Features of *ATXN2* Expression Control

Daniel R. Scoles, Lance T. Pflieger, Steven T. Hansen, Khanh K. Thai, Stefan-M. Pulst
Department of Neurology, University of Utah, Salt Lake City

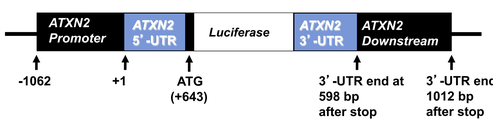
Introduction

SCA2 is an autosomal dominant cerebellar ataxia characterized by progressive degeneration of the cerebellum and brain stem. SCA2 is caused by polyglutamine expansion in the SCA2 protein ataxin-2, causing gain-of-function. We hypothesize that reduction of *ATXN2* expression will be therapeutic for SCA2, based on reversible phenotypes in polyQ models and lack of neuro-degeneration in SCA2 knockout mice.

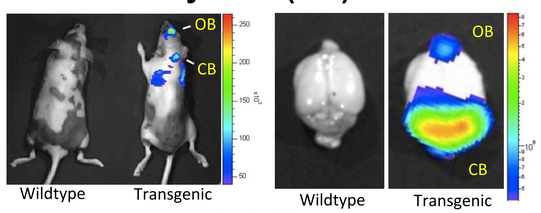
Objectives

To characterize features of *ATXN2* that might be exploited therapeutically for treating SCA2.

ATXN2-luc reporter construct

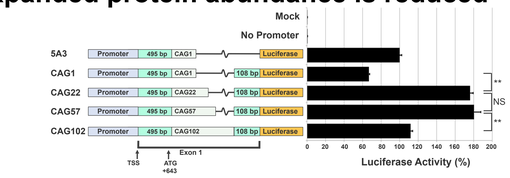


Similar to endogenous *Atxn2*, *ATXN2-luc* is expressed in cerebellum (CB) and olfactory bulb (OB)



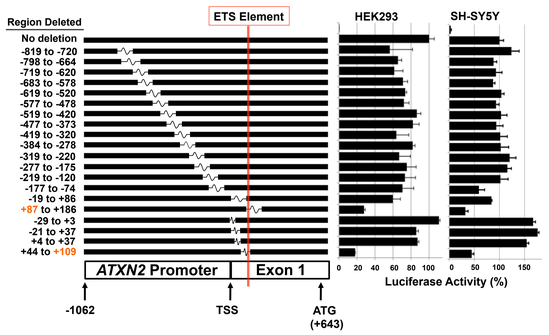
Images display photons/sec/cm²/sr determined by *in vivo* imaging on a Xenogen IVIS 100.

The presence of the CAG repeat supports *ATXN2-luc* expression, but if it is highly expanded protein abundance is reduced

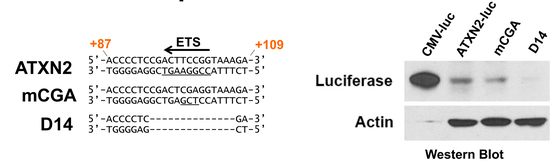


Not shown is nearly identical data for full-length *ATXN2* with varying CAG repeats.

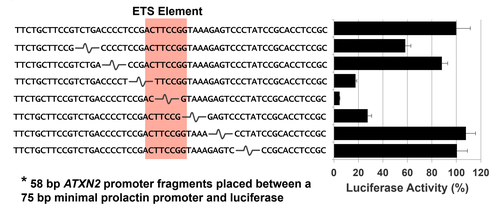
Deletion of an ETS element in the *ATXN2* promoter inhibited *ATXN2-luc* expression



The *ATXN2* ETS site has 100% match with the ETS consensus and when mutated in *ATXN2-luc* expression was reduced

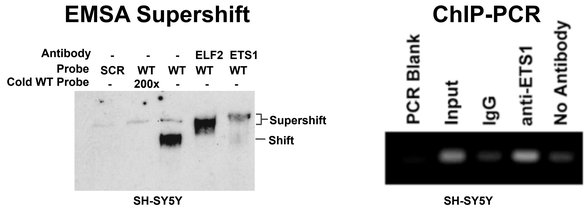


5 bp deletions in *ATXN2* reveal lowest expression with ETS core elimination

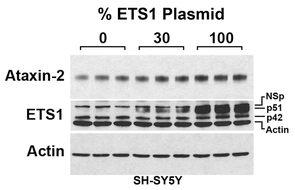


* 58 bp *ATXN2* promoter fragments placed between a 75 bp minimal prolactin promoter and luciferase

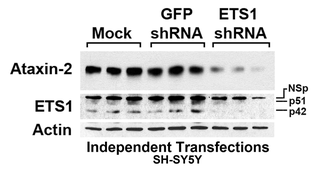
ETS1 binds the ETS element in *ATXN2*



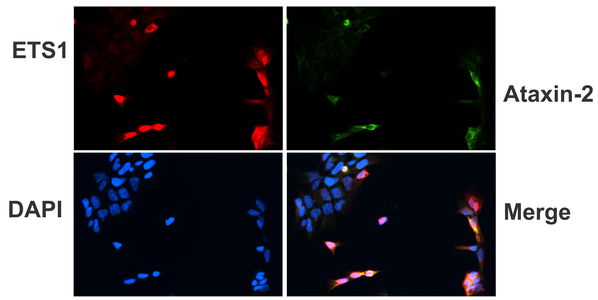
ETS1 overexpression increased ataxin-2



ETS1 underexpression decreased ataxin-2



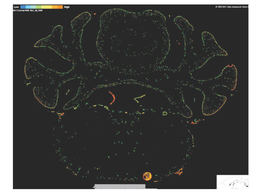
Immunofluorescent labeling showed ETS1 overexpression increased endogenous ataxin-2 expression in SH-SY5Y cells



ETS1 transfected cells (red) make more ataxin-2 (green)

Conclusions

- ***ATXN2-luc*** **expression mimicked predictions for endogenous *Atxn2* (seen in Allen Brain Atlas), suggesting this *ATXN2* fragment possesses promoter elements required for tissue-specific expression.**
- ***Ets1*** **in the Allen Brain Atlas shows high expression in Purkinje cells, suggesting ETS1 may support *ATXN2* expression in this cell type.**



Ets1 in situ **hybridization.**
Reproduced with permission of the Allen Mouse Brain Atlas [Internet]. Seattle (WA): Allen Institute for Brain Science. ©2009. Available from: http://mouse.brain-map.org.

- **The presence of the CAG repeat increased protein abundance without increasing transcription while very long CAG tracts in *ATXN2* reduced protein abundance. We hypothesize that support of *ATXN2* expression may involve RNA binding proteins binding the CAG repeat or increased ataxin 2 stability, and reduced protein abundance when CAG is expanded may reflect RNA or protein aggregation.**
- **The ETS element supports the majority of *ATXN2* expression, therefore ETS1 may represent a therapeutic target for SCA2.**
- ***ATXN2*** **expression might be targeted by decoy oligonucleotides containing the *ATXN2* ETS element and short flanking sequences.**

Acknowledgements

We thank Barbara Graves for advice on ETS transcription factors and Steven Lessnick for assistance with IVIS imaging. This work was supported by grants 1RC4NS073009 & 5R01NS033123 from NINDS.