Neurodegenerative Diseases:

A genetic Approach

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Presented for Dr. Pulst by Daniel Scoles

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Highlights on Spinocerebellar Ataxia Type 2 in the Pulst Laboratory

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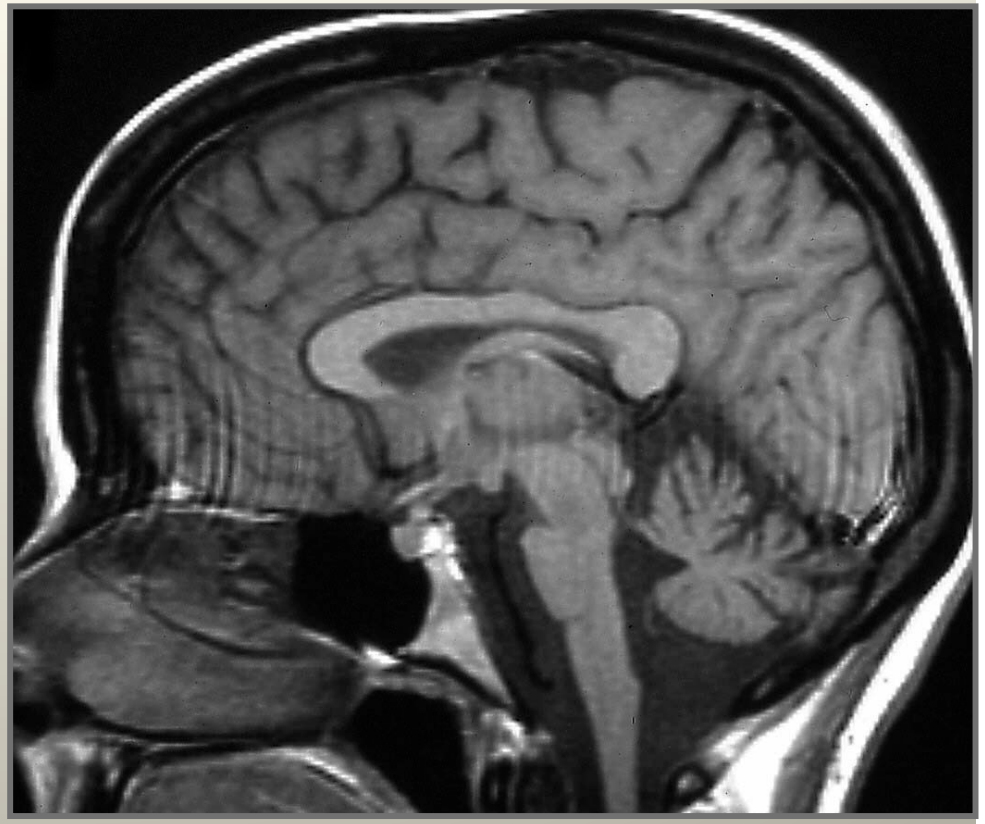
What are SCAs?

- Spinocerebellar Ataxias
- Neurodegenerative Disorders
- Affect primarily cerebellum
- Often Purkinje cells
- Autosomal dominant

The SCA2 Gene

Normal: 22Q
Mutant: ≥32Q

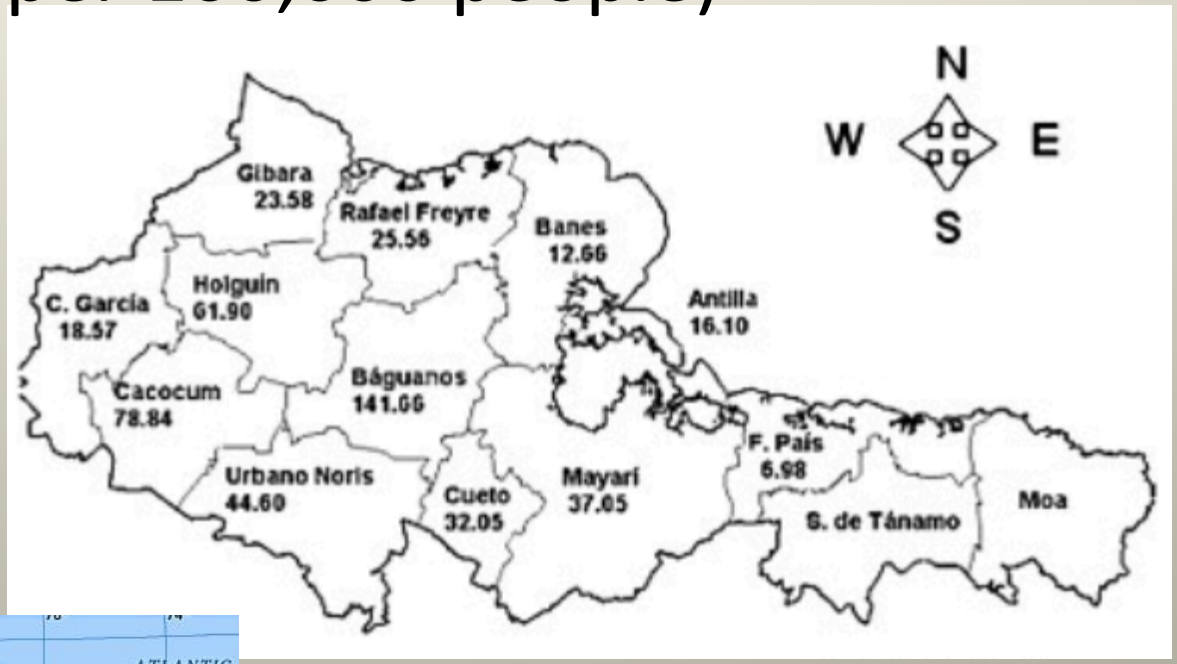


- CAG Repeat codes for glutamine (Q).

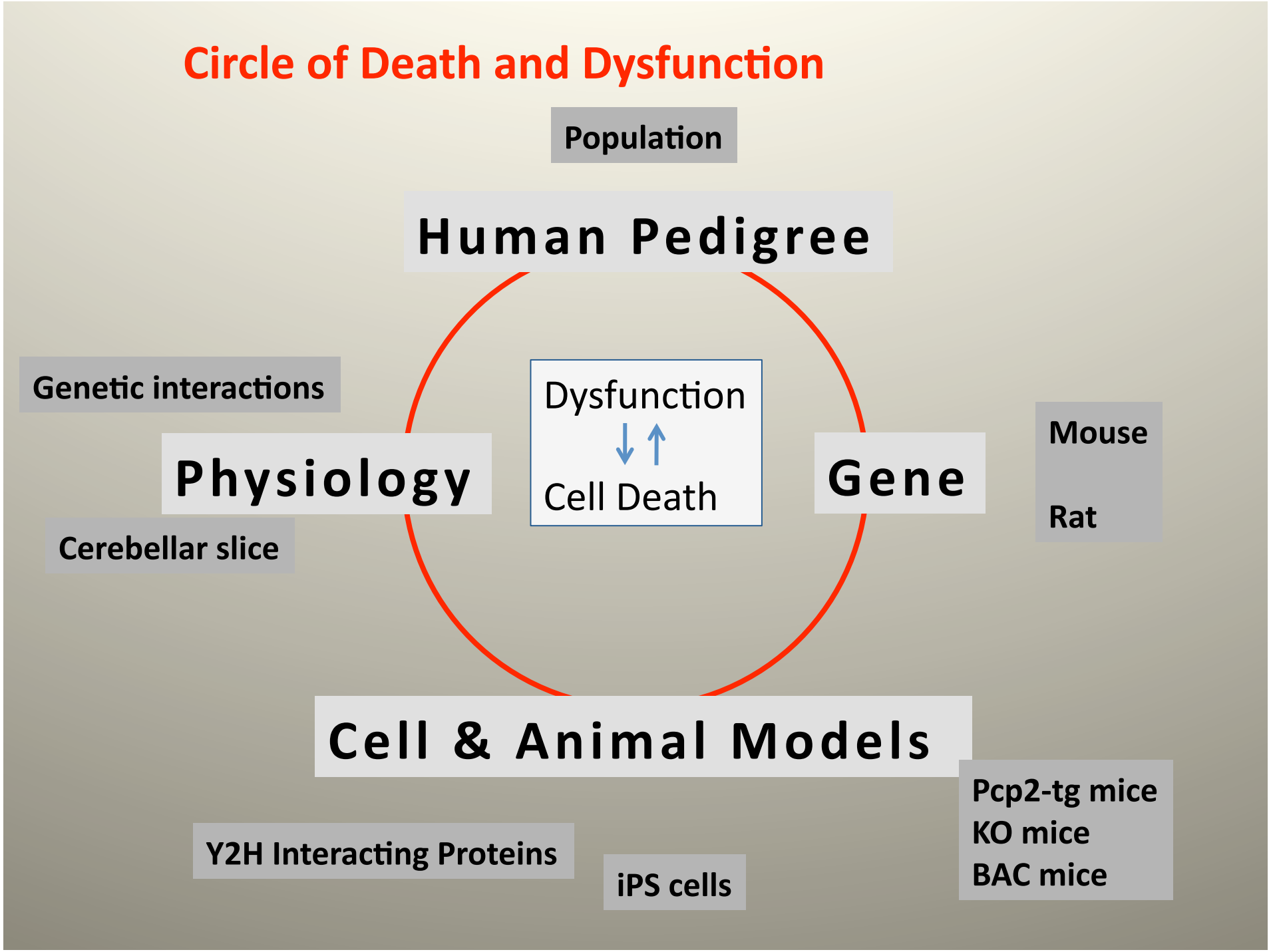
Ataxia Plus
Slow saccadic eye movements
More or less pure
DOPA-responsive PD
ALS-like

Pulst et al, Nature Genet 1996

Prevalence rate in Eastern Cuba (per 100,000 people)



Velazquez Perez et al. Neurosci. Lett. 454:157-60; 2009.


Circle of Death and Dysfunction
Population
Human Pedigree
Genetic interactions
Dysfunction
Cell Death
Physiology
Gene
Mouse
Rat
Cerebellar slice
Cell & Animal Models
Pcp2-tg mice
KO mice
BAC mice
Y2H Interacting Proteins
iPS cells

SCA2

- Caused by mutation in the *ATXN2* gene, encoding the protein "ataxin-2"
- The nature of the mutation is encoded CAG repeat expansion in exon 1 → polyQ
- The normal gene has CAG repeat of structure interrupted by CAAs:

  $(CAG)_8CAA(CAG)_4CAA(CAG)_8$

- The mutant *ATXN2* has pure CAGs of lengths from 33 to >200 repeats

  $(CAG)_n$ Stablity is lost

- The normal ataxin-2 interacts with RNA binding proteins:

  A2BP1/FOX1, PABP1, the ALS protein TDP-43

ATXN2: 152 kDa on gels
144 kDa predicted, 1312 aa (published)
126.8 kDa predicted, 1152 aa (based on my finding that translation starts further ds)
Chr 12q14.1

SCA2

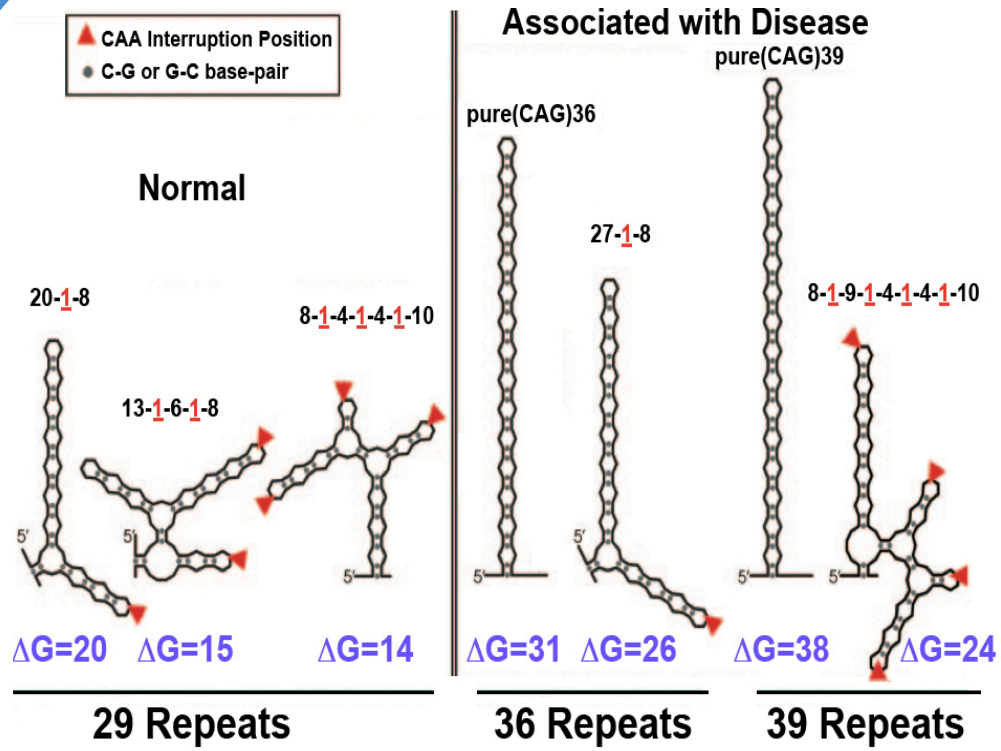
The mutated ataxin-2 is characterized by gain-of-function or gain-of-toxicity

Expanded polyQs gain protein interactions
ITP3R, glycolosis proteins…

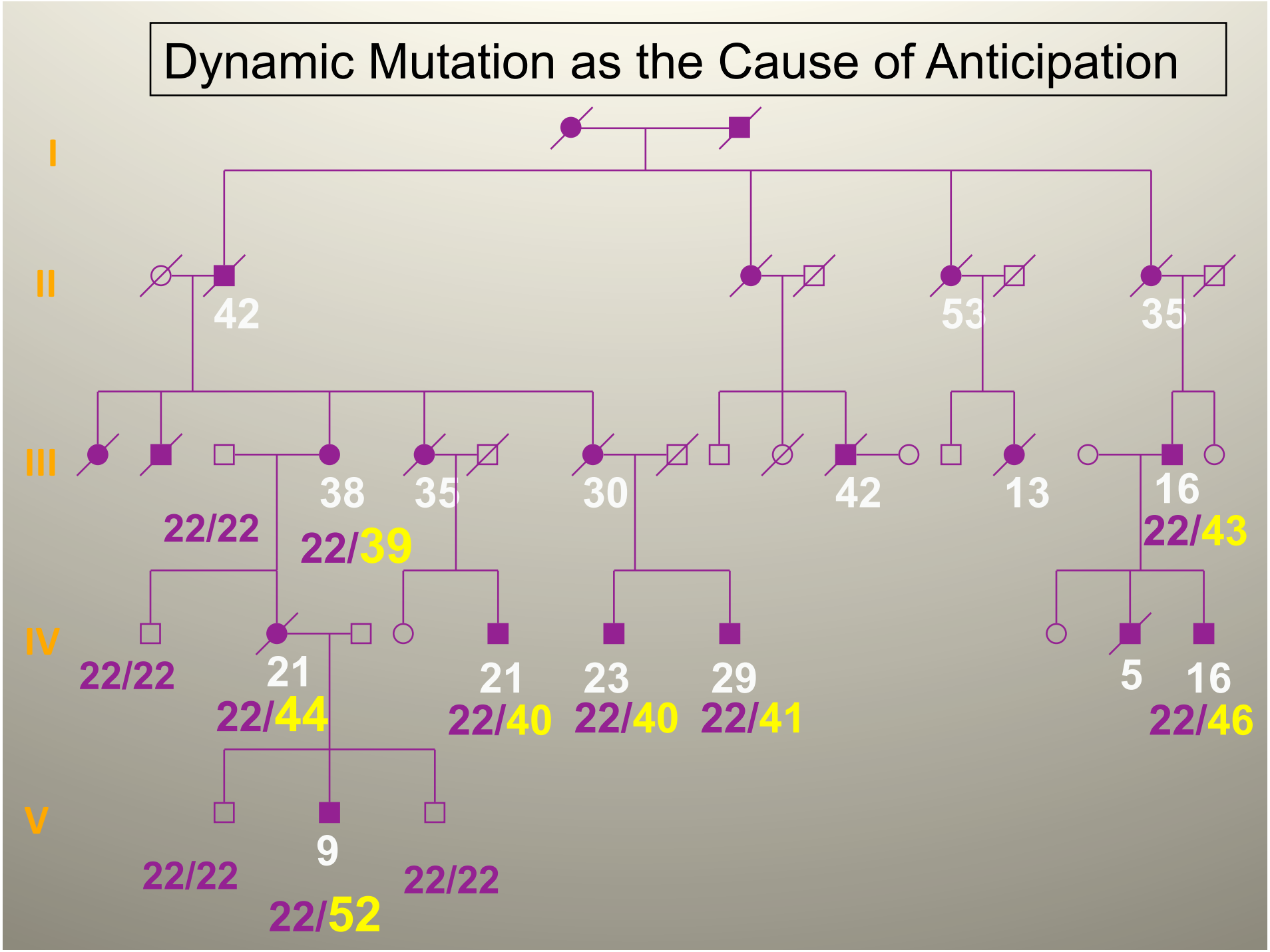
ATXN2 RNAs with expanded CAGs gain RBPs

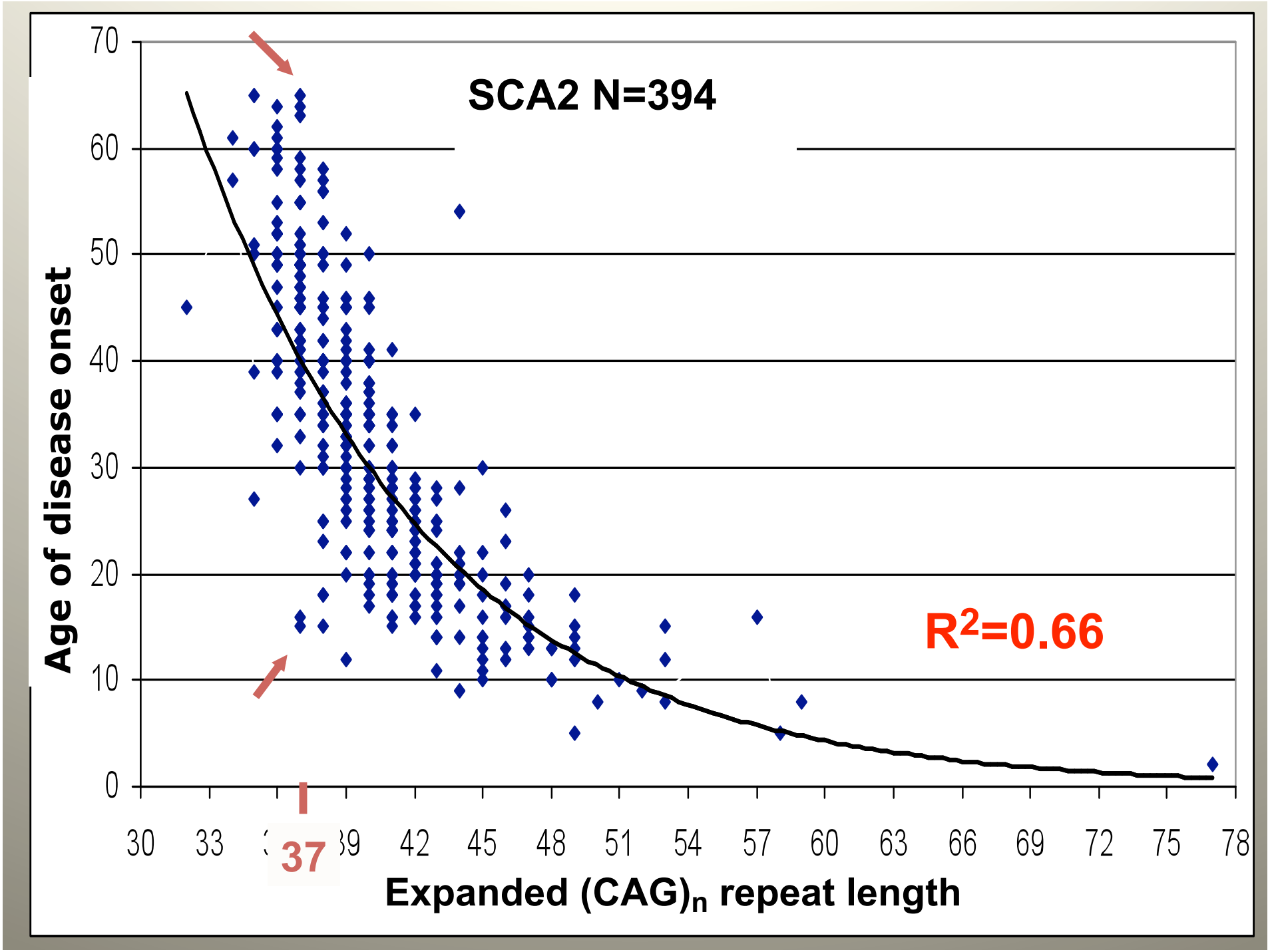
Moderate expansions (near normal)
with CAA interruptions
cause PD and ALS

RNA structure changes RBP binding

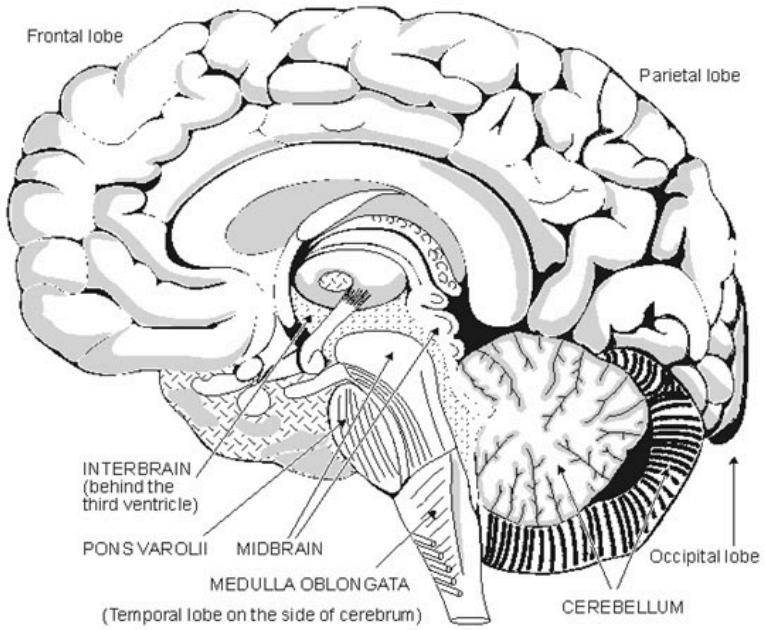


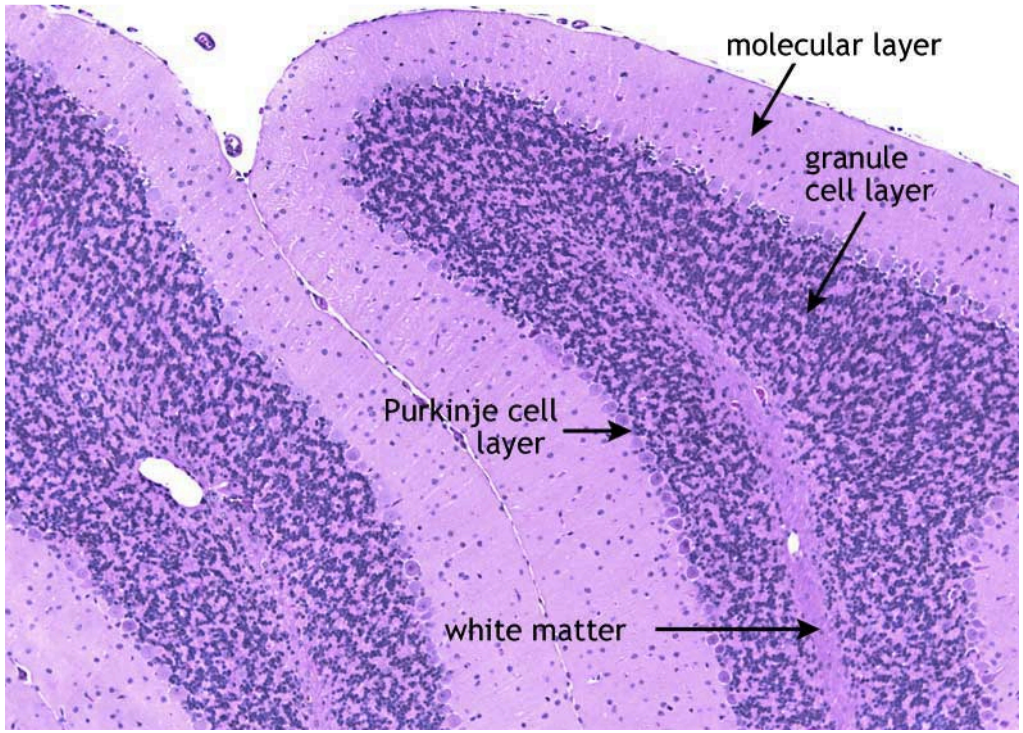
Dynamic Mutation as the Cause of Anticipation




SCA2 N=394
Age of disease onset
70
60
50
40
30
20
10
0
30
33
37
42
45
48
51
54
57
60
63
66
69
72
75
78
Expanded (CAG)n repeat length
R2=0.66

Cerebellum


Frontal lobe
Parietal lobe
INTERBRAIN
(behind the
third ventricle)
PONS VAROLII
MIDBRAIN
MEDULLA OBLONGATA
(Temporal lobe on the side of cerebrum)
Occipital lobe
CEREBELLUM


molecular layer
granule
cell layer
Purkinje cell
layer
white matter

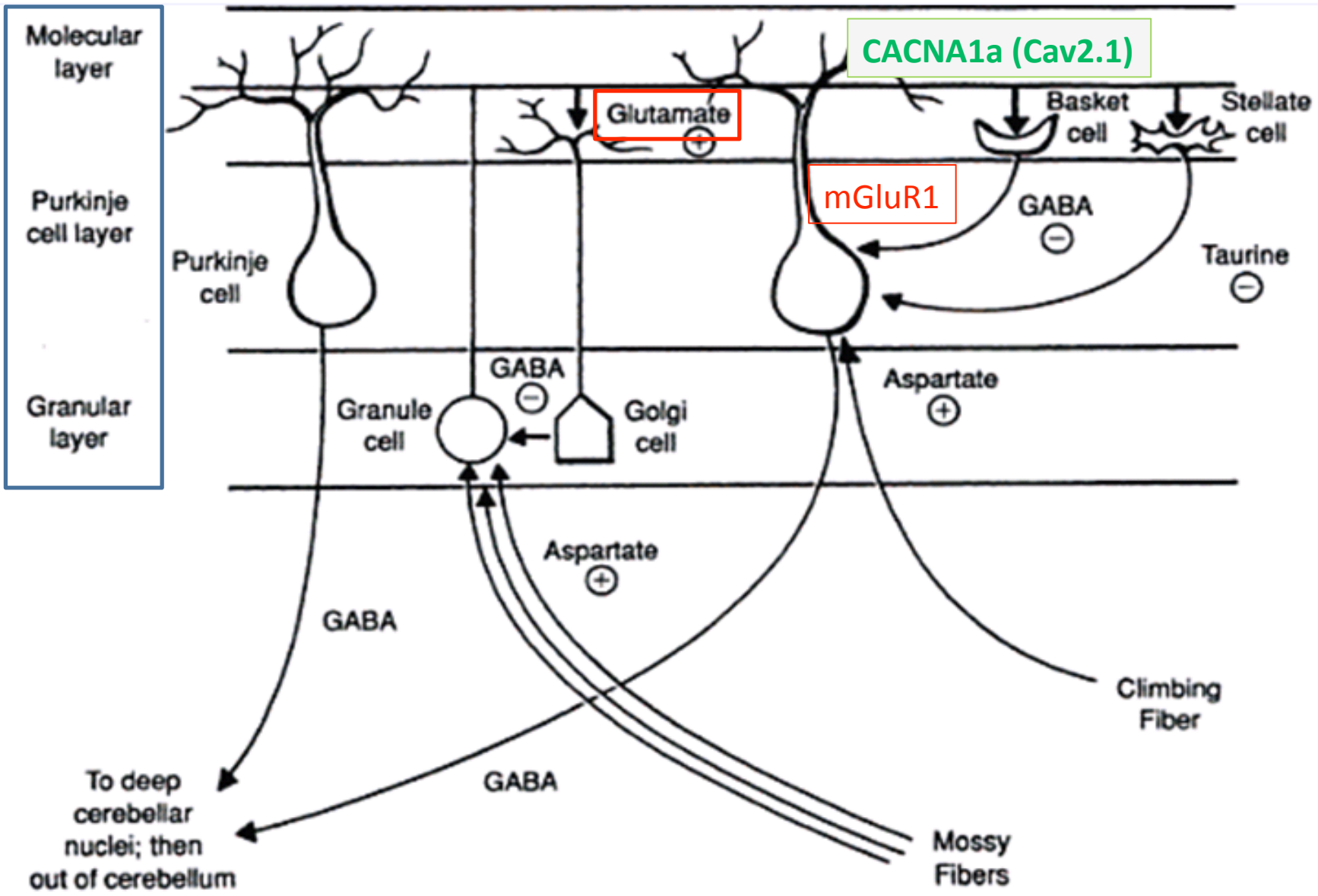
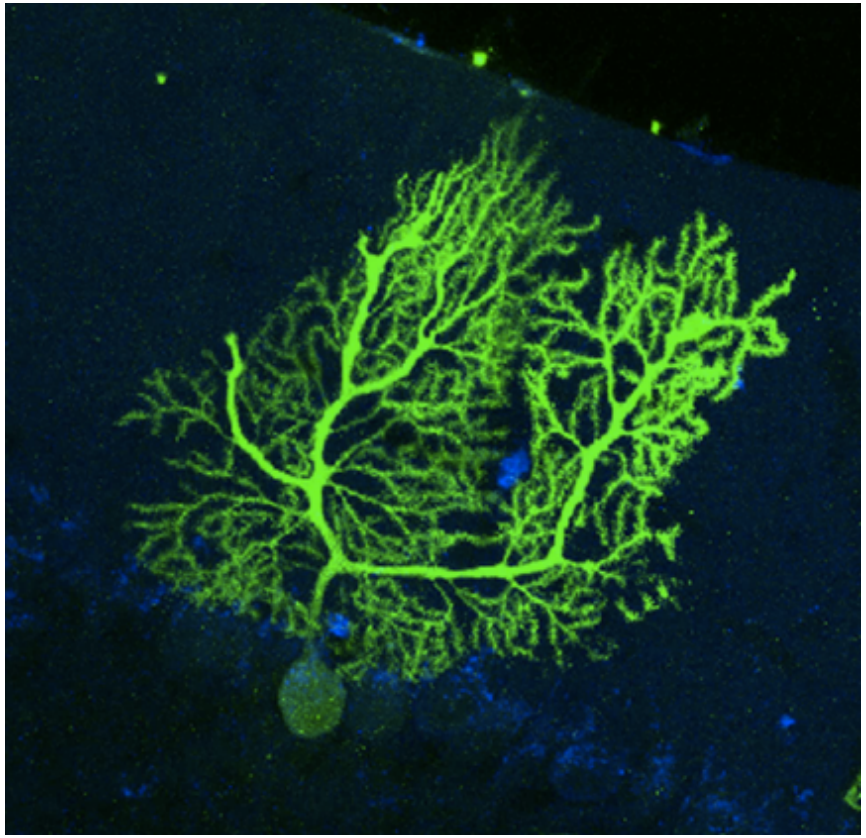
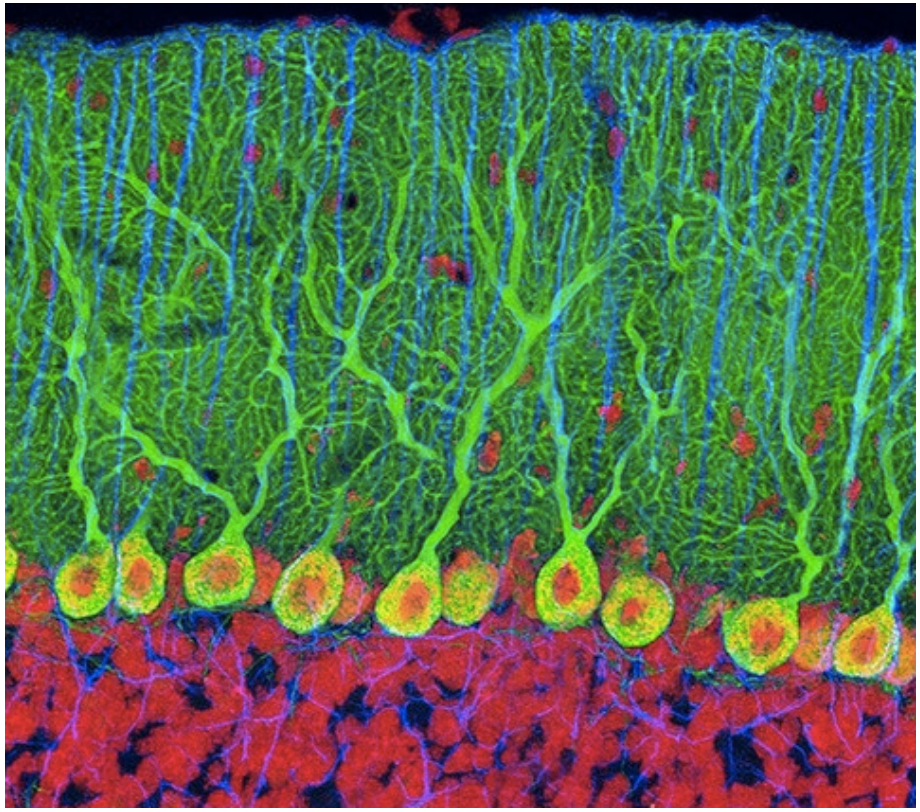


FIGURE 2-1. Cerebellar cortex: microscopic.

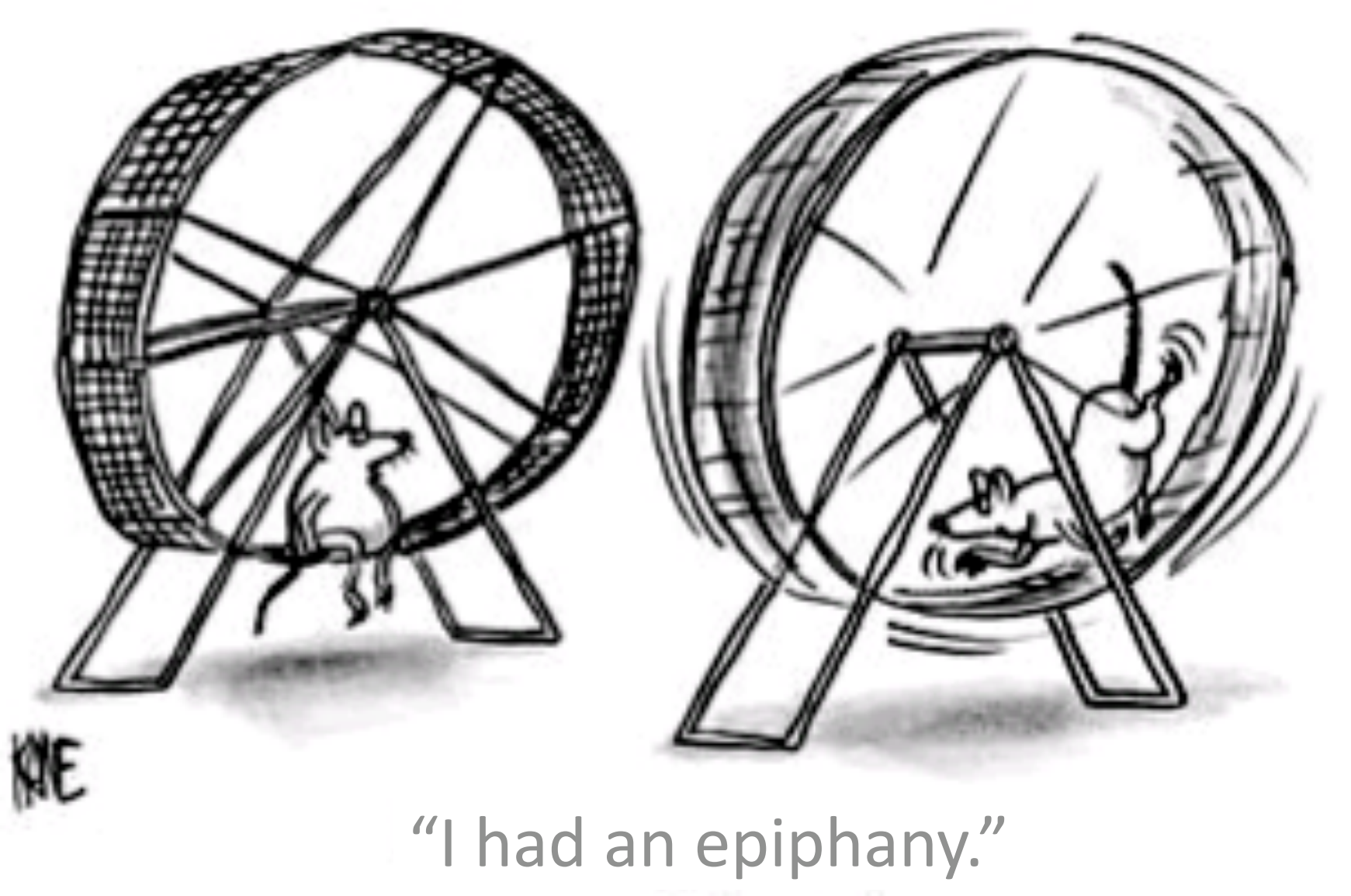
Purkinje Cells

- Largest neurons in the brain
- 200,000 parallel fiber synapses, excitatory, glutamatergic
- 500 climbing fiber synapses, excitatory
- Output is to deep cerebellar nucleus and is inhibitory



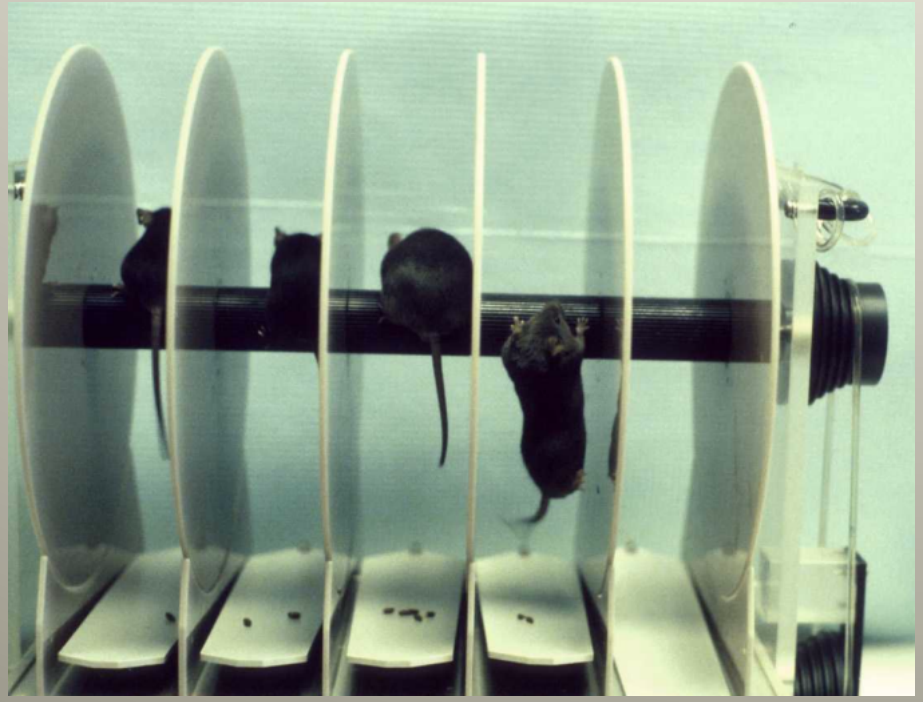


Models

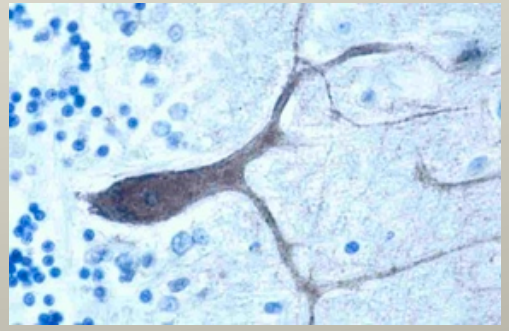


"I had an epiphany."

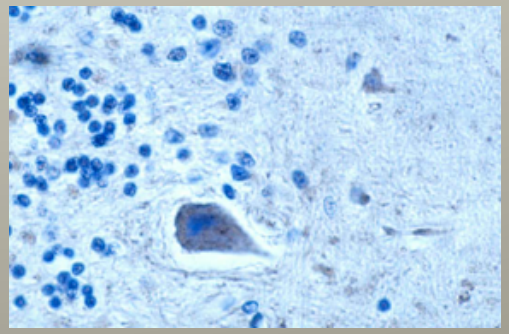
B6:D2 tg-Pcp2-cDNA($ATXN2_{Q58}$)



Calbindin Staining



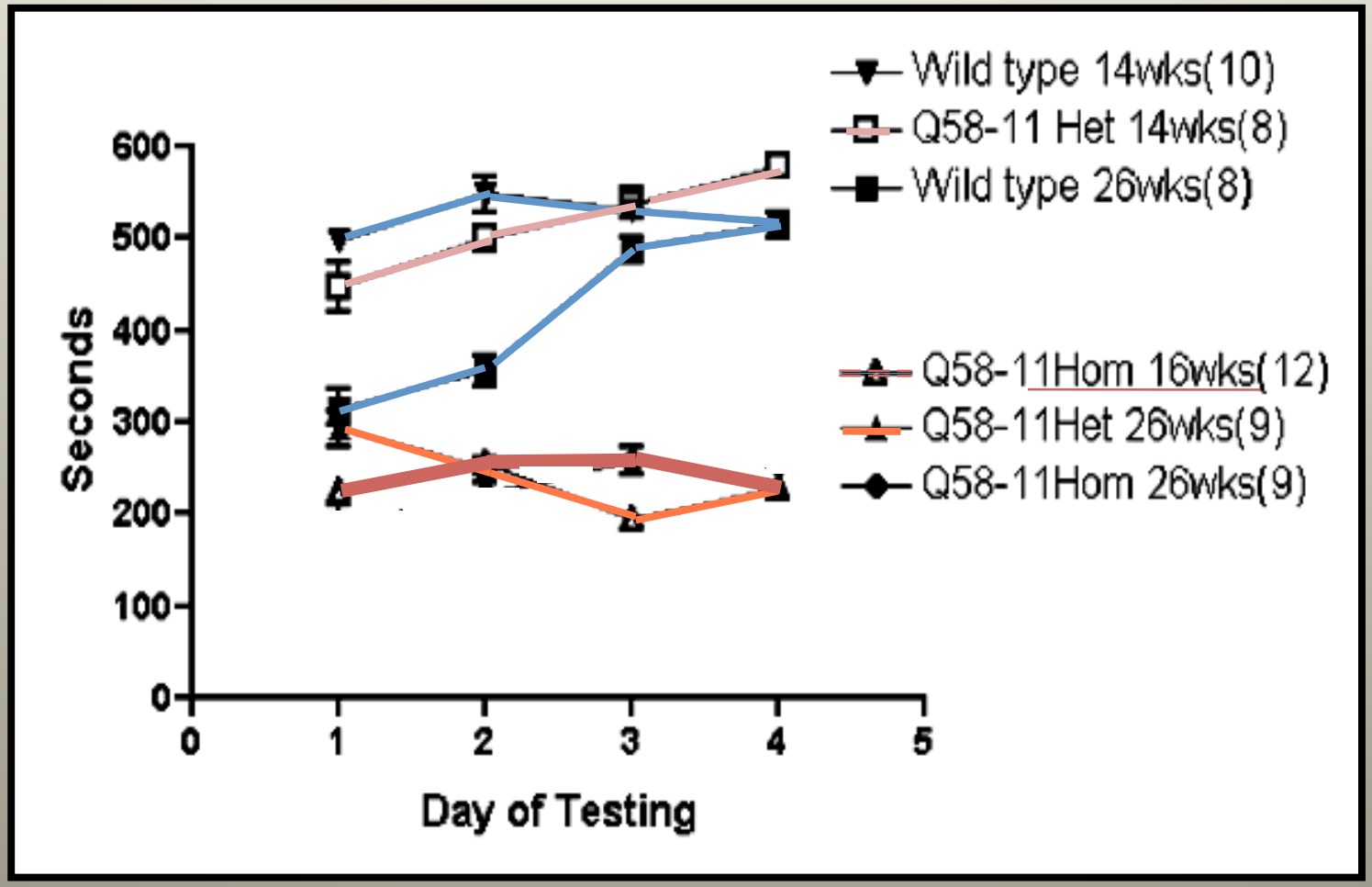
Wt



Tg

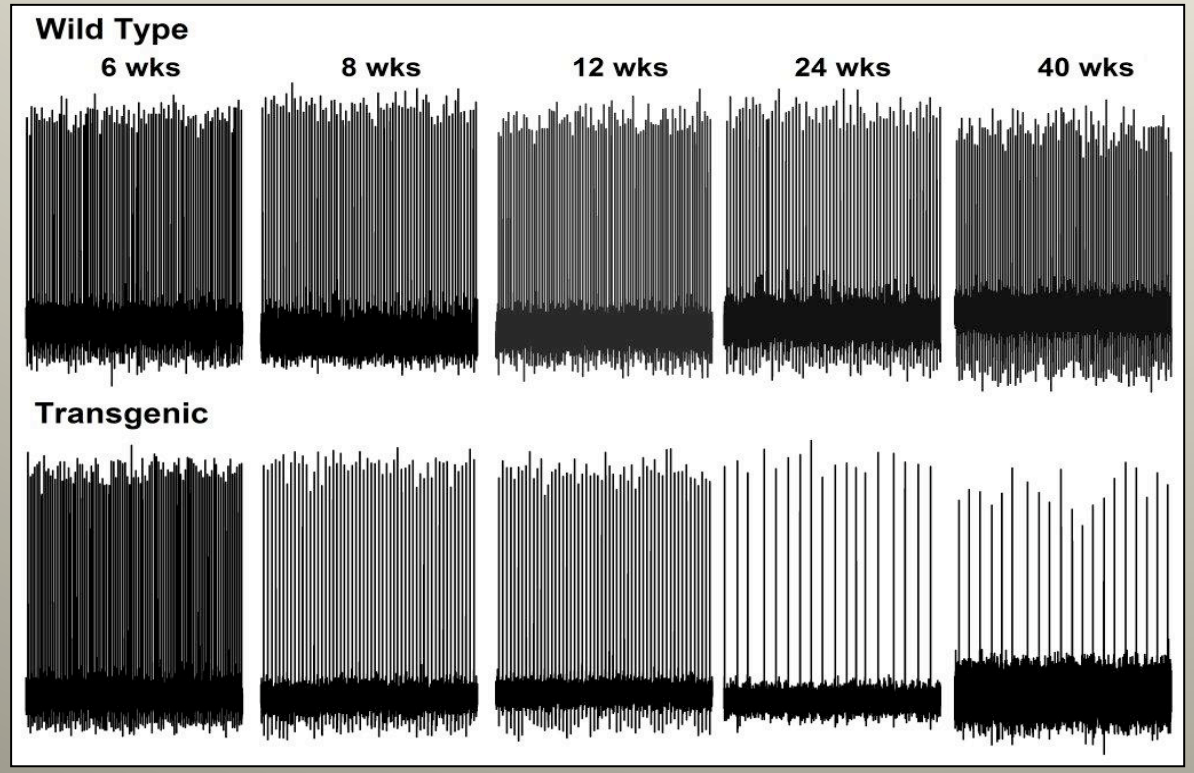
Huynh et al Nat Genet 2000

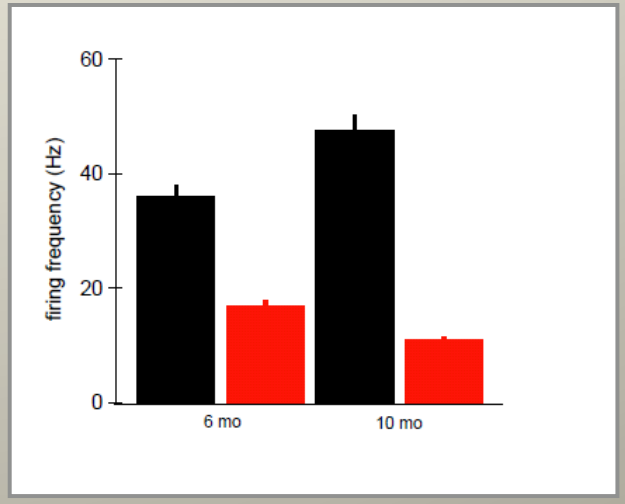
Functional Analysis



Huynh et al Nat Genet 2000

PC Firing is abnormal in SCA2 tg Mice.



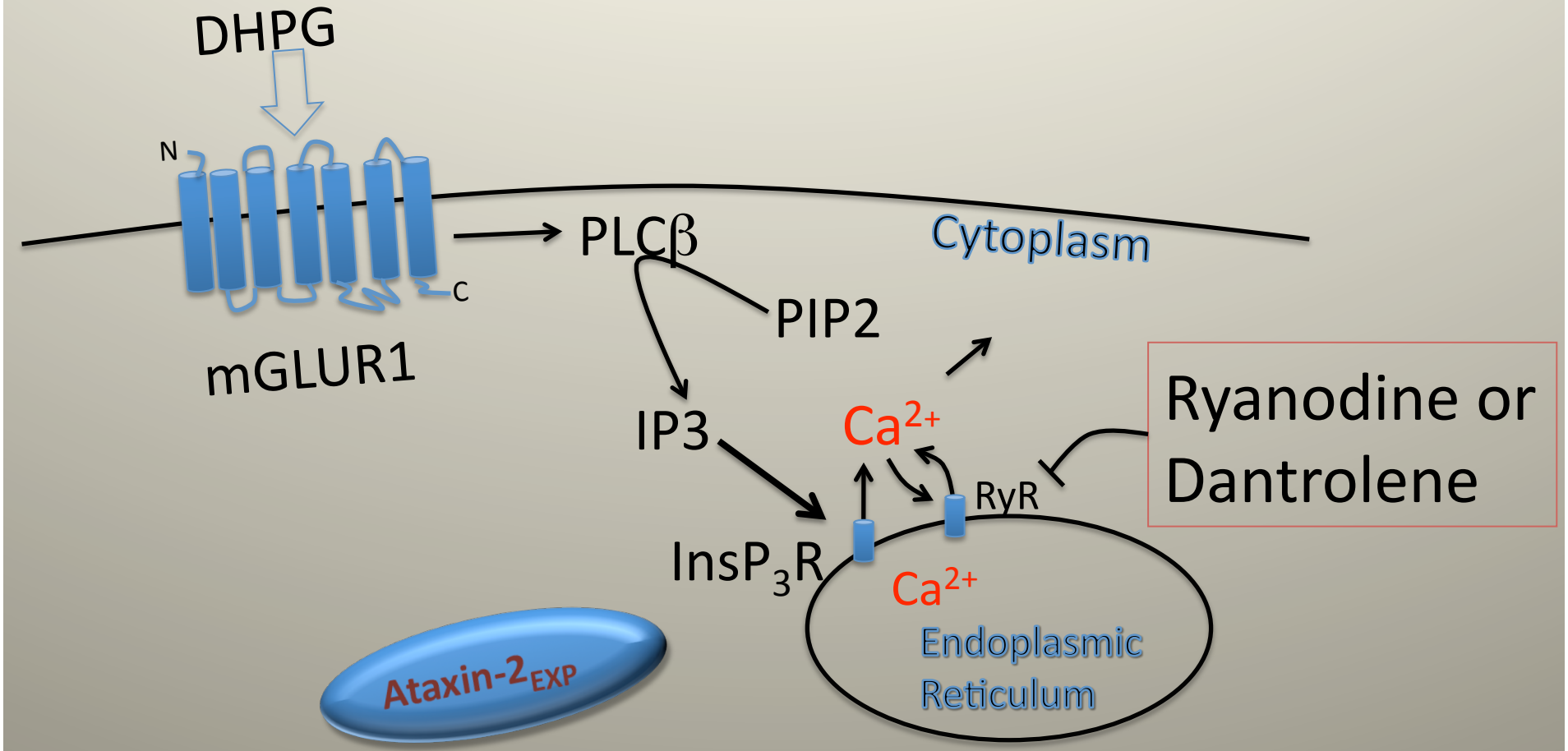


Red=Q128
Black=wt

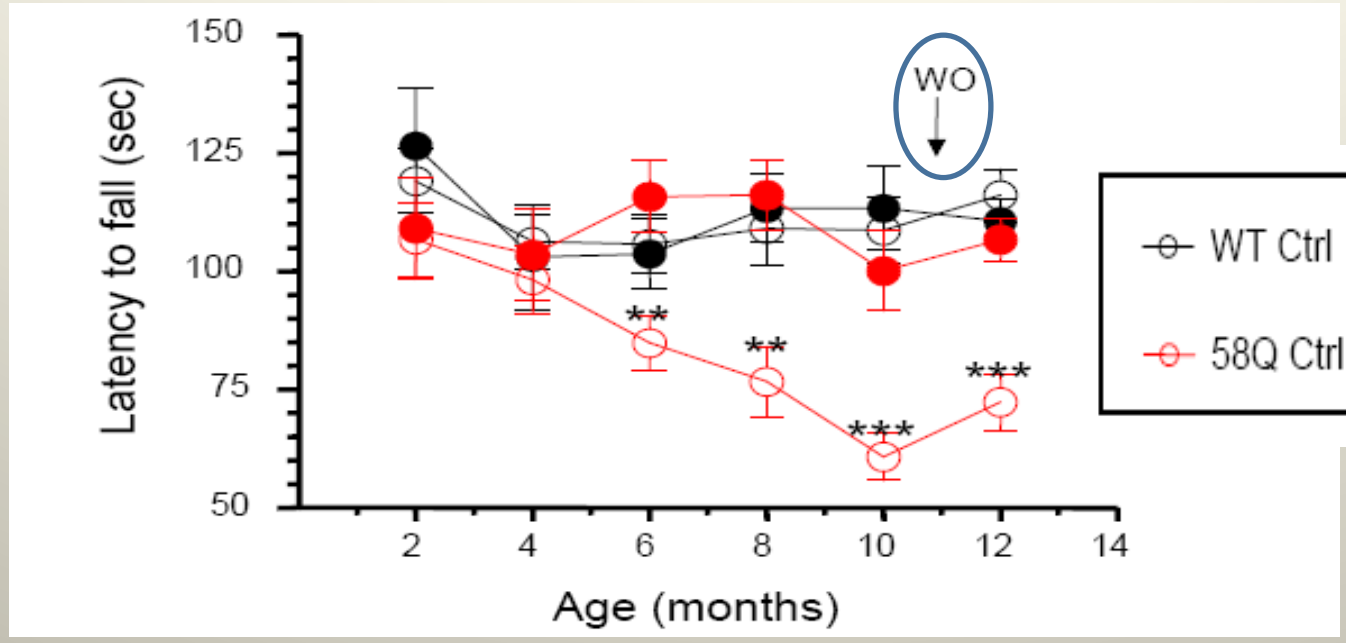
Treatments

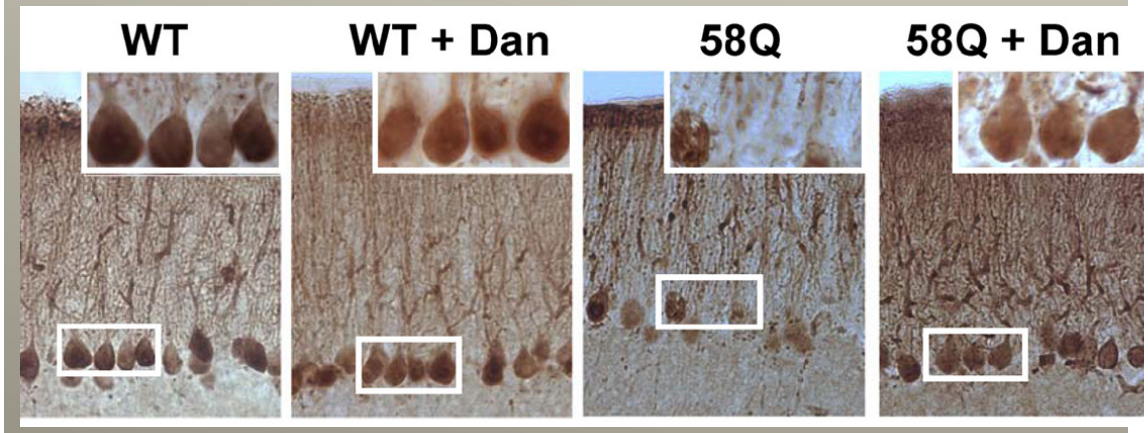
"Discouraging data on the antidepressant"

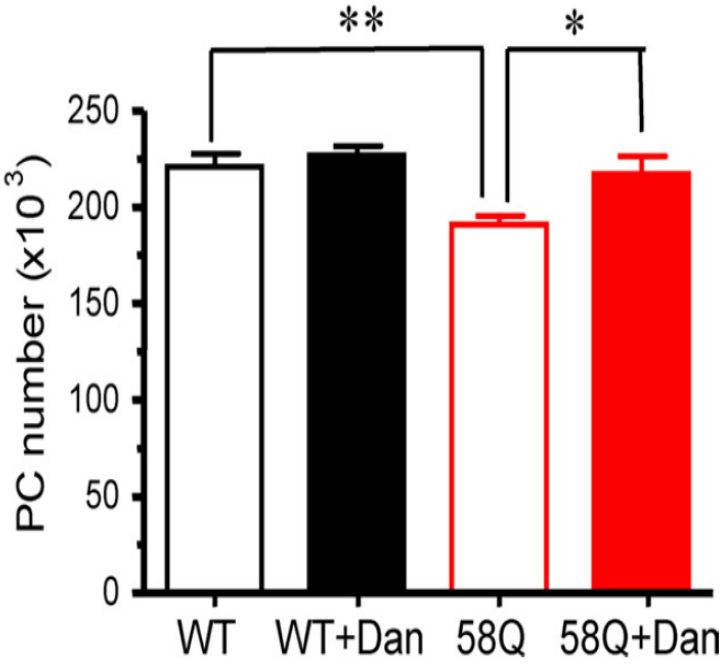
Ataxin-2 action on Ca^{2+} movement in cultured primary Purkinje cells from *ATXN2* transgenic mice



Liu J et al J Neurosci 2009

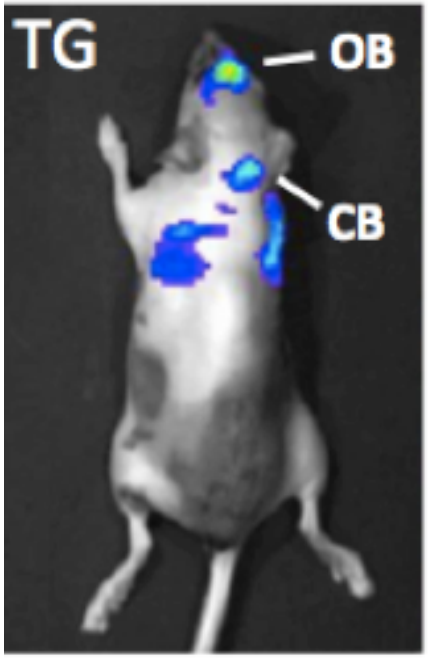

Latency to fall (sec)
150
125
100
75
50
2
4
6
8
10
12
14
Age (months)
WO
**
**
***
***
WT Ctrl
WT Dan
58Q Ctrl
58Q Dan

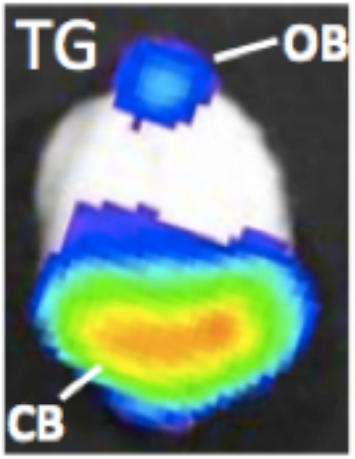

WT
WT + Dan
58Q
58Q + Dan


**
*
PC number (x10 3)
250
200
150
100
50
0
WT
WT+Dan
58Q
58Q+Dan

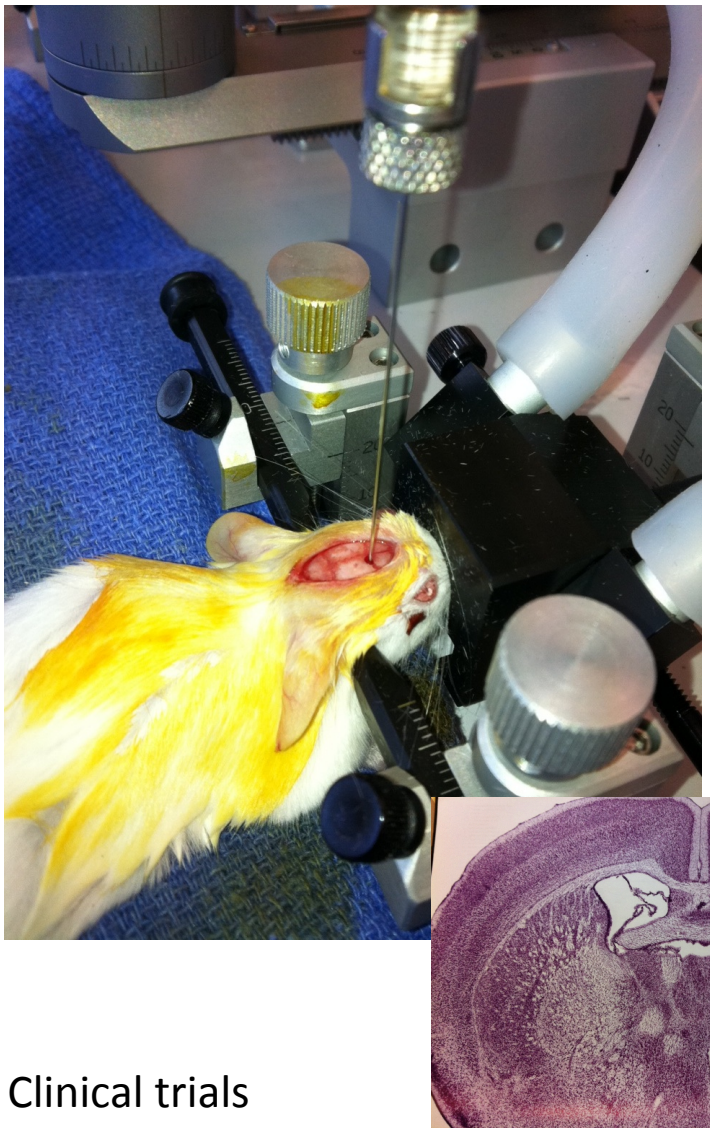
ATXN2-promoter-luciferase

Screening for compounds inhibiting luciferase
in collaboration with the NIH Chemical Genomics Laboratory

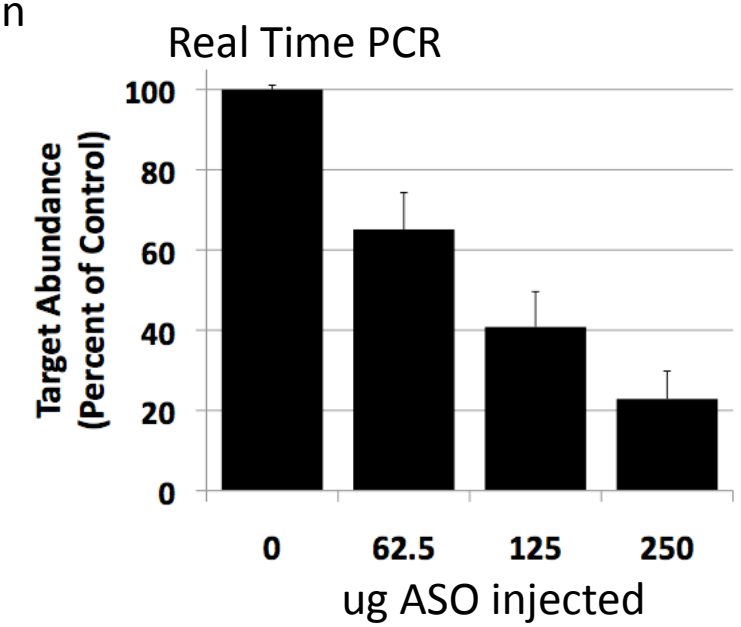


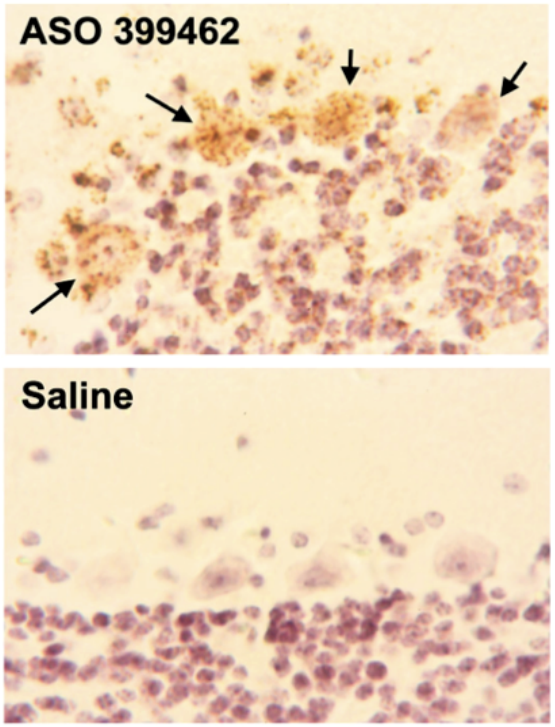


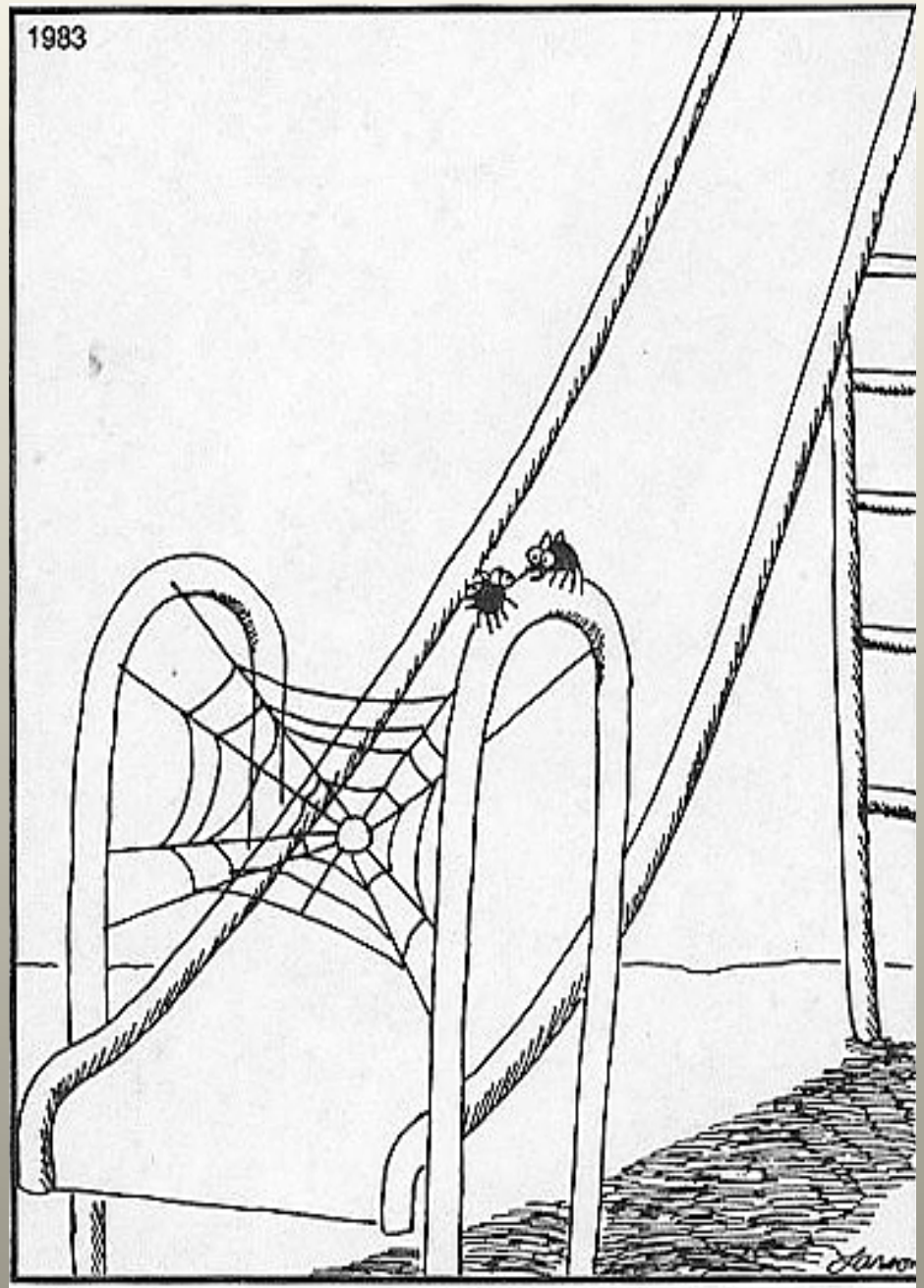
Antisense oligonucleotides inhitibing *ATXN2* expression
Collaboration with ISIS Pharmaceuticals



Clinical trials







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