What we are learning from animal models of hereditary ataxias

PolyQ: a common cause of hereditary ataxias.

- PolyQ diseases are a subset of dominant neurodegenerative diseases due to the instability of multiple CAG triplets in the coding region and translated in a stretch of Q (Glutamine)

- The same mutation in several different ubiquitously expressed proteins leads to different pathologies

Among these diseases are:
- Huntington disease
- dentatorubralpallidoluysian atrophy (DRPLA)
- spinal and bulbar muscular atrophy
- several forms of spino-cerebellar ataxia

- DRPLA is caused by Atrophin-1, a ubiquitous transcriptional co-factor

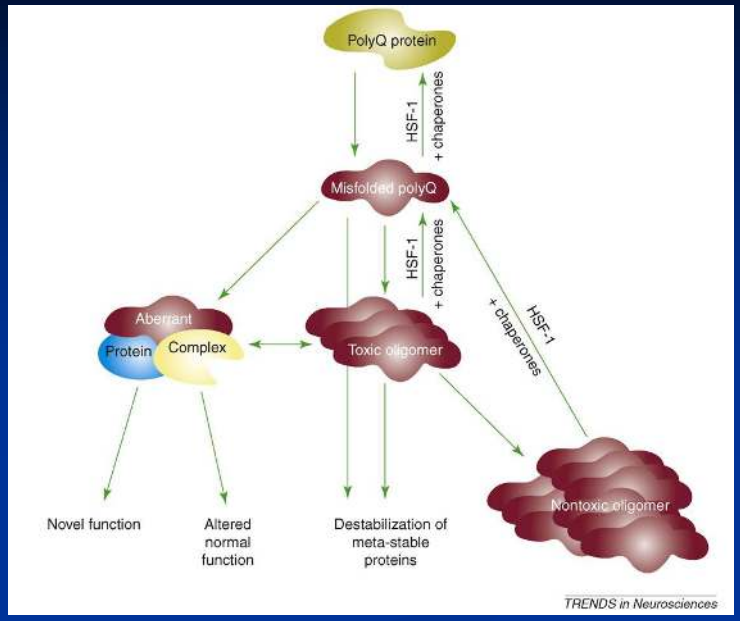
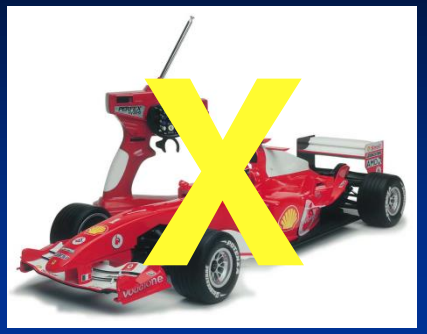


Table 4. Spinocerebellar ataxias (SCA): normal and expanded nucleotide repeat sequences.*

SCA	Nucleotide repeat	Normal	Expanded
SCA 1	CAG	6–39	40–82
SCA 2	CAG	14–31	33–64
SCA 3	CAG	12–42	54–86
SCA 6	CAG	4–18	19–30
SCA 7	CAG	4–27	37–200
SCA 8	CTA/CTG	16–91	107–127
SCA 10	ATTCT	10–21	800–4500
SCA 12	CAG	7–32	55–78
SCA 17	CAG	25–44	47–63
DRPLA	CAG	6–36	49–79

*Modified from Pulst and Subramony[8,18].

Modelling ataxias in animals: does it make sense?



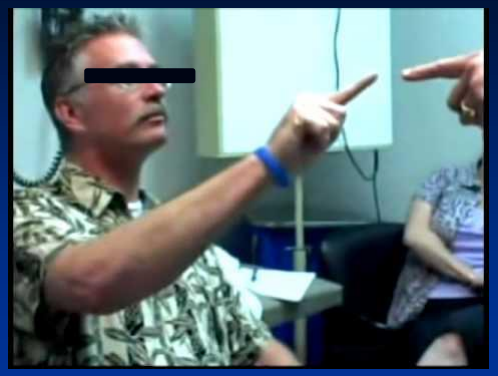
A model is a system that because of its simplicity and knowledge of its parts allow us to establish rules and connections between different elements.

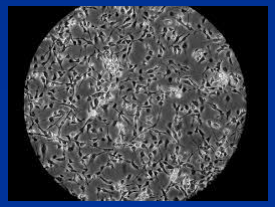
This knowledge can then be translated to more sophisticated models with approximations that balance for the peculiar features of each system

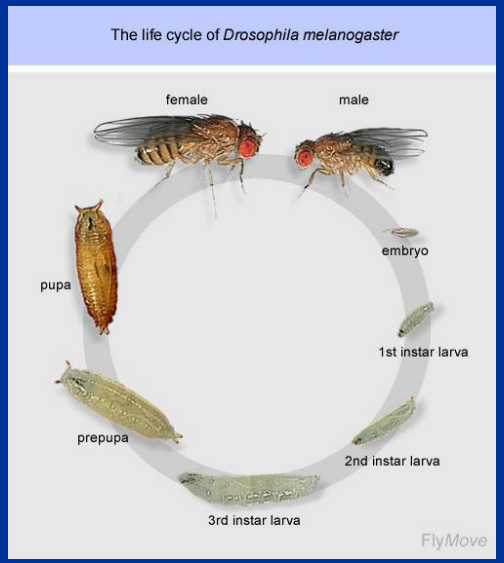
Corollary: There is no ideal model.

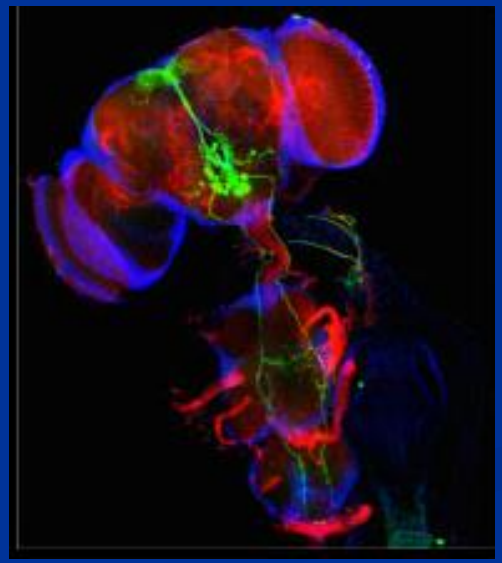
Our pipeline

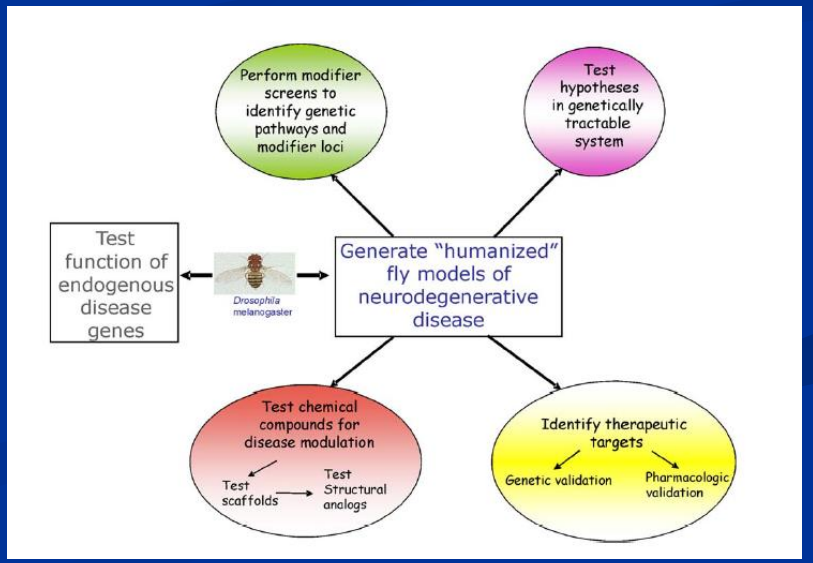
?



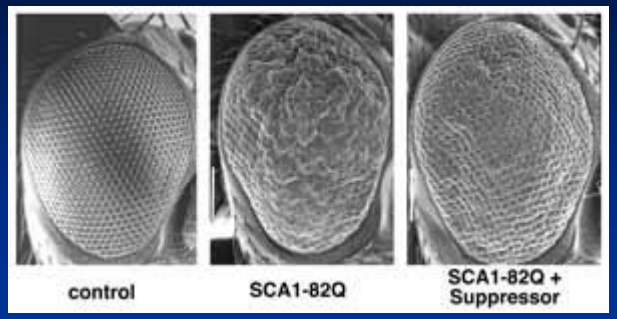


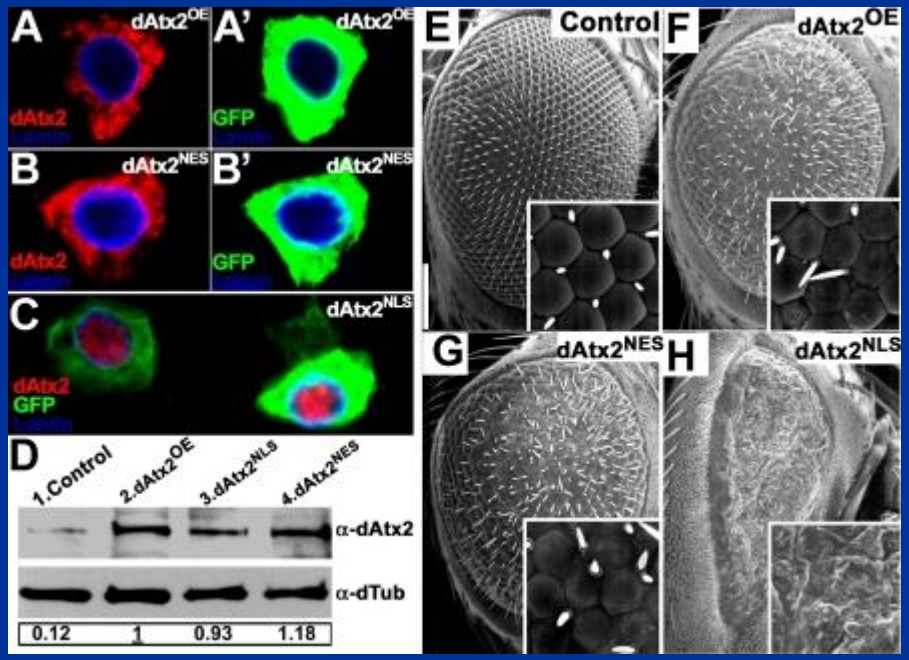


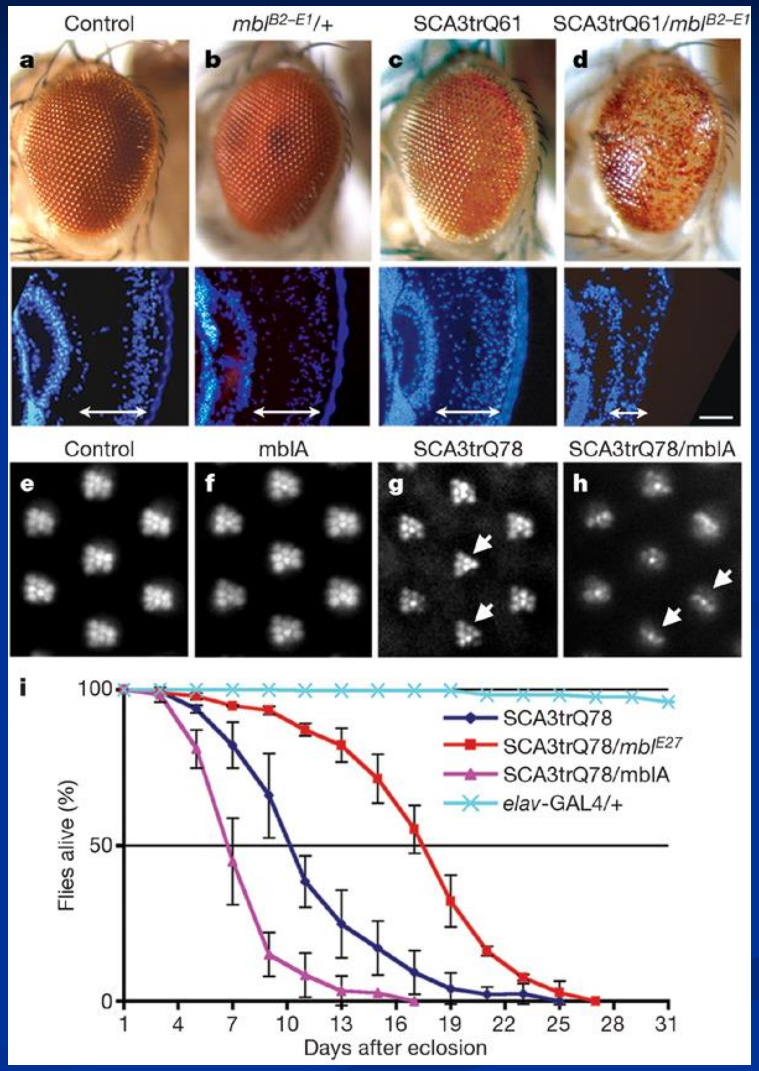




Drosophila models of polyQ ataxias

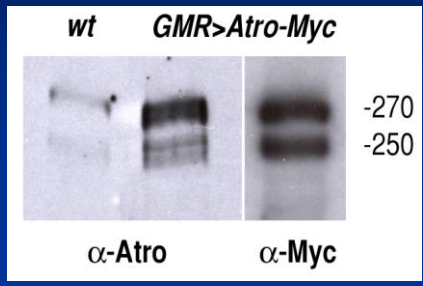






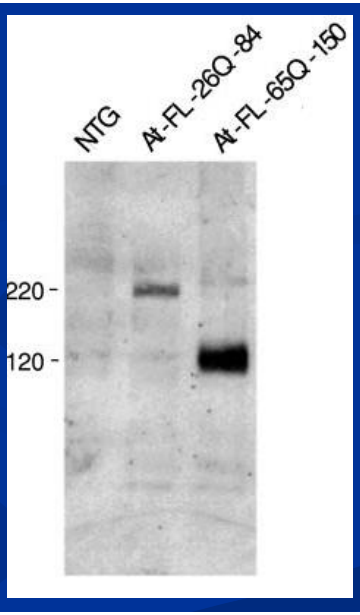
Making of DRPLA models: Atrophins in *Drosophila* and mammals

Drosophila Atro

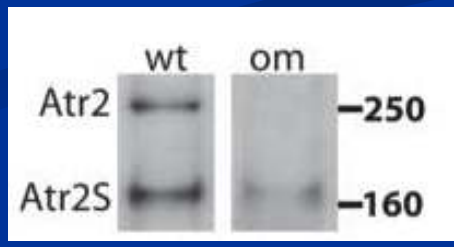


- **Human Atrophin-1 causes DRPLA**
- **Atrophins are transcriptional bimodal cofactors capable of repressing or activating transcription**
- **They are ubiquitously expressed and are present both in the nucleus and in the cytoplasm**

Mammalian Atrophin-1



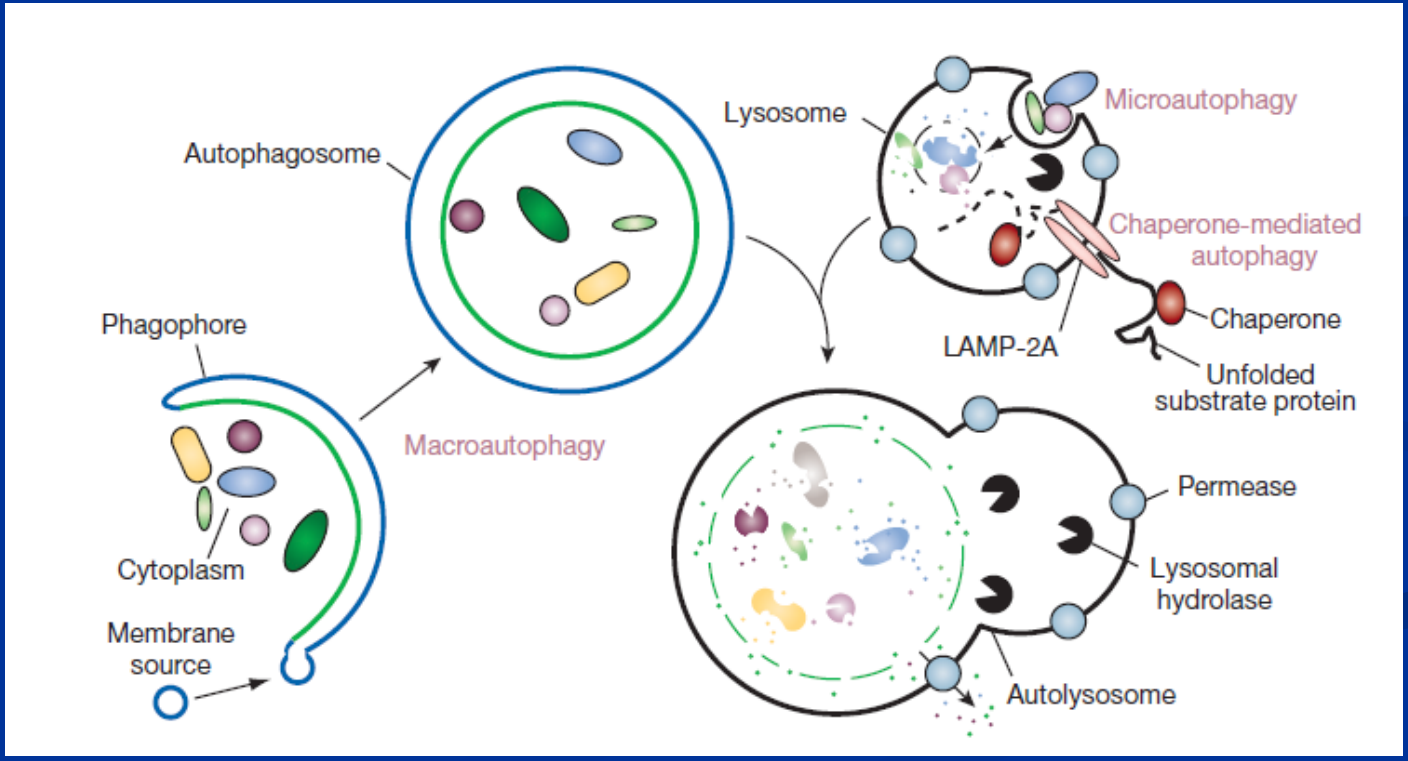
Mammalian Atrophin-2



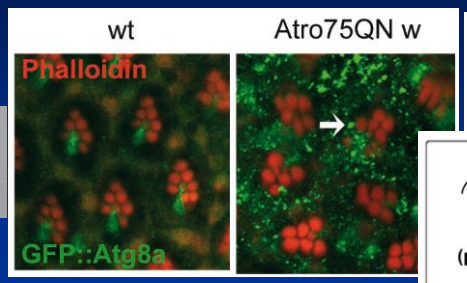
Autophagy

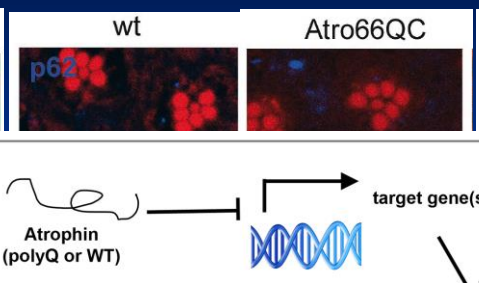
- Autophagy is a catabolic pathway that degrades proteins and cellular components, selectively or bulk, through lysosomal digestion.

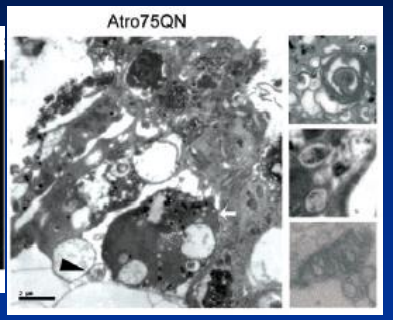
- Regulation of autophagy is critical to long lived cells like neurons and defects can lead to cell death. As such autophagy is a key player in neurodegeneration.



Autophagy defects

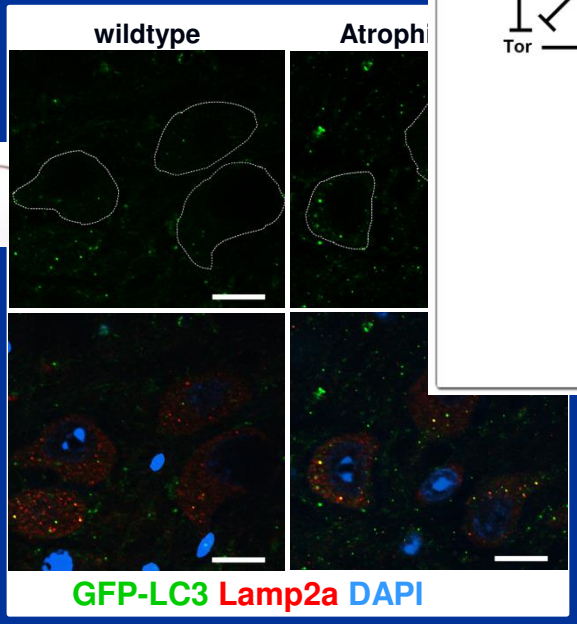


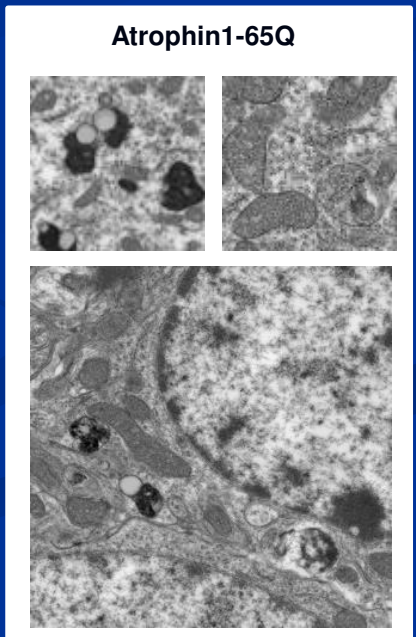




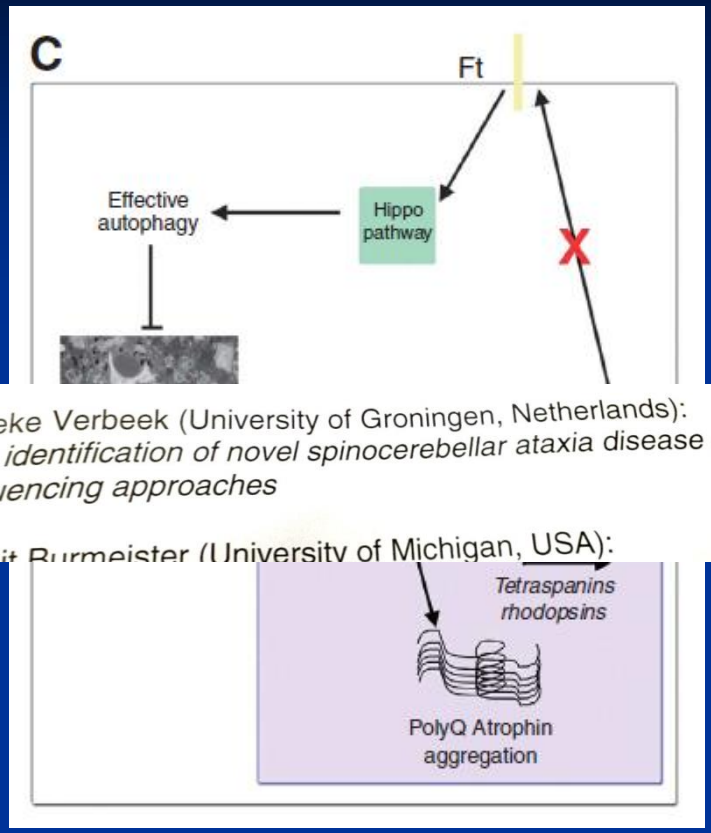
GFP::LC3 (Atg8)
Autophagosomes

EM



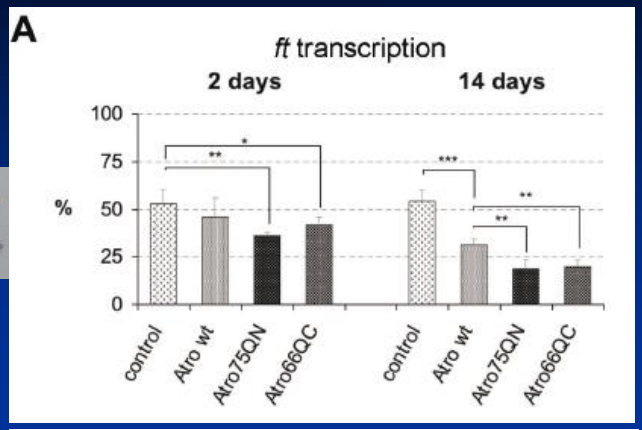


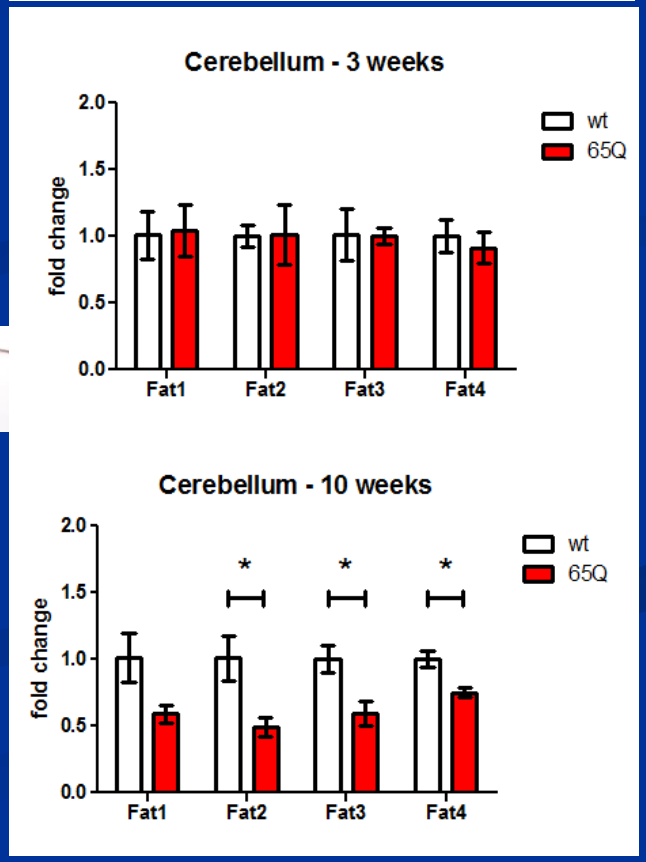
Downregulation of *fat* cadherins



Dineke Verbeek (University of Groningen, Netherlands):
The identification of novel spinocerebellar ataxia disease genes using next generation sequencing approaches

Margit Burmeister (University of Michigan, USA):

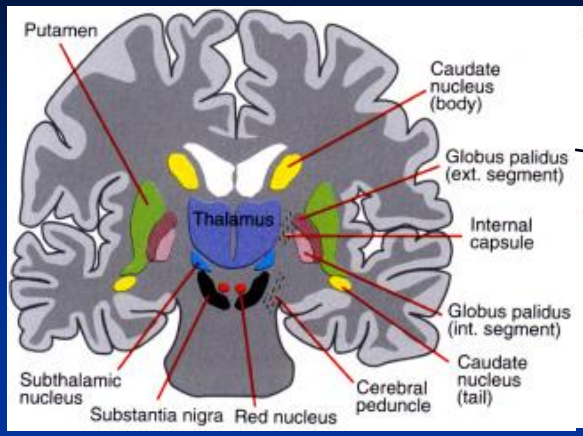


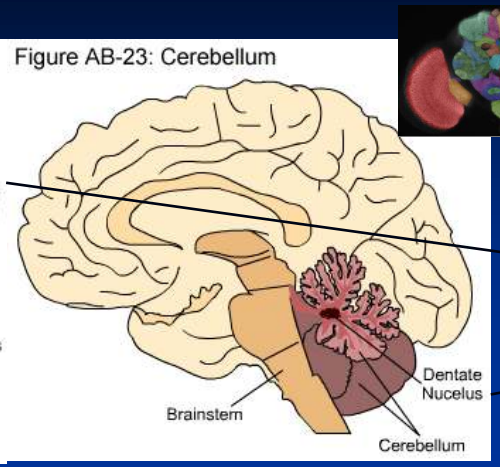


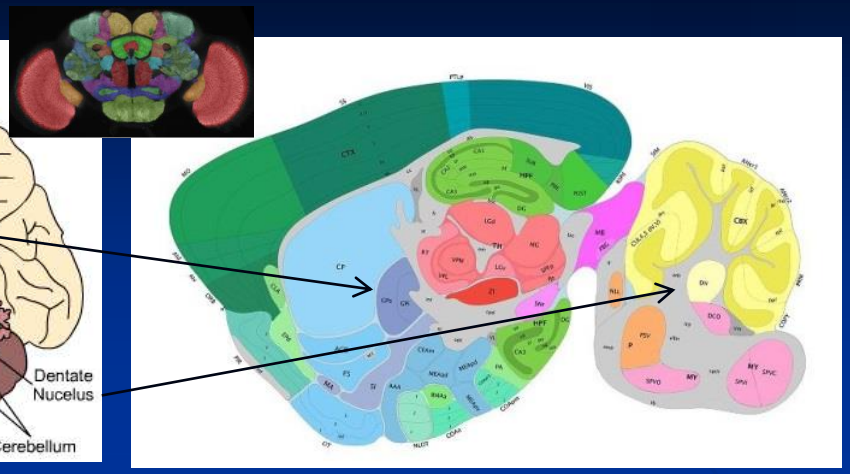
- **Fat2, Fat 3 and Fat4 follow the same pattern of regulation than in *Drosophila*.**

- **Potentially also Fat1 is downregulated but further experiments are required.**

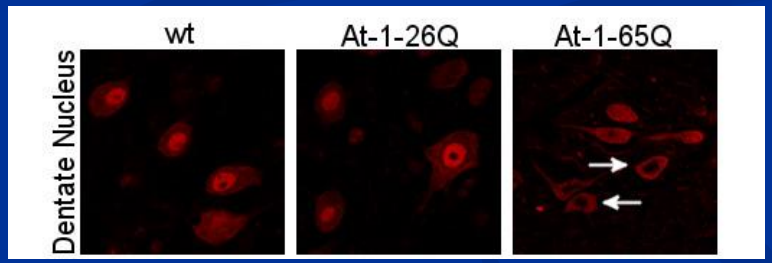
So, do we need mice only to confim flies?





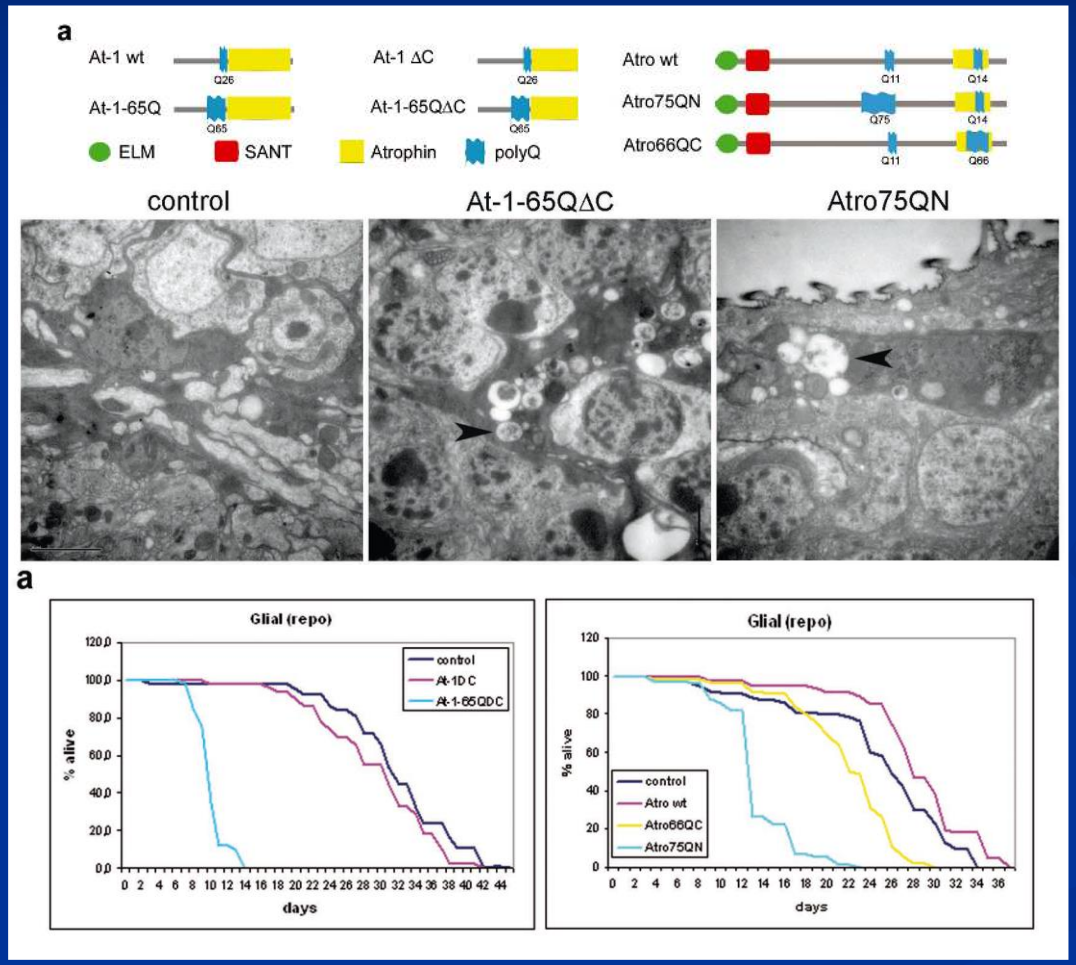


- Mice have anatomical brain areas that are (more) similar to humans, so beside confirmation they provide critical regional specificity.
- So we can address what is specifically wrong in Dentate Nucleus and Red Nucleus



- Rbfox-3 (NeuN) is lost from the nucleus
- Rbfox proteins (-1,-2 and -3) regulate splicing (interestingly of Fat1, Fat2 and Fat3 among others) control, and are controlled by, neuronal excitability.
- Rbfox-1 and Rbfox-2 physically interact with Atrophins (and also Ataxin-1 and -2)
- Rbfox-1 and -2 ko mice have ataxia and epileptic seizures (like DRPLA)

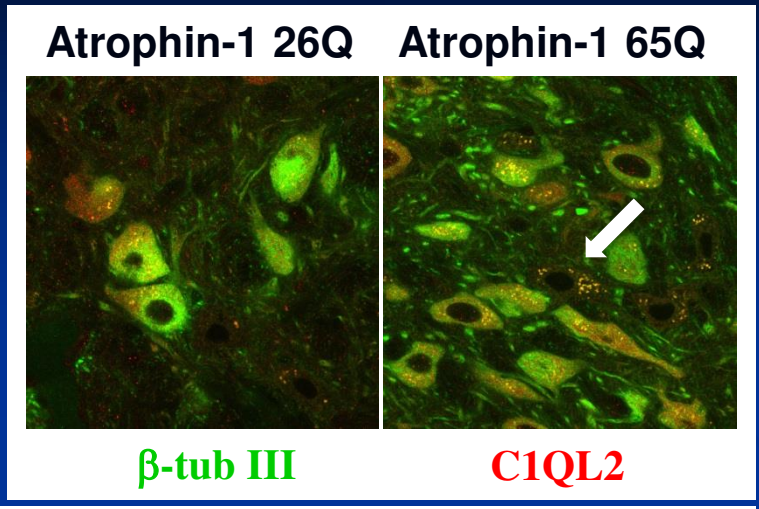
Neuronal (loss of) homeostasis as a result of glial autophagic (dys)-function

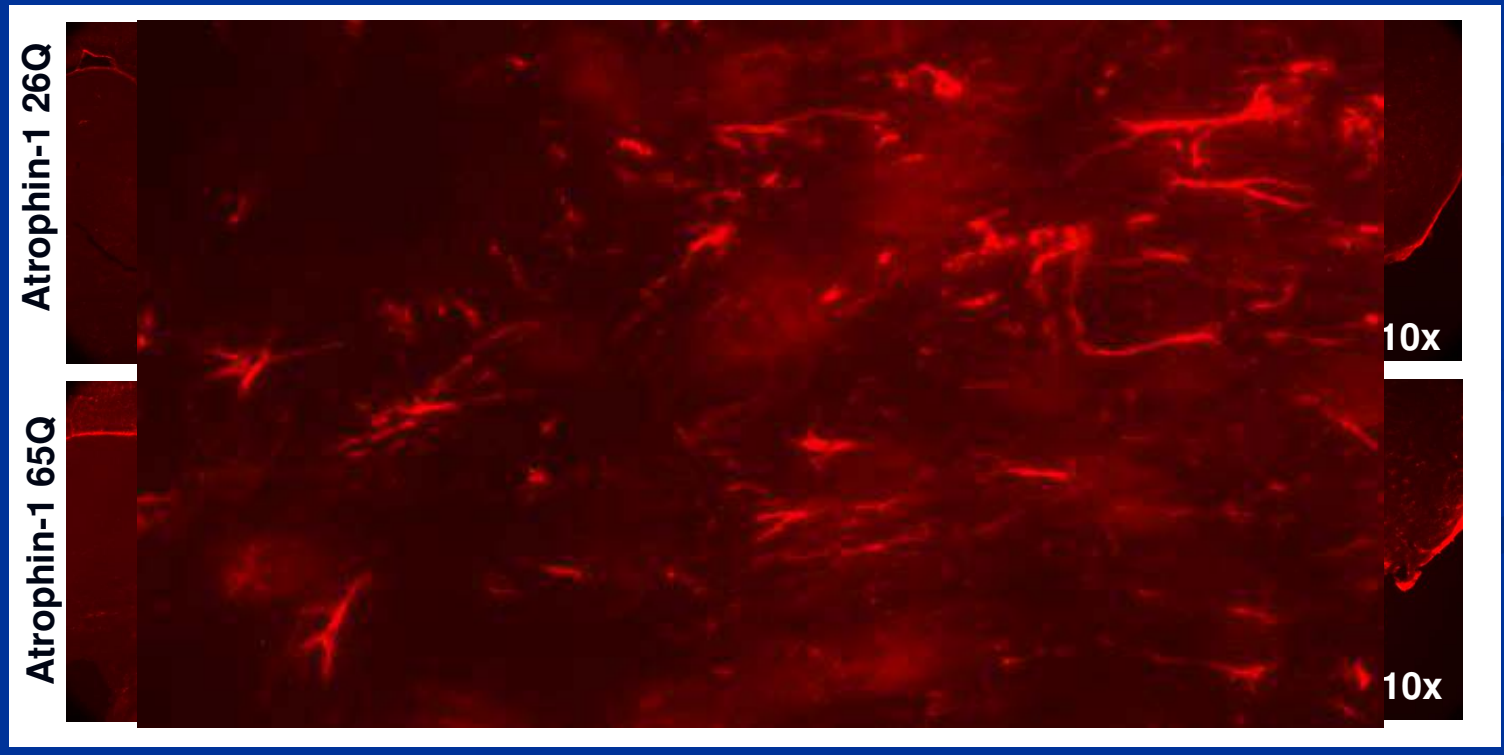


- polyQ Atrophins lead to autophagy defects and degeneration in glial cells too.
- Glial degeneration is accompanied by shortening of lifespan and severe motor deficits.
- Fly death occurs in absence of overt glial cell loss or neuronal cell loss.

Glial pathology and reactivity in the mouse

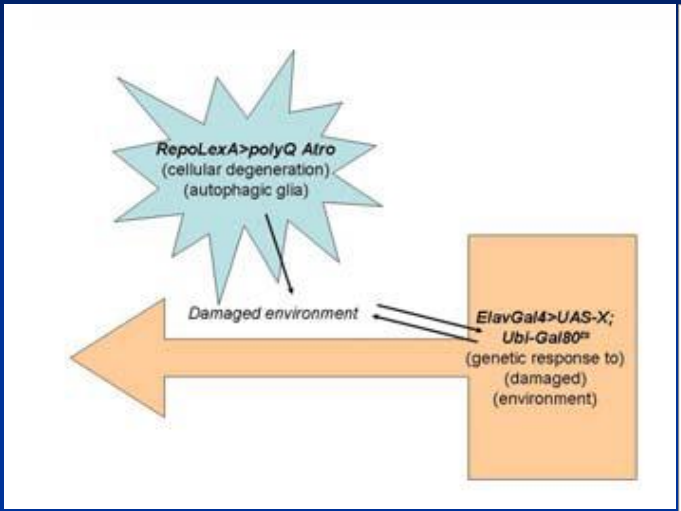
- We detect widespread glial pathology and reactive gliosis in DRPLA mice.

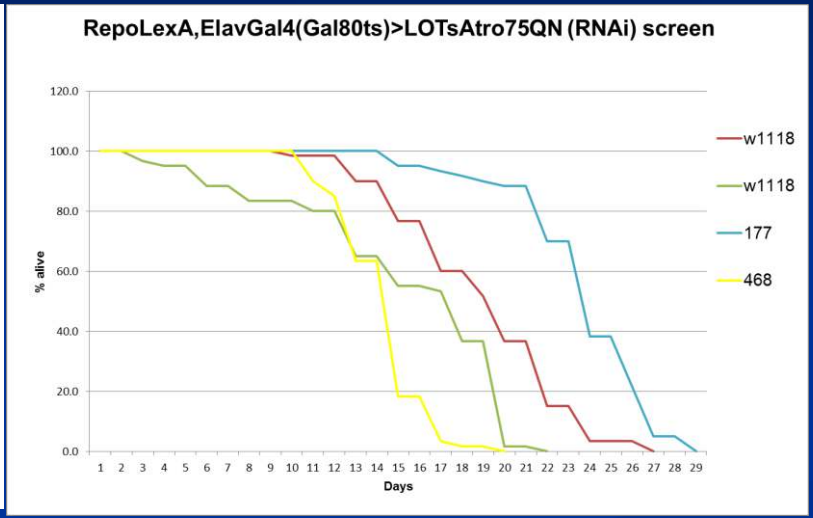




What genes?

- Hypothesis: neurons react to damaged glia and we can identify genes involved in this reaction and at least some of these may be helpful for DRPLA.





Suppressors	Average X^2
VhaSFD	65.2
ZnT77C	63.3
Idgf4	55.5
pum	55
MKP4	47.98
caps	45.96
CG31534	40.33
AnxB10	37.57
CG2926	32.52
Reph	26.7
EcR	27.6
Reph	26.7
dac	21.8
Plod	19.6
mfas	18.9

- We have decided to focus on transmembrane proteins because they might be more accessible to target for a potential therapy.

- mfas is a adhesion molecule of the fasciclin family, which bind ECM.
- the closest human orthologue is TGFβi (aka BIGH3) involved in neuroblastoma and glioblastoma

- caps (and its homologue and binding partner trn) belong to the Leucin Rich Repeat family of cell adhesion molecule.
- closest human orthologue is LRRN-2, also involved in glioma.

Conclusions and perspectives

From *Drosophila* and mouse models of DRPLA we have learnt that:

- cellular degeneration arises through fairly ubiquitous alteration of the autophagic flux arising partly through downregulation of the Fat/Hippo pathway.
  - ➢ we must enhance lysosomal activity as for lysosomal storage disorders.

- the areas most severely affected display additional defects in Rbfox-3 localisation which may be responsible for specificity of the disease.
  - ➢ we need to protect these brain nuclei with additional actions.

- glial cells play a critical role in DRPLA pathophysiology.
  - ➢ we must restore glia function or help neurons cope with glia dysfunction.

Thanks to

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research


Maurice Wohl Clinical Neuroscience Institute

KING'S College LONDON University of London

Ataxia UK

The Henry Smith Charity founded in 1628

MRC Medical Research Council

Collaborators

Annalisa Pastore

Frank Hirth

Gill Bates

Heinz Jungbluth

Mark Wardle/Neil Roberts

Henry Houlden

Patrice Codogno

Sharon Tooze

Eric Lai

Tudor Fulga

Tor Erik Rusten

Angela Giangrande

Jacques Camonis