Rare mendelian diseases: pathways to therapy development.

Stefan-M. Pulst, MD, Dr med
Department of Neurology

Disclosures

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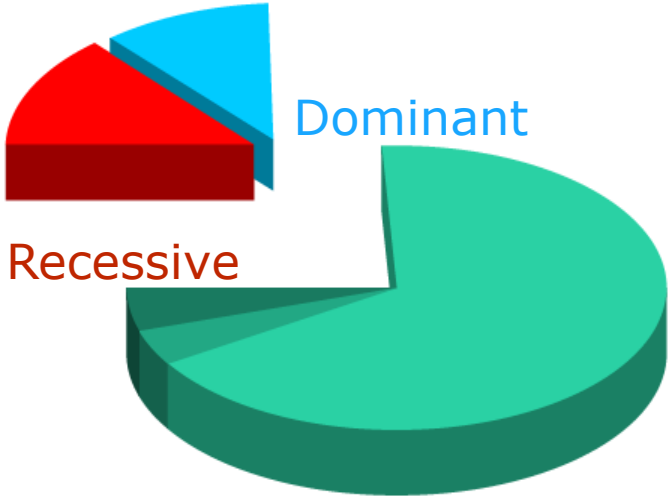
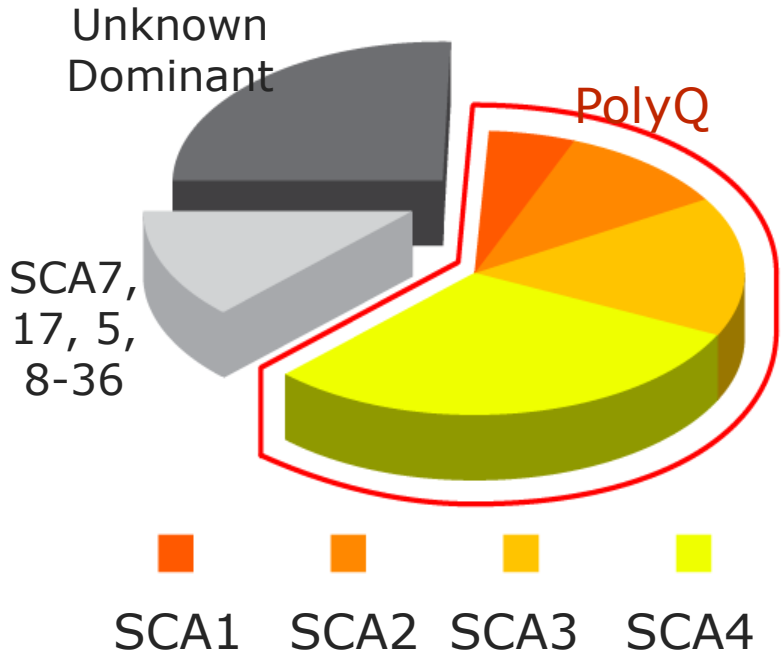
  National Ataxia Foundation

Why study rare genetic diseases?

- Identification of the primary disease-causing step
- Phenotypic definition
- Pathway identification
- Animal (rodent) models
- Mutation or Gene-directed therapies in a homogeneous population

Degenerative Ataxias are common.

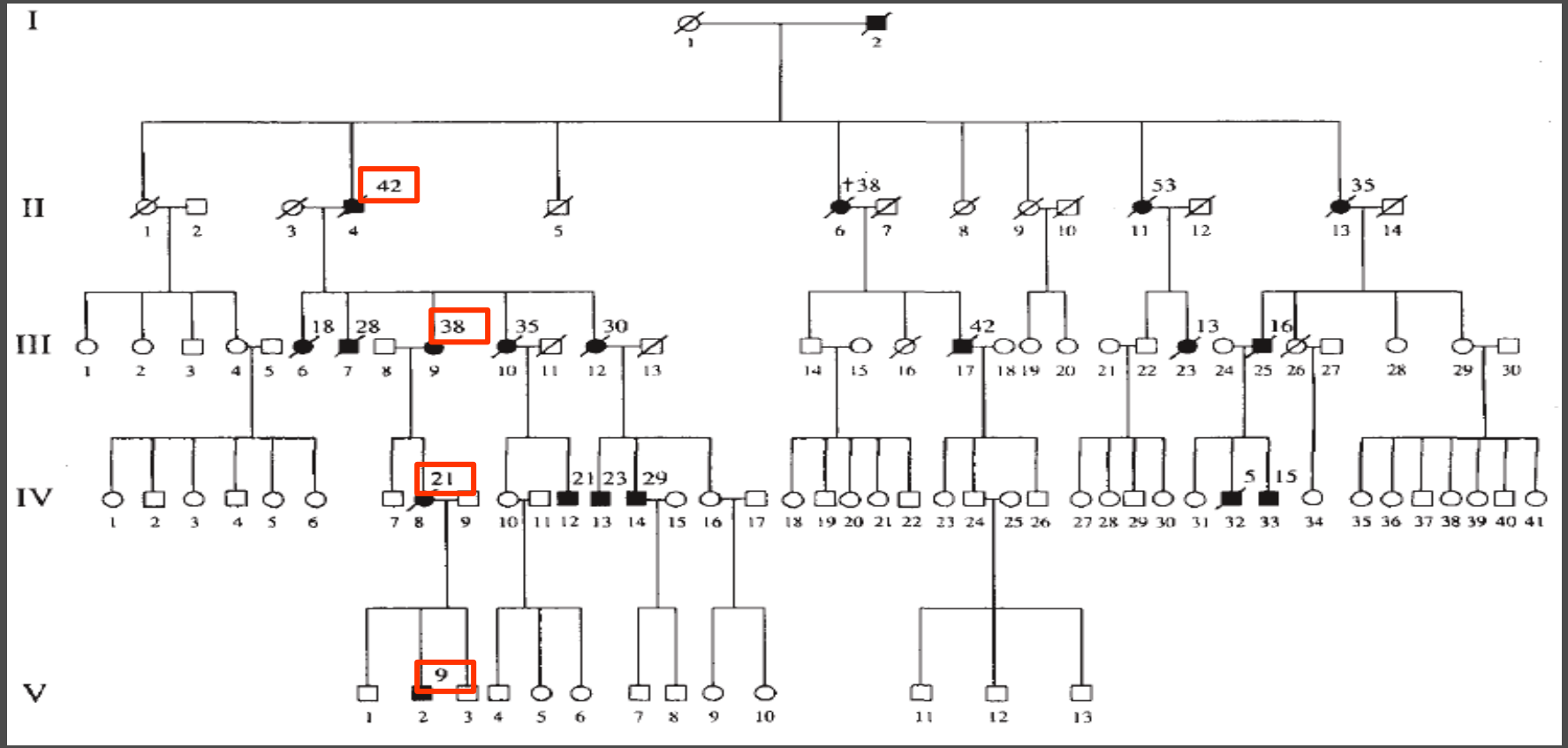




Prevalence rate: 19/100,000

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

Anticipation in the Ataxias



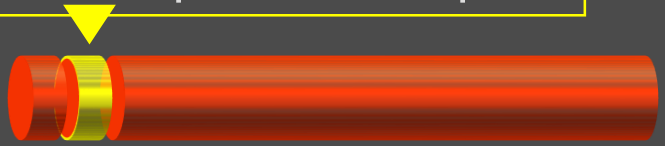
Pulst et al, Nat Genet 1993

SCA Symptoms/Signs

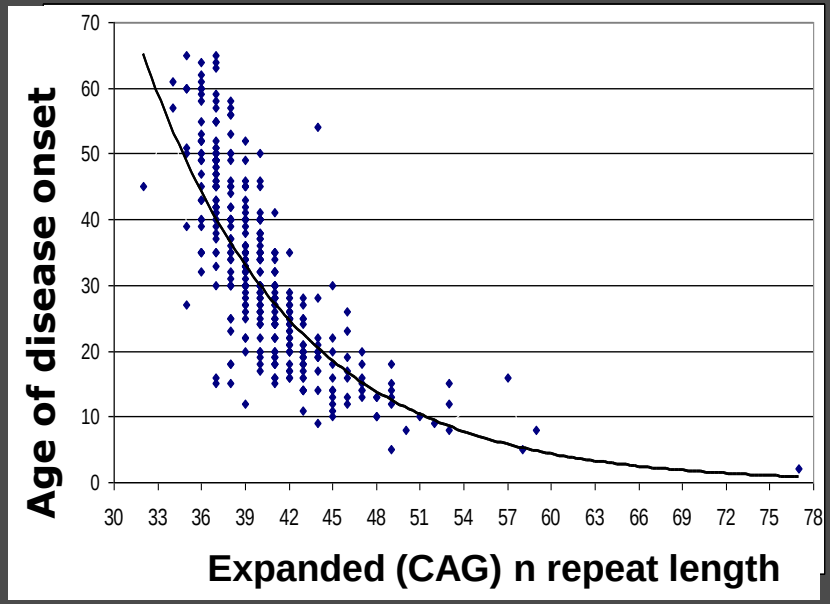
- Cerebellar
  - Gait
  - Appendicular Ataxia (Precision/overshoot, rhythm)
  - Speech
  - Eye movements (nystagmus, overshoot)
- Other
  - Slow saccades
  - Parkinsonism
  - Spasticity
  - Neuropathy
  - ALS-like

SCA2: Repeats and Anticipation

Normal: 22Q
Range: 13 – 32Q
DNA repeat: interrupted



Mutant: ≥33Q

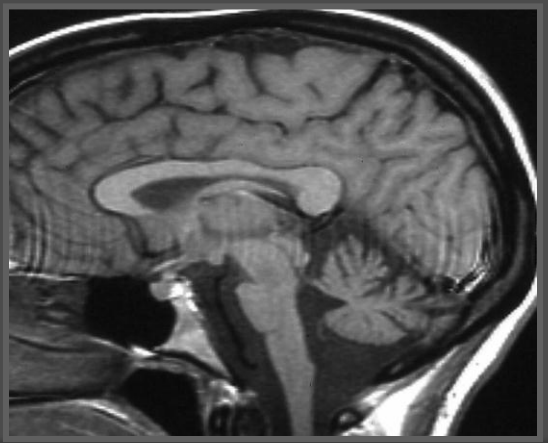


Pulst et al; Imbert et al; Sanpei et al, Nature Genet 1996; Pulst et al Brain 2005

SCA2 is more than cerebellar degeneration.

The usual

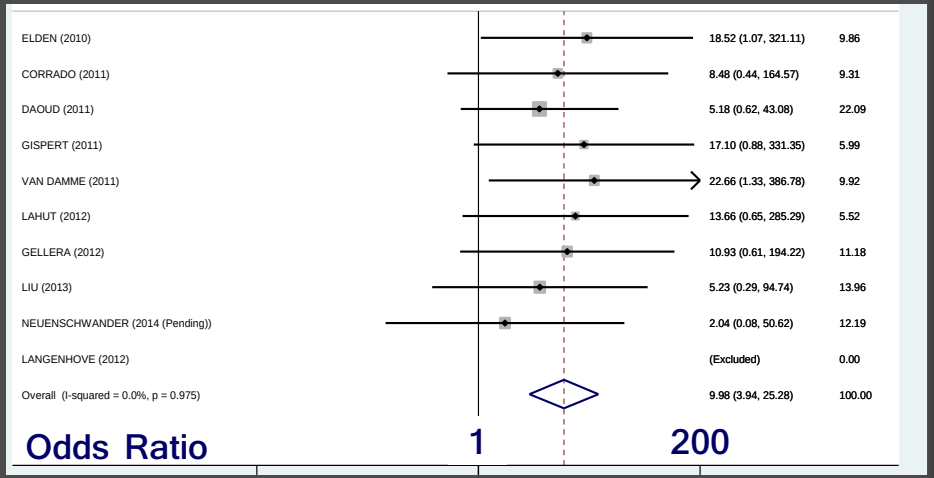
- Slow saccades
- Neuropathy
- Spasticity
- Dystonia
- Frontal-executive dysfunction



The Unusual

- L-DOPA responsive PD
- ALS

Increased ALS risk with ATXN2^{CAG32}



Neuenschwander et al subm.

Models

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.

SCA2 Animal Models

- Transgenic:

  - Pcp2 → targeting of expression to PCs

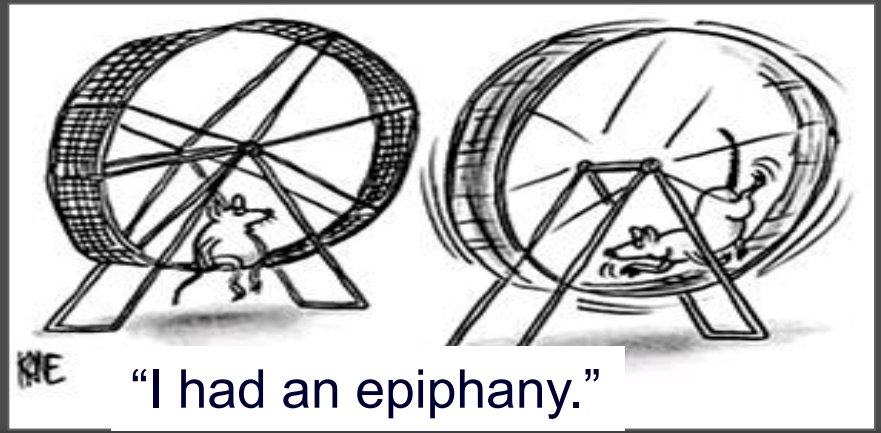
Humanized SCA2: BAC transgenics

ATXN-/- and ATXN2+/-

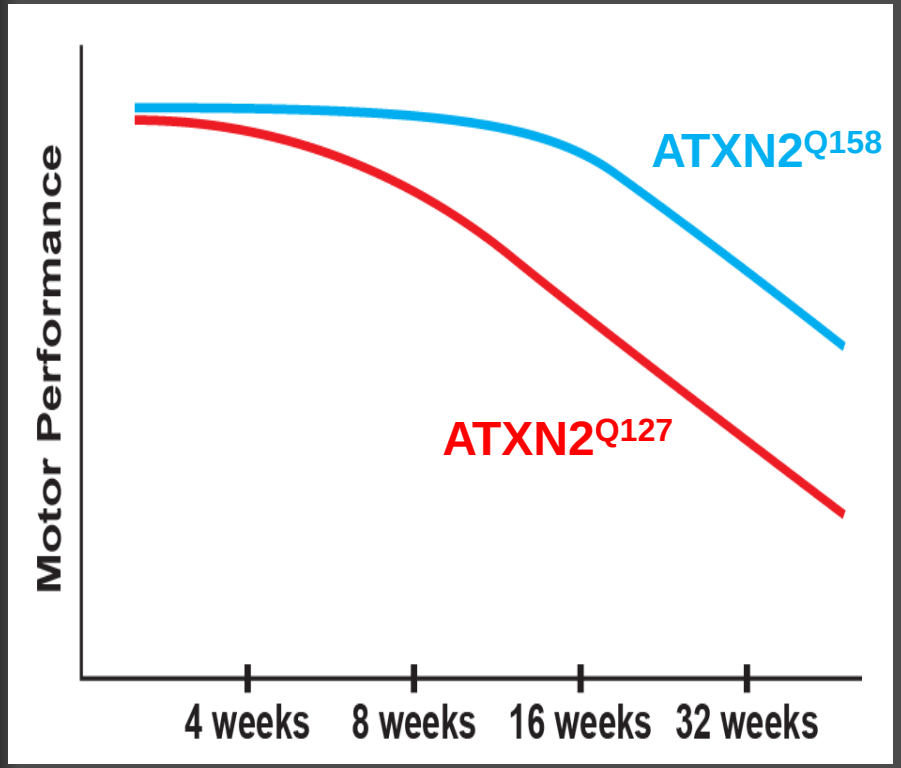
Huynh et al Nat Genet 2000; Hansen et al HMG 2013

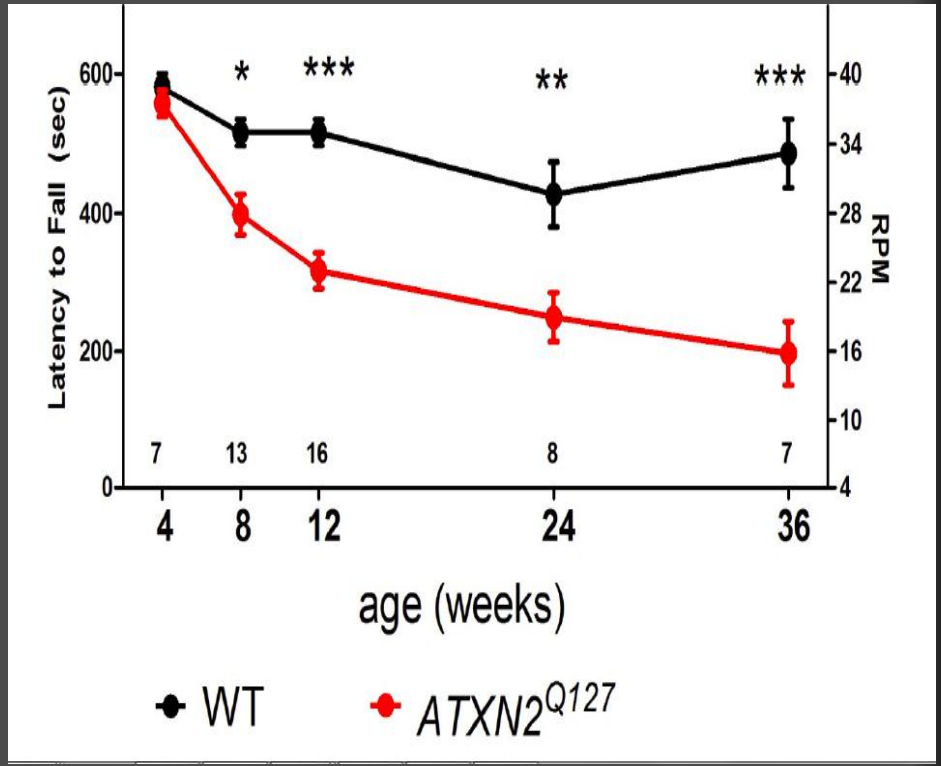
Outcomes: Moutaxia

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

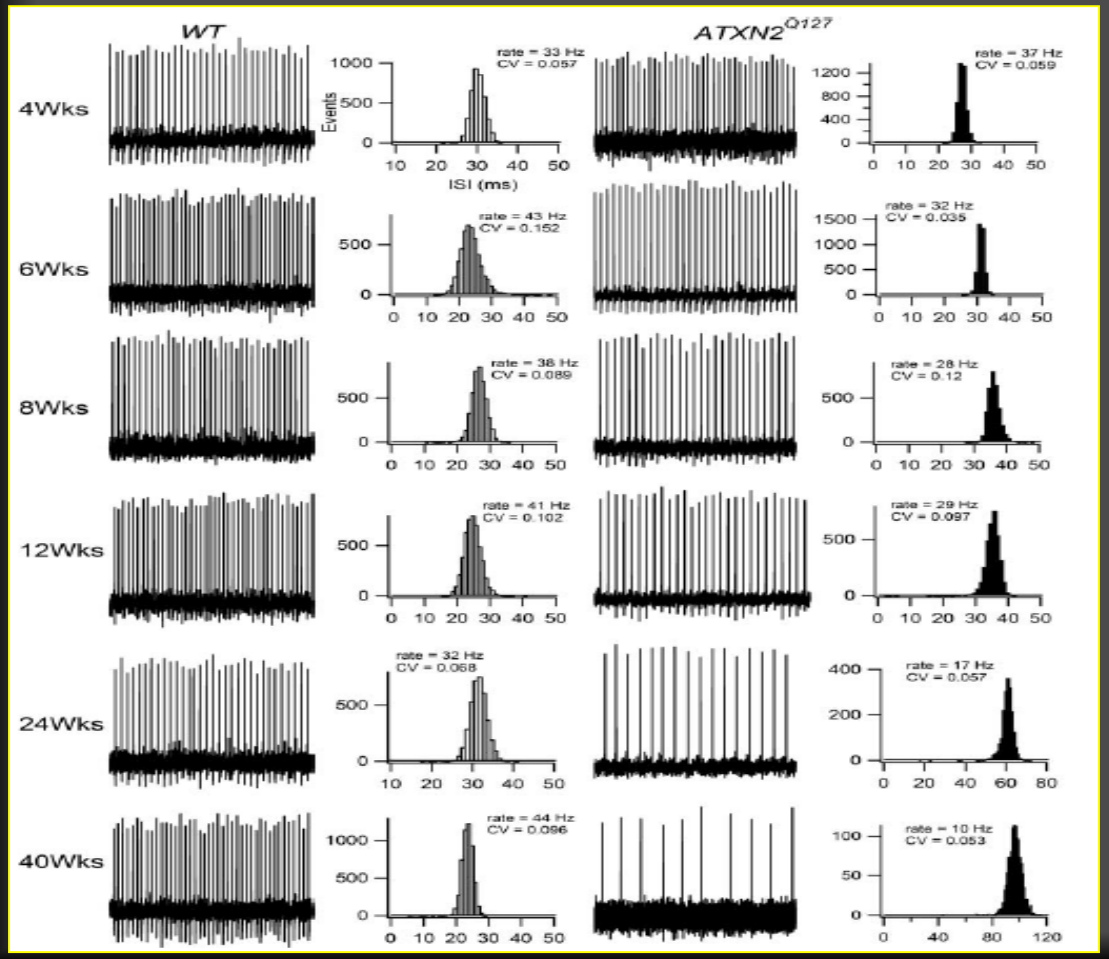


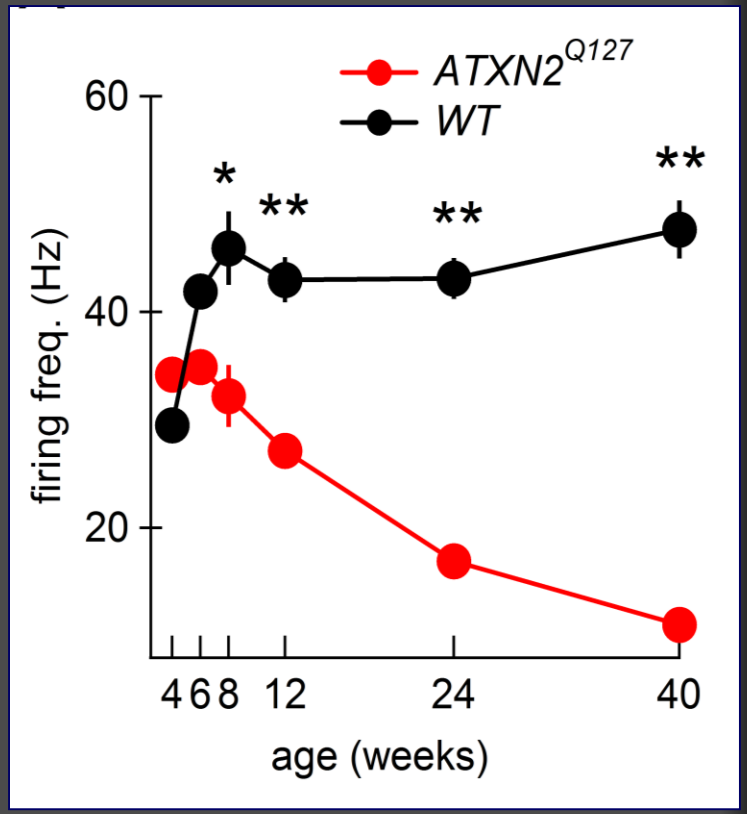
Rotarod behavior is abnormal in mice expressing mutant ATXN2^{Qexp}.





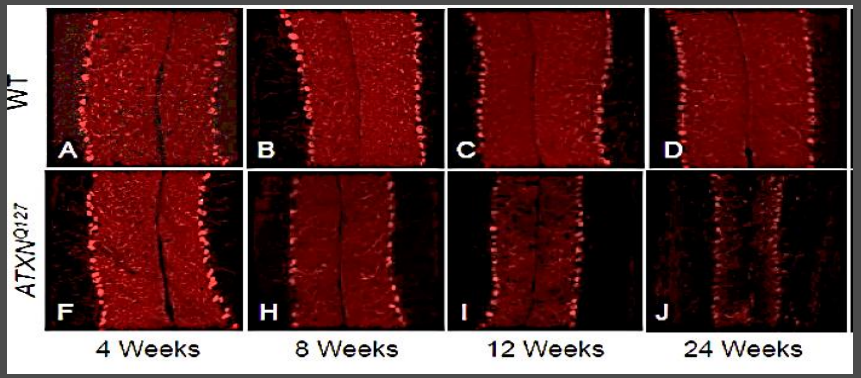
Purkinje cell firing becomes abnormal at onset of motor dysfunction.





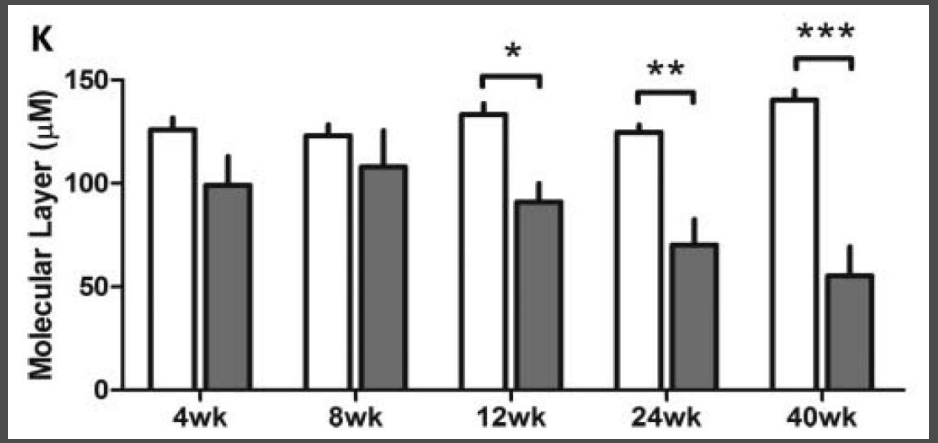
Hansen et al HMG 2013

Morphologic Changes occur late.

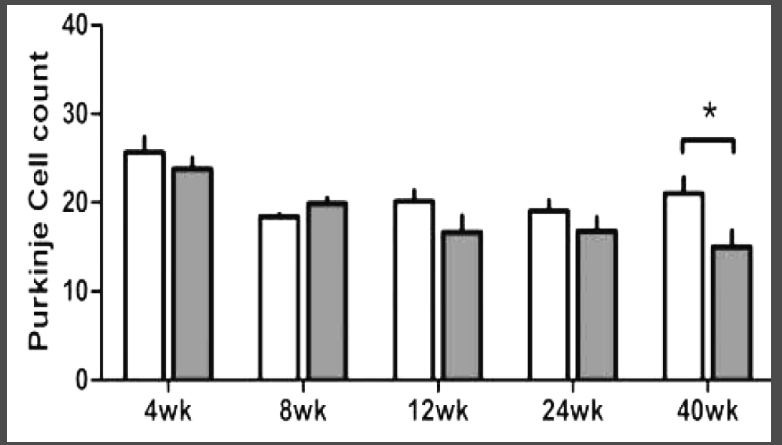


Calbindin28K
(Calb1)

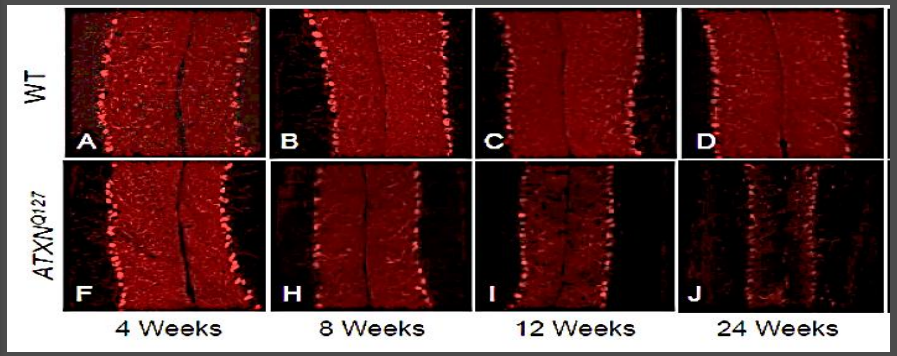
Mol. layer decreases at 12 weeks

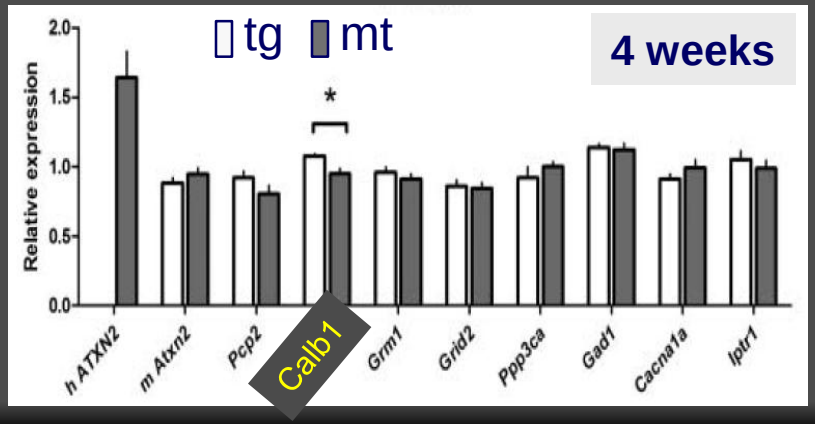


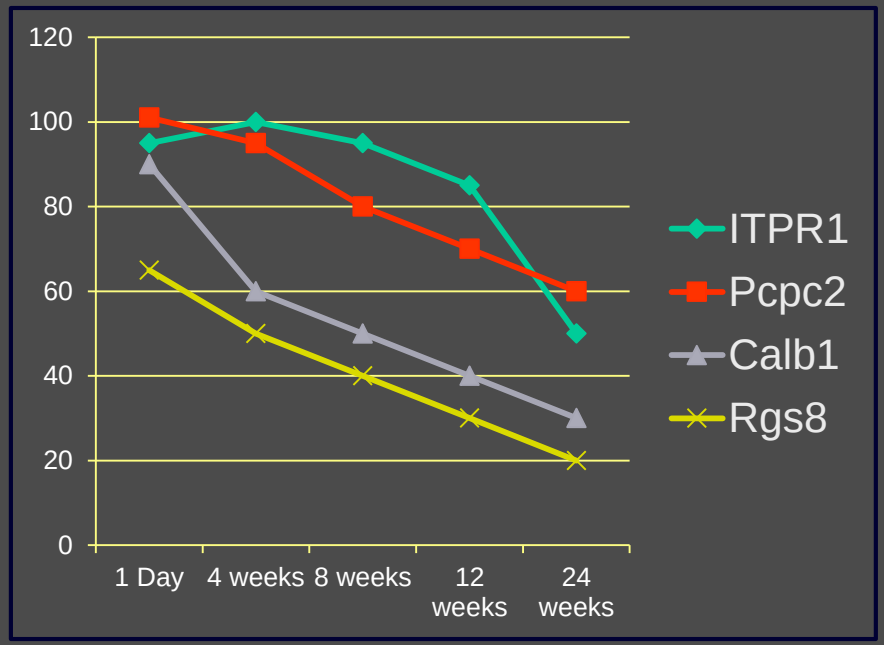
Purkinje cell loss occurs at 40 weeks



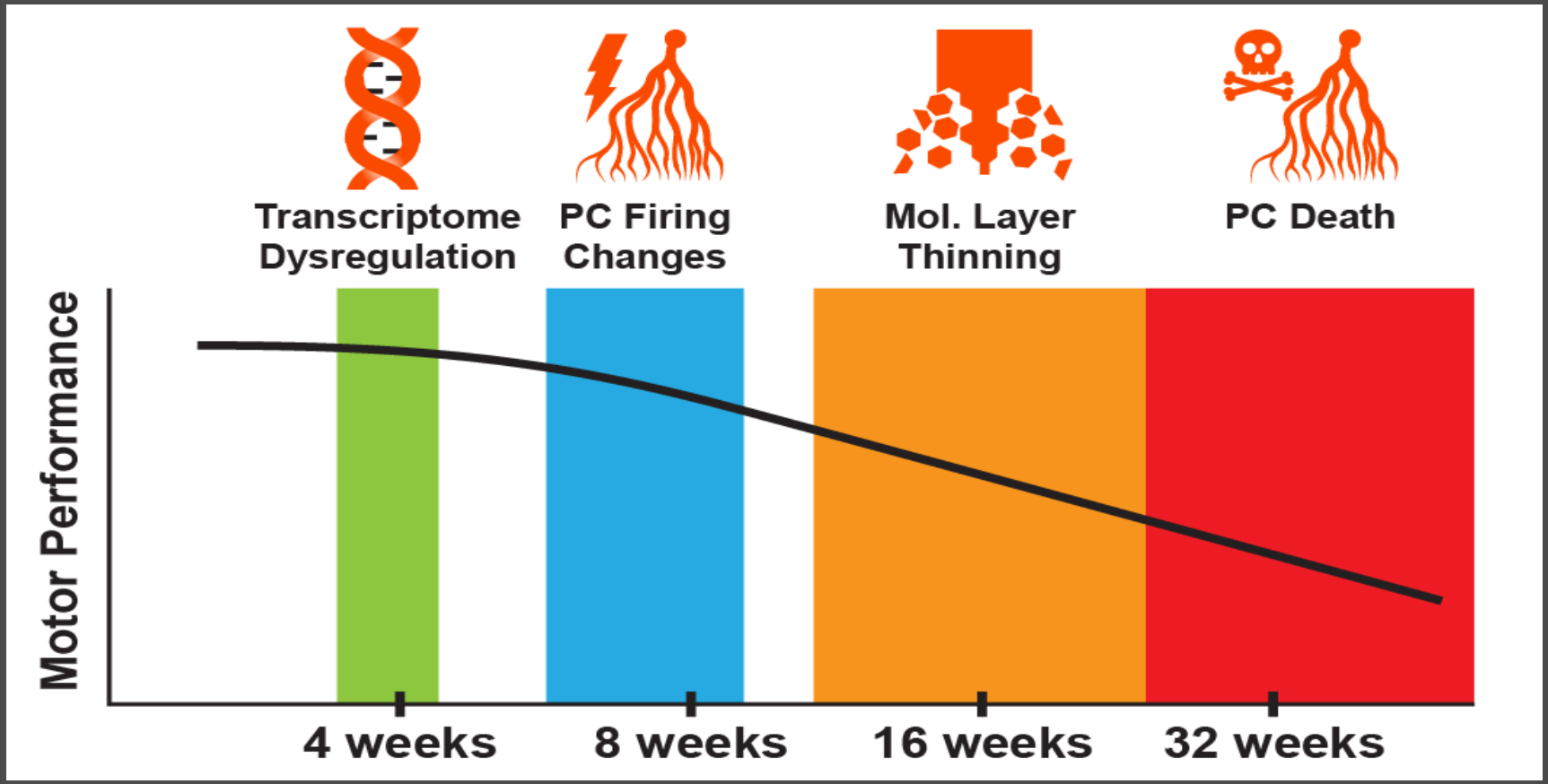
Transcriptome profiling identifies an intermediate biochemical phenotype.



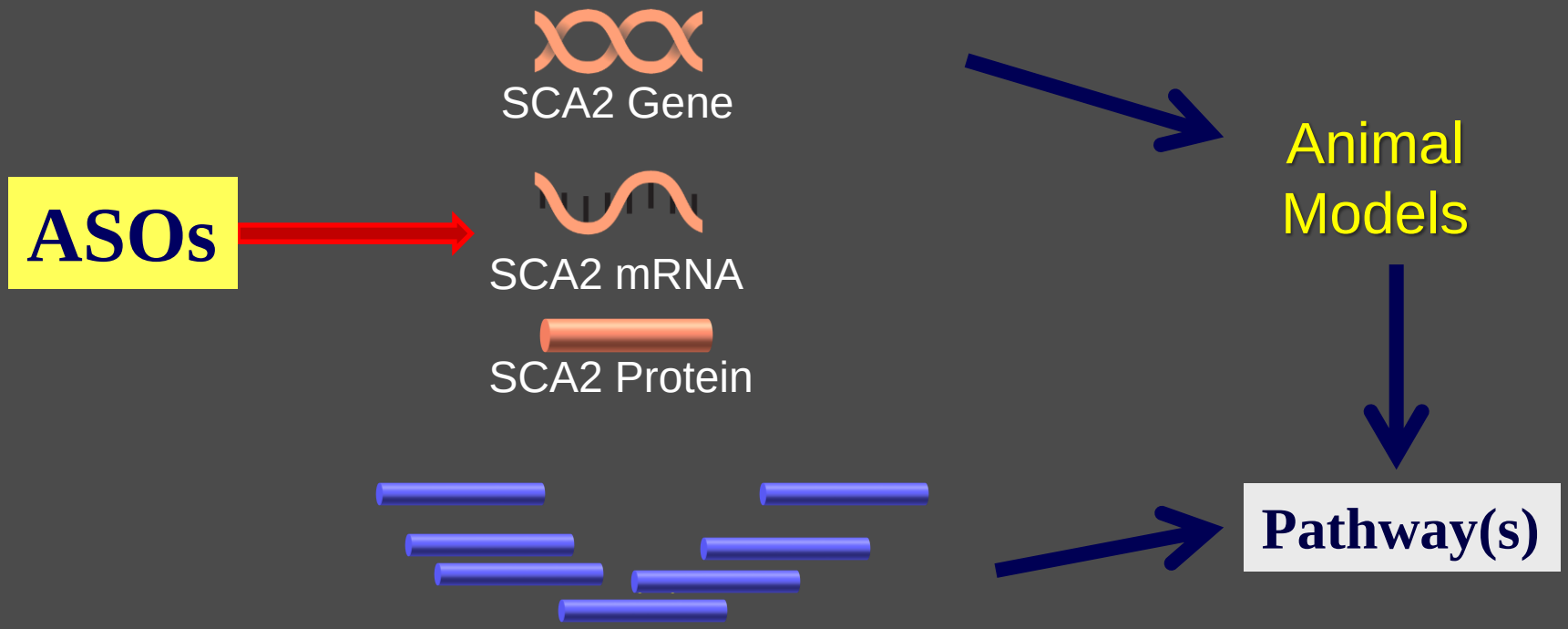




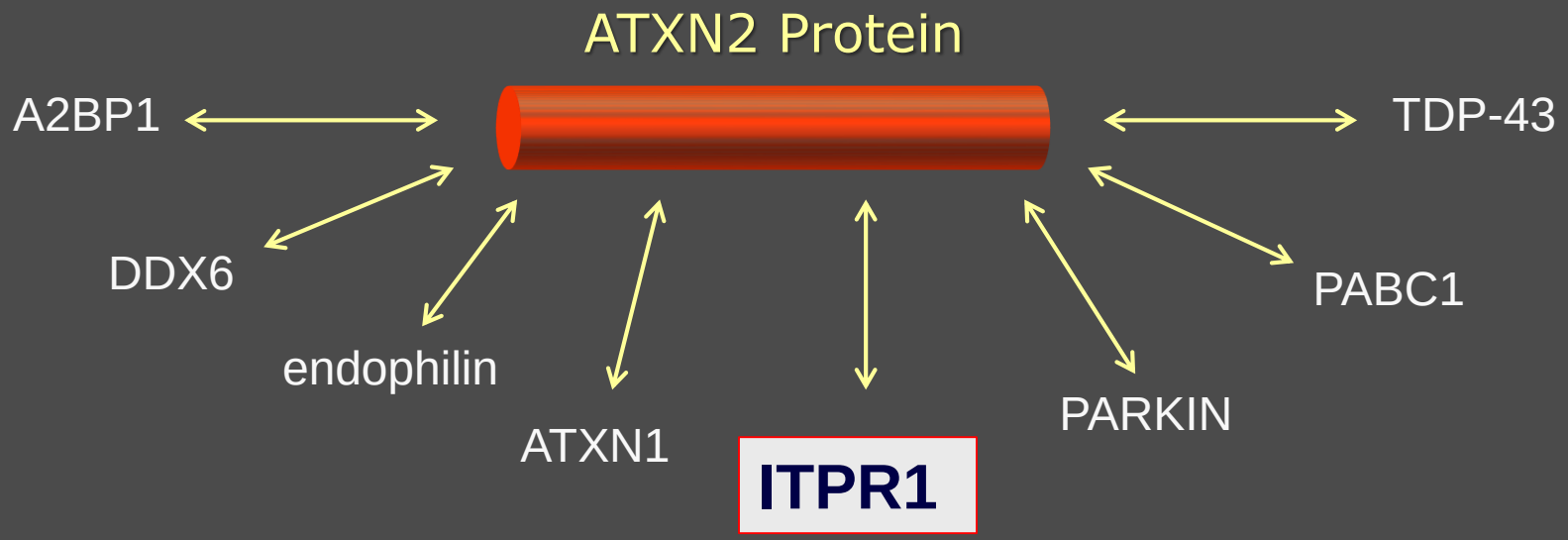
The 4 Stages of PC Dysfunction & Death



Pathway-directed and Gene-directed Therapies

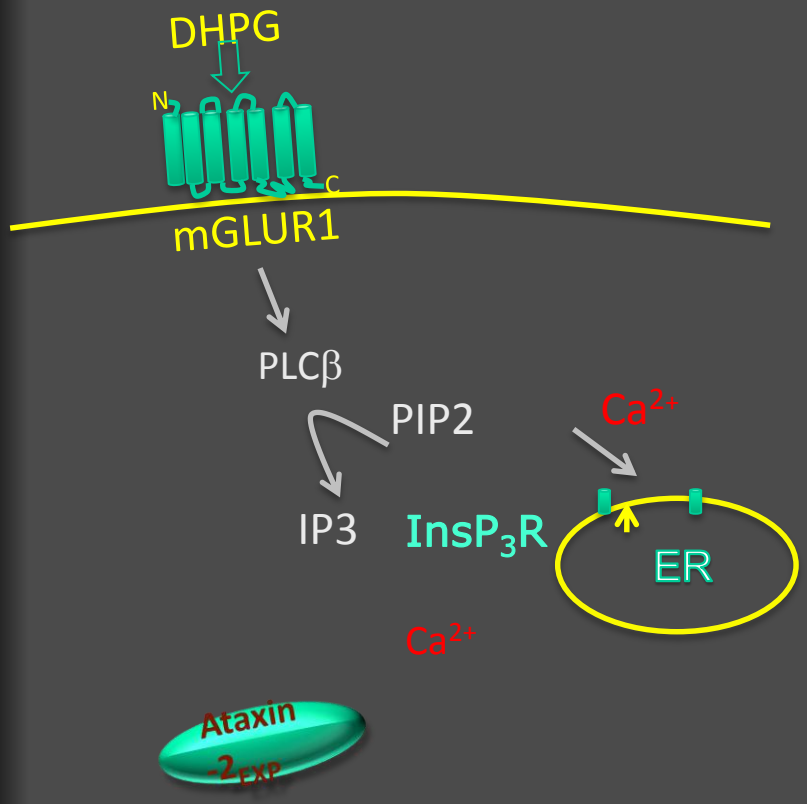


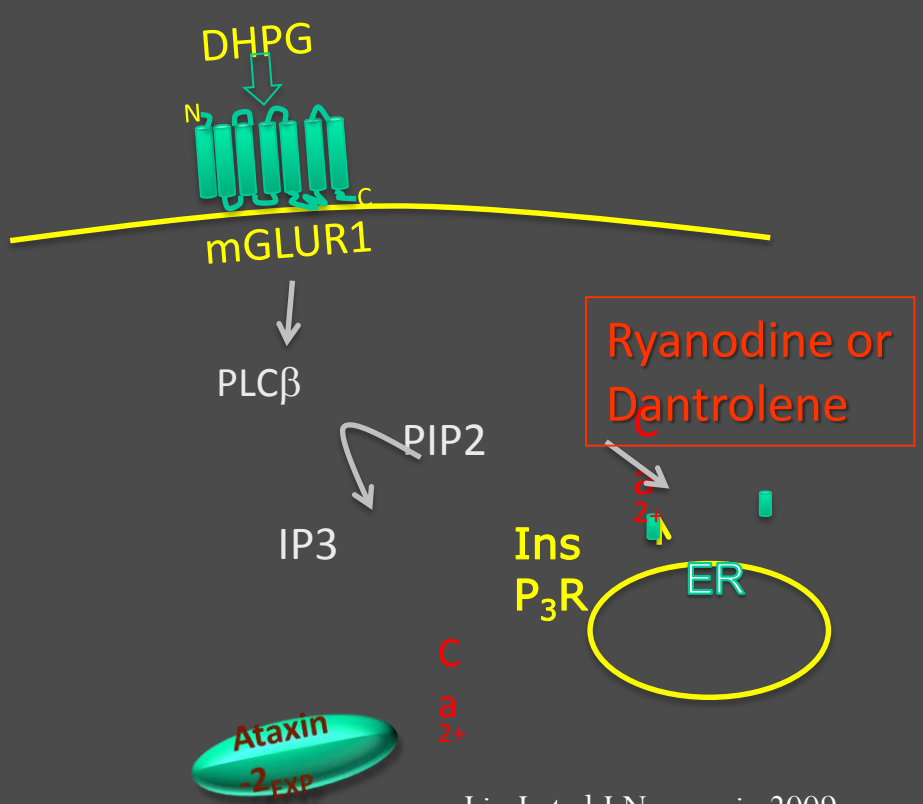
Multiple Proteins interact with ATXN2 !
Which one(s) are relevant in the cerebellum?



Shibata et al HMG 2000; Ralser et al HMG 2005 & JMB 2005; Satterfield&Pallanck HMG 2006; Huynh et al Exp Neurol 2007; Nonhoff et al MCB 2007; Liu et al J Neurosci 2009; Elden et al Nature 2010; Damrath et al PLoS Genet 2012

Mutant ATAXN2 and Ca^{2+} Release





Liu J et al J Neurosci 2009

Mutant ATXN2 causes abnormal Ca^{++} release after stimulation of mGluR1 in cultured Purkinje cells.

Differential interaction for wt and mutant ATXN2 with InsP3R1

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

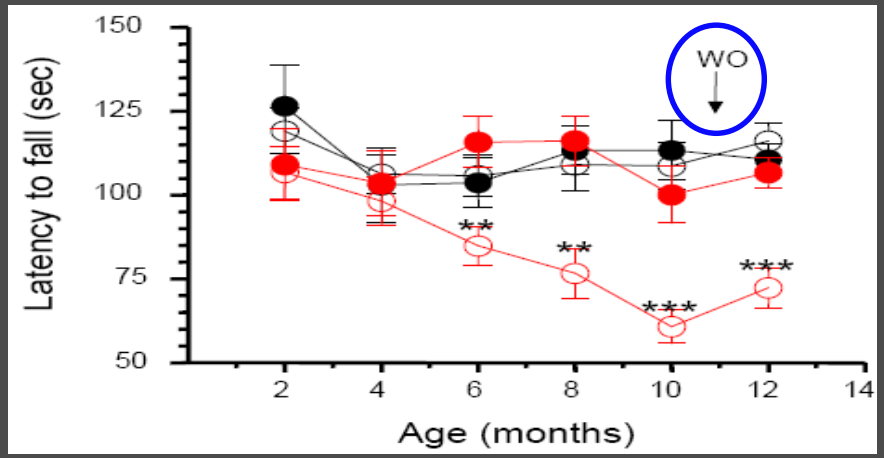
Enhanced Ca signals in 58Q PCs cause Glutamate induced cell death

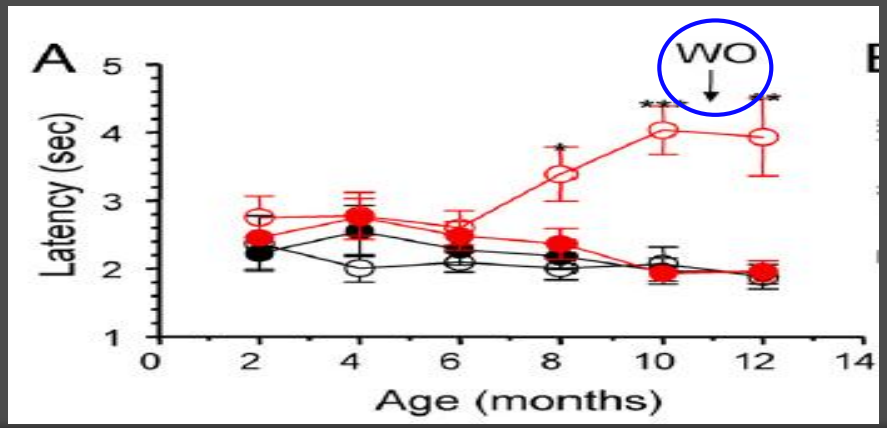
Dantrolene recovery of cellular phenotype in 58Q PCs in vitro

Does dantrolene have an effect *in vivo* ?

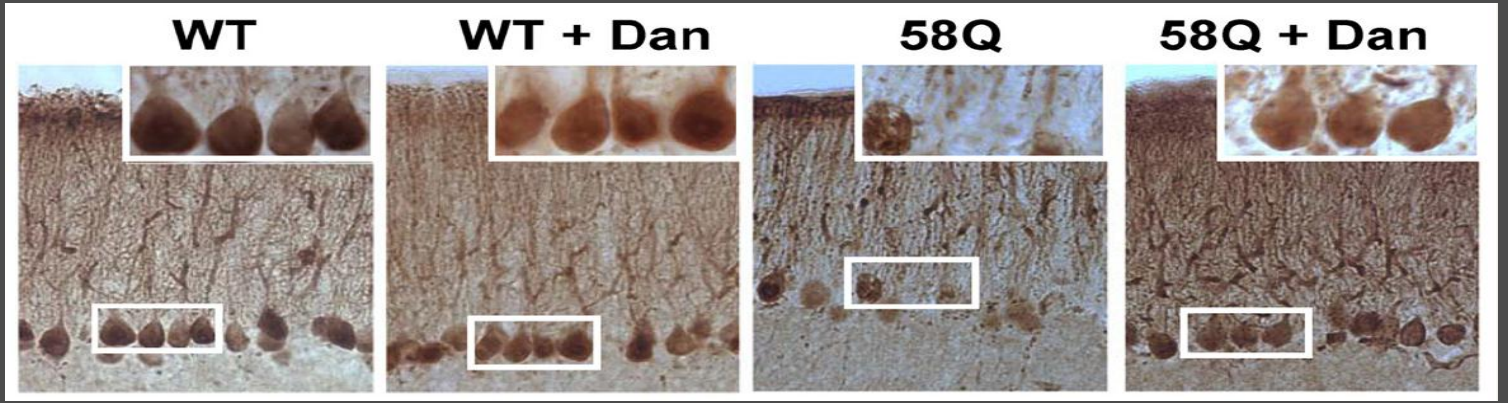
Liu J et al J Neurosci 2009

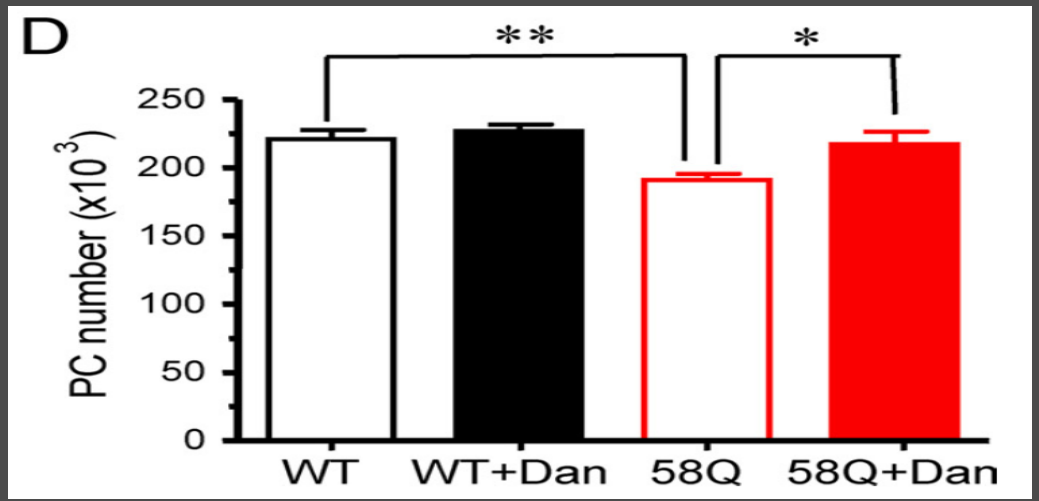
Dantrolene improves motor function in the ATXN2^{Q58} mouse model.





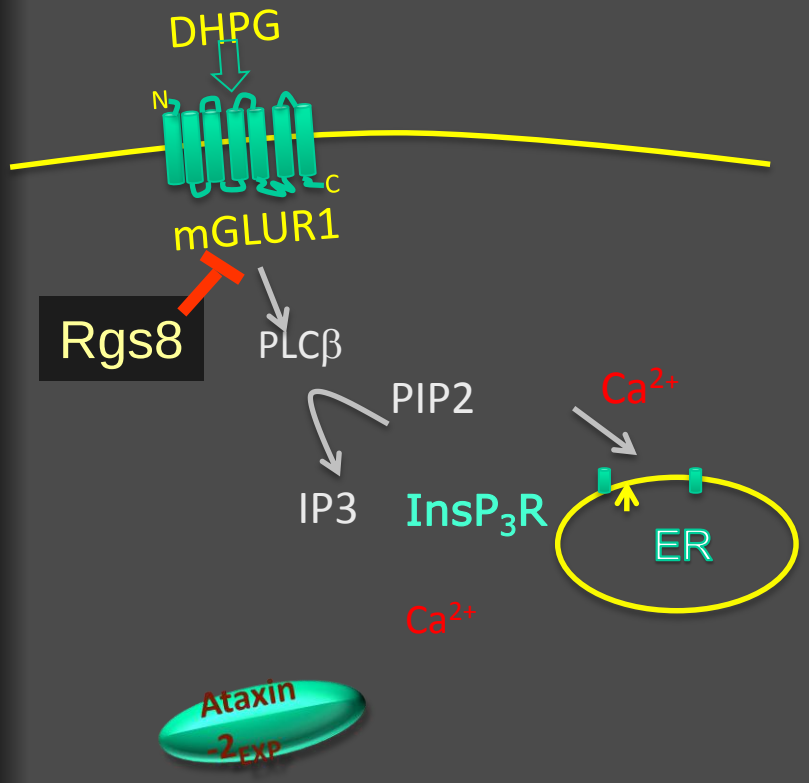
Dantrolene is neuroprotective in the ATXN2^{Q58} mouse model.





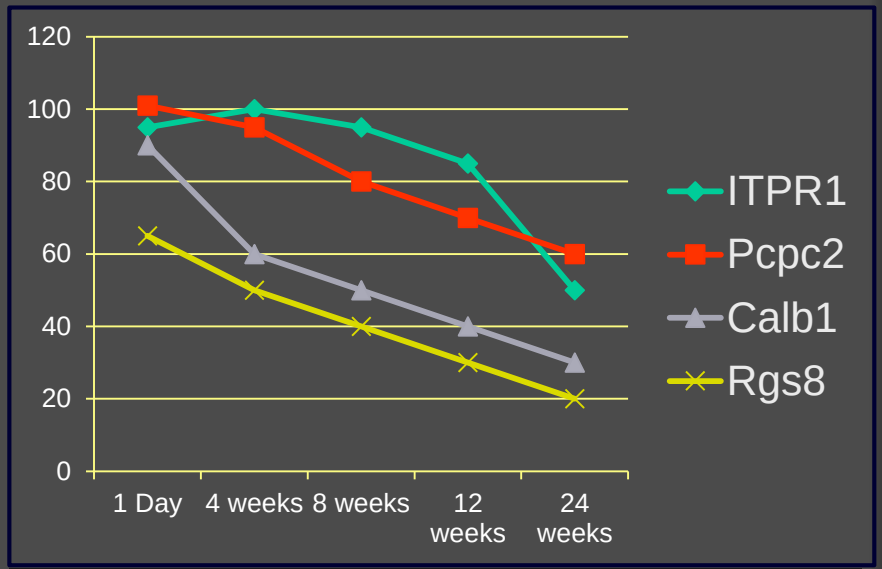
Liu J et al J Neurosci 2009

The mGluR1-ITPR-Ca^{++}model of PC death



Deep RNA-seq

Expression Networks

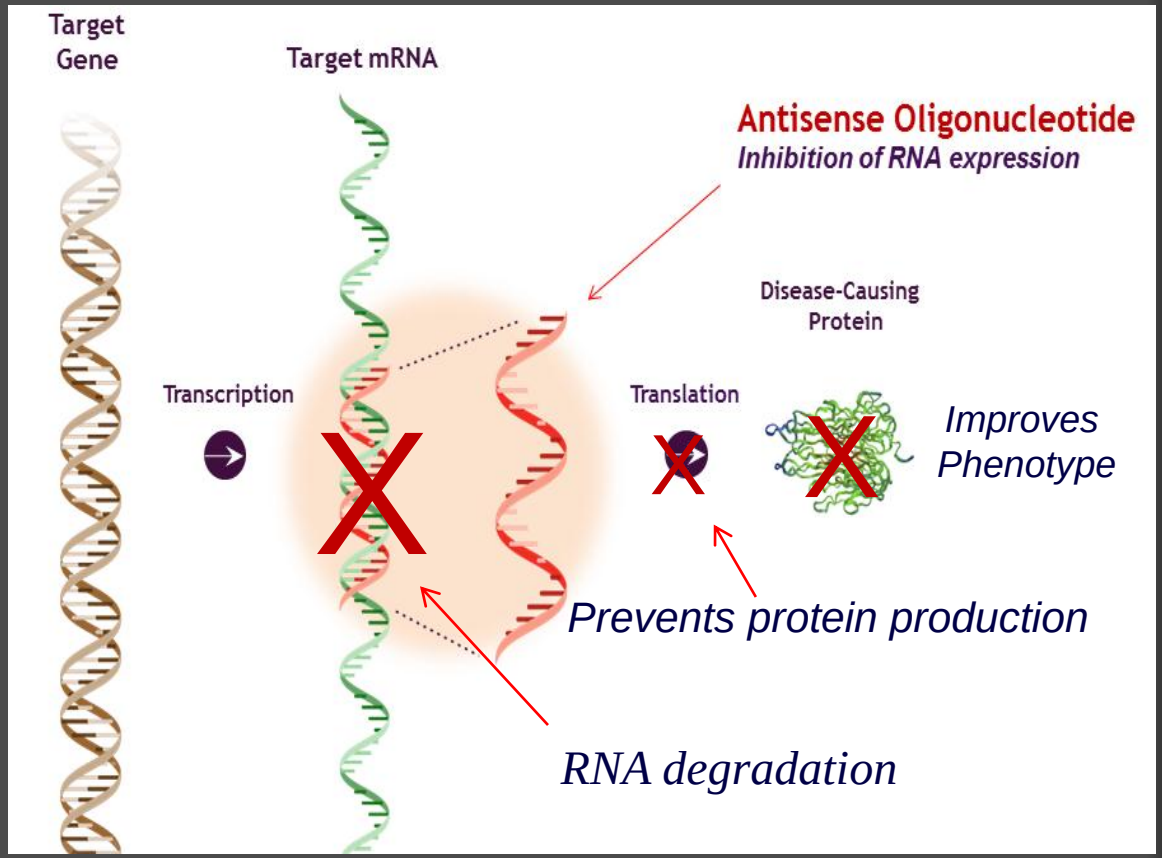


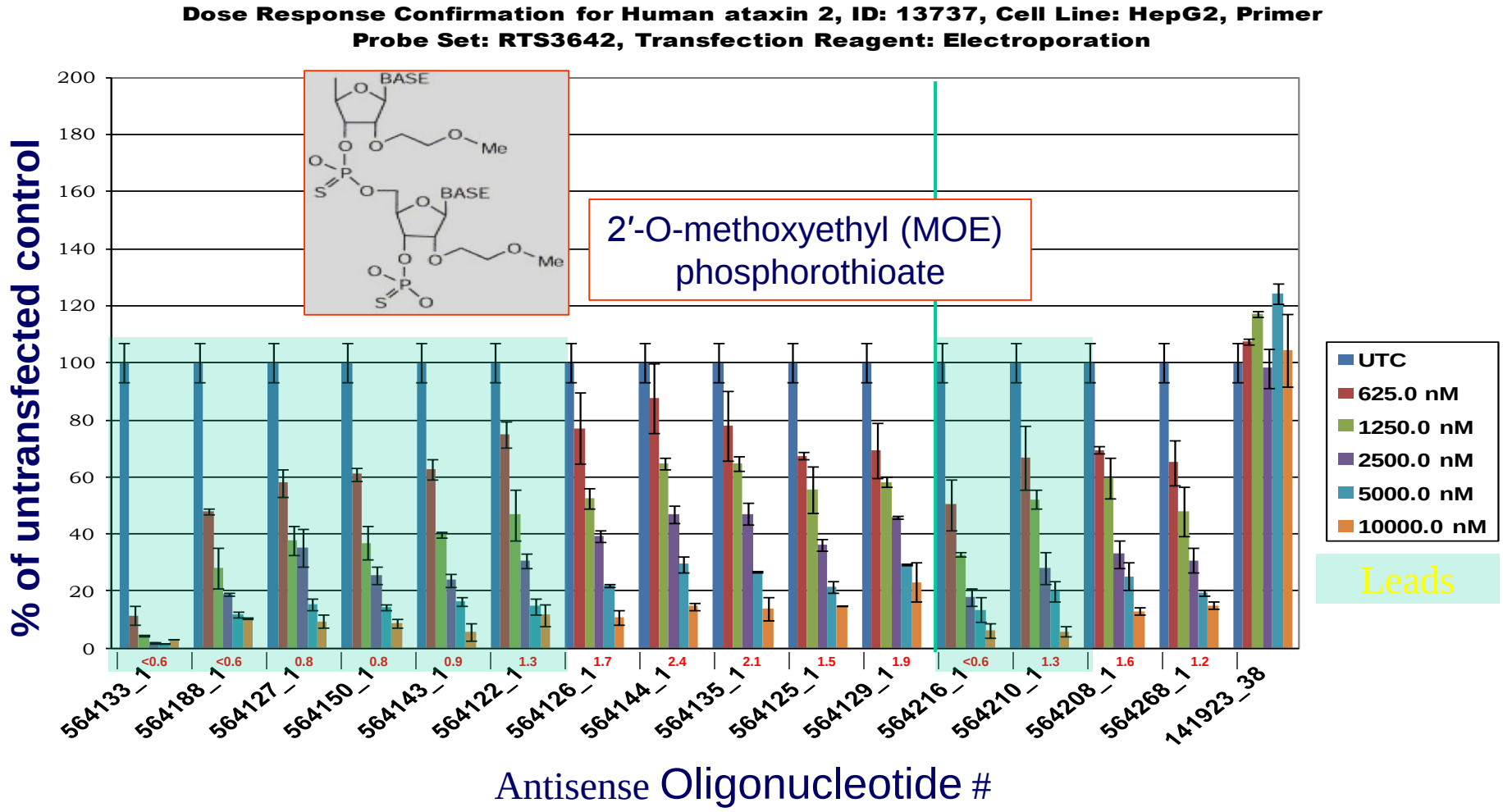
Regulator of G-protein Signaling 8

Antisense drugs target RNA and can inhibit production of disease causing proteins.

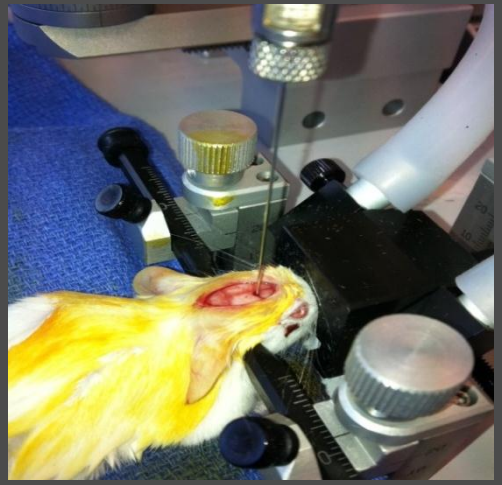
Rationale:

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



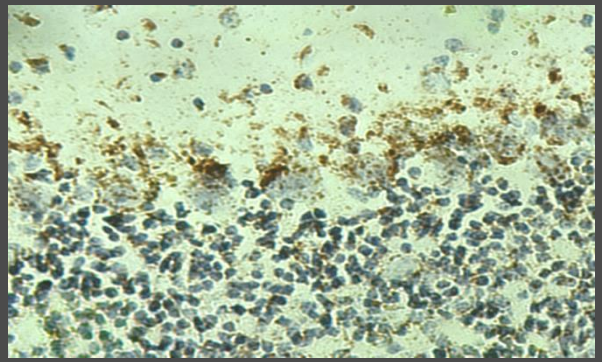

Dose Response Confirmation for Human ataxin 2, ID: 13737, Cell Line: HepG2, Primer Probe Set: RTS3642, Transfection Reagent: Electroporation
% of untransfected control
200
180
160
140
120
100
80
60
40
20
0
BASE
Me
BASE
Me
2′-O-methoxyethyl (MOE) phosphorothioate
UTC
625.0 nM
1250.0 nM
2500.0 nM
5000.0 nM
10000.0 nM
Leads
<0.6
<0.6
0.8
0.8
0.9
1.3
1.7
2.4
2.1
1.5
1.9
<0.6
1.3
1.6
1.2
564133_1
564188_1
564127_1
564150_1
564143_1
564122_1
564126_1
564144_1
564135_1
564125_1
564129_1
564216_1
564210_1
564208_1
564268_1
141923_38
Antisense Oligonucleotide #

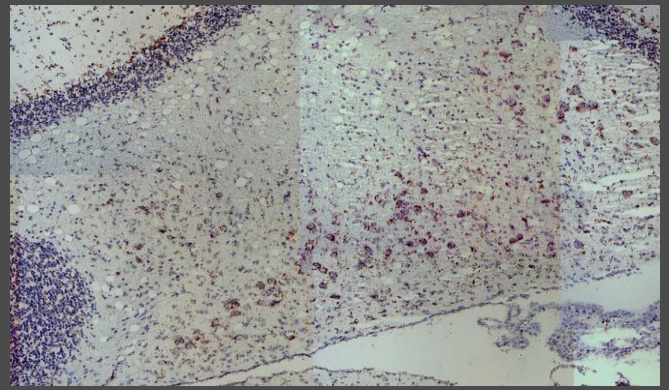
ASO-133 is taken up by PCs & deep cerebellar neurons



Benefits of ASOs

- Diffusible
- Stable
- Reversible

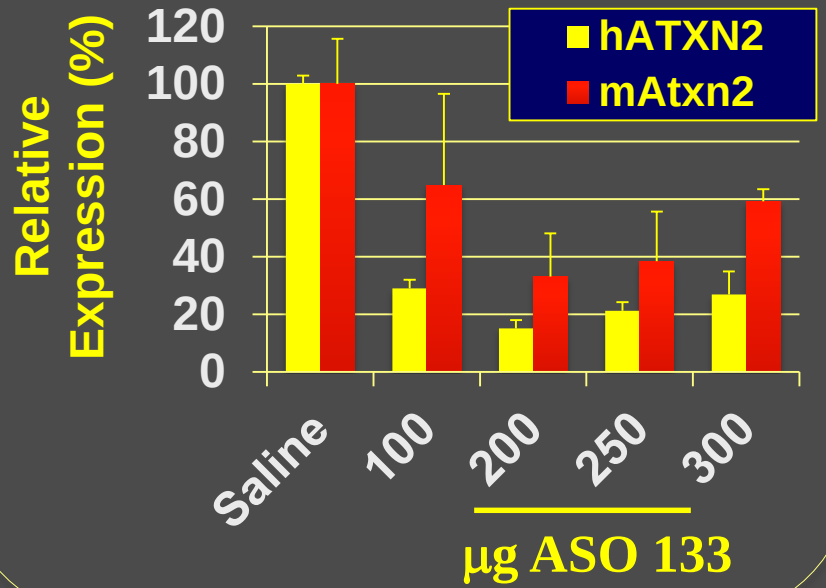




ASOs reduce ATXN2 expression in Tg mice

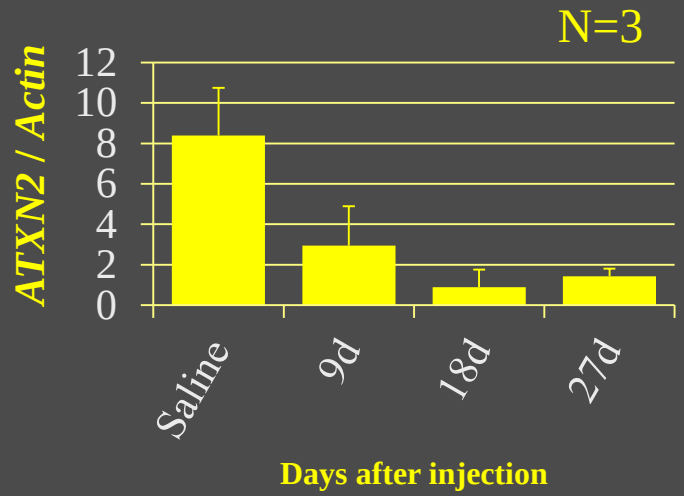
Dosing study:

ICV injection of increasing doses of ASO 133, treated 7 days.



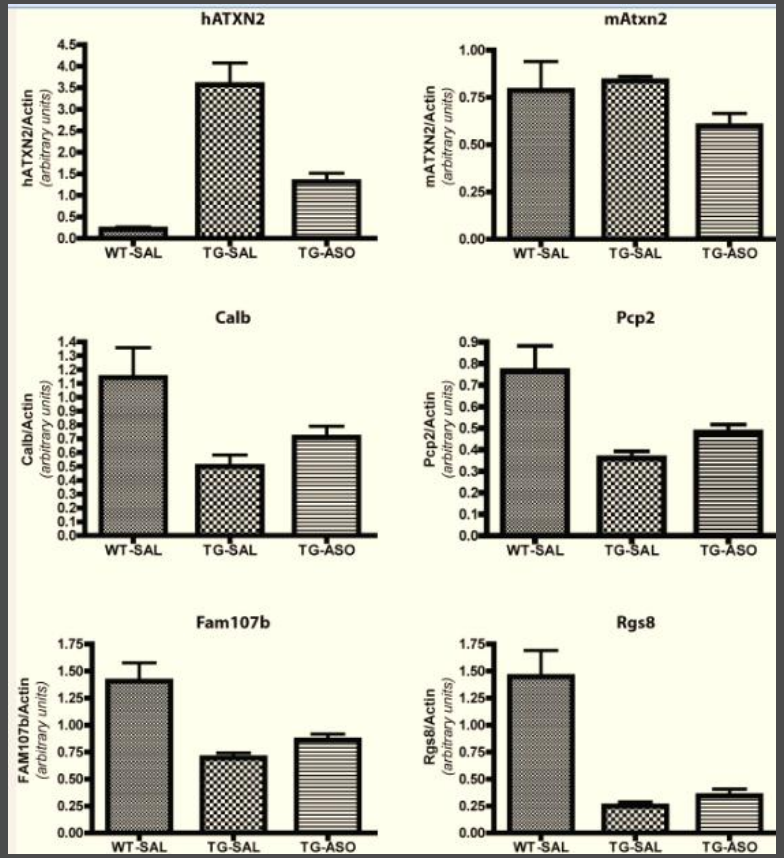
Time course study

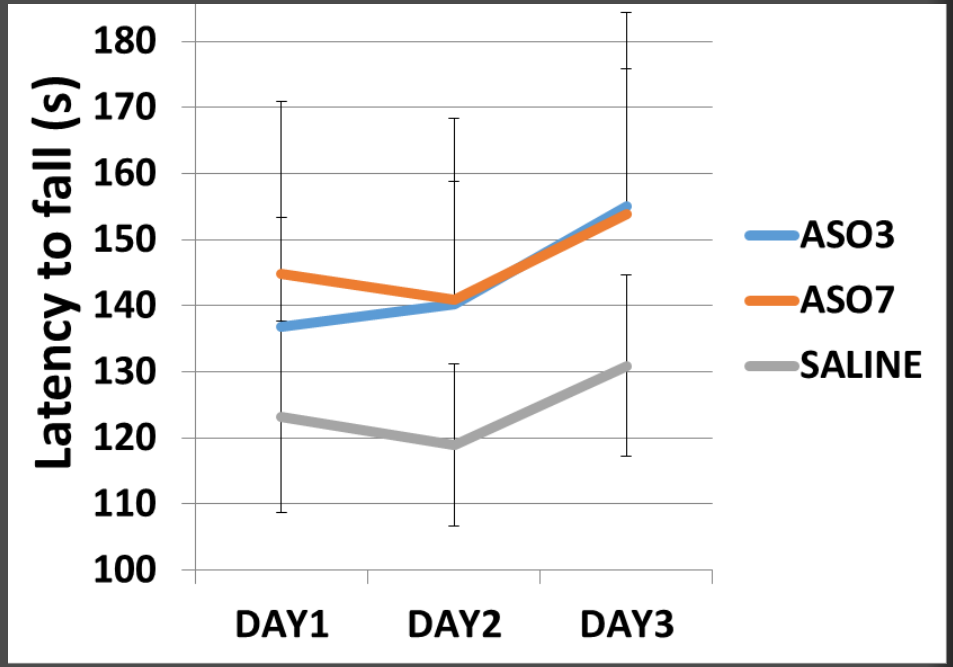
Injected with 200 mg ASO 133 5.7 ml of 35 ml/ml ASO.



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

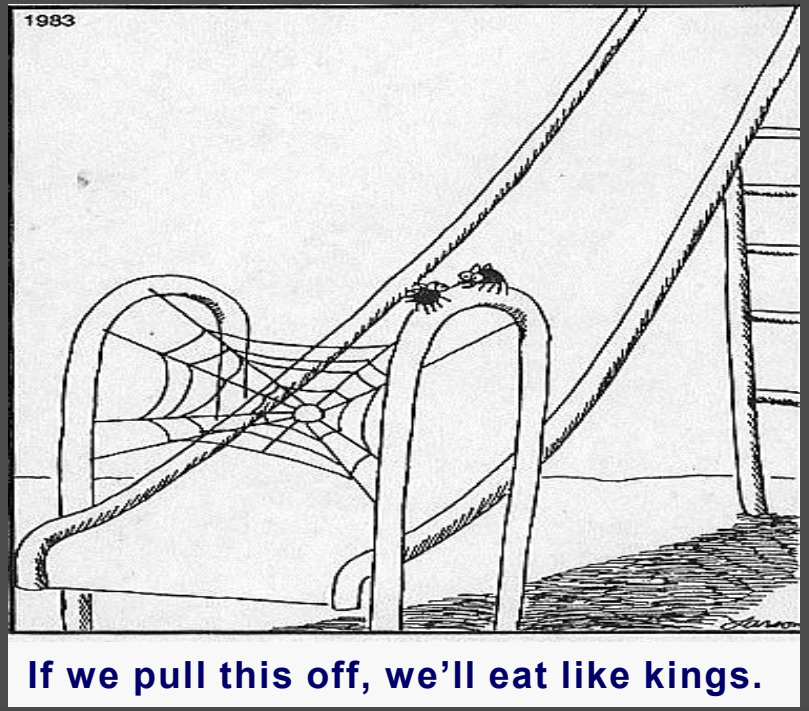
Behavior & Transcriptomes





The $G^1P^1M^1$ Problem

- Adoption of best practices from human clinical trials:
  - Multi-center
  - Randomization
  - Formal blinding
  - Prespecified outcomes
  - Prespecified statistical analysis
  - Multi - PI
- Models
  - Multiple mutational models
  - Multiple genetic backgrounds
  - Use of both sexes.



Summary

- Rare genetic diseases offer unique opportunities for :
  - pathway discovery-
  - Animal modeling
  - Gene-directed therapy.
- Molecular definition discovers a wider phenotypic spectrum.
- Model organisms inform evaluation of therapies.
- Hyperactive mGluR1-ITPR1 calcium axis can be targeted.
- ASO compounds suppress expression of disease proteins.

Collaborators

- Animal Models
  - Pattie Figueroa
  - Duong Huynh, PhD.
  - Stephen Hansen, Ph.D.
  - Warunee Dansithrong, Ph.D.
  - 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
  - Frank Bennett, PhD (ISIS)
  - Gene Hung, PhD (ISIS)