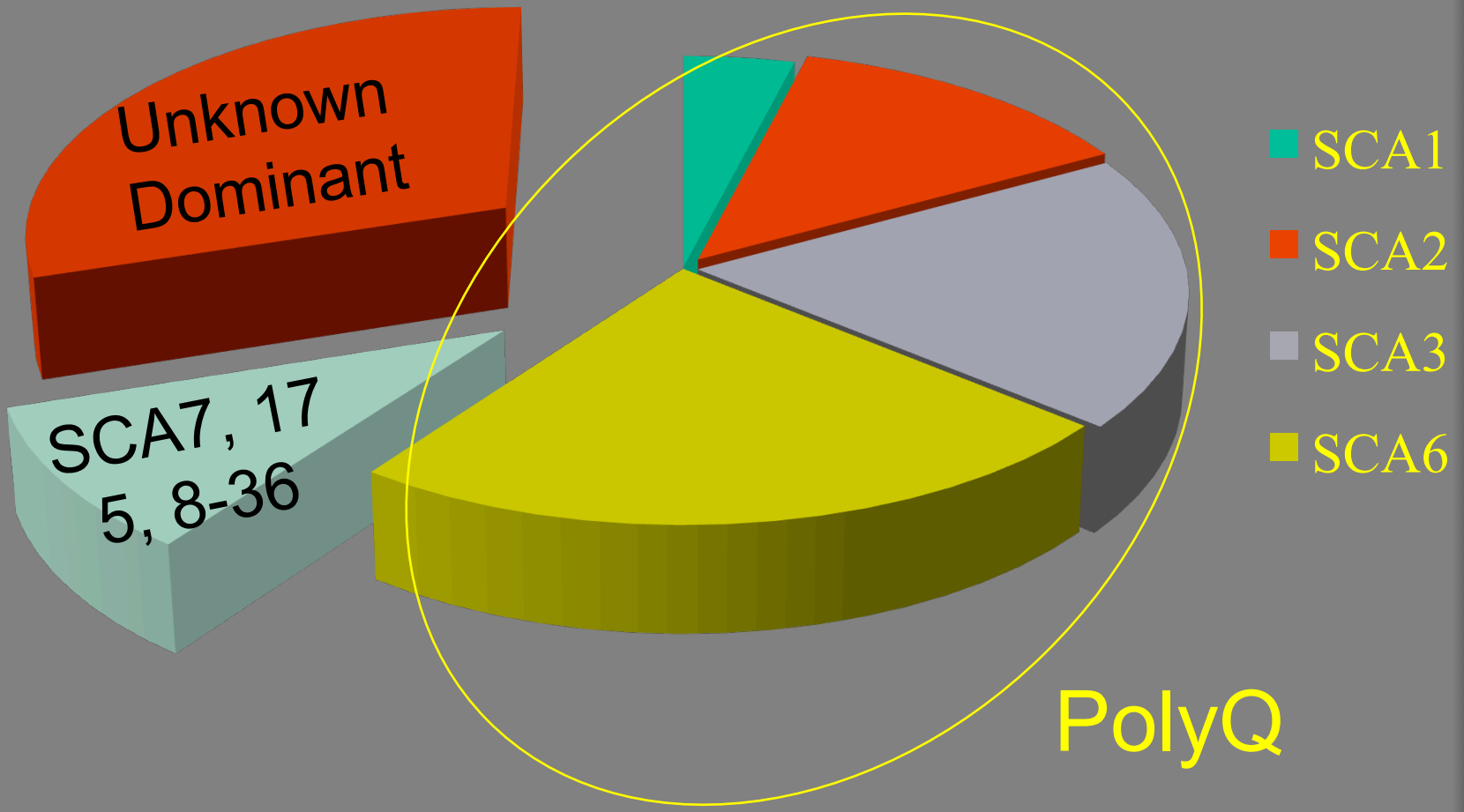
Prevalence rate: 18.6/100,000

Abele et al, Brain 2002
Tsuji et al, Cerebellum, 2008



Genes

Genetic Architecture of SCAs

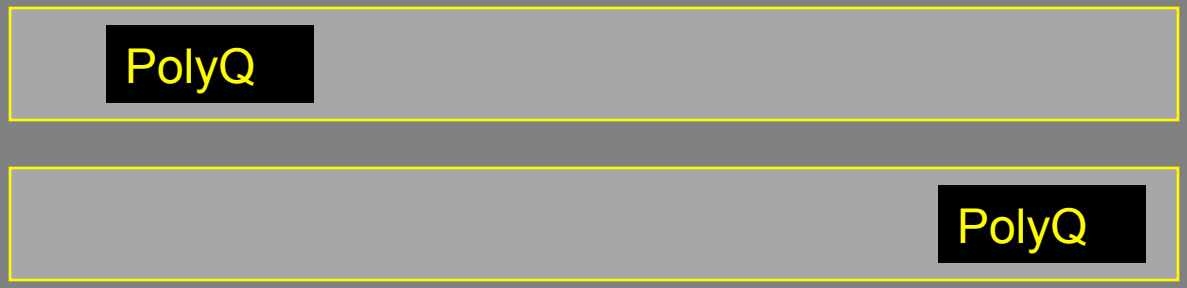


Non-polyQ Ataxias

- EA2 — CACNA1a
- SCA5 — beta3-spectrin
- SCA10 — toxic RNA
- SCA11 — Kinase (TTBK2)
- SCA13 — Voltage- gated K^+ - channel (KCNC3)
- SCA14 — Kinase (PKCγ)
- SCA15/16 — Ca^{++} release (ITPR1 LoF)
- SCA19/22 — Voltage- gated K^+ - channel (KCND3)
- SCA20 — Dup 11q (260kb)
- SCA23 — Prodynorphin
- SCA27 — FGF14 LoF
- SCA28 — mitochondrial AAA protease
- SCA31 & 36 — toxic RNA
- SCA35 — transglutaminase TGM6

Dominant SCAs

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



Repeat is variable in normals

Pathological repeat length different

Repeat unstable→ Anticipation

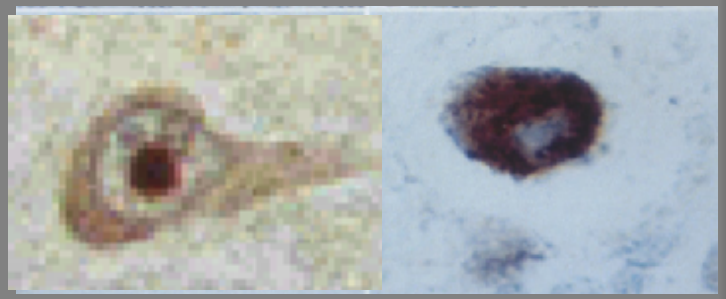
No homologies other than polyQ-tract

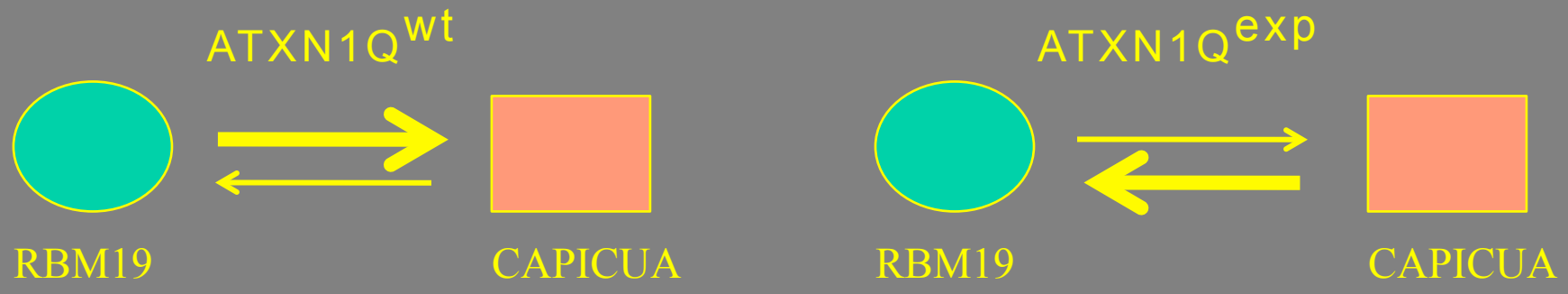
SCA6: Ca^{++} channel (CACNA1A)

SCA17: transcription factor (TBP)

Poly-Q Pathogenesis

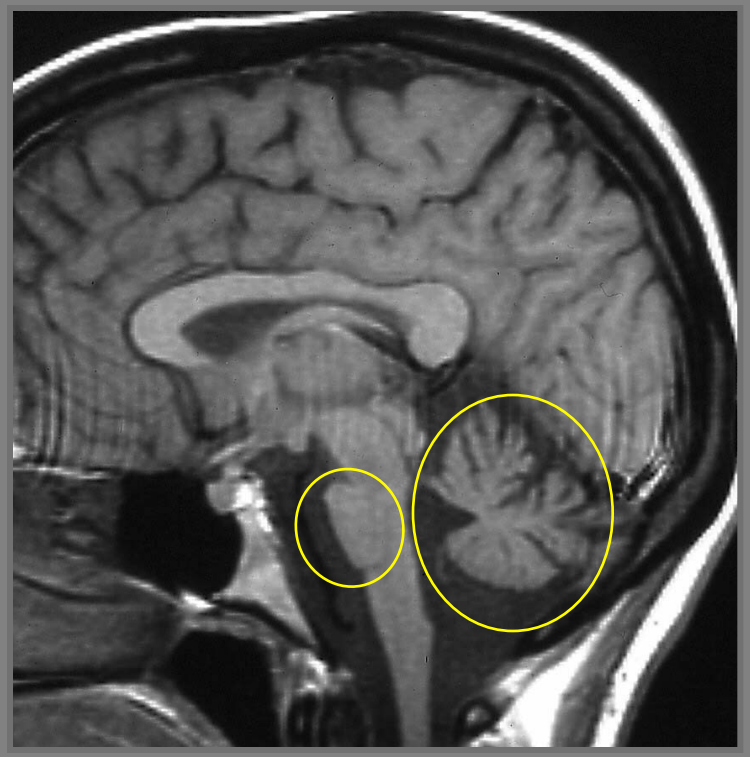
- Gain of Toxic Function
  - Aggregation of misfolded proteins
  - Misfolded toxic oligomeres
- Gain of Normal Function
- Allele-specific Gain/Loss of Normal Function





SCA2: Phenotype & Gene

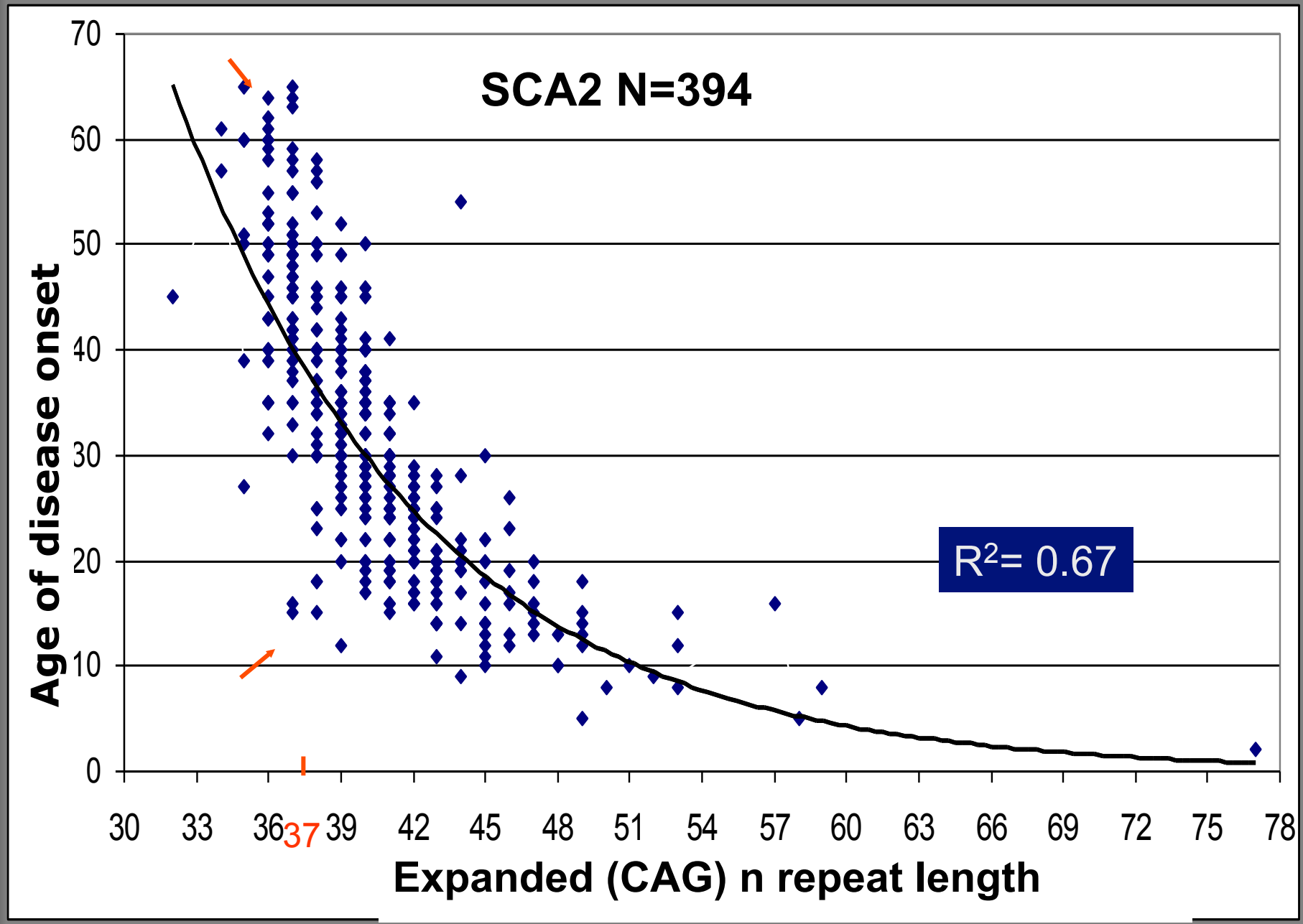
Ataxia
Slow saccades
Neuropathy
Parkinsonian features
Dystonia & Spasticity
ALS-like



Normal: 22Q
DNA repeat: interrupted

Mutant: ≥32Q

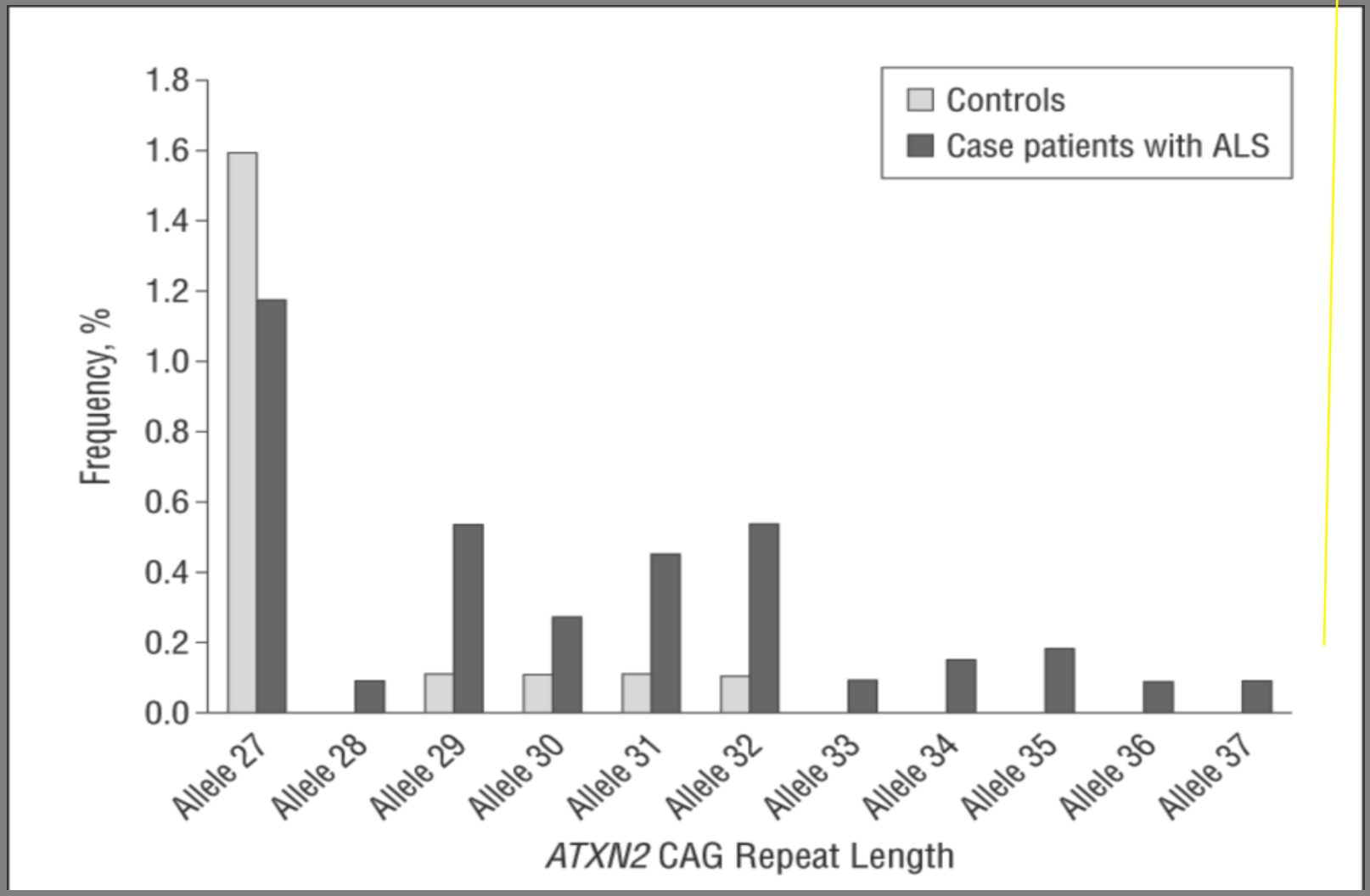
Pulst et al Nature Genet 1996


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

From Phenotypic Diversity to Phenotype Outliers

- Parkinsonian signs → DOPA-responsive PD
- UM signs & neuropathy → ALS
- Usually lower mutant repeat numbers
- Repeat may be interrupted

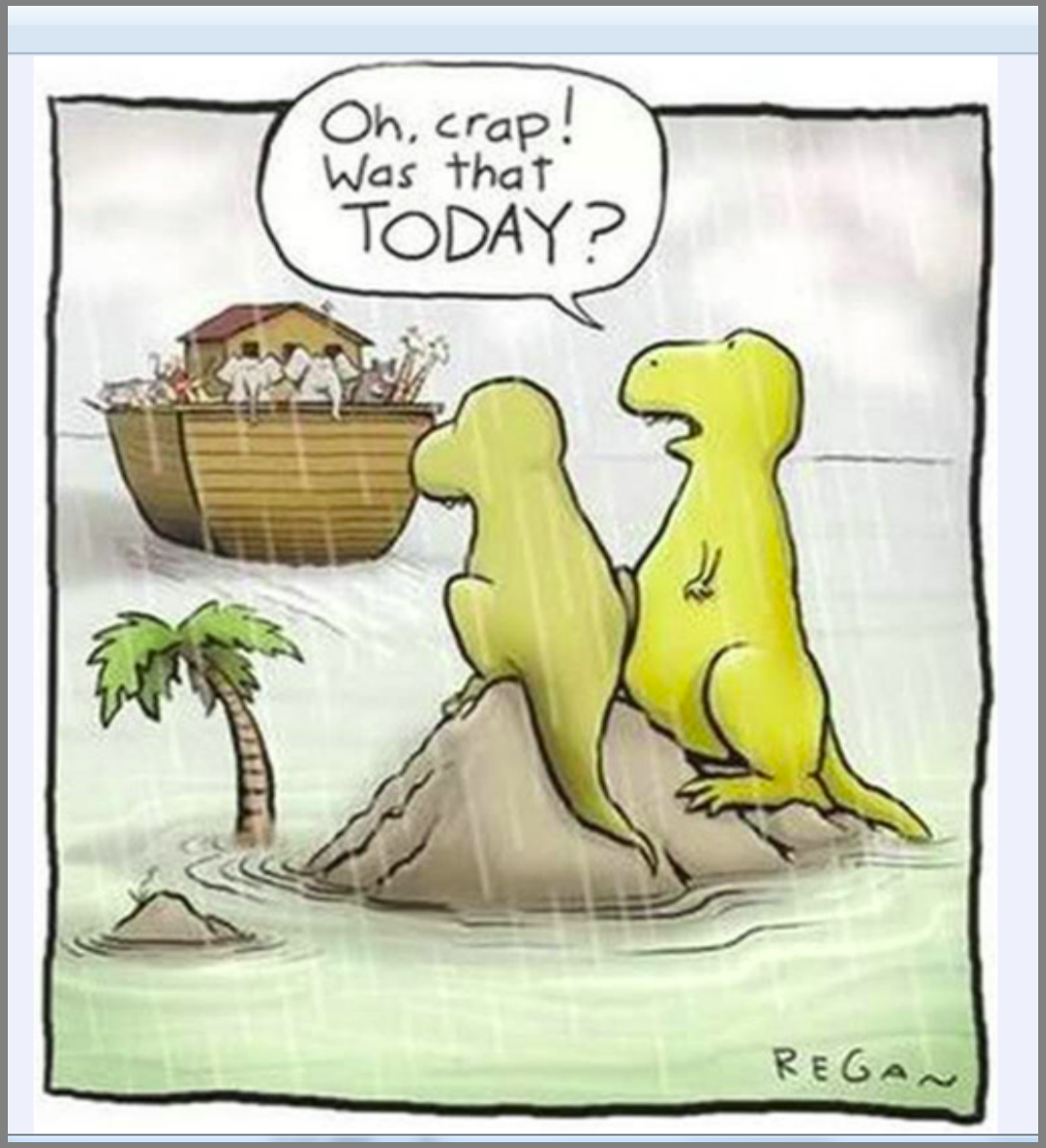
Association of Long ATXN2 CAG Repeat Sizes With Increased Risk of ALS



Arch Neurol. 2011;68(6):739-742. doi:10.1001/archneurol.2011.111

SCA2 Alleles & Phenotypes

- ≤ 24 — Normal
- 29-32 — Predisposed to expansion to full mutation
  Increased ALS risk
- ≥ 33 — Cerebellar Ataxia
  ALS
  PD



SCA2:

Polyglutamine disease

Phenotypes:

Ataxia

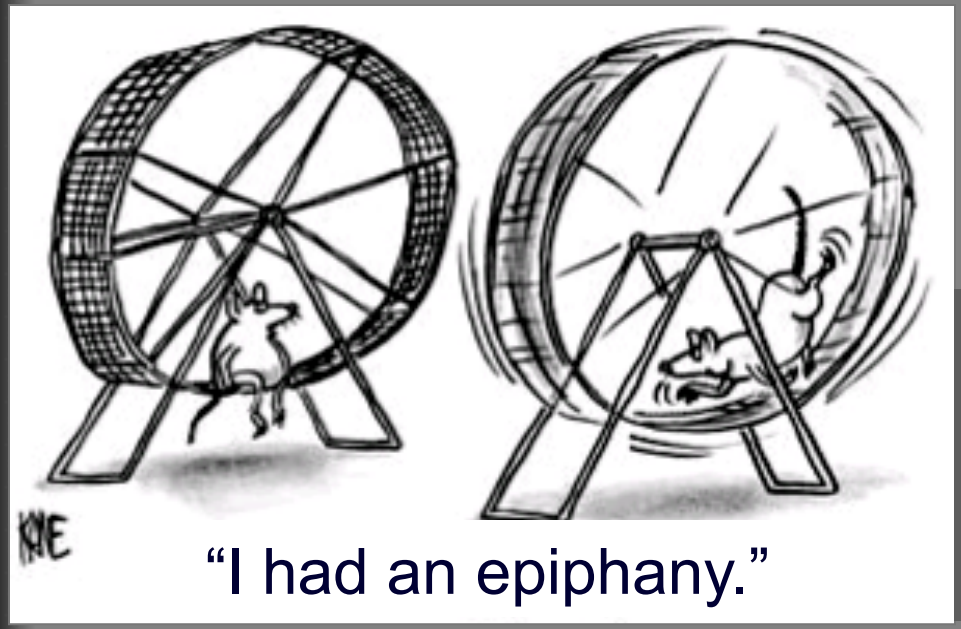
PD

ALS

Why are we wobbling towards translation?

- Human:
- Limited Natural History
- Scales to measure progression and response
- Biomarkers
- Animal
- Clinical trials approach:
- Multiple Models & Backgrounds
  Multiple Centers

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

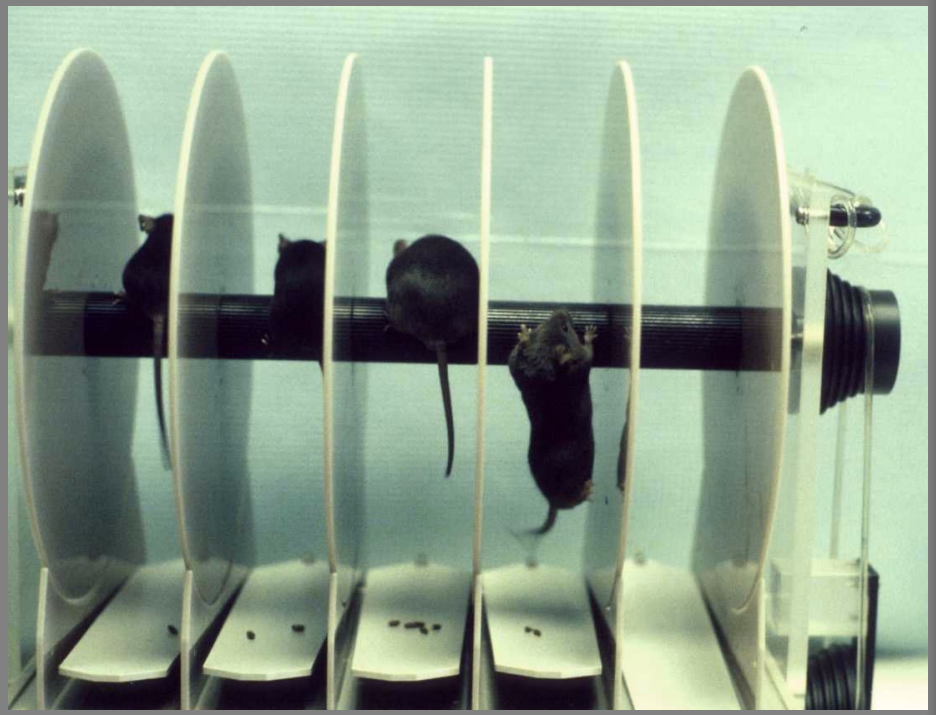
A model is a model is a model.

- 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

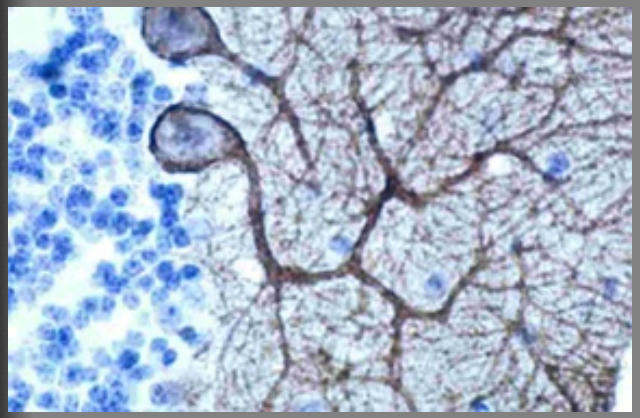


The tg-Pcp2-ATXN2[Q58] mouse

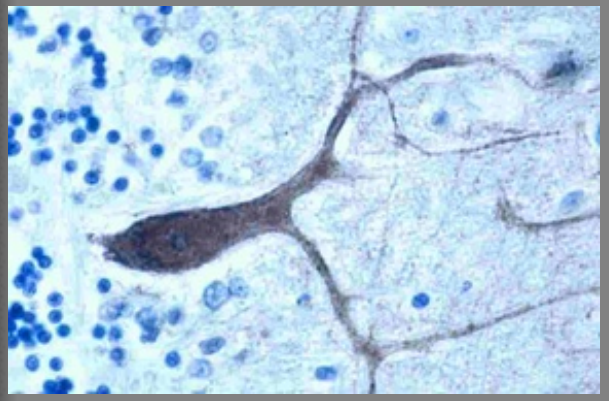
Promoter: Purkinje cell specific PcP2

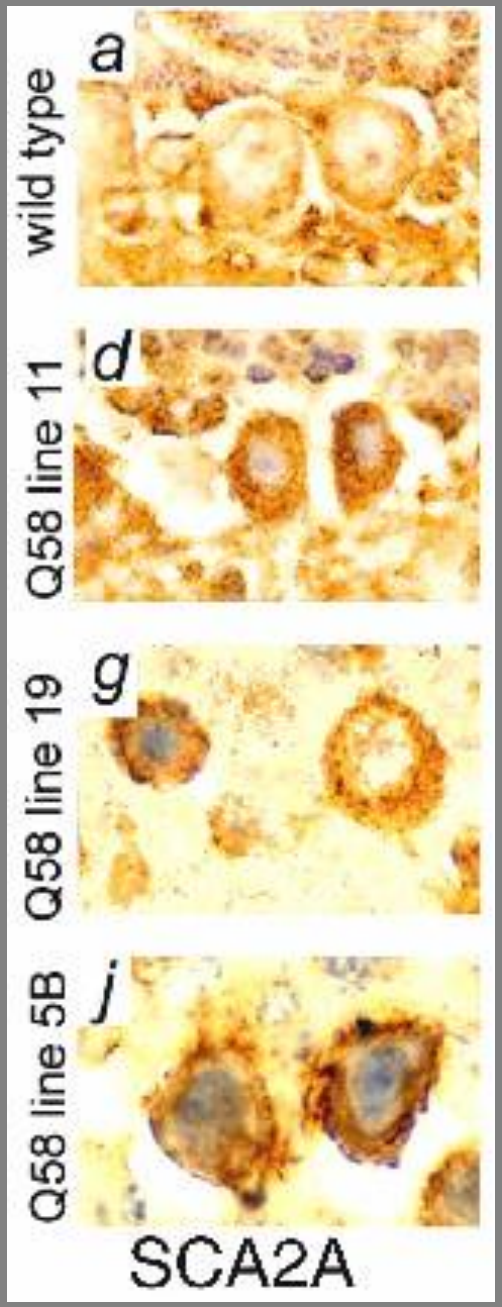
Huynh et al Nat Genet 2000

Human control

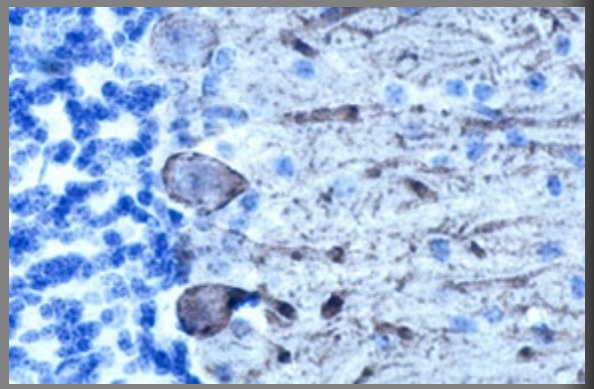


Wildtype Mouse

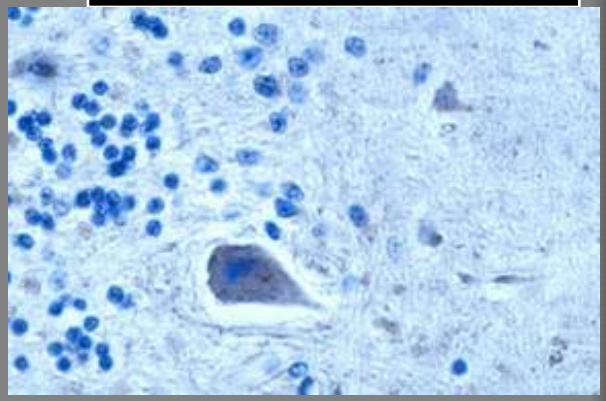




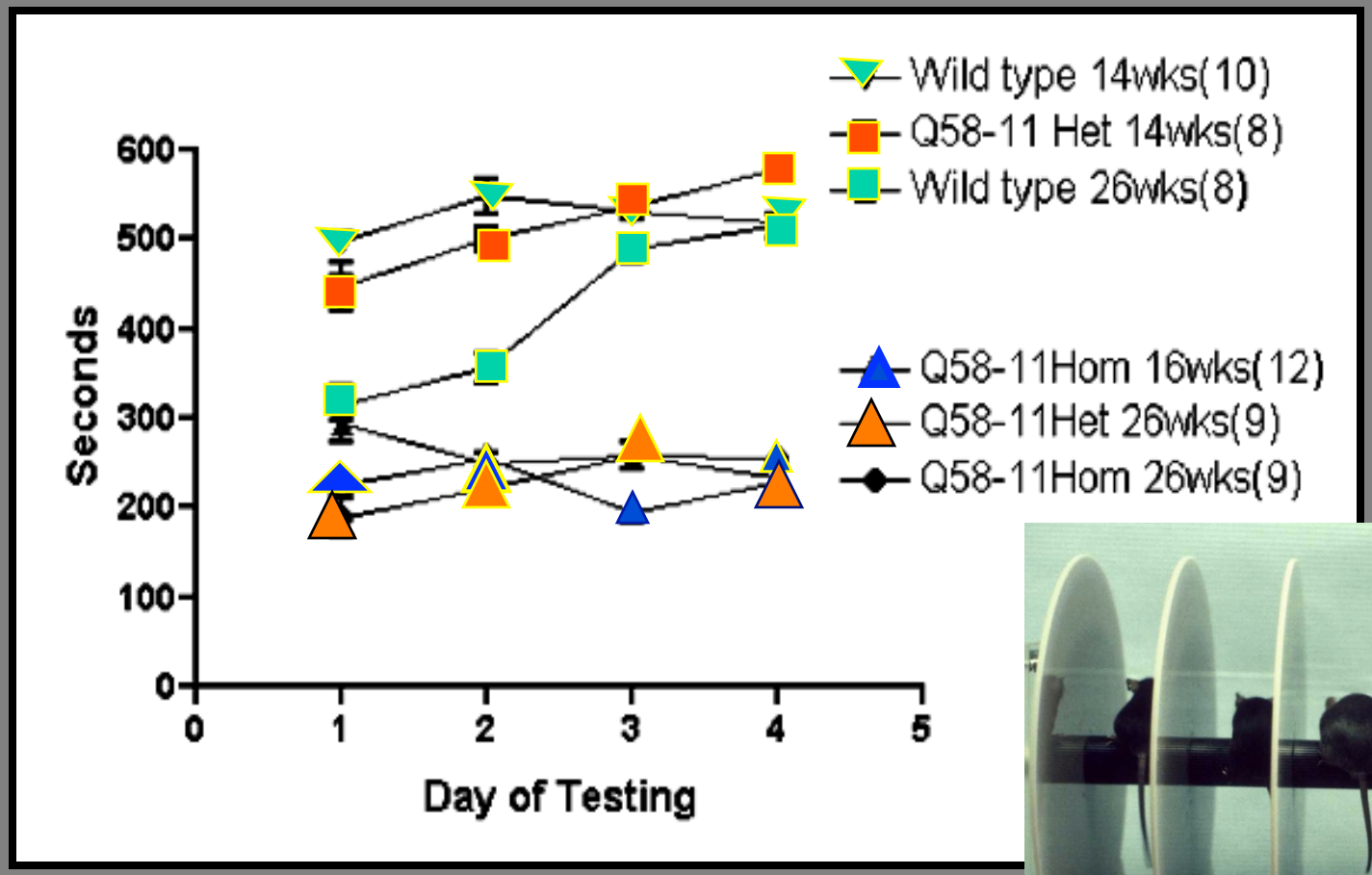
SCA2 patient

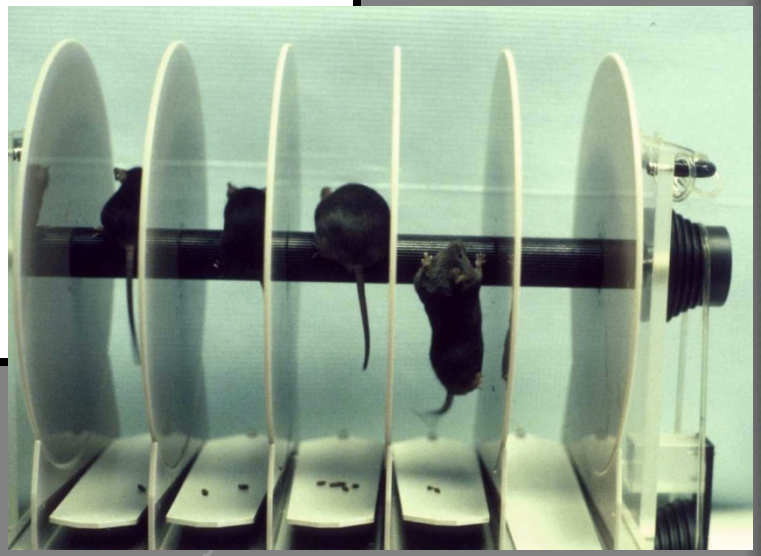


Ataxin-2$_{[Q58]}$ mouse



Functional Analysis





Huynh et al Nat Genet 2000

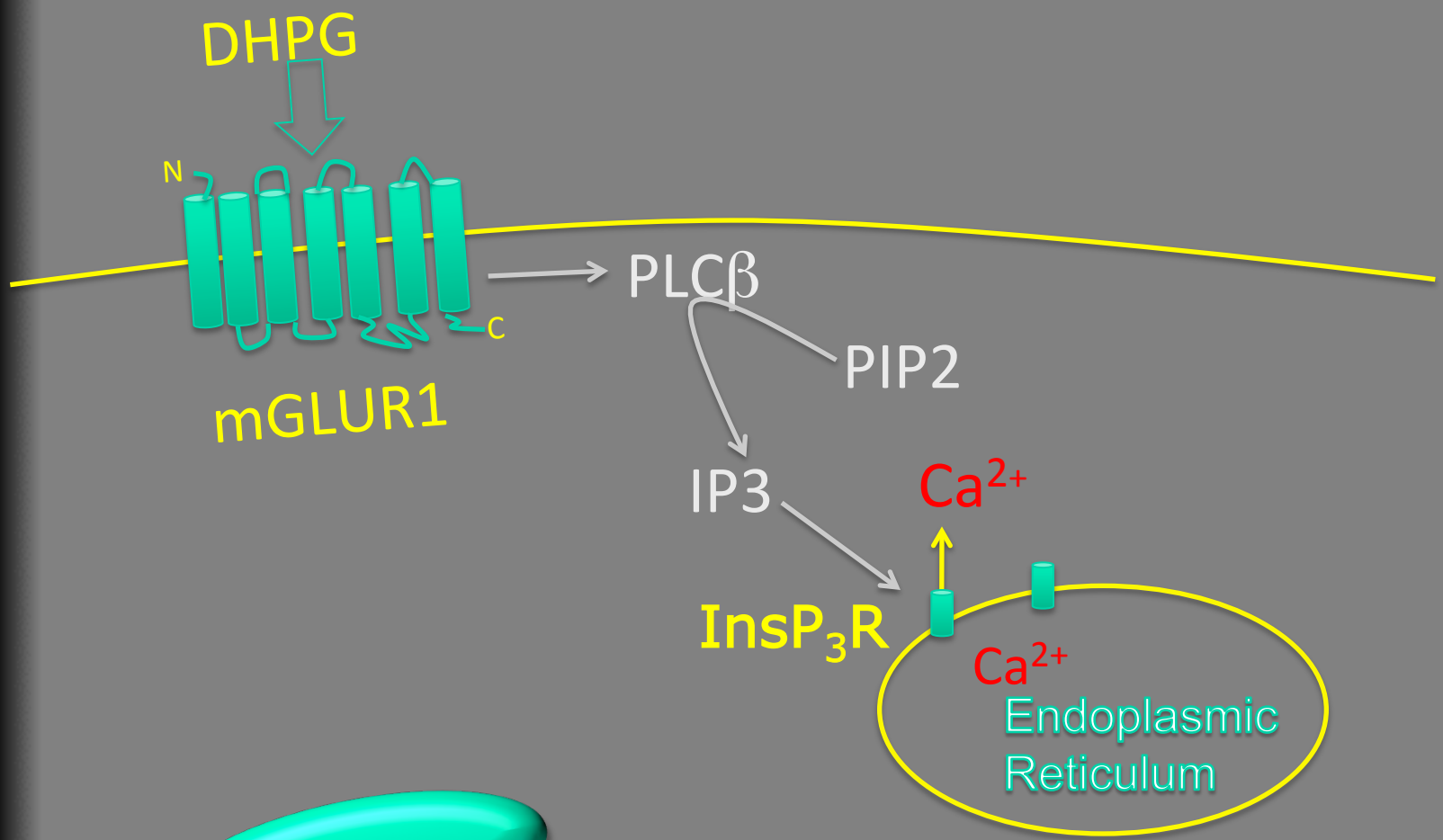
Treatments

"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

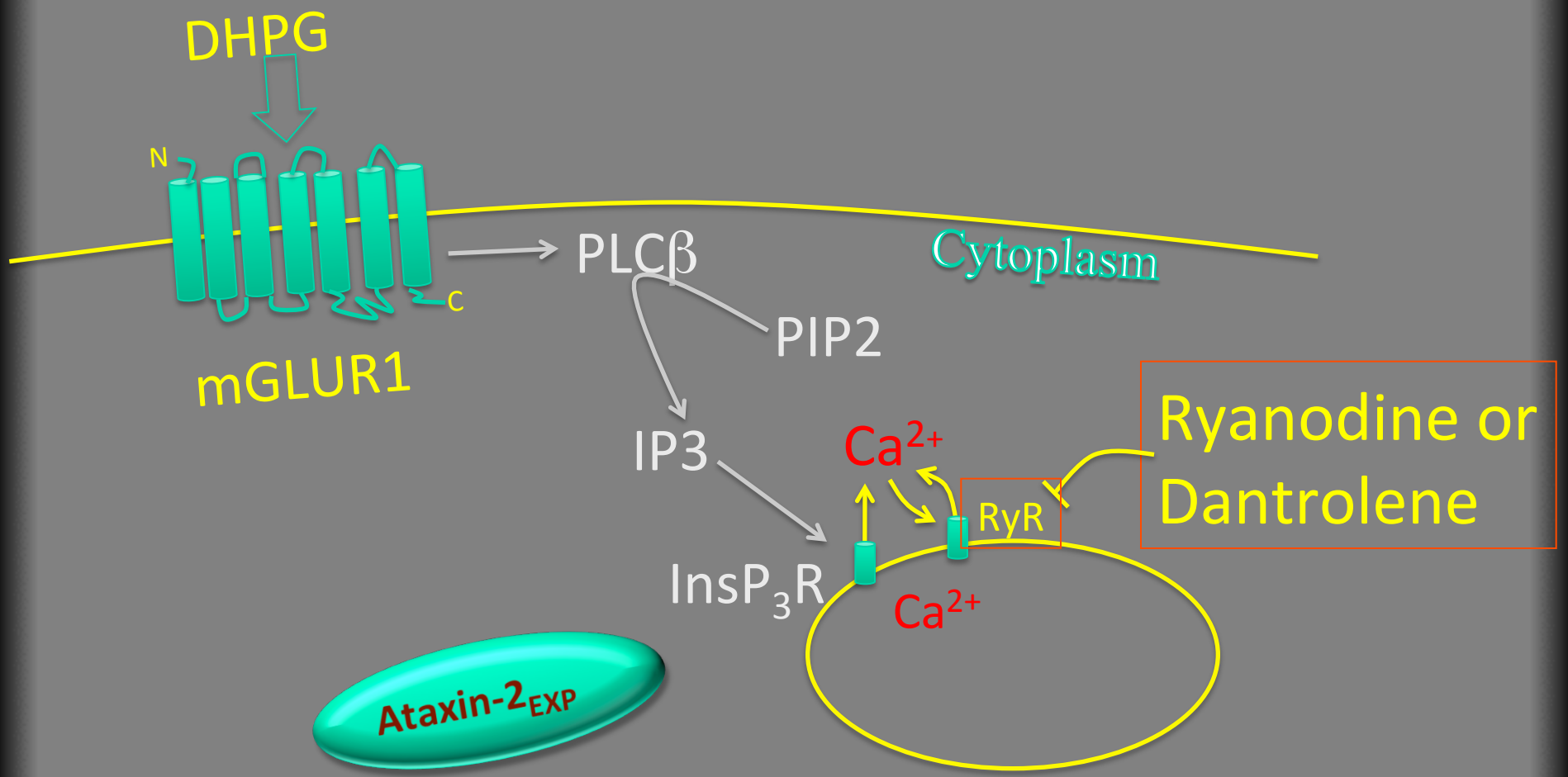
Mutant ATAXN2 and Ca^{2+} Release



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

Liu J et al J Neurosci 2009

Mutant ATAXN2 and Ca^{2+} Release



Liu J et al J Neurosci 2009

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

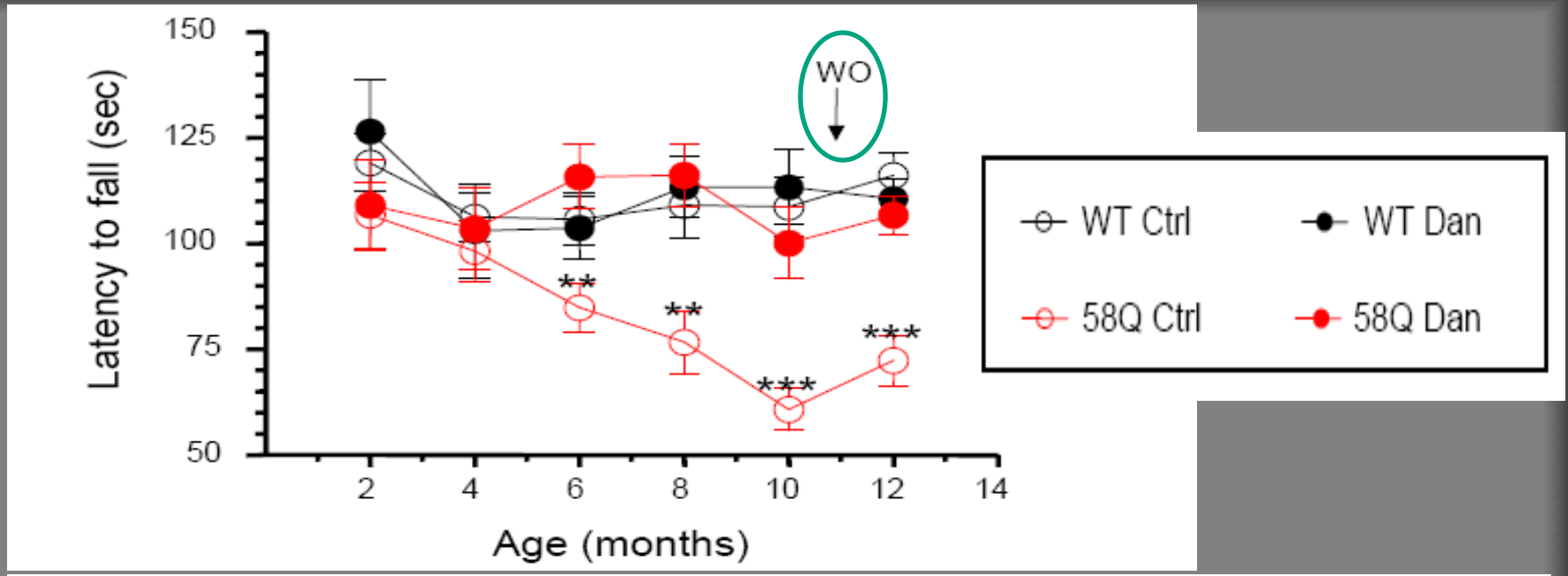
Exaggerated responses in 58Q PCs to DHPG stimulated Ca^{++} release

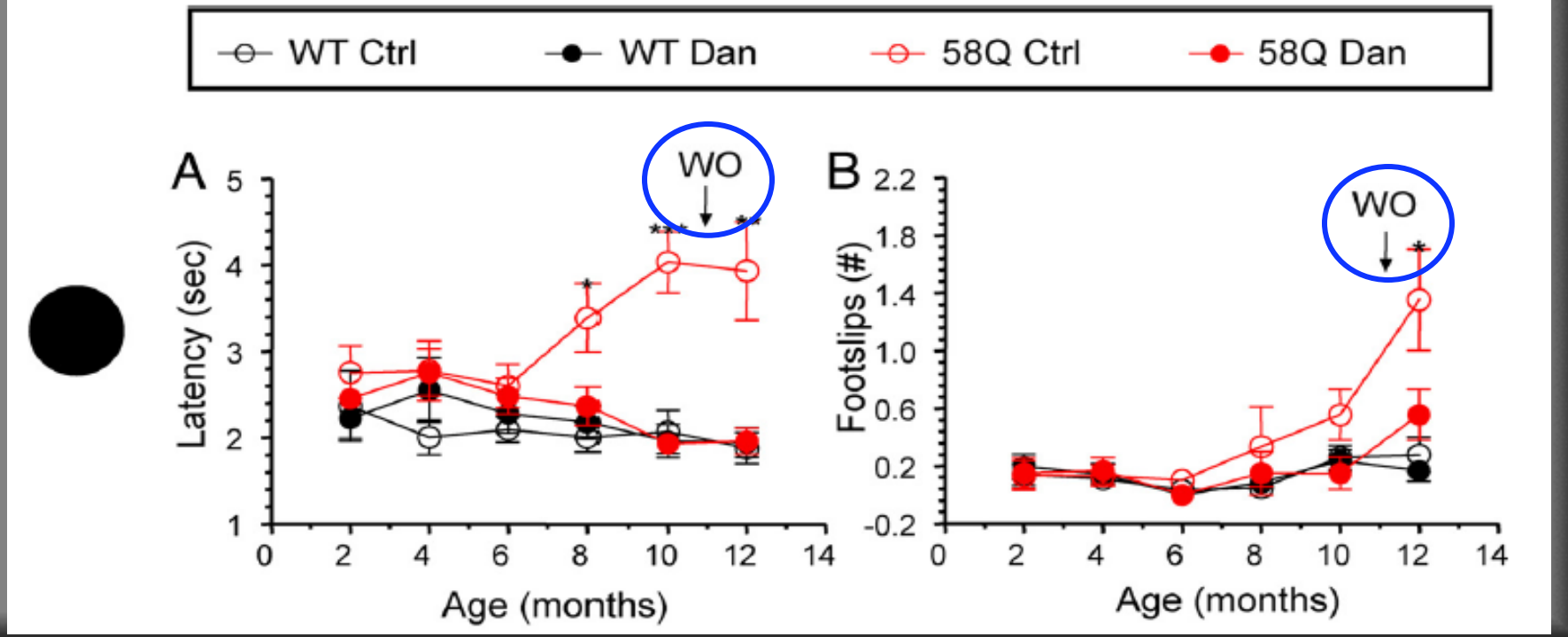
Enhanced Ca signals in 58Q PCs cause Glutamate induced cell death

Dantrolene recovery of cellular phenotype in 58Q PCs in vitro

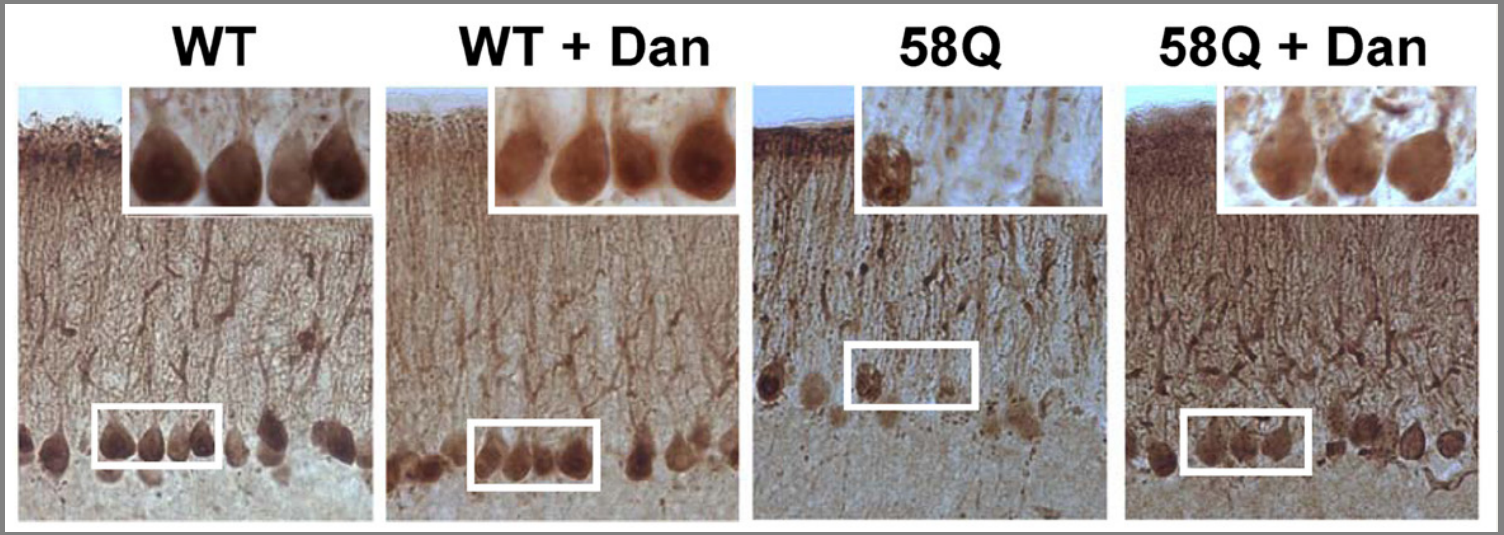
Does dantrolene have an effect *in vivo* ?

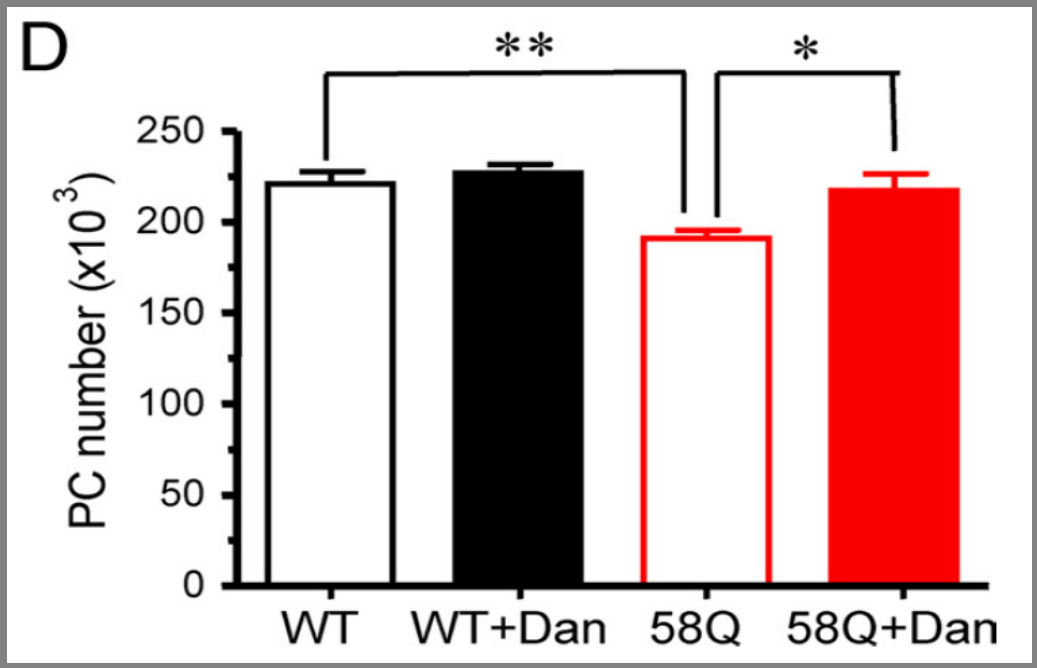
Liu J et al J Neurosci 2009


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
*

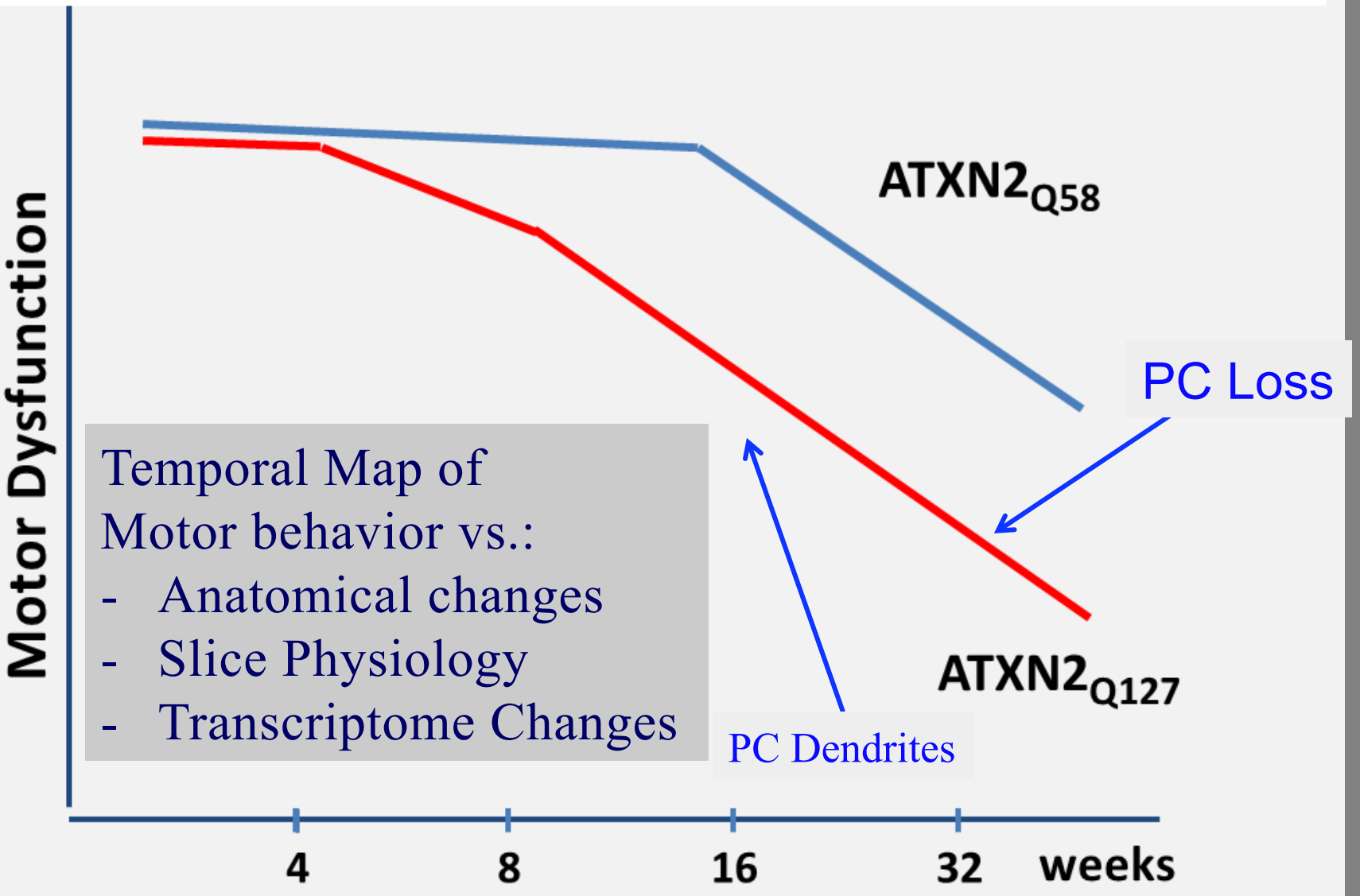
Dantrolene is neuroprotective in the ATXN2Q58 mouse model



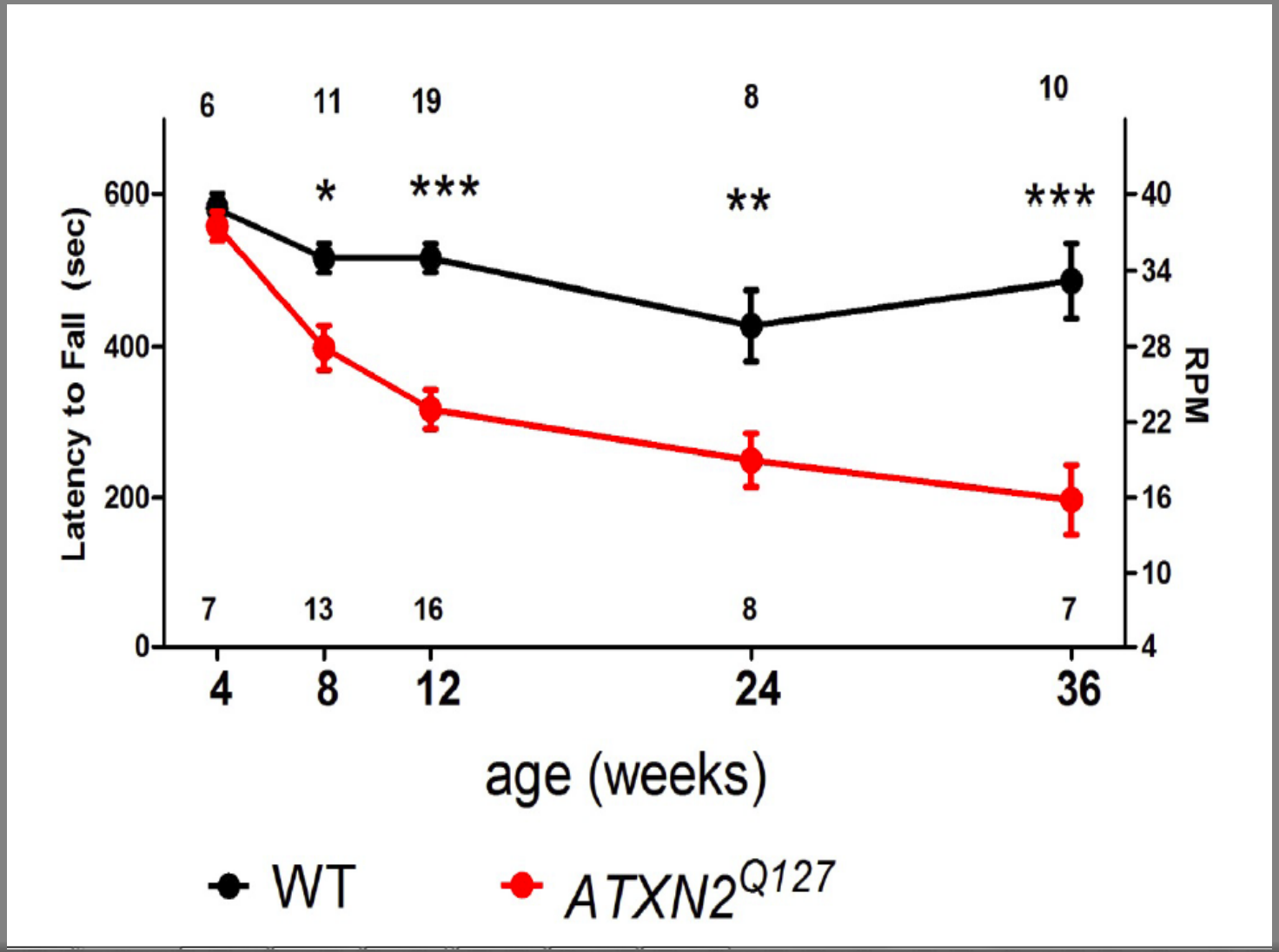


Liu J et al J Neurosci 2009

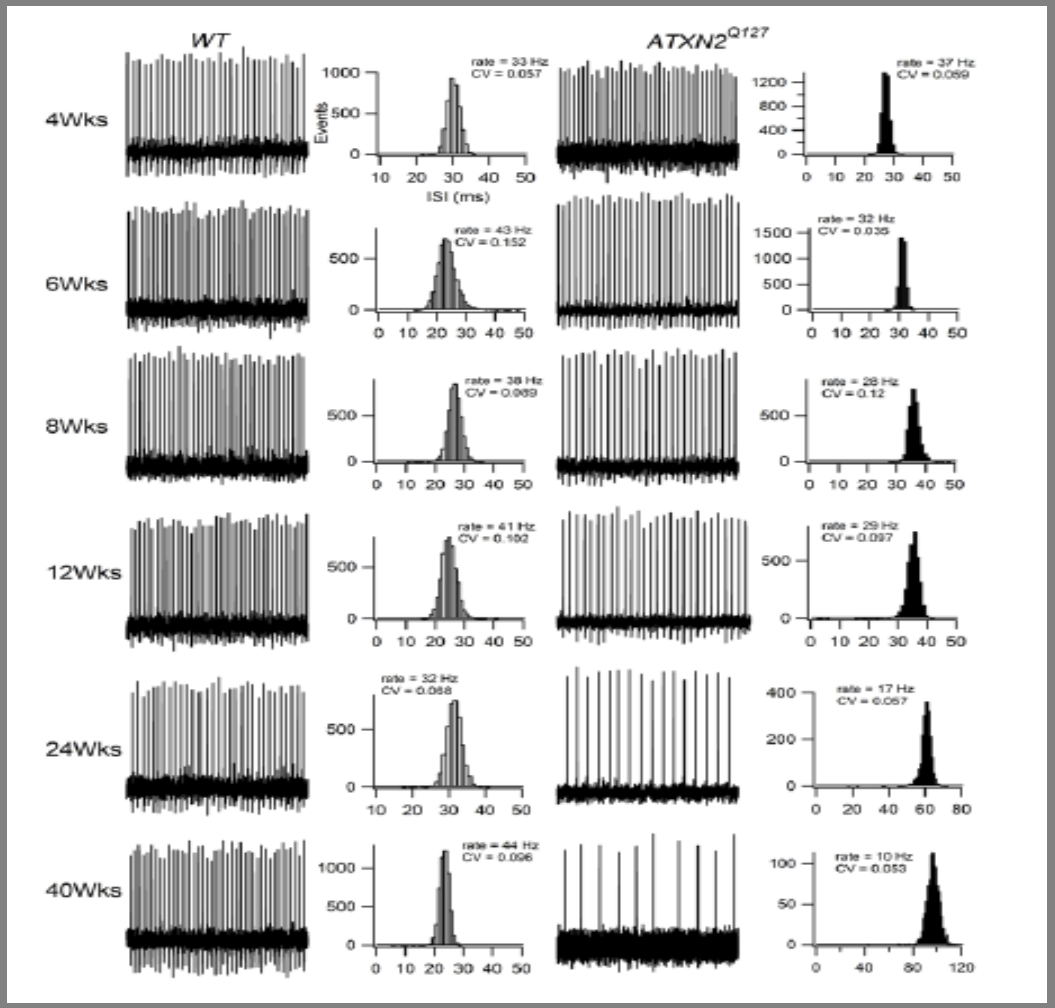
The Pcp2 -tg(ATXN2Q127) mouse has a more severe phenotype.

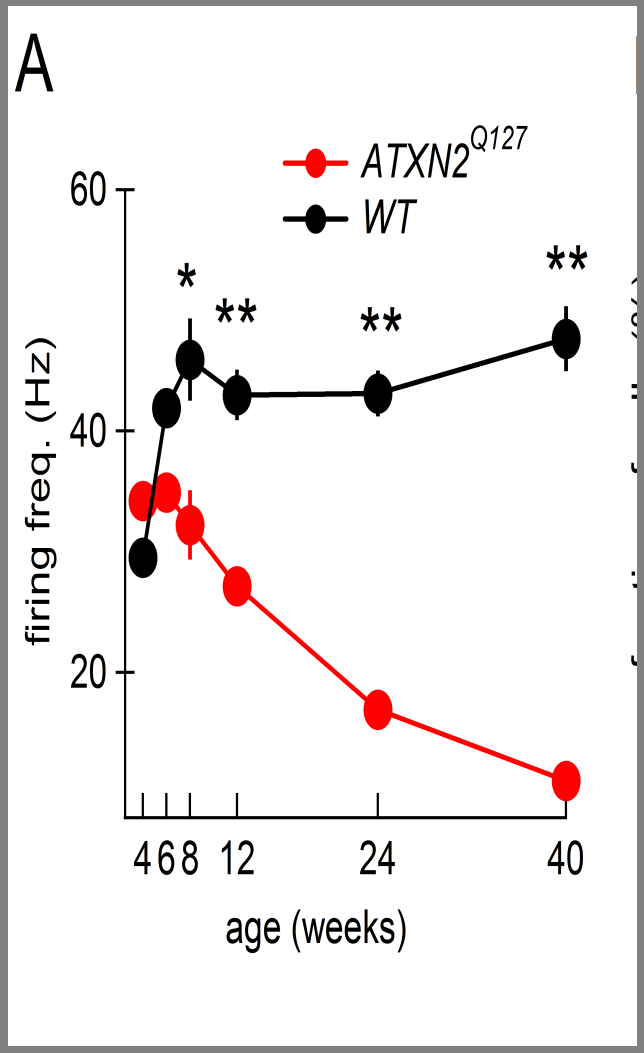


Rotarod behavior is abnormal in the ATXN2[Q127] Mouse

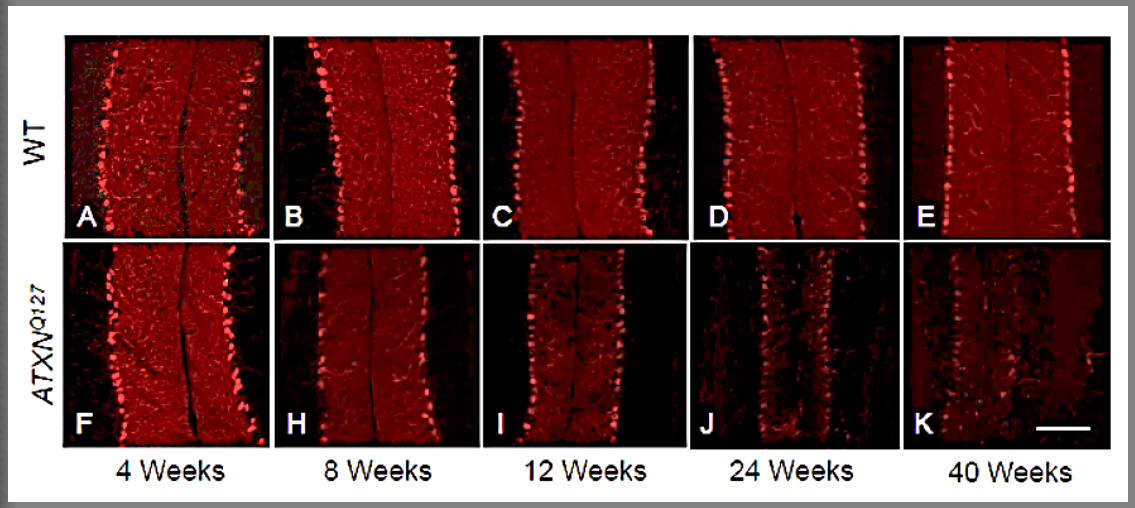


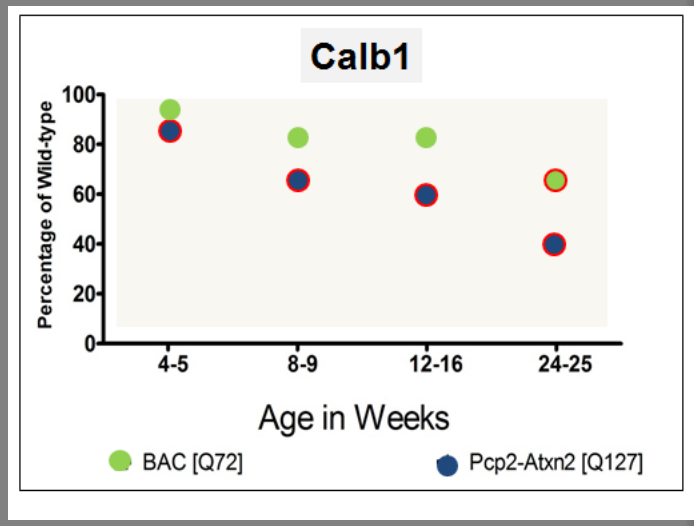
Purkinje cell firing becomes abnormal at onset of motor dysfunction.

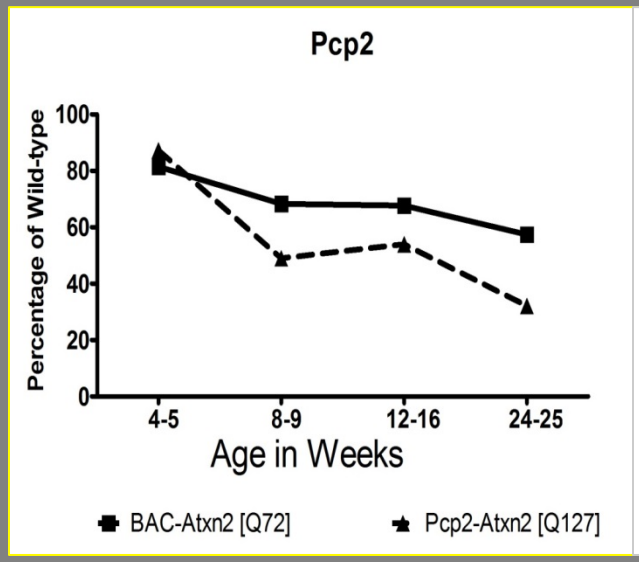


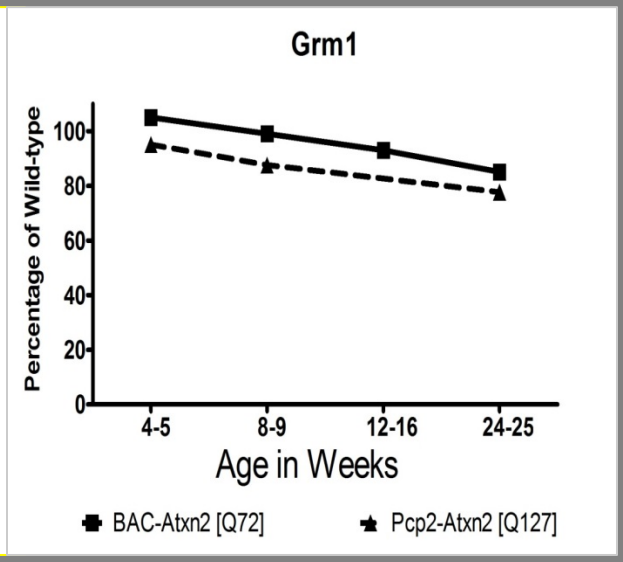


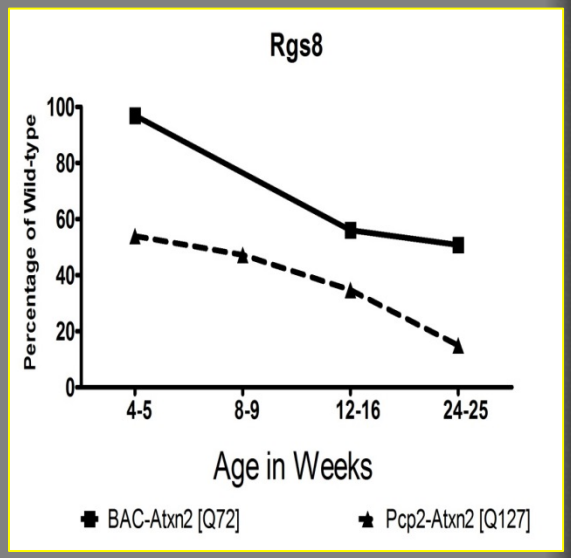
From ICC to qPCR

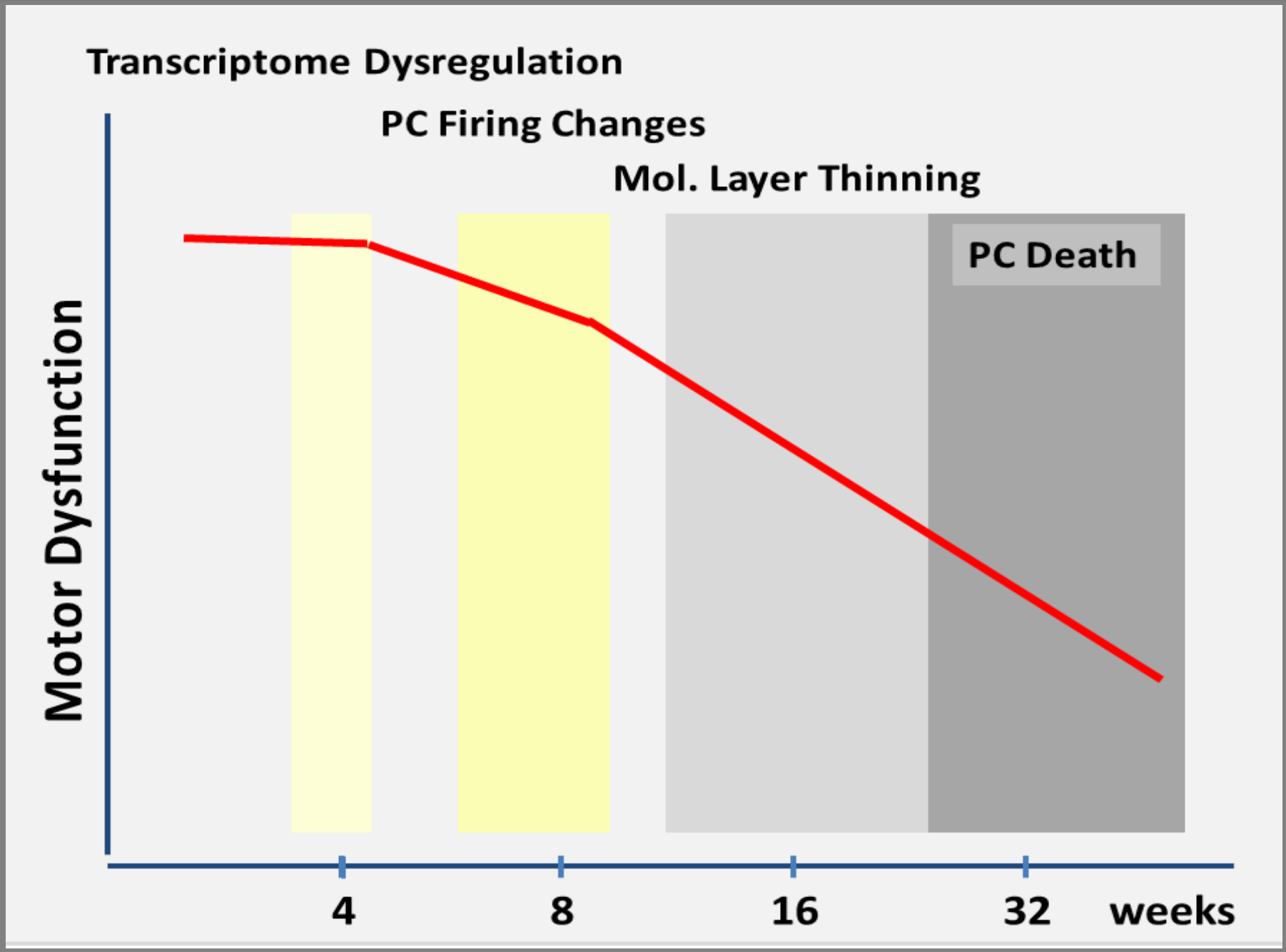










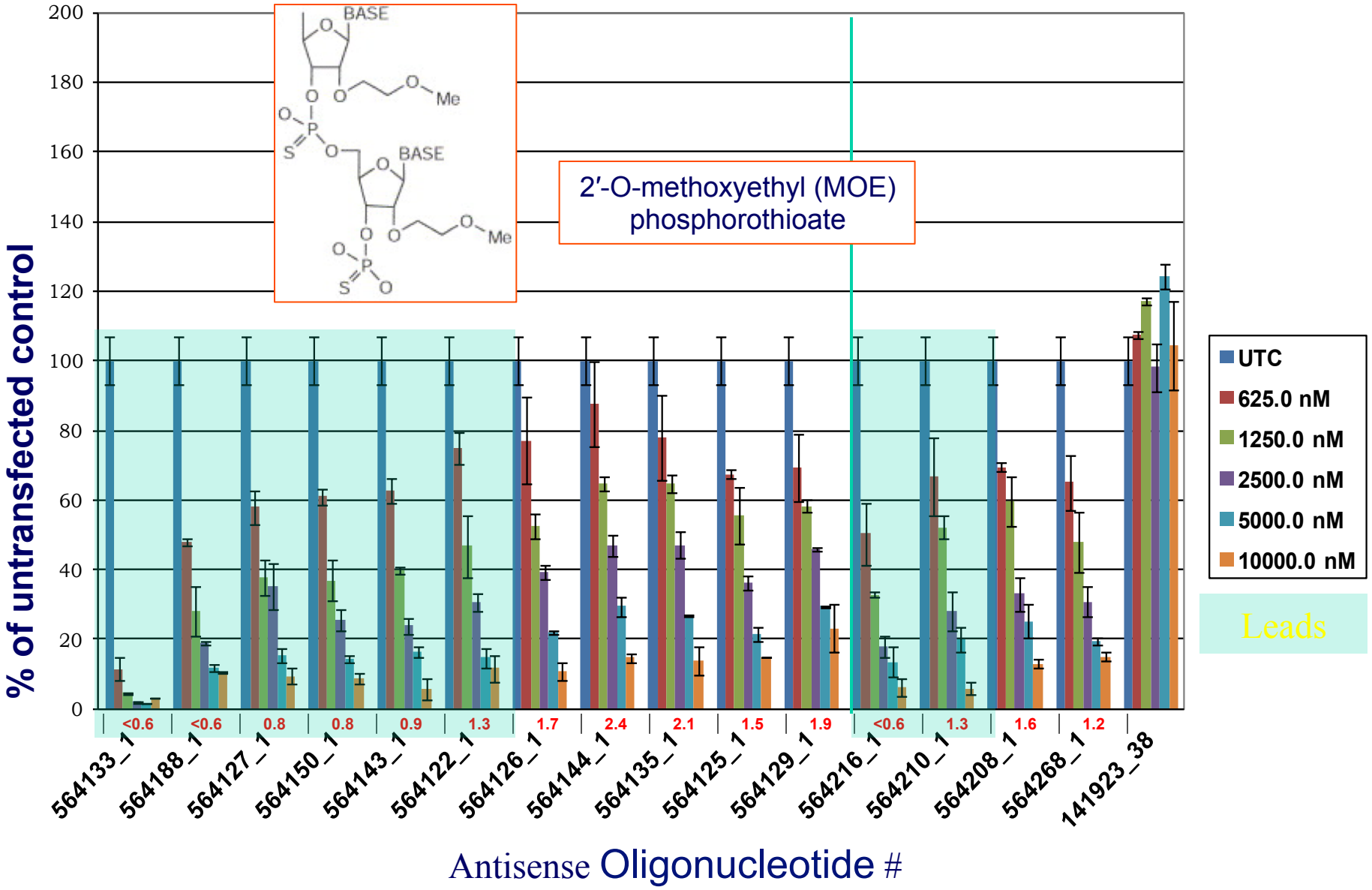

Transcriptome Dysregulation
PC Firing Changes
Mol. Layer Thinning
PC Death
Motor Dysfunction
4
8
16
32
weeks

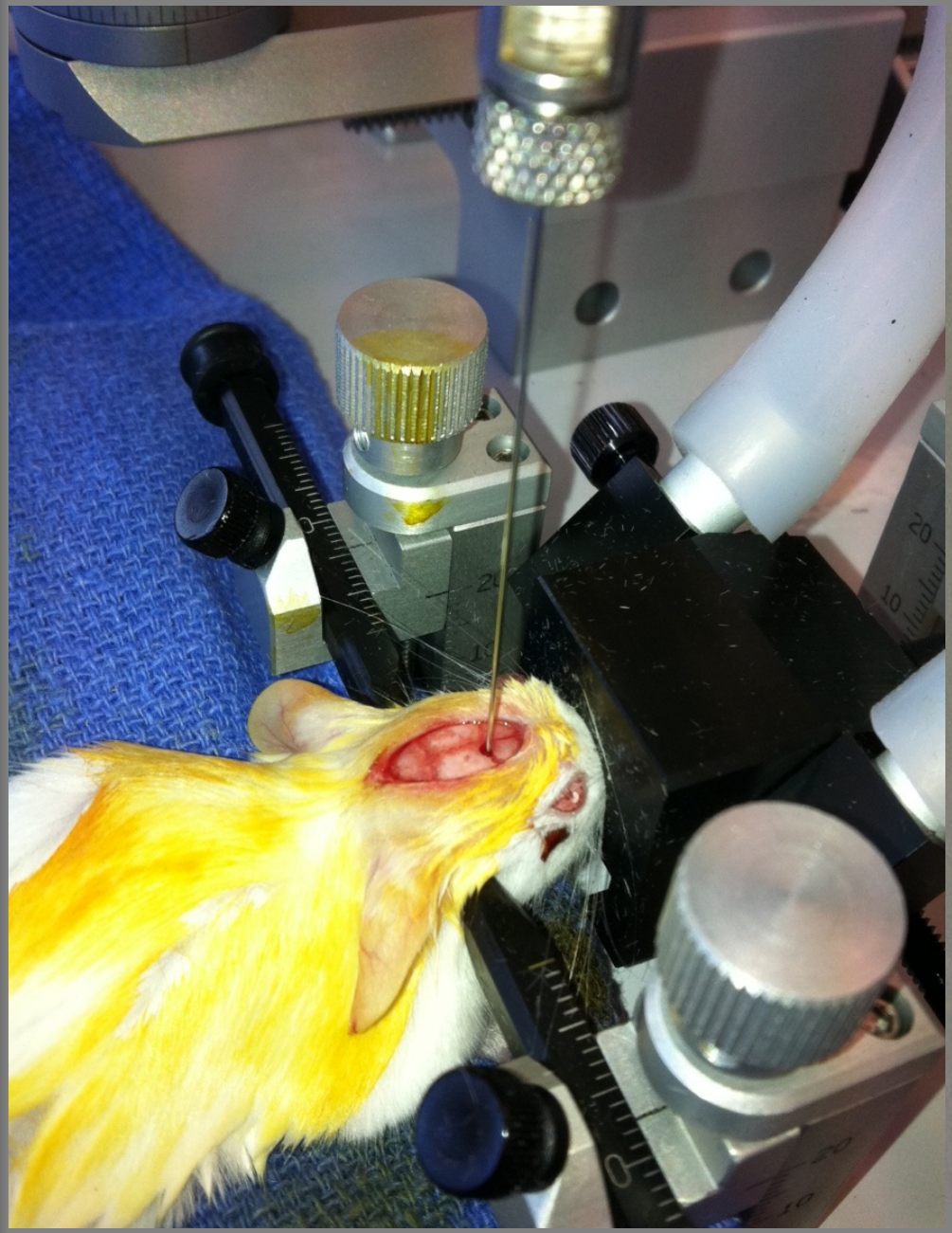
Targeting the Allele or Gene of Interest

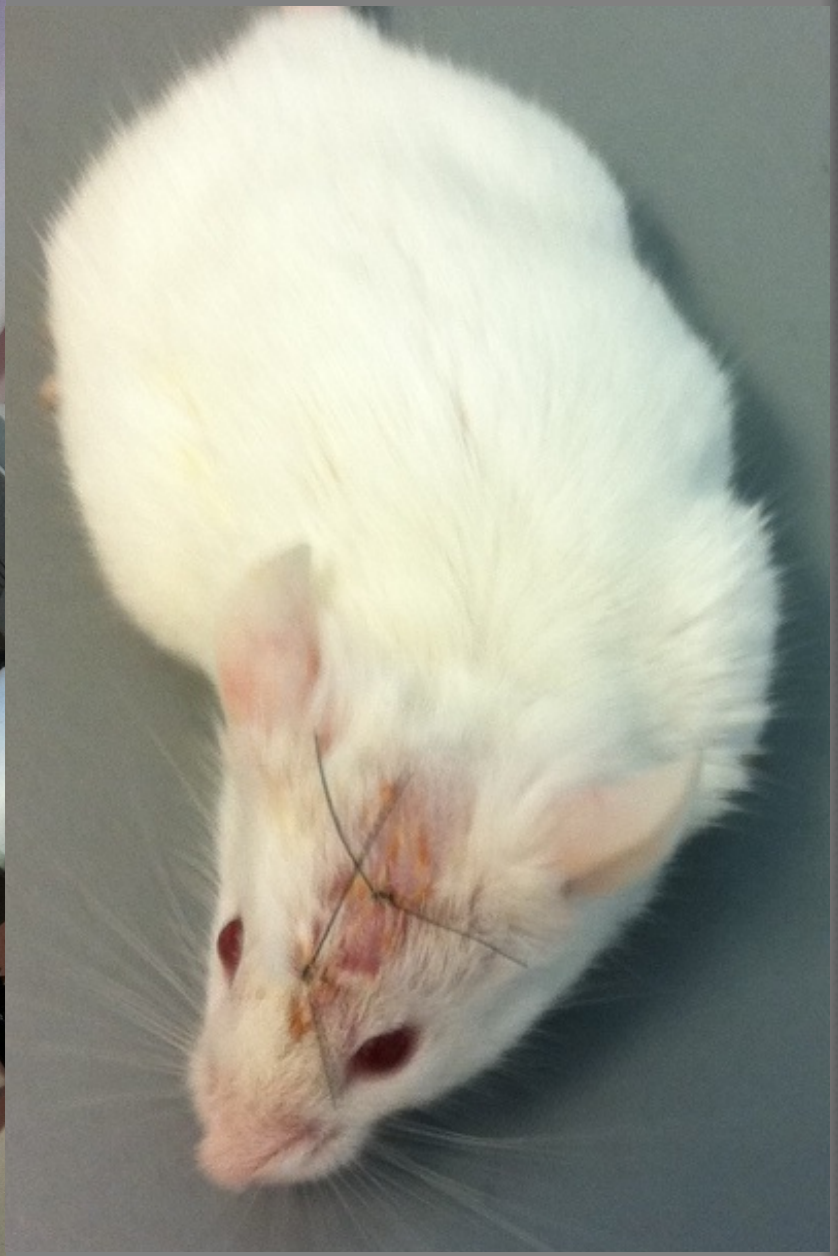
Rationale:

- Human double mutants have more severe disease.
- Fly & Mouse models are dosage sensitive.
- Conditional transgenics
- Knock-out

Dose Response Confirmation for Human ataxin 2, ID: 13737, Cell Line: HepG2, Primer Probe Set: RTS3642, Transfection Reagent: Electroporation

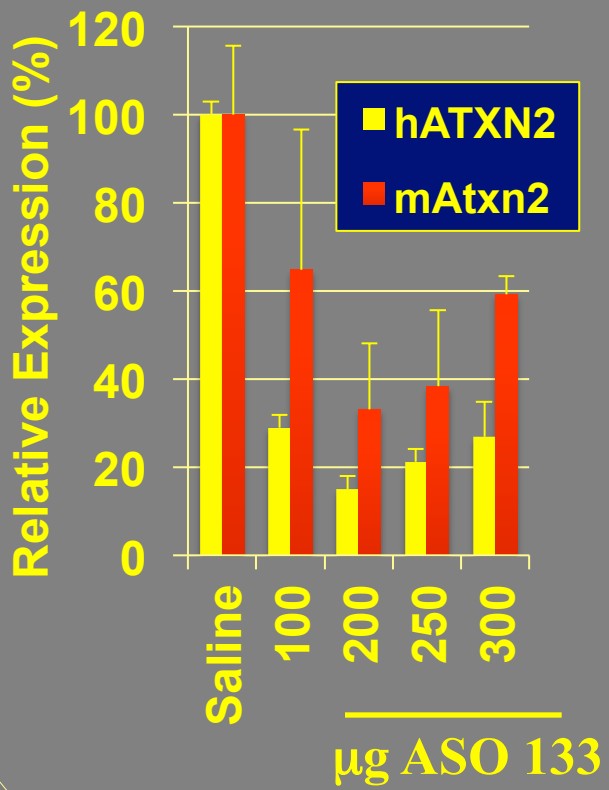




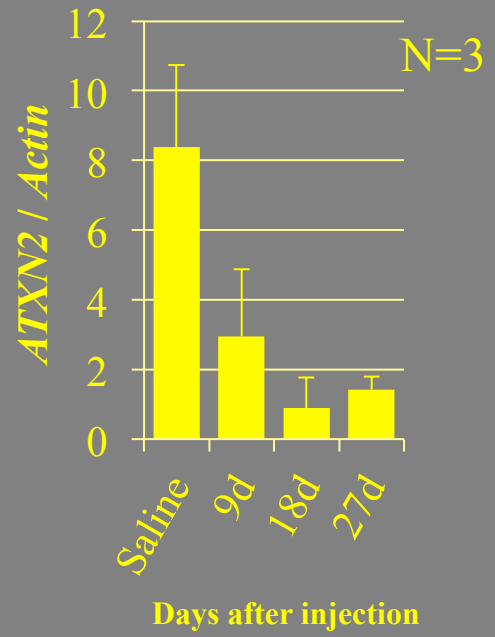


ASO In *ATXN2-Q127* Tg & SCA2-BACQ72 mice

Dosing study
Injected with increasing doses of ASO 133, treated 7 days.

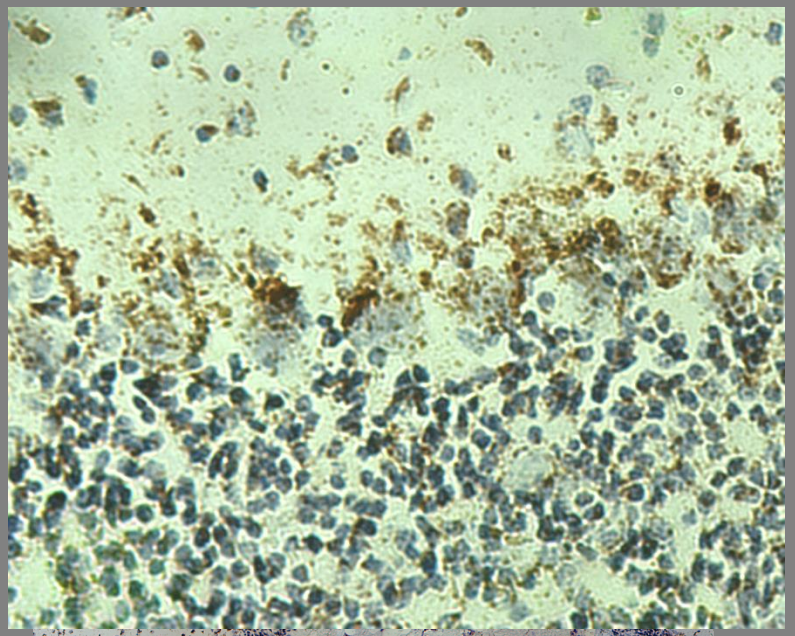


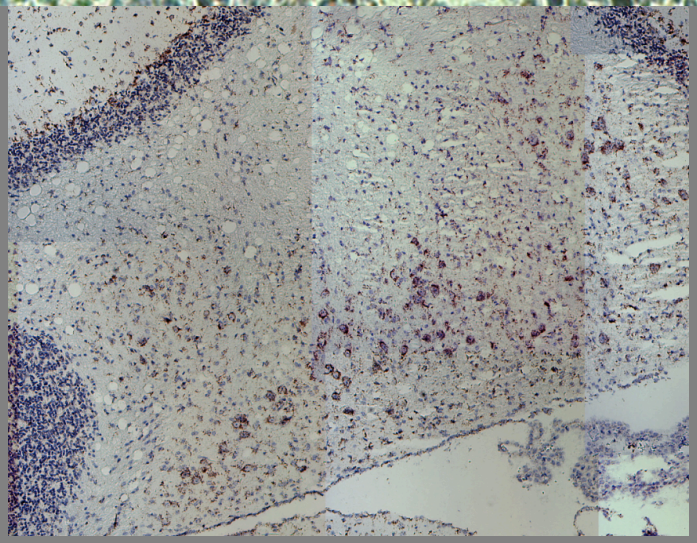
Timecourse study
Injected with 200 µg ASO 133
5.7 µl of 35 µl/ml ASO.



* The saline group consisted of one 9d mouse, one 18d mouse, & one 27d mouse.

ASO-133 localized to deep cerebellar nuclei



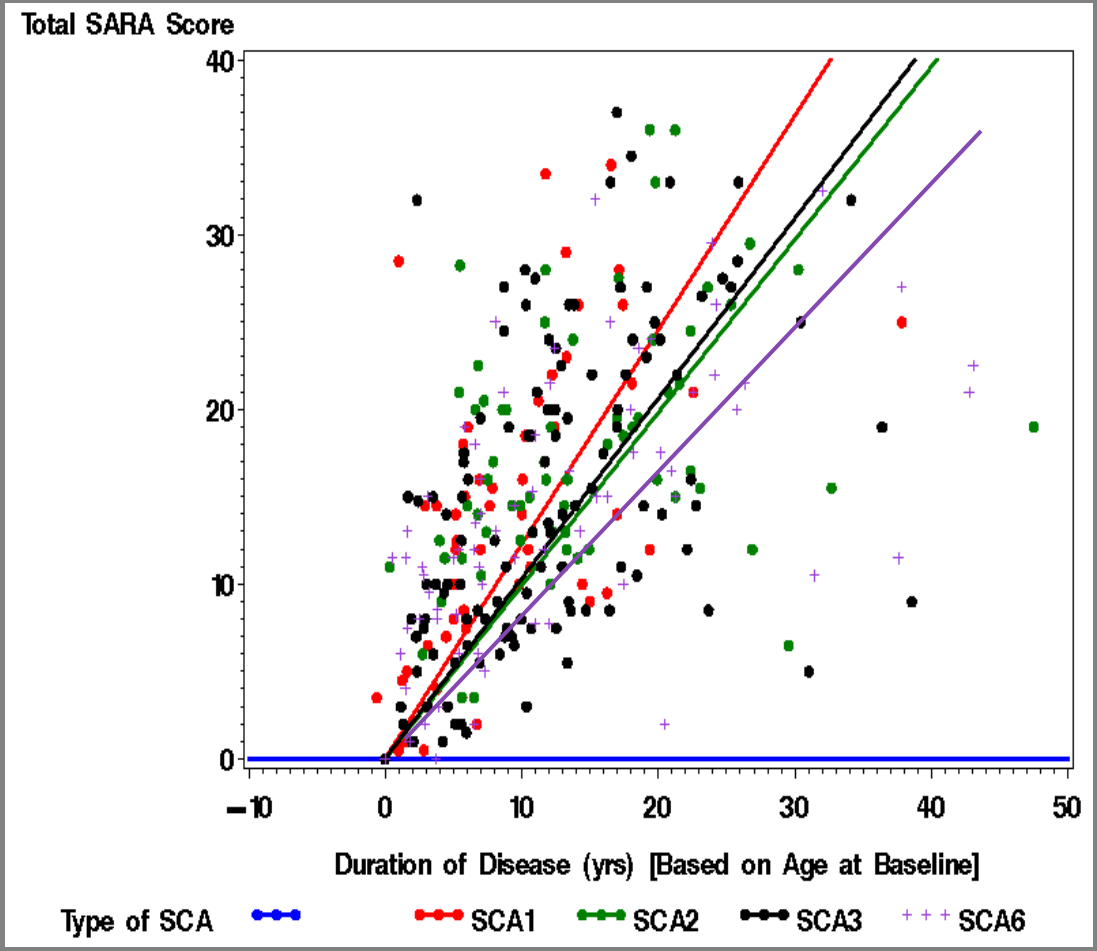


SARA Scale

- Published in 2006
- From 0 (normal) to 40 points

- Gait/Stance
- Sitting
- Speech
- Finger Chase/N-F
- RAM/H-S slide

The NIH SCA-CRC confirms progression rates for the SCAs Shown by EURO-SCA



Translation is bidirectional

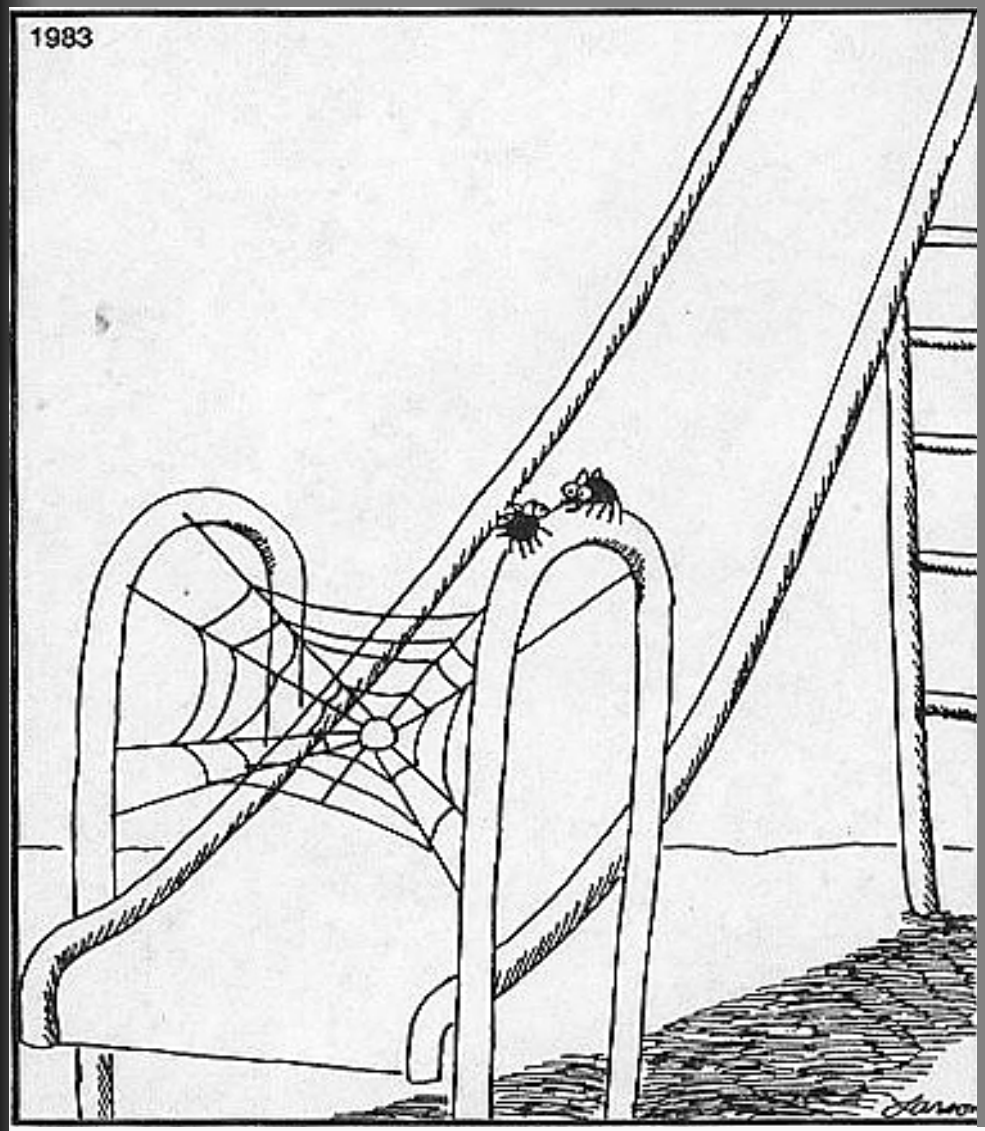
- Bedside to Bench important.
- Culture change from the “one grad-student-one PI” model to multi-center trials.
- Rodent trials can distinguish between symptomatic changes and disease modification.

For rare diseases
Mouse trials are a must !

- Formal Randomization
- Multi-investigator & Multi-center
- Multiple models
- Multiple mouse backgrounds & both sexes
- Pre-specified outcome criteria

- Funding Agencies & Journals

Collaborators



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

- Animal Models
  - Pattie Figueroa
  - Duong Huynh, PhD.
  - Stephen Hansen, Ph.D.
  - Warunee Dansithrong, Ph.D.
  - Marion Schiffmann
  - Don Atkinson
  - Tim-Rasmus Kiehl, MD
- Dantrolene Study
  - Ilya Bezprozvanny, PhD
  - Jing Liu, PhD
  - Emily Herndon, PhD
  - Duong Huynh, PhD.
- Physiology (UCLA)
  - Tom Otis, PhD
  - Meera Pradep PhD
- Small Molecule & ASO Screen
  - Daniel Scoles, Ph.D.
  - Lance Pflieger

Neurology F1's @ the Smoker's "Spleen Girls"