Published in final edited form as:
Science. 2011 November 4; 334(6056): 690–693. doi:10.1126/science.1212673.

Exercise and Genetic Rescue of SCA1 via the Transcriptional Repressor Capicua*

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Abstract

Spinocerebellar ataxia type 1 (SCA1) is a fatal neurodegenerative disease caused by expansion of a translated CAG repeat in Ataxin-1 (ATXN1). To determine the long-term effects of exercise, we implemented a mild exercise regimen in a mouse model of SCA1 and found a considerable improvement in survival accompanied by upregulation of epidermal growth factor and consequential downregulation of Capicua, an ATXN1 interactor. Offspring of Capicua mutant mice bred to SCA1 mice showed significant improvement of all disease phenotypes. Although polyglutamine-expanded Atxn1 caused some loss of Capicua function, further reducing Capicua levels, either genetically or by exercise, mitigated the disease phenotypes. Thus, exercise might have long-term beneficial effects in other ataxias and neurodegenerative diseases.

Spinocerebellar ataxia type 1 (SCA1) is characterized by a progressive loss of motor skills, usually beginning with impaired gait and balance (1). As with other neurodegenerative diseases, the disease protein ATAXIN-1 (ATXN1) is abundantly expressed in most neurons yet some neuronal populations are more vulnerable than others. In SCA1, cerebellar Purkinje cells are first to show dysfunction; eventually other neuronal populations, including deep cerebellar and brainstem nuclei, are affected, leading to premature death (2). While exercise has beneficial effects on many brain functions (3), it is not clear whether it would be protective in SCA1 or would accelerate neuronal demise by increasing the activity and metabolic demands on these already-vulnerable neuronal populations, as has been suggested for other neurodegenerative diseases (4, 5).

To determine the effects of exercise in SCA1, we implemented a mild exercise regimen in the Atxn1^{154Q} knock-in mice, which bear 154 CAG repeats targeted into the endogenous mouse locus to create a model that recapitulates all aspects of SCA1 (6). From 4 to 8 weeks of age, wild-type (WT) or Atxn1^{154Q} mice were placed on a fixed speed rotarod apparatus 5 times/week, while control mice were placed on an immobile rotarod apparatus. At 10 weeks of age we found no significant improvements in motor performance (Figure S1A and B), but Atxn1^{154Q} mice that were exercised showed a remarkable and highly significant extension of lifespan of (Figure 1A).

To determine the molecular mechanism of this rescue, we measured the expression of several growth factors in vulnerable tissues (cerebellum and brainstem) one week after the last exercise session. We found a sustained increase in the level of epidermal growth factor (EGF) in the brainstem but not the cerebellum (Figure 1B and S1). Since Drosophila data indicate that Capicua (Cic) lies downstream of EGF signaling (7), and we previously showed that Cic interacts with Atxn1(8), we measured Cic levels after exercise and found a significant decrease in the brainstem (Figure 1C and D) but not the cerebellum (Figure S1B). Notably, exercise did not affect brainstem Atxn1^{154Q} protein levels (Figure S1F). Additionally, primary brainstem neuronal cultures treated with recombinant EGF for 72 hours showed a dose-dependent decrease in Cic levels (Figure 1E). Thus, EGF regulates Cic levels in vivo and in vitro, and reduction of Cic might modulate the survival of Atxn1^{154Q} mice.

To test formally the effect of reduced Cic levels on SCA1 phenotypes, we generated a loss-of-function allele of Cic and backcrossed these mice onto a C57BL/6 strain (Figure S2A). Two isoforms of Cic, long (Cic-L) and short (Cic-S), are transcribed from alternative promoters. Cic-L$^{-/-}$ mice completely lack the Cic-L isoform with ~10% of Cic-S remaining (Figure S2B). Importantly, Cic-L$^{+/-}$ mice had a ~50% reduction of both Cic-L and Cic-S. Cic protein is reduced in Atxn1$^{-/-}$ cerebellum (8), and we also found a Cic dose-dependent reduction of Atxn1 and its paralog Ataxin-1 Like (Atxn1L) in WT, Cic-L$^{+/-}$, and Cic-L$^{-/-}$ cerebellum (Figure S2B), confirming the interdependency of Cic and Atxn1 paralog proteins in vivo.

To determine whether reducing the level of Cic would affect disease course in SCA1, we bred Atxn1^{154Q} male mice to Cic-L$^{+/-}$ female mice to generate WT, Cic-L$^{+/-}$, Atxn1^{154Q}, and Atxn1^{154Q}; Cic-L$^{+/-}$ mice, all on a pure C57BL/6 background. Because of their motor impairments, Atxn1^{154Q} mice showed less total locomotor activity in the open field assay than WT, but Atxn1^{154Q}; Cic-L$^{+/-}$ mice performed significantly better than their Atxn1^{154Q} littermates (Figure 2A). Reduced Cic levels also significantly improved the motor coordination defects normally seen in Atxn1^{154Q} mice (Figure 2B and C). Reduced Cic levels also improved the learning and memory deficits Atxn1^{154Q} mice normally exhibit in the conditioned fear assay (Figure 2D). These improved phenotypes were accompanied by reduction in neuropathology: Atxn1^{154Q}; Cic-L$^{+/-}$ mice had significantly more Purkinje cells than Atxn1^{154Q} mice at 40 weeks of age (Figure 3A and B). Thus, a 50% reduction of Cic levels is enough to mitigate the behavioral defects and Purkinje cell loss in Atxn1^{154Q} mice.

Reduction of Cic levels also improved phenotypes that could be attributed to other brain regions. Following weaning, Atxn1^{154Q} mice lost weight progressively, but loss of one Cic allele was enough to significantly rescue this weight loss (Figure 3C). As with the exercised Atxn1^{154Q} mice, the median age of survival of the Atxn1^{154Q}; Cic-L$^{+/-}$ mice was significantly older than that of Atxn1^{154Q} mice (Figure 3D). These data likely explain why exercise improved the longevity of Atxn1^{154Q} mice but not the impaired coordination: exercise reduced Cic levels only in the brainstem and not in the cerebellum.

To determine the mechanism by which constitutive reduction of Cic rescues SCA1, we focused on the cerebellum, the primary site of dysfunction. We examined how the Atxn1^{154Q} protein influenced the transcriptional repressor function of Cic by comparing microarrays of Cic-L$^{-/-}$ cerebella (Table S1) with previous microarrays from Atxn1^{154Q} cerebellum (9). We identified many "hyper-repressed" genes (upregulated in Cic-L$^{-/-}$ but downregulated in Atxn1^{154Q}), with >50% containing a Cic motif (10) (Table S2). We selected several of these hyper-repressed target genes and found that genes that were downregulated in Atxn1^{154Q} cerebellum were in fact restored to near WT levels in Atxn1^{154Q}; Cic-L$^{+/-}$ cerebellum, with significantly more Cic bound to their promoters in Atxn1^{154Q} vs WT mice (Figure 4A). This suggests that the mechanism of disease rescue is relief of Cic hyper-repression conferred by polyglutamine-expanded Atxn1.

In *Drosophila*, overexpression of Atxn1^{82Q} is rescued by overexpression of Cic and is exacerbated by Cic reduction (8, 11). Although this is likely due to a titration of the endogenous *Drosophila* Cic away from its normal transcriptional targets, it suggests that polyglutamine-expanded Atxn1 could cause a loss of Cic transcriptional function (de-repression). We identified many "de-repressed" genes (upregulated in both Cic-L$^{-/-}$ and Atxn1^{154Q} cerebella, Table S3), that were in fact even further upregulated in Atxn1^{154Q}; Cic-L$^{+/-}$ cerebellum, with significantly less Cic bound to their promoters in Atxn1^{154Q} vs WT mice (Figure 4B). This suggests that polyglutamine-expanded Atxn1 does indeed cause Cic to lose some transcriptional repressor activity, but this could not explain the genetic rescue; if partial loss of Cic activity was the driving factor in pathogenesis, the disease phenotype would be exacerbated in Atxn1^{154Q}; Cic-L$^{+/-}$ mice. We propose that the polyglutamine-expanded Atxn1 causes Cic to bind more tightly to certain transcriptional targets (hyper-repressing them) while concomitantly causing Cic to bind less to, and thus upregulate (de-repress), other transcriptional targets. Genetic or exercise-induced reduction of Cic relieves the toxic hyper-repressive activity despite the concomitant loss of normal repressive function (Figure S3), consistent with the fact that the gain-of-function mechanism is the driving mechanism for toxicity in SCA1. While other polyglutamine diseases are caused by a gain-of-function mechanism despite partial loss of function of the involved protein (9, 11–13), here we have demonstrated that polyglutamine-expanded protein can cause concomitant gain- and loss-of-function effects on the same native protein partner. The level of polyglutamine-expanded Atxn1 protein was reduced by ~9% in Atxn1^{154Q}; Cic-L$^{+/-}$ mice compared to Atxn1^{154Q} mice (Figure S2C and D), and while this could possibly contribute to the phenotypic rescue, we suggest that the rescue is more likely caused by relief of Cic-dependent hyper-repression. Therapeutics aimed at lowering Cic levels or disrupting the Cic-Atxn1^{154Q} protein interaction could potentially ameliorate the disease.

The effect of exercise on lifespan was long lasting, well after its discontinuation, underscoring the potential value of exercise beyond motor improvements. Thus, SCA1 individuals might benefit from an exercise program early in disease course (14, 15). It is interesting to note that genetic reduction of Cic also increased the lifespan of Atxn1^{154Q} mice (by 3.5 weeks), but the magnitude of the survival effect was greater (~ six weeks) in exercised Atxn1^{154Q} mice. Thus, other pathways such as enhanced growth factor signaling are likely to contribute to the effect. We cannot rule out the possibility that more intense or longer duration exercise could cause a sustained EGF increase and Cic decrease in the cerebellum that could lead to motor improvements. The exercise regimen we chose was quite gentle; a more rigorous or sustained exercise paradigm that engages the cerebellum more intensely might reduce cerebellar Cic levels and improve cerebellar phenotypes. It is encouraging that exercise and the accompanying increase in neuronal activity and metabolic demands do not seem to exacerbate the disease process in vulnerable neuronal populations, which may be important in a variety of neurodegenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

JDF and HYZ conceived of the study and designed experiments. JDF, CMB, ANC, and YG performed behavioral assays and provided input on analysis. JDF and JC-B performed molecular work and analysis. PY, HK, and CS analyzed microarray data. JDF and HYZ wrote the manuscript with input from JC-B, AF, and HTO. We are grateful to Gabriele Schuster for the generation of mutant mice and the members of the HYZ laboratory for comments and discussions on the manuscript. This research was supported by NIH grants NS27699, NS27699-20S1–ARRA and HD24064 (BCM-IDDRC) to HYZ; 1F32NS055545 to JDF; and NS022920 & NS045667 to HTO. HYZ is an investigator with the Howard Hughes Medical Institute.

References and notes

1. Orr HT, et al. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. Nat Genet. 1993; 4:221–226. [PubMed: 8358429]
2. Zoghbi HY, Orr HT. Pathogenic Mechanisms of a Polyglutamine-mediated Neurodegenerative Disease, Spinocerebellar Ataxia Type 1. Journal of Biological Chemistry. 2009; 284:7425–7429. [PubMed: 18957430]
3. Cotman CW, Berchtold NC, Christie LA. Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends Neurosci. 2007; 30:464–472. [PubMed: 17765329]
4. Chiò A, Benzi G, Dossena M, Mutani R, Mora G. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. Brain. 2005; 128:472–476. [PubMed: 15634730]
5. Mahoney DJ, Rodriguez C, Devries M, Yasuda N, Tarnopolsky MA. Effects of high-intensity endurance exercise training in the G93A mouse model of amyotrophic lateral sclerosis. Muscle & Nerve. 2004; 29:656–662. [PubMed: 15116368]
6. Watase K, et al. A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. Neuron. 2002; 34:905–919. [PubMed: 12086639]
7. Astigarraga S, et al. A MAPK docking site is critical for downregulation of Capicua by Torso and EGFR RTK signaling. EMBO J. 2007; 26:668–677. [PubMed: 17255944]
8. Lam YC, et al. ATAXIN-1 interacts with the repressor Capicua in its native complex to cause SCA1 neuropathology. Cell. 2006; 127:1335–1347. [PubMed: 17190598]
9. Crespo-Barreto J, Fryer JD, Shaw CA, Orr HT, Zoghbi HY. Partial loss of ataxin-1 function contributes to transcriptional dysregulation in spinocerebellar ataxia type 1 pathogenesis. PLoS Genet. 2010; 6:e1001021. [PubMed: 20628574]
10. Kawamura-Saito M, et al. Fusion between CIC and DUX4 up-regulates PEA3 family genes in Ewing-like sarcomas with t(4;19)(q35;q13) translocation. Hum Mol Genet. 2006; 15:2125–2137. [PubMed: 16717057]
11. Lim J, et al. Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature. 2008; 452:713–718. [PubMed: 18337722]
12. Thomas PS Jr, et al. Loss of endogenous androgen receptor protein accelerates motor neuron degeneration and accentuates androgen insensitivity in a mouse model of X-linked spinal and bulbar muscular atrophy. Hum Mol Genet. 2006; 15:2225–2238. [PubMed: 16772330]
13. Van Raamsdonk JM, et al. Loss of wild-type huntingtin influences motor dysfunction and survival in the YAC128 mouse model of Huntington disease. Hum Mol Genet. 2005; 14:1379–1392. [PubMed: 15829505]
14. Vaz DV, et al. Treadmill training for ataxic patients: a single-subject experimental design. Clin Rehabil. 2008; 22:234–241. [PubMed: 18057087]
15. Ilg W, et al. Intensive coordinative training improves motor performance in degenerative cerebellar disease. Neurology. 2009; 73:1823–1830. [PubMed: 19864636]

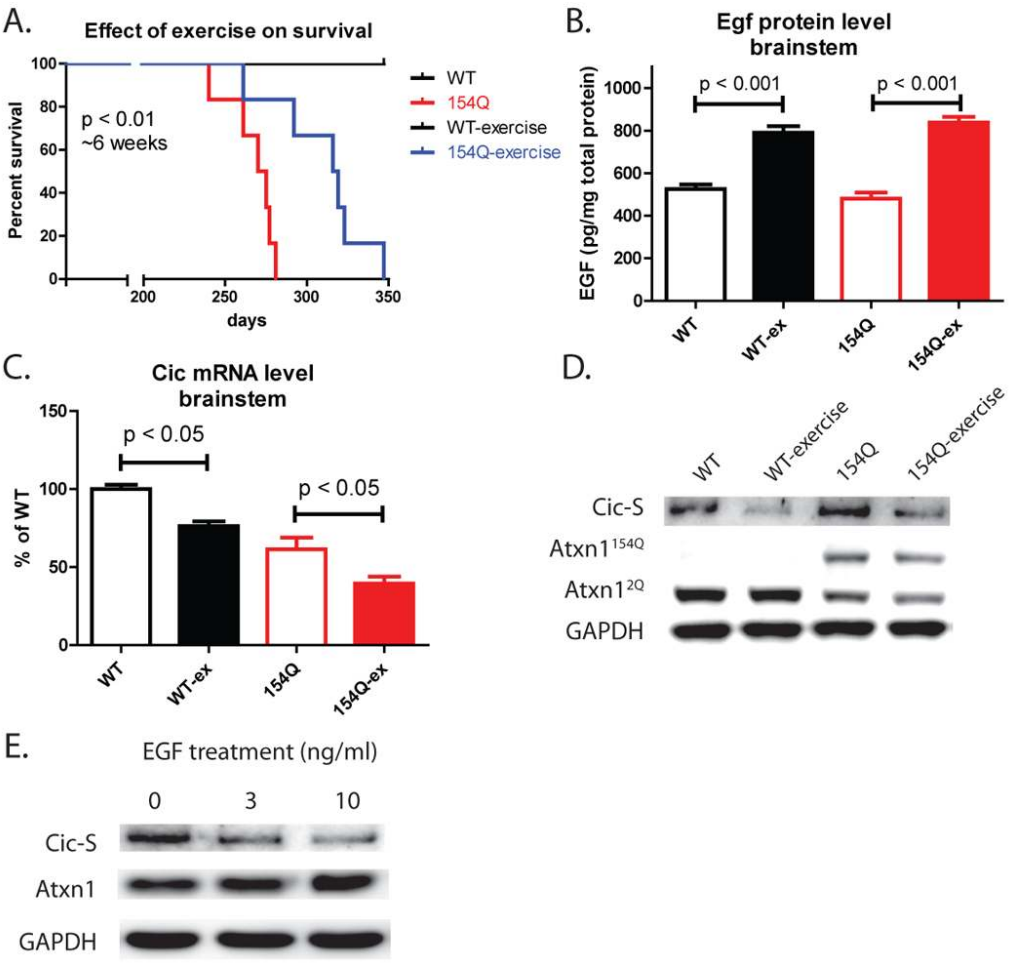

Figure 1.

Exercise extends lifespan in Atxn1^{154Q} mice by upregulating EGF and consequently downregulating of Cic. (A) Median survival was 272 days for non-exercised Atxn1^{154Q} mice vs 317 days for exercised Atxn1^{154Q} mice (45 day extension, N = 6 per group, $p < 0.01$ by log-rank test). (B) Exercise caused a 50% increase in brainstem EGF levels in WT mice and a 74% increase in Atxn1^{154Q} mice measured by ELISA (mean ± SEM, N = 5 per group). (C) Exercise caused a 24% and a 22% decrease in brainstem Cic levels in WT and Atxn1^{154Q} mice, respectively measured by RT-qPCR (mean ± SEM, N = 5 per group). (D) Western blotting for Cic demonstrates an exercise-induced decrease in Cic levels in the brainstem while Atxn1 or Atxn1^{154Q} remain unaffected. (E) Primary brainstem neuronal cultures treated with recombinant EGF for 72 hours show a dose-dependent decrease in the level of Cic but not Atxn1.

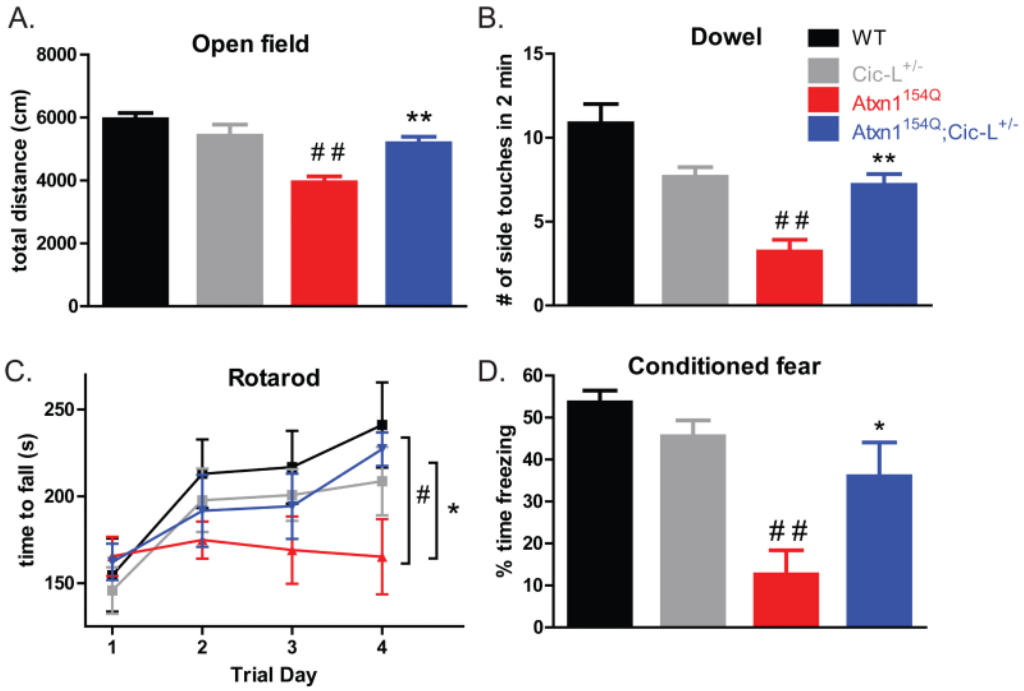

Figure 2.

Reduction of Cic rescues multiple behaviors in SCA1 mice. Reduction of Cic by 50% in Atxn1^{154Q} mice resulted in motor improvements in the open field assay (A), dowel test (B), rotarod (C), and conditioned fear assay of learning and memory (D). Legend for each panel indicated in Panel B. Values represent mean ± SEM with N= 9-10 per genotype. ANOVA with post-hoc t-tests performed where # or ## indicates $p < 0.05$ or $p < 0.01$ between WT and Atxn1^{154Q} mice, and * or ** indicates $p < 0.05$ or 0.01 between Atxn1^{154Q} and Atxn1^{154Q}; Cic-L$^{+/-}$ mice.

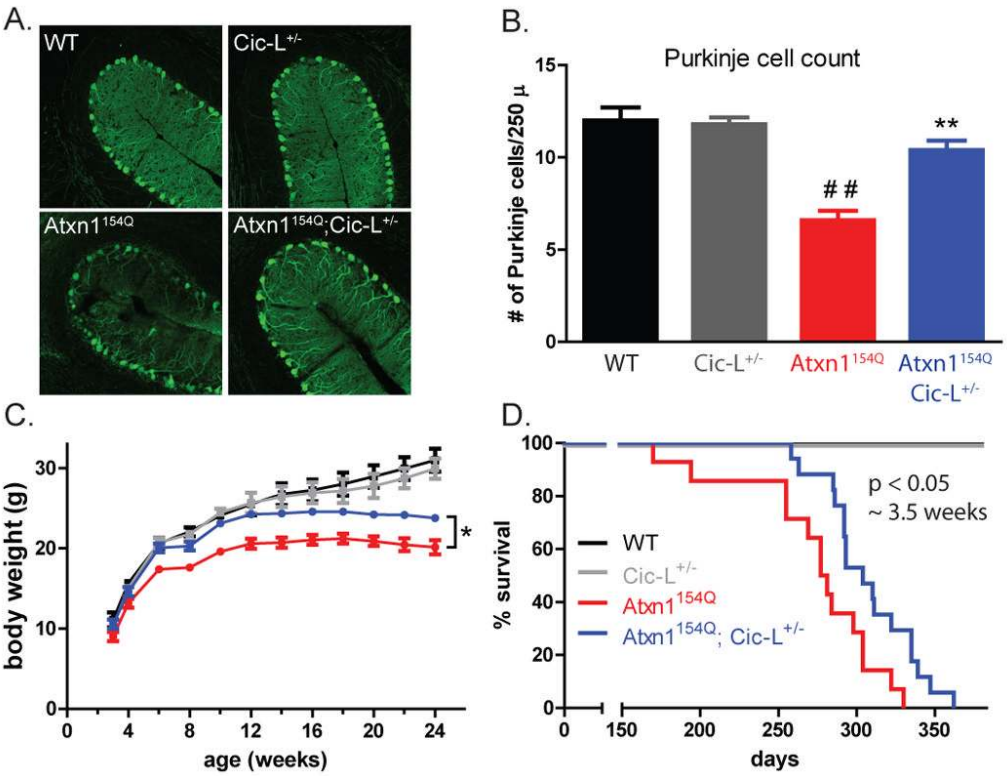

Figure 3.

Reduction of Cic rescues multiple phenotypes in SCA1 mice. A 50% reduction of Cic in Atxn1^{154Q} mice rescued Purkinje cell integrity (A and B, N = 4 per genotype), body weight (C), and premature lethality (D, $p < 0.05$ by log-rank test). The median lifespan of Atxn1^{154Q} mice in this cohort is in close agreement with the median lifespan from the unexercised Atxn1^{154Q} cohort (Figure 1), with some increased variability due to greater numbers of litters being used in this larger cohort. For body weight and lethality, N=11 for WT, N = 12 for Cic-L$^{+/-}$ mice, N=14 for Atxn1^{154Q} mice, N= 16 for Atxn1^{154Q}; Cic-L$^{+/-}$ mice.

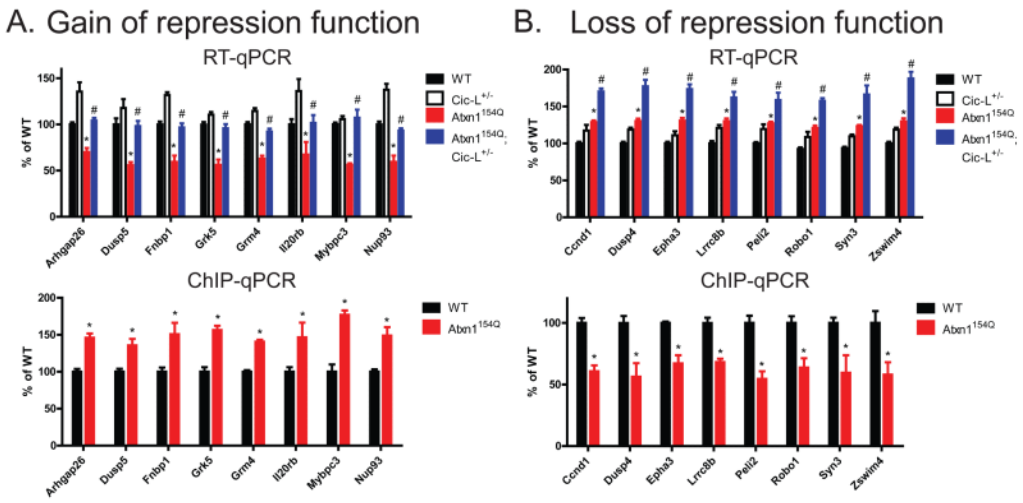

Figure 4.

Atxn1^{154Q} causes concomitant gain and loss of Cic transcriptional function. (A) Atxn1^{154Q} hyper-repressed cerebellar transcripts containing a Cic motif, but these were normalized to near WT levels in Atxn1^{154Q}; Cic-L$^{+/-}$ mice measured by RT-qPCR (mean ± SEM, N = 6 per genotype) with more Cic was bound to their promoters in Atxn1^{154Q} vs WT littermates as measured by chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR, mean ± SEM, N = 4 per genotype). (B) Atxn1^{154Q} also de-repressed cerebellar transcripts containing a Cic motif, but these transcripts were further upregulated in Atxn1^{154Q}; Cic-L$^{+/-}$ mice by RT-qPCR (mean ± SEM, N = 6 per genotype), with less Cic was bound to their promoters in Atxn1^{154Q} vs WT littermates measured by ChIP-qPCR (mean ± SEM, N = 4 per genotype). Data analyzed by ANOVA with post-hoc t-test where * indicates $p < 0.05$ between WT and Atxn1^{154Q} and # indicates $p < 0.05$ between Atxn1^{154Q} and Atxn1^{154Q}; Cic-L$^{+/-}$ mice.