Evaluation of Spinocerebellar Ataxia Type 2 (SCA2) Gene Expression in *ATXN2* Promoter-Luciferase Transgenic Mice

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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, and is characterized by gain of normal or toxic function. Based on partial reversibility of phenotypes after transgene silencing in polyQ models and lack of major deleterious effects upon *ATXN2* knockout in the mouse, we hypothesize that reduction of *ATXN2* expression will be therapeutic for SCA2. To support this effort we are characterizing the *ATXN2* promoter.

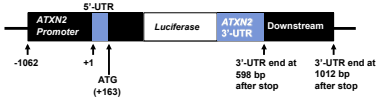
Objectives

To characterize *ATXN2* promoter-luciferase reporter expression in mouse tissues that are relevant to SCA2 to support use of this construct in high-throughput screening for identifying compounds inhibiting *ATXN2-luc* expression.

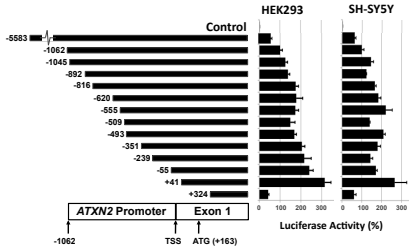
Methods

- **We cloned the *ATXN2* expression control regions flanking luciferase.**
- **We characterized deletions in the *ATXN2* upstream-5'-UTR region on *ATXN2-luc* expression, relative to *SV40-Renilla luc* in HEK293 and SH-SY5Y cells.**
- **We created an *ATXN2-luc* transgenic mouse and characterized *ATXN2-luc* tissue distribution.**

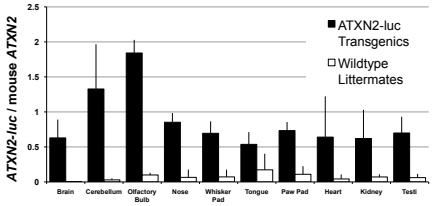
ATXN2-luc reporter construct



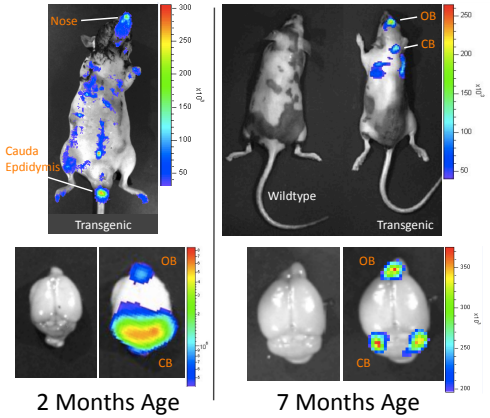
Ever-increasing expression upon progressive removal of the *ATXN2* upstream sequence suggests inhibitory upstream elements for tissue specificity



Expression of *ATXN2-luc* among tissues by real time PCR



ATXN2-luc is expressed in cerebellum (CB), olfactory bulb (OB), and nose



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

Progression of the *ATXN2-luc* construct to a cell-based HTS in collaboration with the NIH Chemical Genomics Center (NCGC)

Primary Assay:

350,000 compounds tested for luc inhibition in SH-SY5Y expressing *ATXN2-luc*

Secondary Assays:

Positive hits tested for:

a) Inhibition of recombinant luc *in vitro*
b) Absence of toxicity
c) Inhibition of *ATXN2-luc* in a positive readout system based on the lac repressor.
d) Absense of inhibition of *CMV-luc*

Tertiary Assays:

Positive hits tested for:

a) Inhibition of endogenous *ATXN2* in patient lymphoblasts by real time PCR and WB.
b) Absence of CYP 2D6 and CYP 34A inhibition.
c) Permeability of Caco-2 colorectal cells.
d) Compound stability in plasma.
e) Compound stability in the presence of liver microsomes.

Validated compounds will be tested in *ATXN2* BAC-transgenic mice for reduction of *ATXN2* expression and phenotype amelioration

Discussion

Previous studies demonstrated that reductions of mutant proteins for HD, SCA1, and SCA3 in inducible mice were associated with reversals of disease phenotypes (Yamamoto et al. 2000; Zu et al. 2004; Boy et al. 2009). Additionally, non-allele specific silencing of the SCA3 gene (MJD1) reduced neuropathology in a rat model (Alves et al., 2010). Thus, targeting total ataxin-2 may be an effective strategy for the treatment of SCA2.

Conclusions

- **New reporter system for studying control of *ATXN2* expression.**
- **Evidence for inhibitory upstream elements for tissue specificity.**
- ***ATXN2-luc* expression mimicked predictions for endogenous *ATXN2* (seen in Allen Atlas) supporting its use in compound screening.**
- ***ATXN2-luc* expression in nose and olfactory bulb may explain development of obesity in *ATXN2* knockout mice (Kiehl et al).**

Acknowledgements

We thank Steven Lessnick for assistance with IVIS imaging and Khanh K. Thai for contributing to *ATXN2* promoter evaluation. This work was supported by grants 1RC4NS073009 & 5R01NS033123 from NINDS.