The Spinocerebellar Ataxias: From Gene Discovery to Treatments

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Nagoya

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Treatment Strategies for SCAs

- SCA-type specific
  - siRNA knockdown (SCA1)
  - Small molecules (SCA2)
- Directed at potentially shared mechanisms
  - Correcting deranged gene expression: SCA1 Lithium
  - Glutamate-stimulated Ca-release: SCA2 & SCA3, Dantrolene

What are SCAs?

- Neurodegenerative Disorders
- Autosomal dominant
- Affect primarily cerebellum
  - Often Purkinje cells
- Other neurologic systems as well
- Treatments:
  - Symptomatic NONE
  - Disease-modifying NONE

SCA Symptoms & Signs

- Gait ataxia
- Appendicular Ataxia, Dysmetria
- Dysarthria
- Pontine & bulbar dysfunction
- Parkinsonian features
- Spasticity neuropathy
- Dementia & Frontal Executive Dysfunction

Dominant SCAs

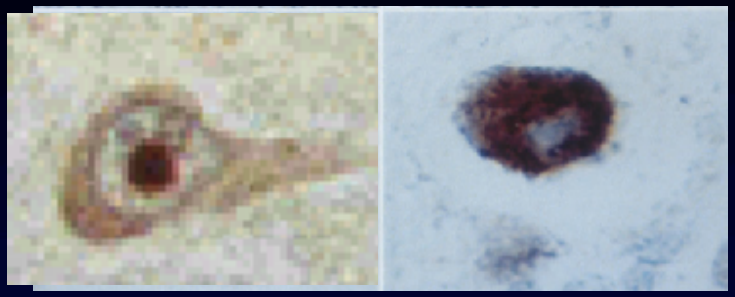
- 60%: Coding CAG repeat expansions: SCA1, 2, 3, 6, 7, SCA17

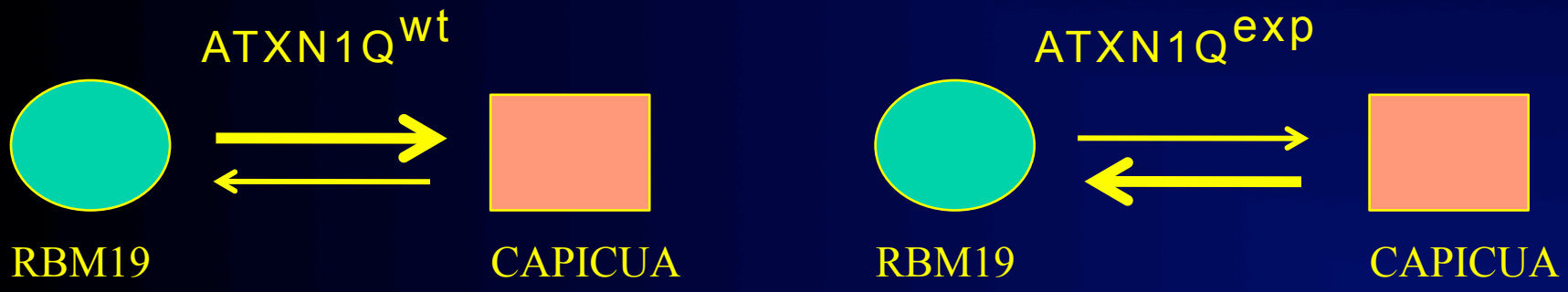
10%: Other SCAs:

- Non-coding repeat mutations
- Point mutations, INS/DEL mutations in a number of genes

Poly-Q Pathogenesis

- Gain of Toxic Function
- Gain of Normal Function
- Allele-specific Gain/Loss of Normal Function





Why the Mouse ?

- Cheaper & Faster than human trials
- Cells do not have a Cerebellum
- Cerebellar Circuits very similar in Mouse and Human.
- Precise timing of disease onset and treatment.
- Differentiation between symptomatic and disease-modifying treatments potentially easier.

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**

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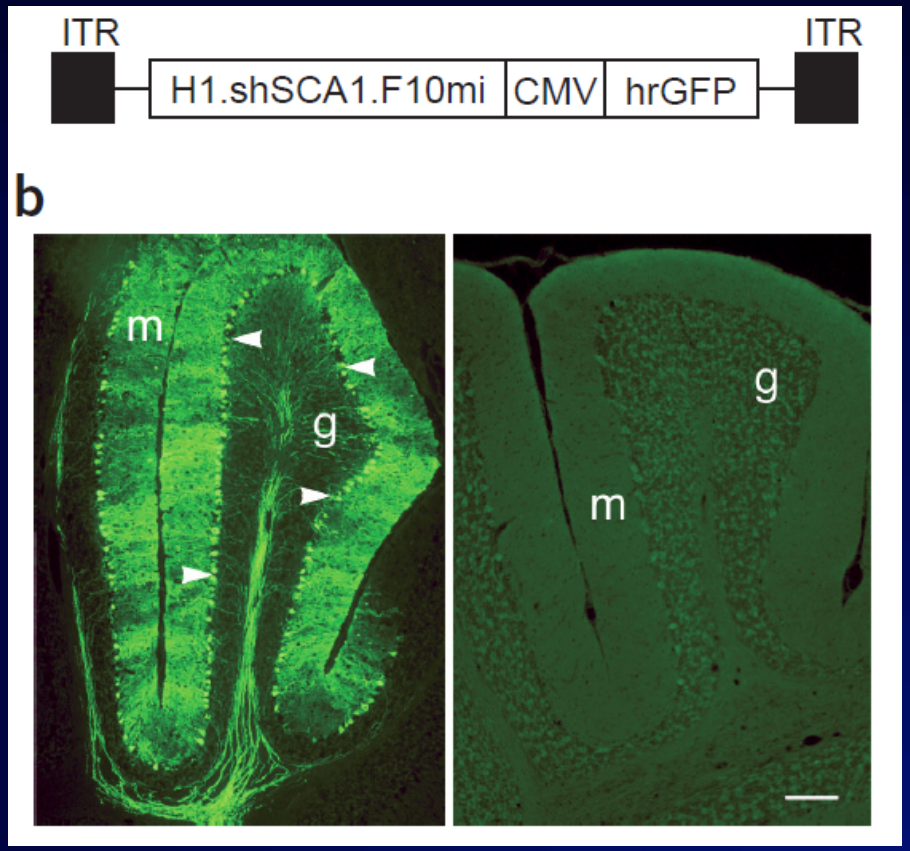
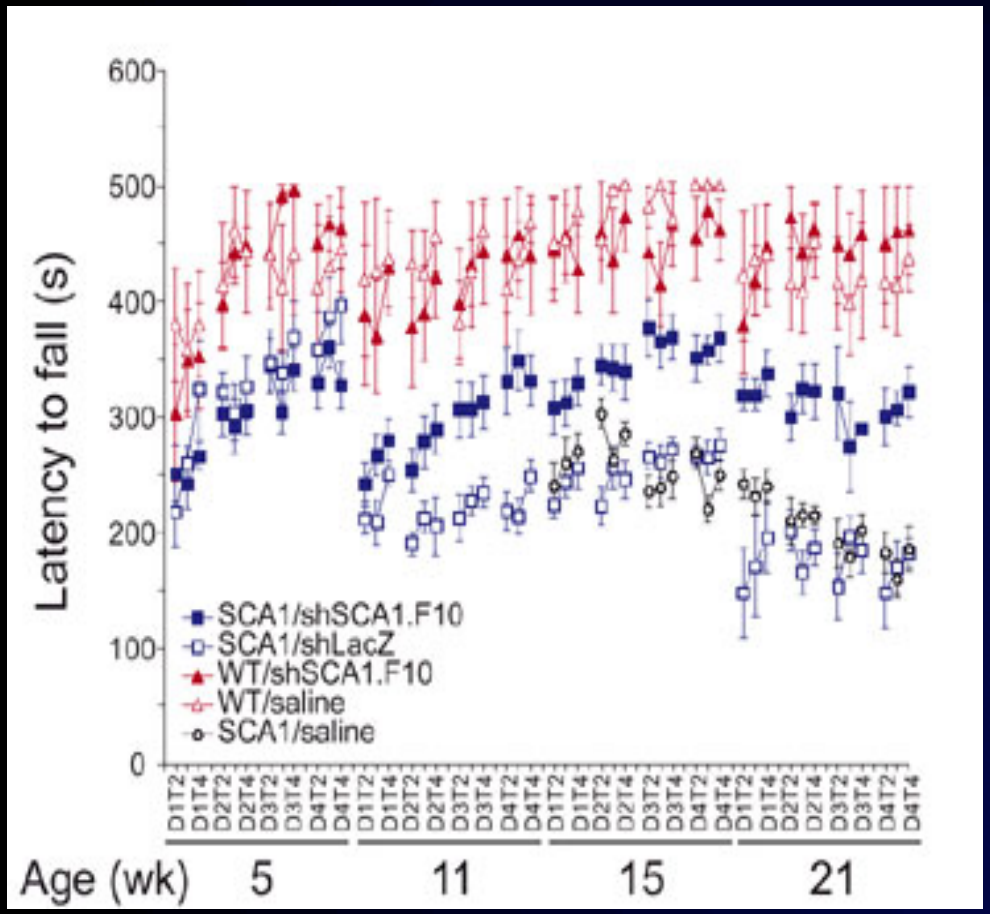
RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia.

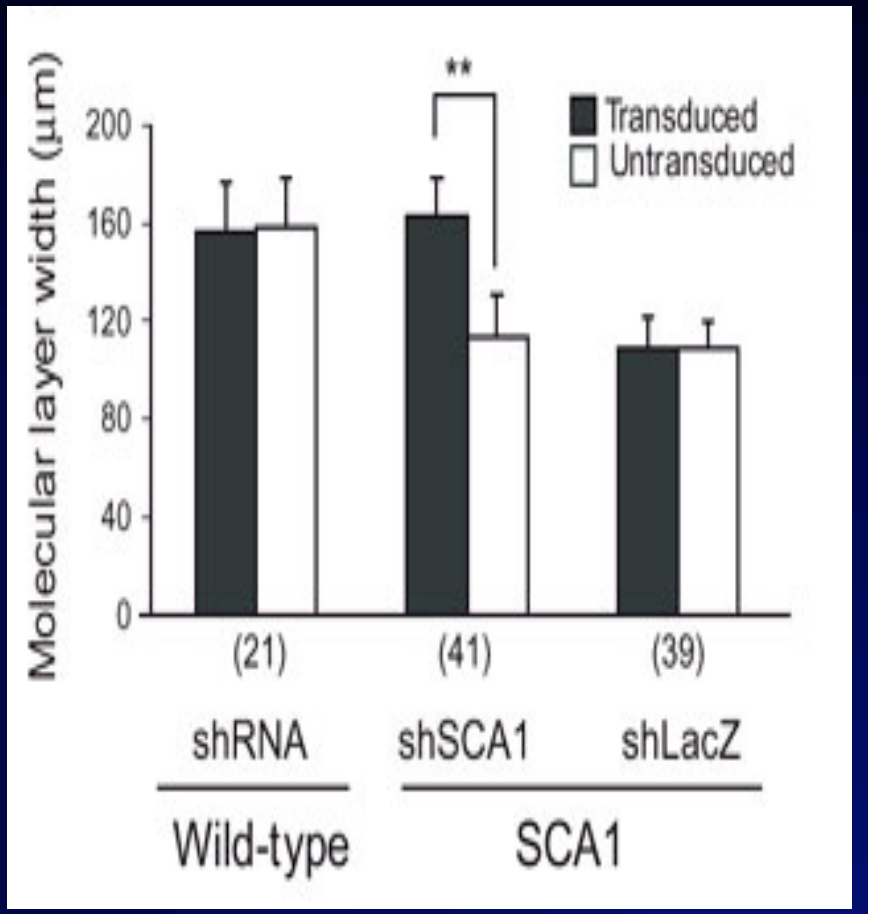
Haibin Xia et al. Nature Med 2005

- Pcp2-ATXN1[Q82]
- AAV-shRNA
- Small HairpinRNA
- GFP marker



Functional Recovery and Morphological protection.





siRNA-mediated Knock-down SCA1

- Pros
- Potential for selective knock-down of mutant allele
- Most rational therapy
- Cons
- Purkinje cell limited disease model
- Viral delivery to multiple sites
- Viral persistence

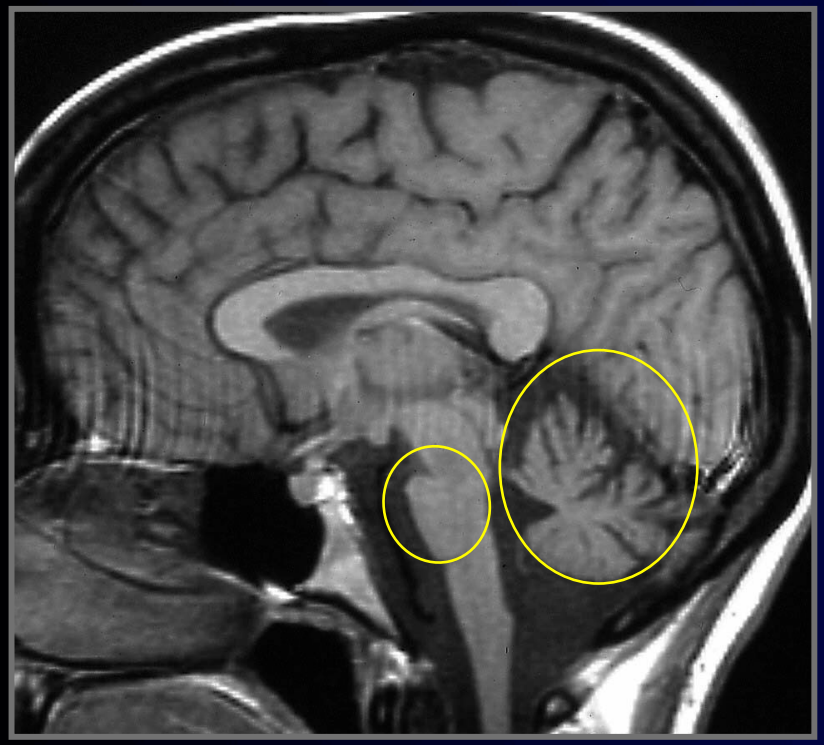
Can small molecules be identified that down-regulate *ATXN2* expression?

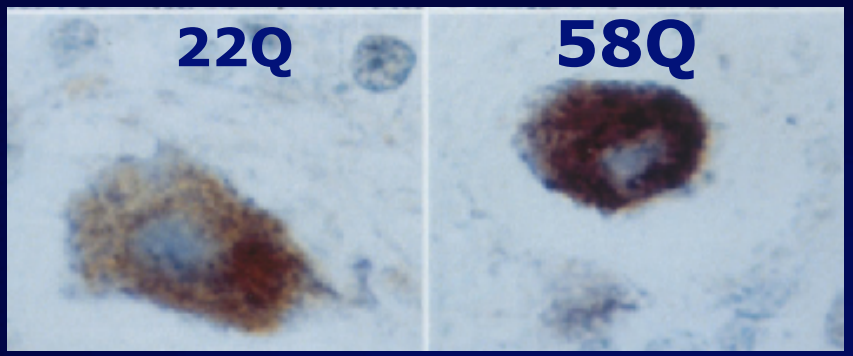
- Potential targets:
  - Regulatory elements (promoter)
  - 5'- UTR
  - 3'- UTR
- Emphasis on expression level, not on downstream normal or toxic functions

SCA2: Phenotype & Gene

Normal: 22Q

Mutant: ≥32Q





Pulst et al Nature Genet 1996

Evidence for Dosage

- Models:

  Double transgenics sicker

- Humans:

  Rare homozygotes: earlier onset

- Other diseases:

  - Duplication of wildtype APP and α-synuclein

- Reversal of expression of mutant allele reverses Phenotype even after disease onset !

  SCA1: Zu et al. J Neurosci (2004)

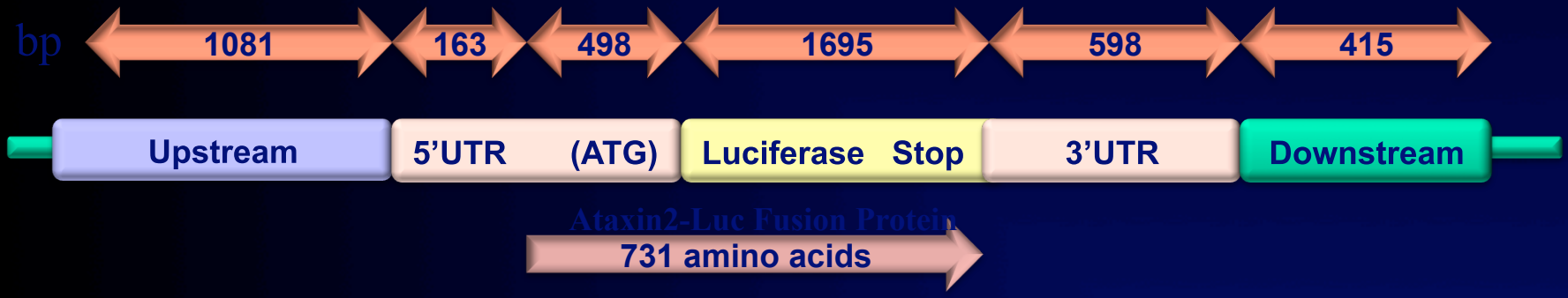
  SCA3: Boy et al. Hum Mol Genet (2009)

Sca2 Knock-out

- Subtle behavioral deficits
- No Neurodegeneration

Kiehl et al 2006, Huynh et al 2009

HEK Cells stably expressing pGL2-ATXN2-Luciferase



Stably expressing HEK and SHY cell lines

Libraries Screened and Hits

Number of compounds = 68,297, all at 10μM final conc.

Library	Source	Plates	Compounds	Hits (3SD)
Enzymes	Biomol International	1	300	7
Lipids	Biomol International	1	204	0
PW	Prestwick Chemical (FDA approved)	4	1120	27
MS	Microsource Spectrum	7	2000	41
TAR	Targeted Library	27	9504	19
NIH	BioFocus DPI (NIH Clinical Collection)	2	607	15
ES	Unpublished	4	1408	7
S	Unpublished	4	1408	2
DL	Asinex (Platinum Collection)	53	18,656	74
UCLA	ChemBridge Corp.	94	33,088	171

Total hits(3SD)=363 Hit rate=0.5%

Further Evaluation of Lead Compounds

- In vitro
- Titration in HEK cells
- Mapping onto deletion constructs

- Neuronal cell lines
- Tests in animal model
- Luciferase mouse
- BAC model

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Lithium Therapy Improves Neurological Function and Hippocampal Dendritic Arborization in a Spinocerebellar Ataxia Type 1 Mouse Model.

Watase et al. PLoS Medicine 2007

- Lithium suppresses neurodegeneration induced by various kinds of insults:

  - Alzheimer disease amyloid-β peptide
  - Glutamate
  - Ischemia

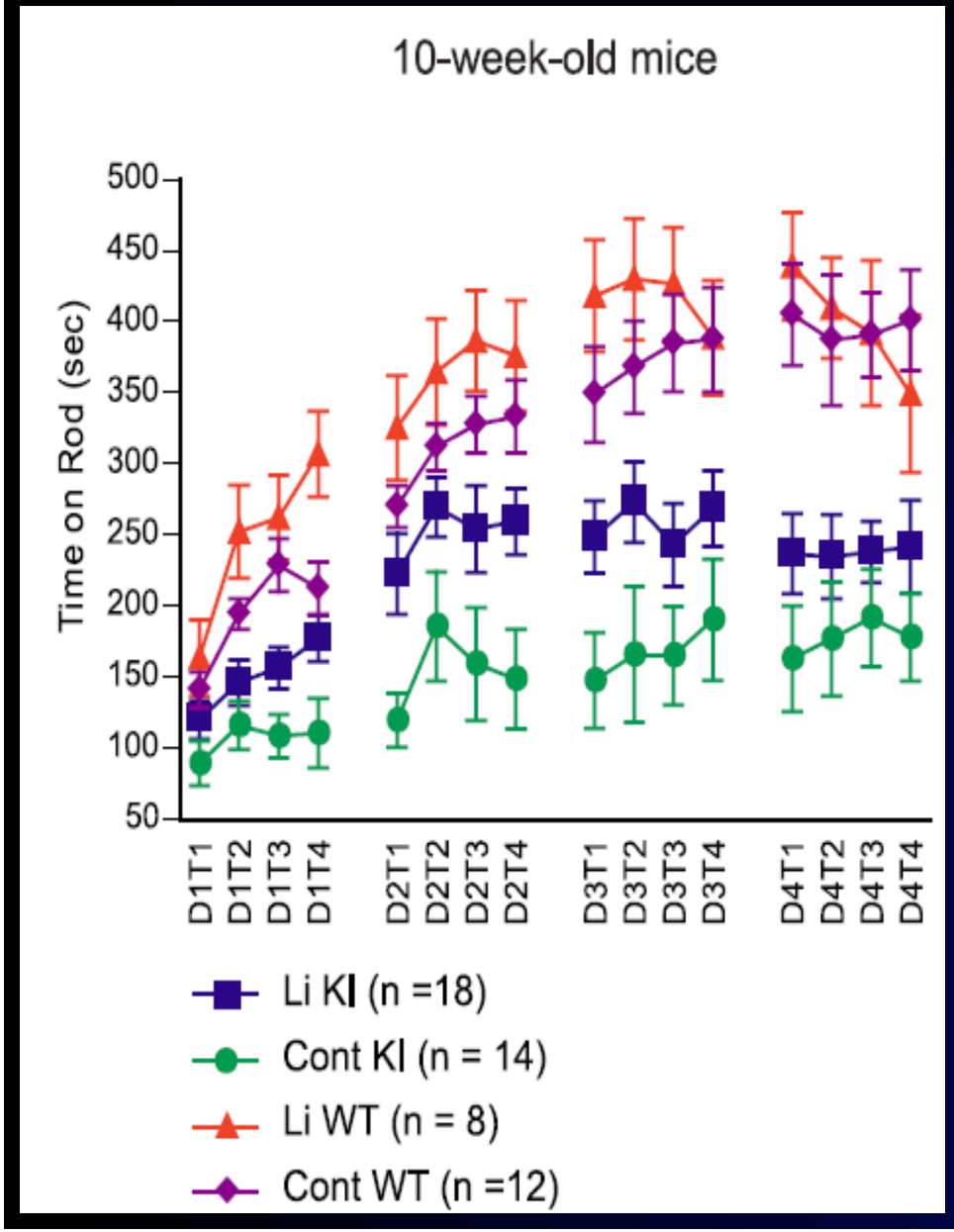
Lithium

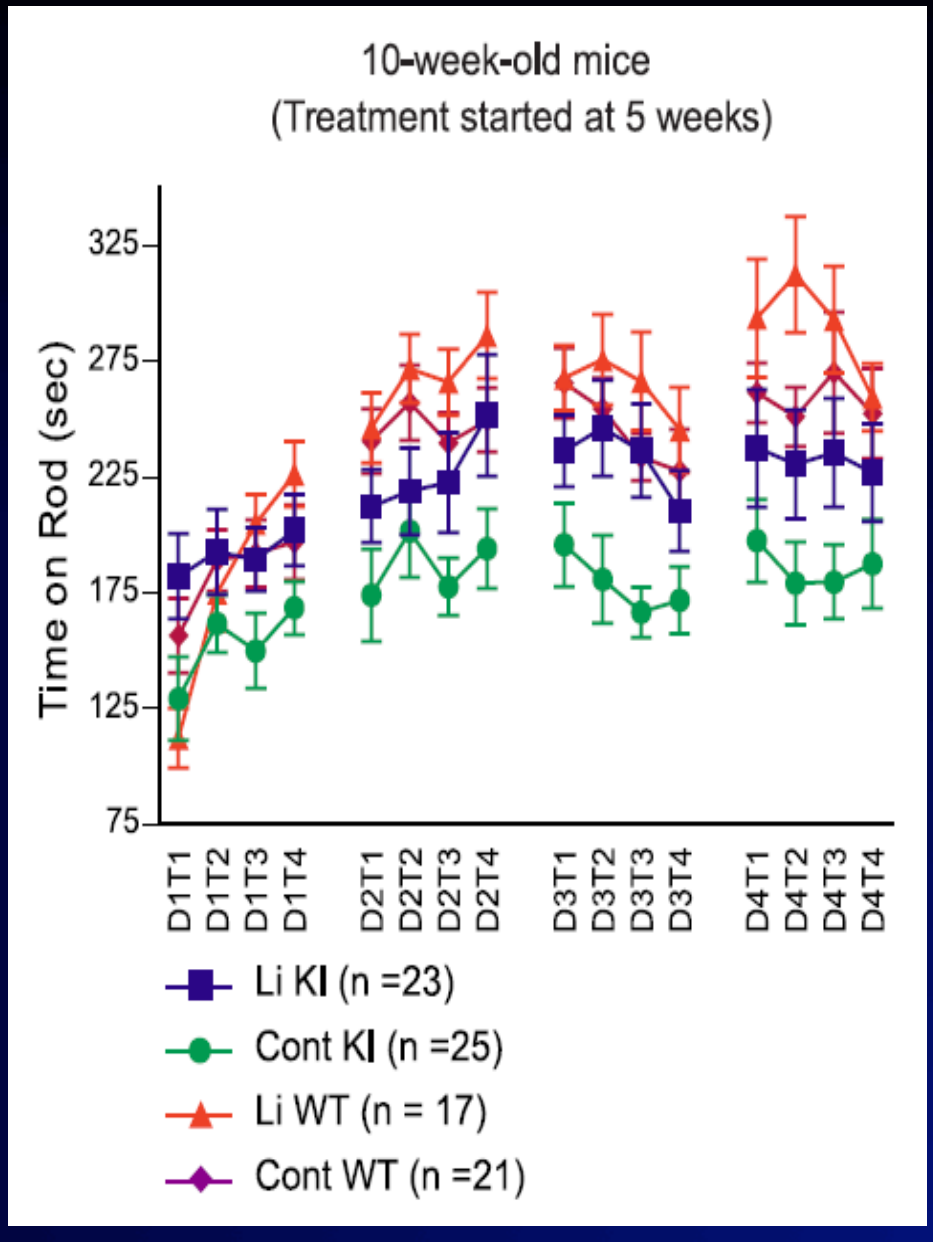
- Model:
- SCA1 knock in CAG 154

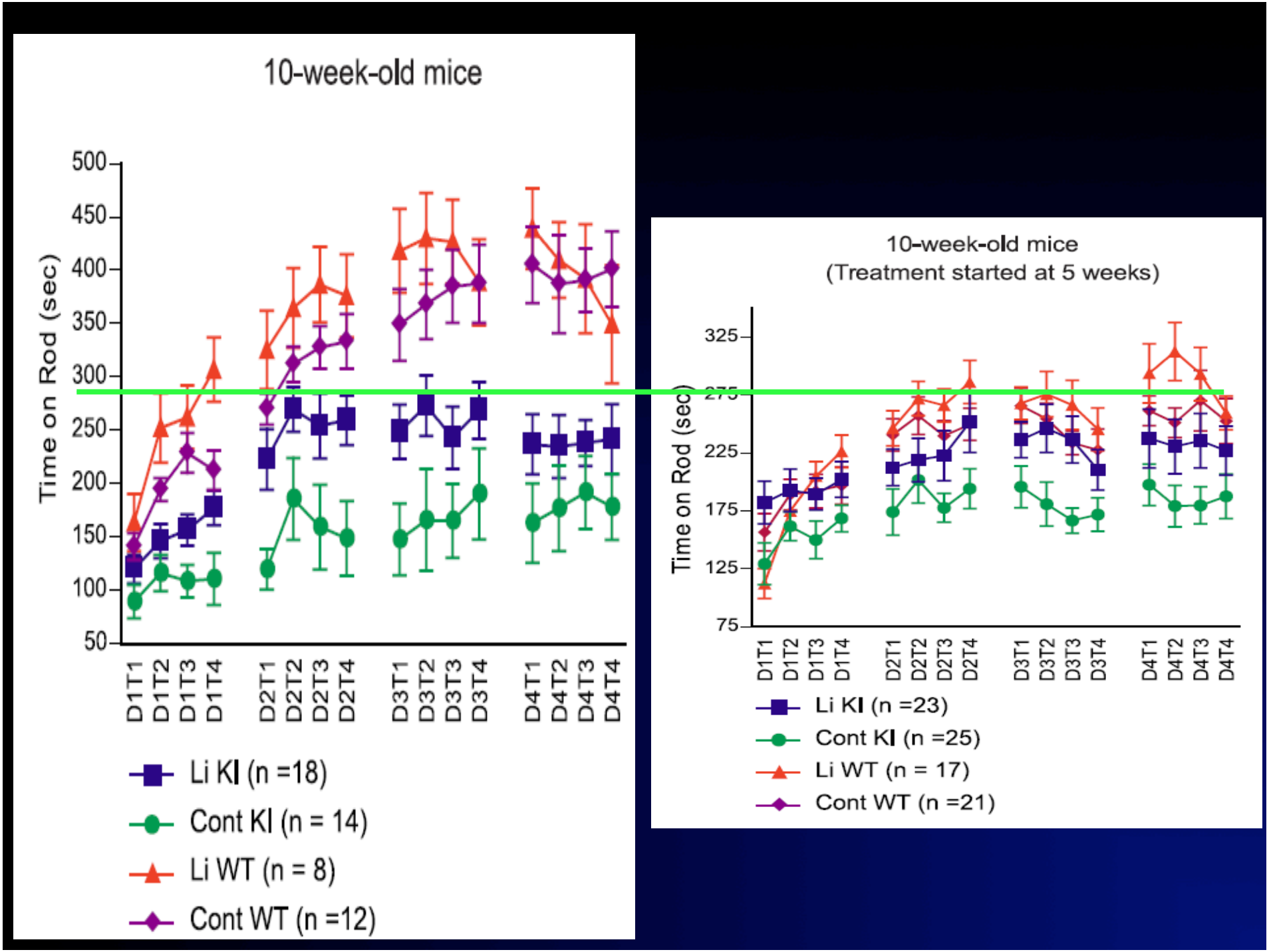
- Outcome
- Morris Water Maze
- Rotarod
- Intermediate phenotypes: βGST, Pccmt

Watase et al. Plos Medicine 2007

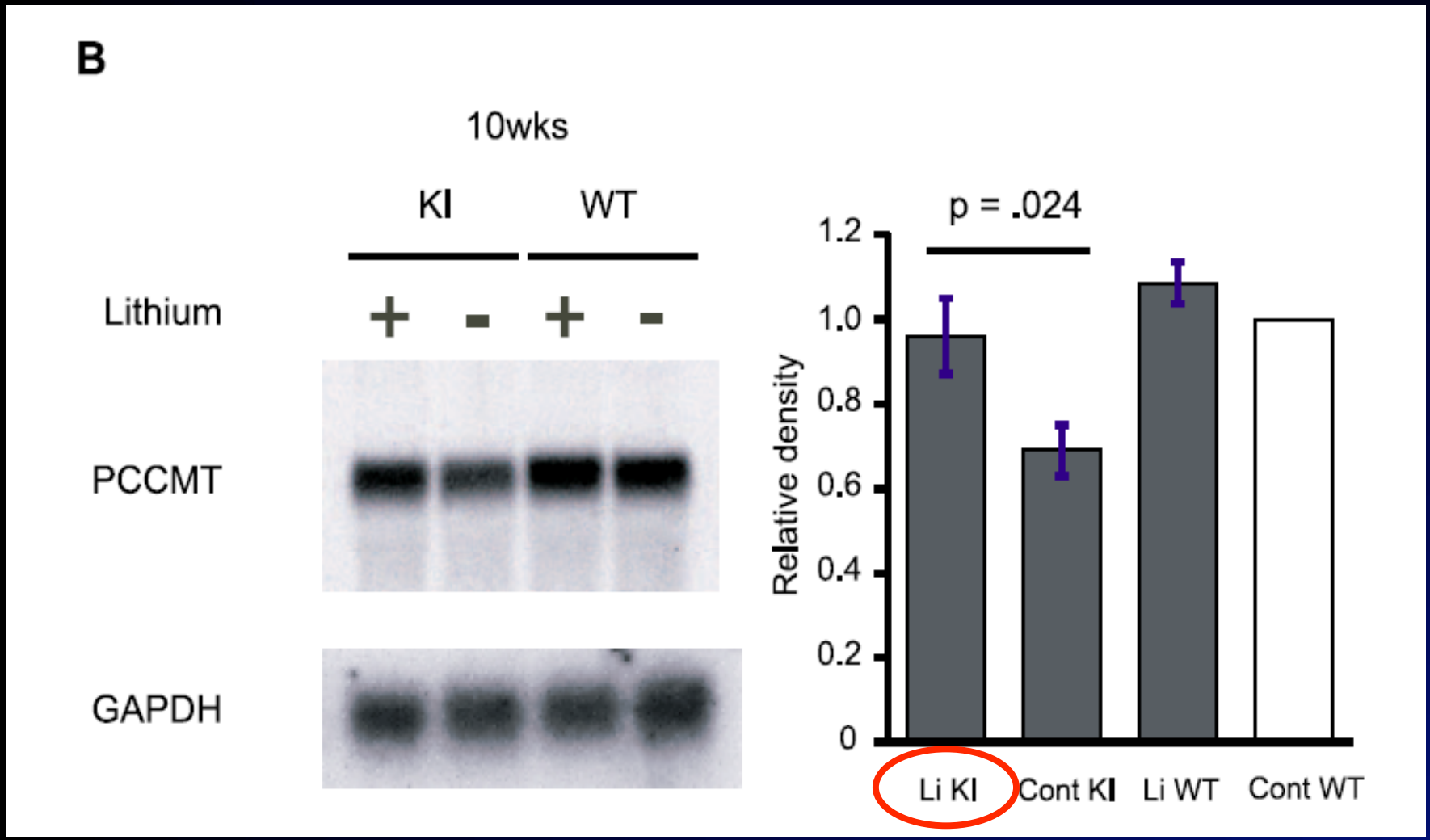
Rotarod Performance with Li Treatment






10-week-old mice
Time on Rod (sec)
500
450
400
350
300
250
200
150
100
50
D1T1
D1T2
D1T3
D1T4
D2T1
D2T2
D2T3
D2T4
D3T1
D3T2
D3T3
D3T4
D4T1
D4T2
D4T3
D4T4
Li KI (n =18)
Cont KI (n = 14)
Li WT (n = 8)
Cont WT (n =12)
10-week-old mice
(Treatment started at 5 weeks)
Time on Rod (sec)
325
225
175
125
75
Li KI (n =23)
Cont KI (n =25)
Li WT (n = 17)
Cont WT (n =21)

Lithium increases cerebellar Pccmt levels.



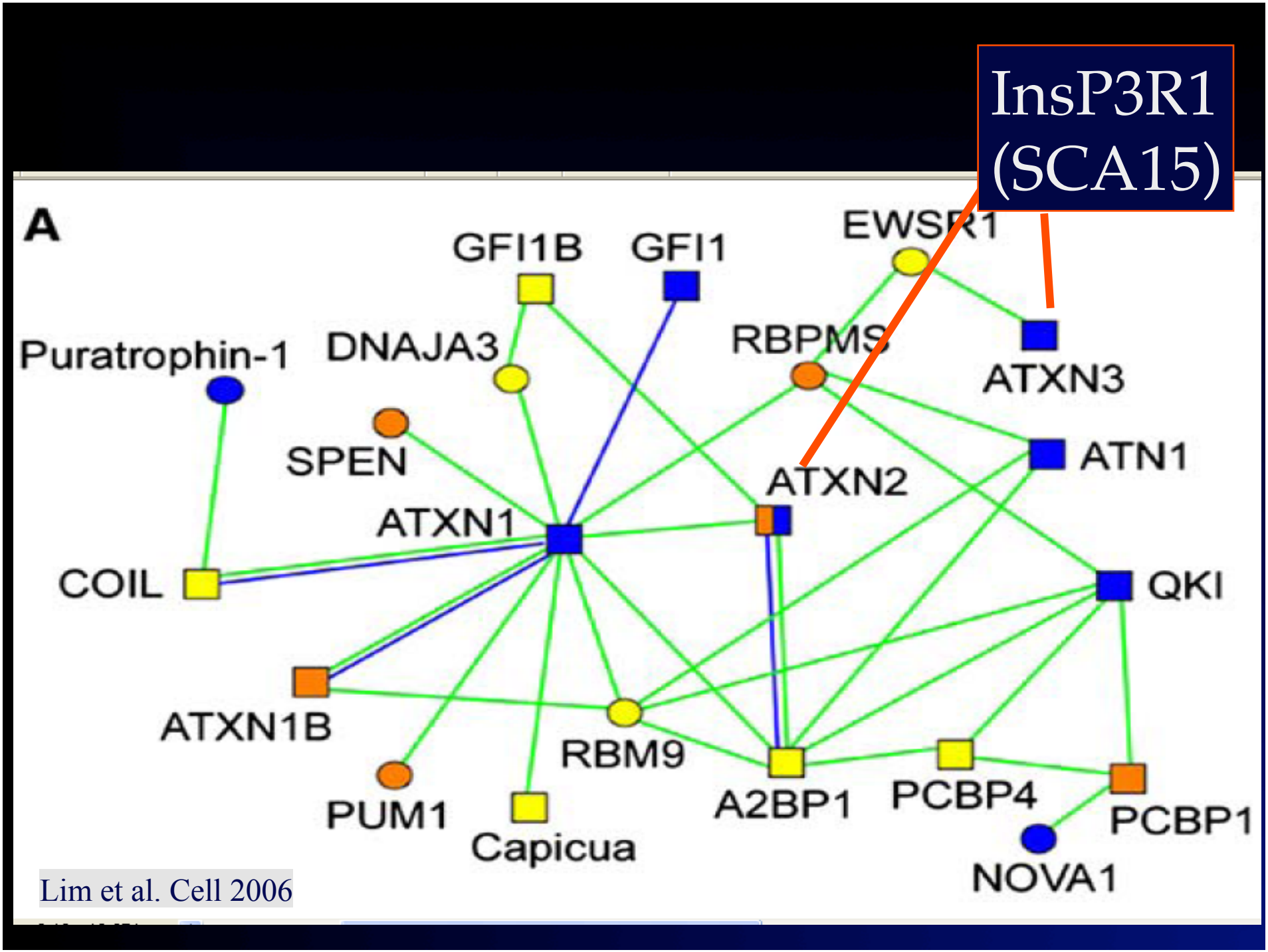
- Pros:
- Knockin model
- Effect on intermediate phenotypes and multiple functional outcomes
- Effect in two strain backgrounds
- Lithium in human use
- Cons:
- Non-physiologic CAG repeat length
- Dendritic loss in PCs unchanged
- Lithium is PC-toxic at high levels in humans

Dantrolene in SCA2 Purkinje cells and the tg-Pcp2-atxn2[Q58] mouse model.

Deranged Calcium Signaling and Neurodegeneration in Spinocerebellar Ataxia Type 2.

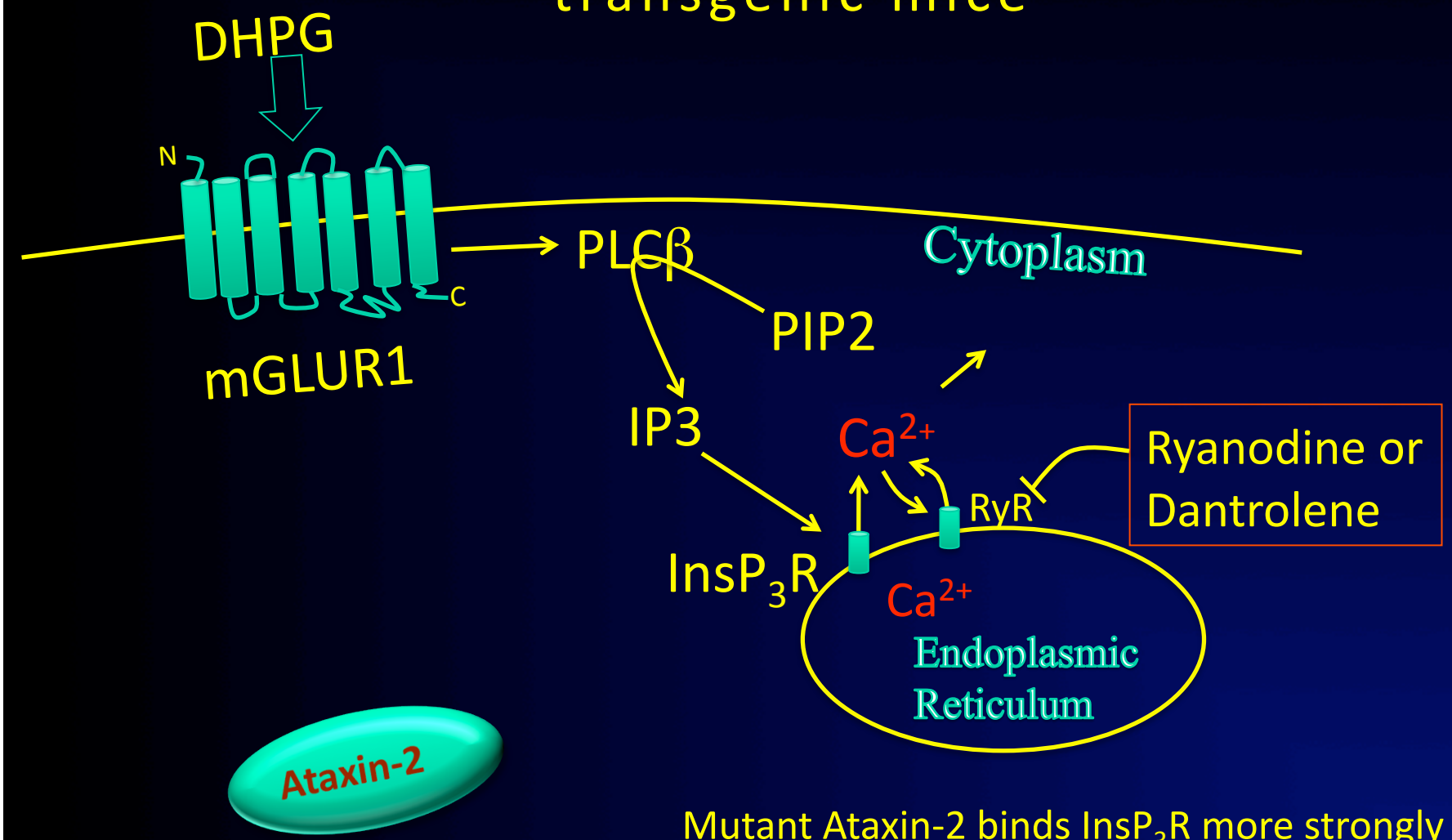
Jing Liu Tie-Shan Tang, Huiping Tu, Omar Nelson, Emily Herndon, Duong P. Huynh, Stefan M. Pulst and Ilya Bezprozvanny

J Neurosci (2009)



Lim et al. Cell 2006

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



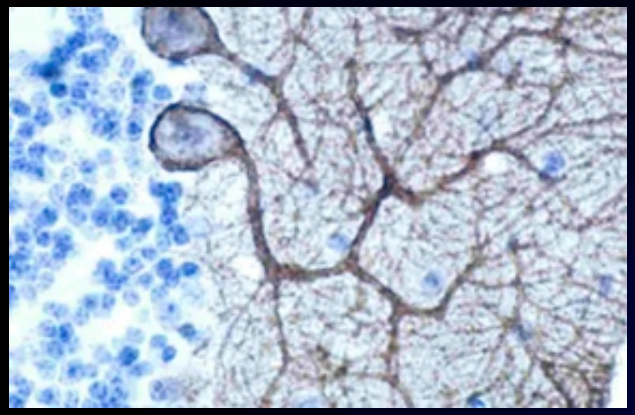
Mutant Ataxin-2 binds $InsP_3R$ more strongly to mediate Ca^{2+} exit from ER .

The tg-Pcp2-ATXN2[Q58] mouse

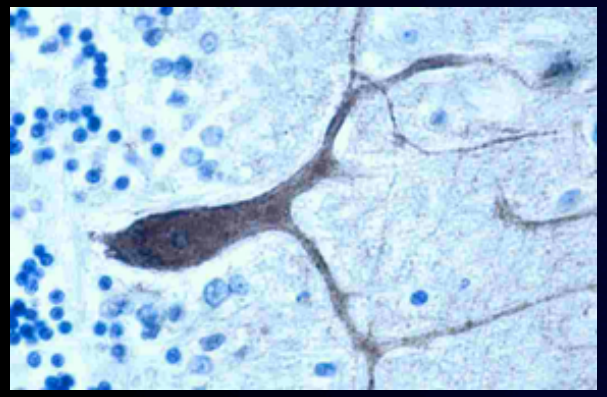
Promoter: Purkinje cell specific PcP2

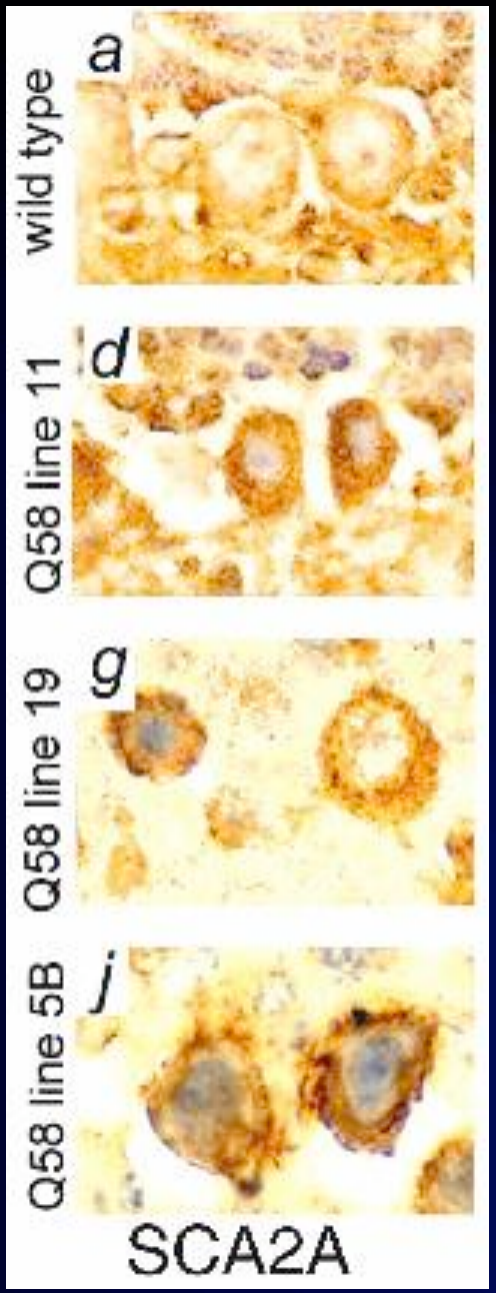
Huynh et al Nat Genet 2000

Human control

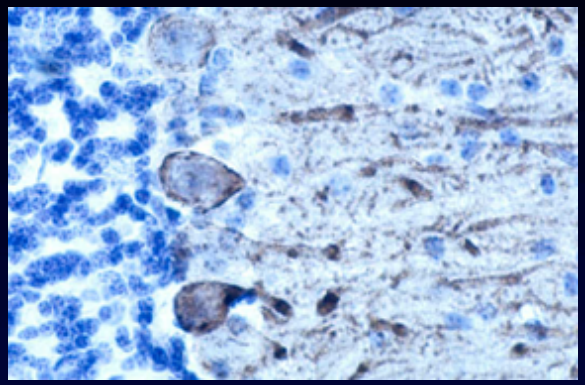


Wildtype Mouse

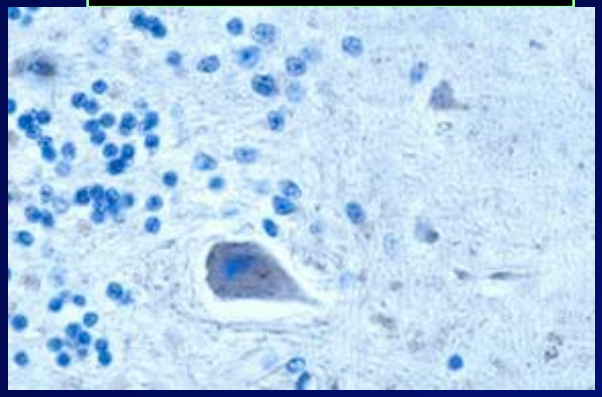




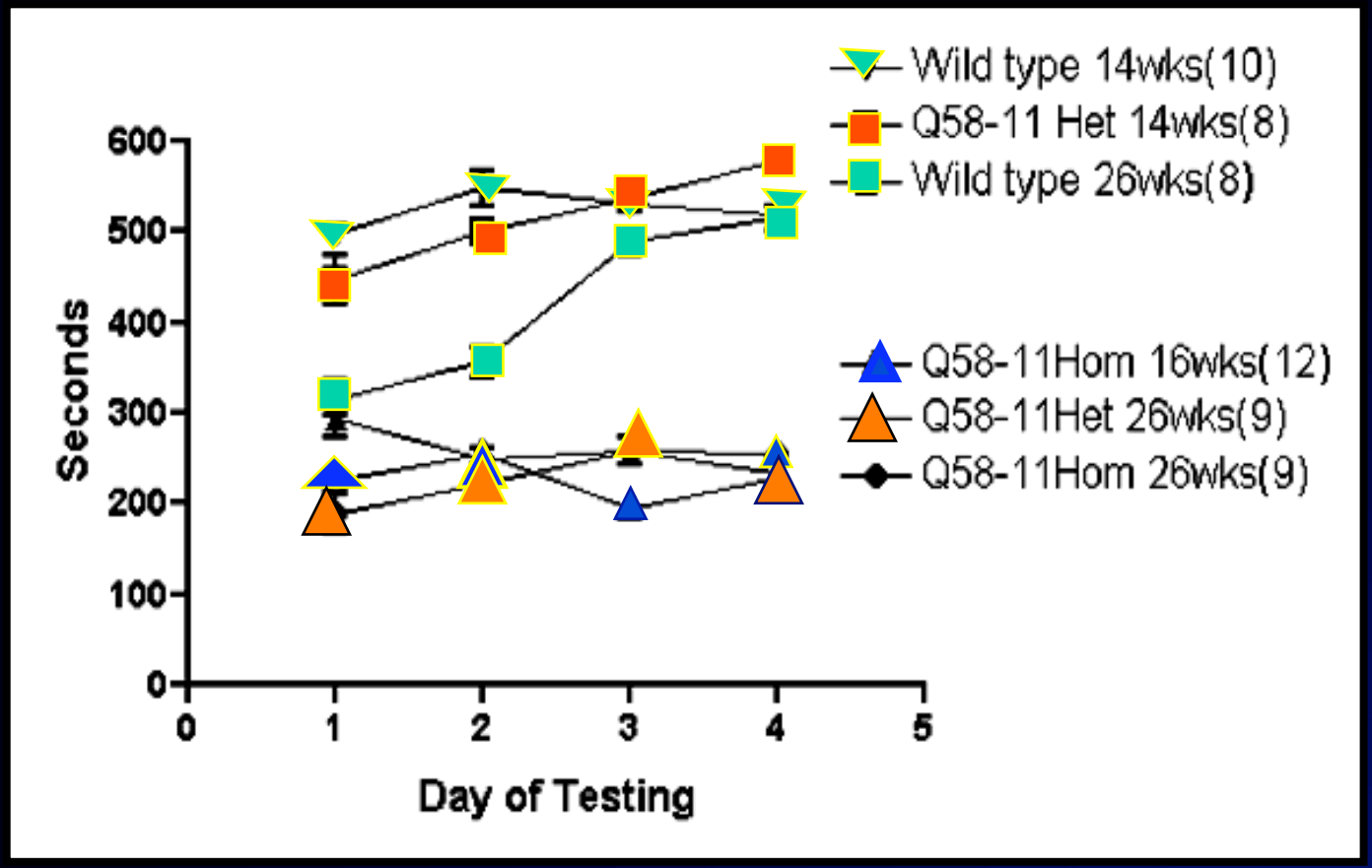
SCA2 patient



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



Functional Analysis



Huynh et al Nat Genet 2000

Differential interaction for wt and mutant ATXN2 with InsP3R1

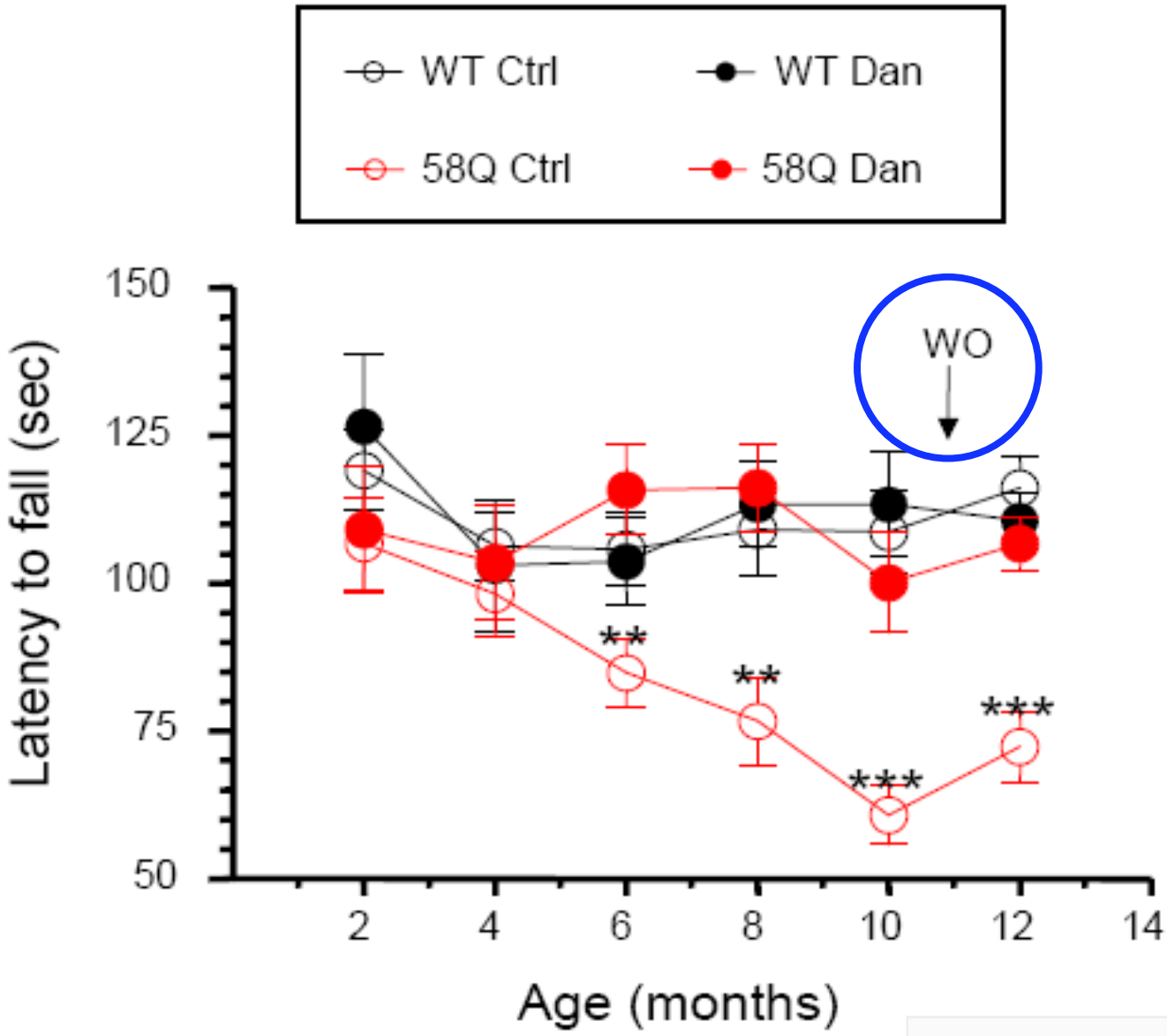
Exaggerated responses in 58Q PCs to DHPG stimulated Ca release

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

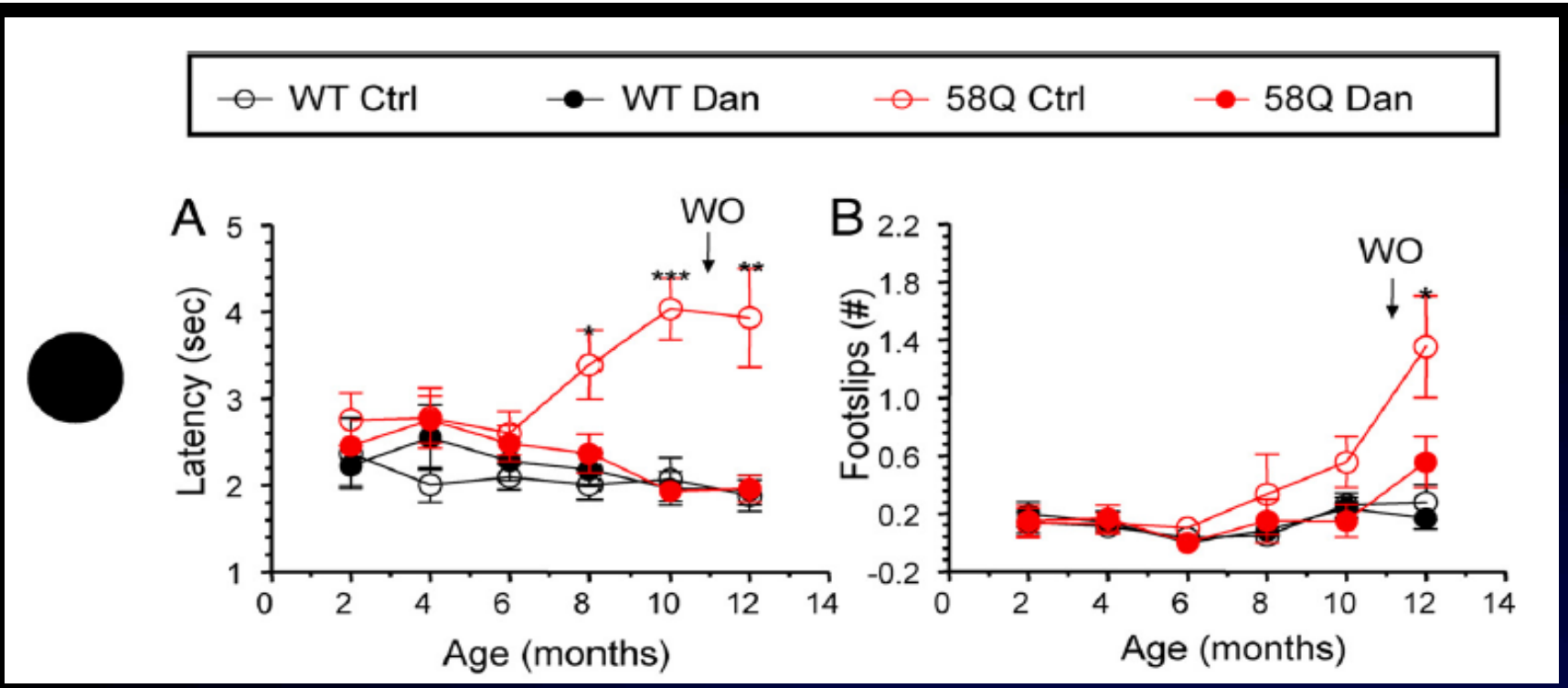
Dantrolene recovery of cellular phenotype in 58Q PCs in vitro

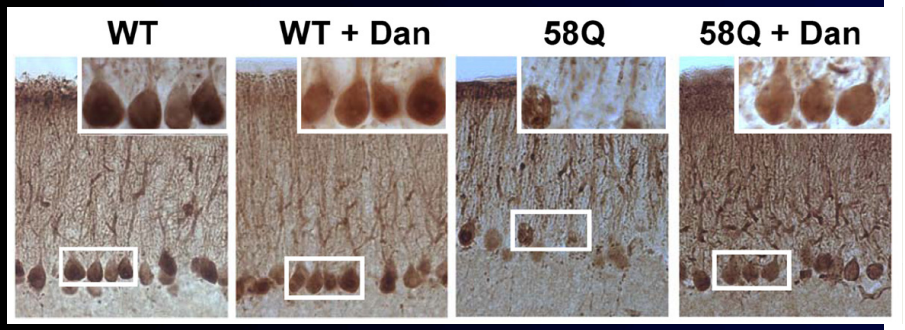
Does dantrolene have an effect *in vivo* ?

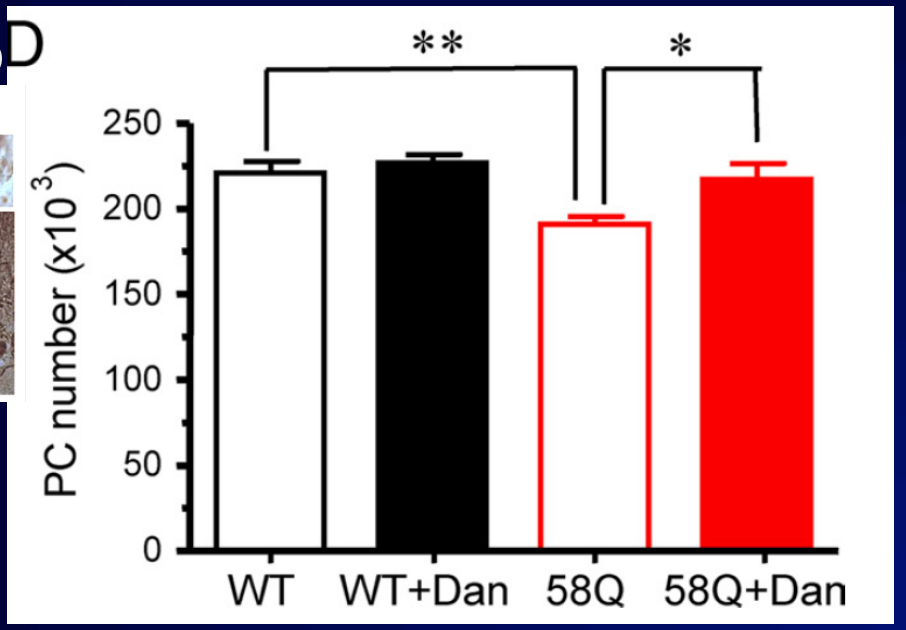
Liu J et al J Neurosci 2009



Liu J et al J Neurosci 2009







Liu J et al J Neurosci 2009

Dantrolene Trial in SCA2:

- Pros
- Effects on motor function and PC number
- Wash-out period
- Dantrolene used in humans
- Cons:
- Treatment started before symptom onset
- PC-specific model
- Wash-out potentially not long enough
- Dantrolene causes Muscle weakness in humans

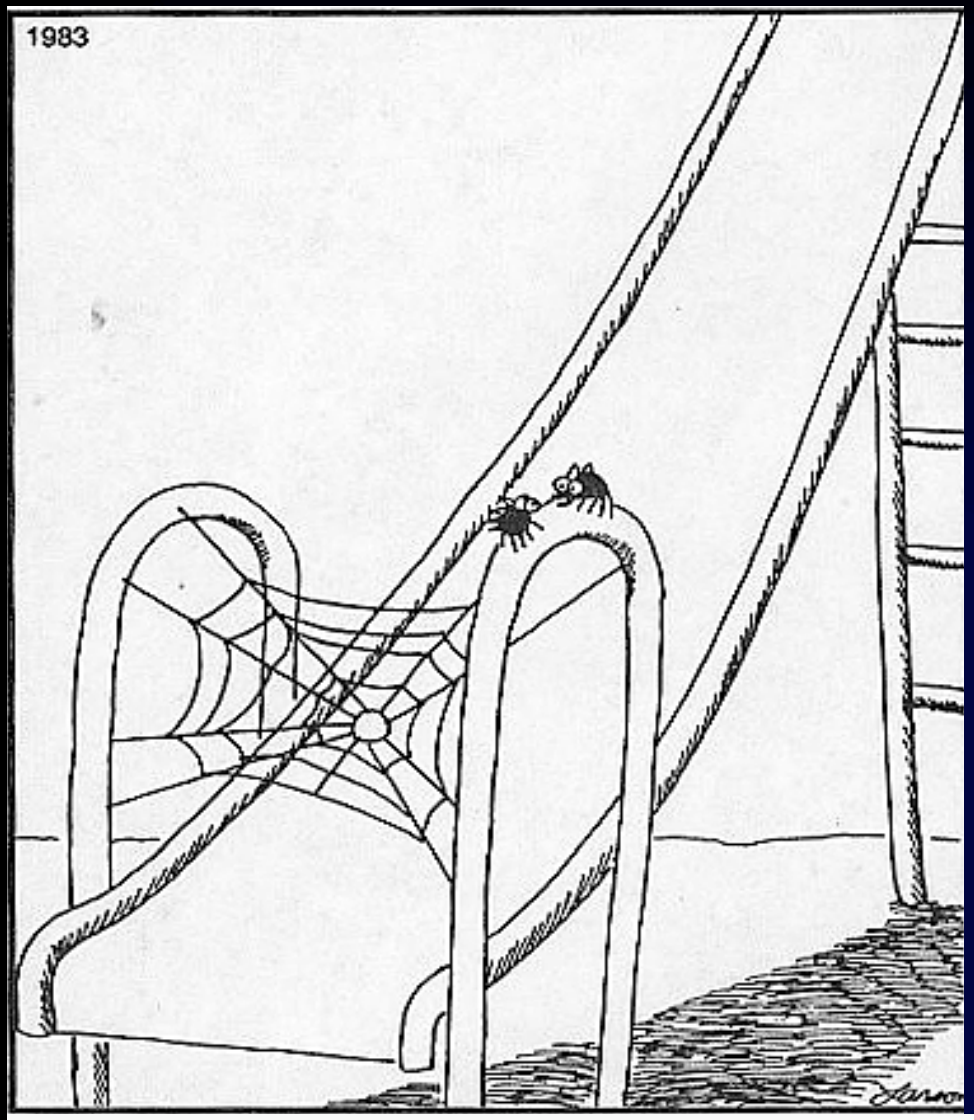
Mice now have unprecedented choices for the treatment of their ataxia !

- Gene therapy with shRNAs
- Small compounds
- ? Ready for Phase 1in humans
- Lithium
- Dantrolene

Criteria for Ataxia Mouse Clinical Trials

- Registration with pre-specified outcome criteria and power analysis
  - ≥ 2 models
  - ≥ 2 outcome tests
  - ≥ two backgrounds
  - Multi-center (conducted in ≥ 2 labs)
- Publication of Negative results
- “Validation” of model(s) by human trials

Collaborators



- Small Molecule Screen
  - Daniel Scoles, Ph.D.
- 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.