Partial Loss of Ataxin-1 Function Contributes to Transcriptional Dysregulation in Spinocerebellar Ataxia Type 1 Pathogenesis

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Abstract
Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disease caused by expansion of a CAG repeat that encodes a polyglutamine tract in ATAXN1 (ATXN1). Molecular and genetic data indicate that SCA1 is mainly caused by a gain-of-function mechanism. However, deletion of wild-type ATXN1 enhances SCA1 pathogenesis, whereas increased levels of an evolutionarily conserved paralog of ATXN1, Ataxin-1-Like, ameliorate it. These data suggest that a partial loss of ATXN1 function contributes to SCA1. To address this possibility, we set out to determine if the SCA1 disease model (Atxn1154Q/+ mice) and the loss of Atxn1 function model (Atxn1−/− mice) share molecular changes that could potentially contribute to SCA1 pathogenesis. To identify transcriptional changes that might result from loss of function of ATXN1 in SCA1, we performed gene expression microarray studies on cerebellar RNA from Atxn1−/− and Atxn1154Q/+ cerebella and uncovered shared gene expression changes. We further show that mild overexpression of Ataxin-1-Like rescues several of the molecular and behavioral defects in Atxn1−/− mice. These results support a model in which Ataxin-1-Like overexpression represses SCA1 pathogenesis by compensating for a partial loss of function of Atxn1. Altogether, these data provide evidence that partial loss of Atxn1 function contributes to SCA1 pathogenesis and raise the possibility that loss-of-function mechanisms contribute to other dominantly inherited neurodegenerative diseases.

Introduction
Polyglutamine diseases are caused by the expansion of an unstable translated CAG repeats that encode a polyglutamine tract in unrelated proteins [1,2]. There are nine dominantly inherited neurodegenerative disorders caused by expanded polyglutamine tracts: Huntington’s disease (HD), spinobulbar muscular atrophy (SBMA), dentatorubropallidoluysian atrophy (DRPLA), and six spinocerebellar ataxias (SCA1–3, 6, 7 and 17) [1–7]. Several genetic studies have revealed that loss of the involved proteins in humans and mice does not cause neurodegeneration, leading to the conclusion that the polyglutamine expanded protein causes disease by a dominant gain-of-function mechanism whereby it confers toxic properties to the host proteins [8–14]. The importance of protein context and sub-cellular localization has been highlighted in SCA1 pathogenesis because expansion of the polyglutamine tract is necessary but not sufficient to cause neurodegeneration. For example, overexpression of polyglutamine-expanded ATXN1 that has a single serine residue mutated to alanine (S776A) does not lead to Purkinje cell degeneration, and overexpression of polyglutamine-expanded ATXN1 lacking a functional nuclear localization signal or lacking the ANX domain is not toxic in mice [15–17]. These data revealed key domains in ATXN1 that are critical for SCA1 pathogenesis, and indicated that mutant ATXN1 must be localized in the nucleus to exert toxicity. Furthermore, these data suggest that perhaps normal interactions or functions of ATXN1 are relevant to SCA1 pathogenesis.

Several protein interactors of ATXN1 have been identified to date. Among these, there are various transcriptional regulators, including the Capicua homolog CIC, SMRTER, HDAC3, GFI-1 and RORγ. Some of these factors modify the pathogenesis of SCA1 in mice and fly models [15,18–20]. For instance, RORγ haploinsufficiency results in enhanced pathogenesis in SCA1 transgenic mice [20]. Furthermore, SCA1 transgenic mice share common gene expression changes with the staggerer mice, which have a spontaneous mutation in the Rorγ gene that leads to cerebellar defects and ataxia [20–22]. Recent evidence shows that altered interactions of ATXN1 with its native partners contribute to SCA1 pathogenesis. Studies in the knock-in mouse model of SCA1, Atxn1154Q/+, show that polyglutamine-expanded Atxn1 prefers the formation of a protein...
Author Summary

Spinocerebellar Ataxia type 1 (SCA1) is one of nine neurodegenerative diseases caused by an increase in the number of the amino acid glutamine in their respective proteins. Genetic studies have pointed to the fact that the glutamine expansion in Ataxin-1 causes SCA1 by causing Ataxin-1 to gain some function(s). Here, we demonstrate that in addition to the toxic gain-of-function mechanism, partial loss of the normal functions of Ataxin-1 contributes to SCA1. Ataxin-1 forms protein complexes with Capicua, a protein that silences expression of other genes, and we found that in SCA1 mouse models the levels of these complexes are reduced, resulting in increased expression of some genes. We also demonstrate that increased levels of Ataxin-1-Like, a protein that is similar to Ataxin-1 and protects against mutant Ataxin-1 in mice, rescues molecular and behavioral defects in mice deficient in Ataxin-1. These results show that Ataxin-1-Like compensates for loss of Ataxin-1 and that Ataxin-1 and Ataxin-1-Like share some normal functions. Together, these findings suggest that rescue of SCA1 symptoms by Ataxin-1-Like could be partly due to restoration of lost normal functions of Ataxin-1 in mice that express the mutant polyglutamine-expanded Ataxin-1.

Loss-of-Function of Ataxin-1 Contributes to SCA1

Results

Atxn1<sup>-/-</sup> mice share cerebellar transcriptional alterations with Atxn1<sup>154Q/154Q</sup> mice

To identify gene expression changes in SCA1 that might be due to partial loss of function of Atxn1, we surveyed transcriptional changes in Atxn1<sup>-/-</sup> and Atxn1<sup>154Q/154Q</sup> mouse cerebellum using the Affymetrix mouse Exon Array ST 1.0 and searched for shared expression alterations. These arrays potentially enable the detection of even small fold changes due to the existence of multiple probes sets for most transcripts. Early symptomatic (7-week-old) Atxn1<sup>154Q/154Q</sup> mice were used to reflect early changes in SCA1 pathogenesis (Figure 1A). Due to a lack of overt phenotypes in 7-week-old Atxn1 null mice [13], 16-week-old Atxn1<sup>-/-</sup> mice were chosen in order to maximize the potential number of gene expression changes detected. Each mutant allele was studied and compared to age-matched wild-type littermates controls.

We observed a highly significant rate of concordance and overlap between cerebellar expression profiles of Atxn1<sup>-/-</sup> and Atxn1<sup>154Q/154Q</sup> mice (<i>z</i> = 11.9483, <i>P</i>-value<sub>corrected</sub> < 2.2e-16, Kendall-t test). Applying a very stringent cutoff value (false discovery rate (FDR)-corrected < 0.01, Fold change > = ±0.1) log<sub>2</sub>, we identified 197 transcripts that are significantly dysregulated in both mouse models (Figure 1A). These 197 transcriptional changes account for 22.1% of all gene expression changes detected in Atxn1<sup>154Q/154Q</sup> cerebella (Figure 1A and Table S1). Remarkably, a majority of the differentially regulated transcripts (135 out of 197, 68.5%) are in the same direction in both mouse models. (Figure 1B). A preponderance of down-regulated genes in both models is observed (90 out of 197), followed by genes up-regulated in both models (45 out of 197), with only less than a third (62 out of 197) altered in an opposite direction (Figure 1B). These data suggest that at least 15.1% (135 out of 892) of the cerebellar transcriptional changes found in the SCA1 knock-in model (Atxn1<sup>154Q/154Q</sup>+) could be attributed to loss of Atxn1 function.

To verify the microarray results, we selected 15 of these shared gene expression changes for independent validation by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) using cerebellar RNA samples from an independent set of 16-week-old Atxn1<sup>-/-</sup> and Atxn1<sup>154Q/154Q</sup> mice, and their respective littermates. Ten out of 15 gene expression changes, or 66.6%, were positively validated in Atxn1<sup>-/-</sup> mice, while 13 out of 15 genes, or 86.7%, were validated in Atxn1<sup>154Q/154Q</sup> cerebella (Table 1). Most importantly, of the 10 genes validated in Atxn1<sup>-/-</sup> cerebella, 9 were also validated in Atxn1<sup>154Q/154Q</sup> cerebella (Table 1). Taken together, the comparison of cerebellar microarray studies of Atxn1 null and polyglutamine-expanded Atxn1 knock-in mouse models demonstrate that a significant amount of transcriptional changes are shared between these models, supporting the notion that some loss of Atxn1 endogenous function contributes to disease.

In order to gain insight into the potential molecular pathways commonly affected in Atxn1<sup>-/-</sup> and Atxn1<sup>154Q/154Q</sup> mice, we performed Gene Ontology (GO) analysis and pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Figures S1, S2, S3, S4, S5, S6, and Table S2). GO analysis revealed some biological functions that are enriched in commonly dysregulated genes. For example, cell junction and synapse, guanyl-nucleotide exchange factor activity and GTPase activity categories were enriched in the common set of up-regulated genes (Figures S1, S2, S3). In the down-regulated gene set, genes encoding for calcium ion binding were the most significantly affected both in Atxn1<sup>154Q/154Q</sup> and Atxn1<sup>-/-</sup> cerebella (Figures S4, S5, S6). Using KEGG pathway analysis, among the top enriched categories for genes commonly down-regulated in
Both Atxn1\(^{-/-}\) and Atxn1\(^{154Q/+}\) mice are the phosphatidylinositol and calcium signaling, Long Term Depression (LTD) associated genes, and Alzheimer’s disease pathways (Table S2). These results strongly suggest that loss of Atxn1 results in transcriptional changes that are potentially pathogenic, since in addition to the enrichment for genes involved in neurodegenerative disease, the phosphatidylinositol and calcium signaling pathways are also known to be dysregulated in SCA1 models [26–28]. Interestingly, commonly up-regulated genes in Atxn1\(^{-/-}\) and Atxn1\(^{154Q/+}\) mice include genes that are involved in cancer pathways (Table S2). This could potentially point to a novel function of Atxn1 that remains to be clarified in proliferating cells.

The categories enriched for genes going in opposite directions between Atxn1\(^{-/-}\) and Atxn1\(^{154Q/+}\) mice involve citrate cycle and ubiquitin-mediated proteolysis. These genes going in opposite directions are of interest, since they might reflect potential differences reflecting the main gain of function mechanism in SCA1 pathogenesis.

Expression changes present in staggerer mice

Given the significant overlap between the transcriptional profiles of Atxn1\(^{-/-}\) and Atxn1\(^{154Q/+}\) cerebella, we were interested in examining whether these transcriptional changes are due to
Atxn1 function being affected in both mouse lines and not simply due to cerebellar dysfunction. Previous studies showed that ATXN1 and RORα interact genetically and biochemically, and deficiency of RORα enhances phenotypes in a transgenic model of SCA1 [20]. Interestingly, we observed a significant overlap between the genetic profiles of Atxn1154Q mice, and those common between staggerer (Table 2) [20,21]. Most of these shared changes between staggerer and SCA1 mice are not present in cerebella that have been knocked down in mouse model for SCA7 [27], suggesting that most of these transcriptional regulation is altered by loss of ATXN1 and RORα [20], these results could potentially indicate that RORα enhances phenotypes in a transgenic model of Atxn1154Q mice and SCA1 transgenic mice [18,23].

To test the hypothesis that RORα-deficiency of Atxn1154Q/+ mice cerebella. These results could reflect the fact that Cic protein levels are diminished both in Atxn1154Q/− mice and Atxn1154Q/+ mice [18,23].

**Table 1.** Real-time qRT–PCR validation of genes commonly altered in Atxn1154Q/+ and Atxn1154Q/− cerebella.

| Gene symbol | Atxn1154Q/+ p-value | Atxn1154Q/− Fold Change | Atxn1154Q/+ p-value | Atxn1154Q/− Fold Change |
|-------------|-----------------|----------------------|-----------------|----------------------|
| Igfbp5      | 0.0422          | −1.39                | 1.20E-05        | −3.55                |
| Nab2        | 0.7402          | −1.04                | 0.0004          | −2.03                |
| Rasa1       | 0.0095          | −1.45                | 2.78E-06        | −2.47                |
| AWS1984     | 0.1273          | 2.36                 | 0.8609          | −1.12                |
| Ldb2        | 0.5421          | 1.15                 | 0.2139          | −1.36                |
| Svep1       | 0.0099          | 1.27                 | 0.0003          | −1.49                |
| Apba2hyp    | 0.0288          | 1.28                 | 0.0001          | −1.51                |
| Synpr       | 0.4993          | 1.26                 | 0.0588          | 1.71                 |
| Adcyap1r1   | 0.1396          | 1.29                 | 0.7922          | 1.05                 |
| Robo1       | 0.0002          | 1.62                 | 0.0033          | 1.44                 |
| Tgfβ3       | 4.76E-05        | 1.32                 | 0.0254          | 1.16                 |
| Creb5       | 0.0012          | 1.44                 | 0.0143          | 1.38                 |
| Ccnd1       | 4.61E-05        | 1.34                 | 0.0018          | 1.23                 |
| Paah1b3     | 4.09E-07        | 1.86                 | 0.1448          | 1.12                 |
| Tiam2       | 2.09E-05        | 1.47                 | 0.0149          | 1.17                 |
| Etv5        | 0.0078          | 1.35                 | 0.8350          | 1.03                 |

P-values for significant genes are represented in bold.
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**Table 2.** RORα-responsive genes altered in SCA1 mouse models and Atxn1154Q/− mice.

| Gene symbol | RORα (impreg) | SCA1/82Q | Atxn1154Q/− |
|-------------|--------------|----------|-------------|
| Rora        | down         | down     | down        |
| Car8        | down         | down     | down        |
| Inpp5a      | down         | down     | down        |
| S10a6       | down         | down     | down        |
| Actb6a      | down         | down     | down        |
| Ccna2       | down         | down     | down        |
| Atb2a2      | down         | down     | down        |
| Itp1        | down         | down     | down        |
| Grid2       | down         | down     | down        |
| Mela        | down         | down     | down        |
| Calb1       | down         | down     | down        |
| Grm1        | down         | down     | down        |
| Nek2        | down         | down     | down        |
| Id2         | down         | down     | down        |
| Spinb3      | down         | down     | down        |
| Ppp2        | down         | down     | down        |
| Ppp4        | down         | down     | down        |
| Kitl        | down         | down     | down        |
| Sif         | up           | up       |             |

*Gold et al 2003 [21].
*Serra et al 2006 [20].
*Gatchel et al 2008 [27].
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Atxn1 and Capicua associate at the promoter regions of genes altered in Atxn1154Q/− and Atxn1154Q/+ cerebella

ATXN1 forms stable complexes in vivo with the Capicua homolog (CIC), a transcriptional repressor that exhibits reduced levels in Atxn1154Q/+ mice [18,23]. To test the hypothesis that reduced Atxn1-Cic complexes lead to dysregulated gene expression in Atxn1154Q/+ mice, we searched our microarray data for up-regulated genes that are direct targets of Cic. Microarray analysis revealed at least 5 significantly up-regulated genes (False Discovery Rate-corrected P<0.05) in Atxn1154Q/− cerebella that have been identified as direct targets of Cic-mediated repression, namely Evi1, Evi5 [29], and Cnd1 (Fryer and Zoghi, unpublished data). Interestingly, the microarray data show that Evi5 and Cnd1 are also significantly up-regulated in Atxn1154Q/+ mice cerebella. These results could reflect the fact that Cic protein levels are diminished both in Atxn1154Q/− and Atxn1154Q/+ mice [18,23].
Since Atxn1 and Cic form stable complexes in vivo, we rationalized that both proteins should bind the promoter regions of target genes if they mediate transcriptional repression together as a complex. To test this possibility, we performed chromatin immunoprecipitation analysis, followed by PCR for the promoter regions of Ets5 and Cond1 (ChIP-PCR). ChIP-PCR analysis using antibodies against Cic confirmed that Cic is present on the promoters of Ets5 and Cond1 in vivo (Figure 2A). To determine if Atxn1 binds to the promoters of Cic targets and if the binding is altered due to polyglutamine expansion, we prepared cross-linked chromatin from mice expressing one wild-type Atxn1 allele (Atxn1\(^{+/+}\)) and compared it to mice expressing one polyglutamine-expanded Atxn1 allele (Atxn1\(^{154Q}\)). We used Atxn1\(^{-/-}\) mice as a negative control for testing Atxn1 antibody specificity. As predicted, in chromatin extracts prepared from Atxn1\(^{154Q}\) cerebella and immunoprecipitated using Atxn1 antibody, wild-type Atxn1 was detected on the promoters of Ets5 and Cond1 (Figure 2B). In contrast, we could not detect any specific signal for Atxn1\(^{154Q}\) above background levels in Atxn1\(^{154Q/-}\) cerebellum immunoprecipitations using Atxn1 antibodies (compared to Atxn1\(^{-/-}\)) and pre-immune sera controls (Figure 2B). These data suggest that there is minimal association of polyglutamine-expanded Atxn1\(^{154Q}\) to the promoters of target genes in vivo. Alternatively, it is possible that a conformational change in Atxn1\(^{154Q}\) renders it inaccessible for immunoprecipitation, resulting in reduced signal.

Given that Atxn1\(^{154Q}\)-specific signal is not detected on the promoters of Ets5 and Cond1, we next asked if Cic protein can be detected at these promoters in Atxn1\(^{154Q/-}\) mice using Cic antisera. We detected Cic binding to the promoter regions of Ets5 and Cond1 (Figure 2C) on all genotypes tested (Atxn1\(^{+/+}\), Atxn1\(^{154Q/-}\), and Atxn1\(^{-/-}\)), despite the fact that Cic protein levels are reduced in Atxn1\(^{-/-}\) and Atxn1\(^{154Q/-}\) mice [18,23]. To better quantify potential differences in Cic binding between the different genotypes, we performed ChIP followed by quantitative PCR. Primer sets designed for conserved regions in the promoters of Ets5 and Cond1 that contain or are adjacent to Cic binding sites (CBS) (TGAATGAA or TGAATGGA) were able to amplify with no significant difference in all three genotypes (Atxn1\(^{+/+}\), Atxn1\(^{154Q/-}\), and Atxn1\(^{-/-}\)), both for Ets5 and Cond1 (Figure 2D–2E). To test for the specificity of Cic-binding to its consensus sequences, primers were designed for regions lacking predicted Cic-binding sites sequences, either upstream or downstream of the CBS-containing regions in Ets5 and Cond1 promoters (Figure 2D–2E). As expected, quantitative PCR for these regions show less binding relative to the corresponding positive regions of Ets5 and Cond1 by quantitative PCR. These findings suggest that although Cic still binds the promoter, its function in repression is less efficient in the absence of wild-type Atxn1 or that Atxn1L partially compensates for the loss of Atxn1 at the promoters. The gene expression and ChIP-PCR data suggest that polyglutamine-expanded Atxn1 is less efficient in Cic-dependent repression. More importantly, they provide evidence for a model in which loss of Atxn1/Cic function can result in transcriptional dysregulation, contributing to SCA1 pathogenesis.

**Atxn1L\(^{dp/+}\) rescues Cic levels in Atxn1\(^{-/-}\) mice by forming functional Atxn1L-Cic complexes**

Given that mild overexpression of the Atxn1 paralog, Atxn1L, can displace both wild-type and polyglutamine-expanded Atxn1 from large native complexes in a dose-dependent manner, we wondered if Atxn1L could partially replace Atxn1 as a Cic binding partner, especially because Atxn1L interacts with Cic in wild-type cerebellum [19,24]. This led us to propose that in addition to competing with Atxn1\(^{154Q}\) in the large native complexes [24], increased Atxn1L levels can suppress a putative loss of Atxn1 function in Atxn1\(^{154Q/-}\) mice by directly substituting for Atxn1 in Cic-containing complexes. To test this possibility, we generated Atxn1\(^{-/-}\) and Atxn1\(^{154Q/-}\); Atxn1L\(^{dp/+}\) littermates, and performed western blot analysis for Cic. As shown in Figure 3A, Atxn1L overexpression results in restoration of Cic levels back to wild-type levels in Atxn1\(^{-/-}\) cerebellum. We then tested if overexpression of Atxn1L increased formation of Atxn1L-Cic complexes, thus stabilizing Cic. For this, we performed co-immunoprecipitation studies using Cic antibody on cerebellar extracts of Atxn1\(^{-/-}\) mice with or without the Atxn1L\(^{dp/+}\) allele, followed by western blot analysis for Atxn1L. As expected, Atxn1L co-immunoprecipitates with Cic in wild-type cerebella (Figure 3B). However, despite the reduced levels of Cic protein in Atxn1\(^{-/-}\) cerebella (Figure 3A and [18]), the relative fraction of Atxn1L co-immunoprecipitated with Cic in Atxn1\(^{-/-}\) protein extracts was greater than in wild-type cerebella (Figure 3B). Atxn1L overexpression further increased the levels of Atxn1L-Cic co-immunoprecipitations (Figure 3C). Interestingly, co-transfection of constructs expressing Cic and Atxn1L resulted in synergistic repression of the Cic-responsive luciferase reporter (Figure 3C). These results strongly suggest that Atxn1L-Cic complexes are functionally redundant in Cic-dependent transcriptional repression. Thus, we conclude that mild overexpression of Atxn1L in Atxn1\(^{-/-}\) mice might partially rescue a loss of Atxn1 endogenous function related to reduced Atxn1-Cic complexes.

**Atxn1L\(^{dp/+}\) restores some gene expression alterations in Atxn1\(^{-/-}\) mice**

Given that mild Atxn1L overexpression rescues Cic levels in Atxn1\(^{-/-}\); Atxn1L\(^{dp/+}\) mice, we predicted that Atxn1L should rescue some of the transcriptional changes in Atxn1\(^{-/-}\) cerebella if it can functionally substitute for Atxn1. To test this, we performed qRT-PCR on cerebellar RNA isolated from Atxn1\(^{-/-}\); Atxn1L\(^{dp/+}\) mice and Atxn1\(^{-/-}\) littermates at 16 weeks of age. We focused our qRT-PCR analysis on the 9 genes that are commonly dysregulated in Atxn1\(^{-/-}\) and Atxn1\(^{-/-}\) cerebella (Table 1), and also on the 4 down-regulated Ror genes validated by qRT-PCR in Atxn1\(^{-/-}\) cerebellum (Figure S7). Interestingly, 5 out of 13 genes tested (Cond1, Igfbp5, Apo2l2, Robo1 and Grid2) were partially or completely restored back to wild-type levels in Atxn1\(^{-/-}\); Atxn1L\(^{dp/+}\), compared to Atxn1\(^{-/-}\) cerebella (Figure 4A–4E), Cond1, one of the up-regulated genes that is potentially a direct target of Atxn1-Cic complexes (Figure 4A), and Igfbp5, an early key pathogenic marker in SCA1 disease (Figure 4B), were among the genes rescued by Atxn1L overexpression in Atxn1\(^{-/-}\) mice. Additionally, the Ror gene Grid2 is also significantly rescued in Atxn1\(^{-/-}\); Atxn1L\(^{dp/+}\) mice. Thus, mild overexpression of Atxn1L in vivo results in partial rescue of several transcriptional changes related to SCA1 pathogenesis in a loss-of-function model of Atxn1. Since Atxn1L binds to transcriptional regulators that interact with ATXN1, such as CIC and SMRT-NCoR, it is possible that Atxn1L rescues transcriptional changes by functionally replacing polyglutamine-expanded Atxn1 in these transcriptional complexes.
Loss-of-Function of Ataxin-1 Contributes to SCA1

A

B

C

D  

E

Promoter-CBS

Fold Enrichment over Preimmune

N.S.  

***

Promoter-No CBS

N.S.

Promoter-CBS

Fold Enrichment over Preimmune

N.S.  

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Promoter-No CBS

N.S.
Partial rescue of behavioral defects in Atxn1Ldp/+ mice by mild overexpression of Atxn1L

Although Atxn1Ldp/+ mice do not exhibit overt ataxia phenotypes or progressive neurodegeneration, they do exhibit a variety of neurobehavioral deficits ([13] and Figures S8, S9, S10, S11). Interestingly, Atxn1Ldp/+ mice exhibit deficits in spatial learning and memory, and in motor learning and coordination, phenotypes shared with Atxn1154Q/+ mice ([13] and Figures S10 and S11). This raises the possibility that partial loss of Atxn1 function could also contribute to these phenotypes in Atxn1Ldp/+ mice. The fact that the Atxn1Ldp/+ allele can suppress behavioral phenotypes in Atxn1514Q/+ mice [24] also raises the possibility that Atxn1L suppresses SCA1 pathogenesis by functionally replacing those Atxn1 functions altered by polyglutamine-expanded Atxn1.

To investigate whether Atxn1L and Atxn1 are functionally redundant in vivo, we tested if the Atxn1Ldp/+ allele can ameliorate behavioral defects in Atxn1Ldp/+ mice. We focused on two characteristic phenotypes clinically associated with SCA1 disease: cognitive deficits (learning and memory) and deficits in motor coordination and balance [30,31]. We performed the conditioned fear paradigm on Atxn1Ldp/+ mice carrying the Atxn1Ldp/+ allele and compared them to Atxn1Ldp/- littermates. As shown in Figure 5A, and in agreement with previous data [32], Atxn1Ldp/- mice exhibited significant deficits, as determined by reduced freezing behavior, in the contextual fear-conditioning test compared to wild-type and Atxn1Ldp/- littermates. Wild-type and Atxn1Ldp/+ mice expressing the Atxn1Ldp/+ allele performed similarly to the controls (Figure 5A). Interestingly, the duplication of Atxn1L rescued the freezing behavior due to loss of Atxn1 (compare Atxn1Ldp/- mice to Atxn1Ldp/+ mice; Figure 5A).

Having shown that mild overexpression of Atxn1L rescues Pavlovian contextual learning in Atxn1Ldp/- mice, we then wanted to assess the effects of increased Atxn1L levels on motor coordination and balance deficits in Atxn1Ldp/- mice, another phenotype common to SCA1 mouse models. In order to discriminate between motor learning impairments and motor coordination deficits, we chose to use the dowel rod and wire hang tests. These paradigms do not rely on consecutive daily training, as does the rotating rod test, therefore we selected them in order to discern motor coordination and balance deficits from cerebellar learning deficits [33,34]. We generated an independent cohort of Atxn1Ldp/- mice and Atxn1+/−; Atxn1Ldp/+ mice, with Atxn1+/− and Atxn1Ldp/+ littermates as controls, and tested them at 8 weeks of age. We measured the latency to reach the side (first touch) and frequency of walking off the rod (number of side touches in 120 seconds) (Figure 5B and 5C). All genotypes tested remained on the dowel for the maximum time (data not shown), but Atxn1Ldp/+ mice moved much less than Atxn1+/− and Atxn1Ldp/-. Atxn1Ldp/+ littermates on the rod. The latency of Atxn1Ldp/+ mice to reach the side for the first time was increased (Figure 5B), and consequently they walked off the rod fewer times (Figure 5C). It is noteworthy that Atxn1Ldp/+ mice are active and travel the same distance as wild-type littermates the open field analysis (Figure S5). Thus the hesitancy to move on the dowel suggests that in addition to learning and memory deficits, Atxn1Ldp/+ mice have balance or motor coordination impairments, evident at an early age (8-week-old). In contrast, Atxn1Ldp/- mice carrying the Atxn1Ldp/+ allele take less time to walk off the dowel (Figure 5B), and they also crossed the dowel more times in 120 s than Atxn1Ldp/- littermates (Figure 5C). Thus, mild Atxn1L overexpression partially rescues the dowel phenotype caused by loss of Atxn1.

The wire-hang paradigm assesses motor coordination and grip strength [34]. In this paradigm, mice are hanging onto the center of an elevated wire from their forepaws, and they need to get to the sides for relief, which requires coordination and normal strength. We measured the time and frequency to reach the sides in a 120 s interval, and found no significant falling off the wire in Atxn1Ldp/- mice compared to the control littermates, suggesting that they have reasonable grip strength (data not shown). However, Atxn1Ldp/- mice showed increased latency to reach the sides for the first time compared to Atxn1+/− and Atxn1Ldp/+; Atxn1Ldp/+ controls (Figure 5D). Additionally, Atxn1Ldp/+ mice reached the sides fewer times than control littermates (Figure 5E). In contrast, Atxn1Ldp/- mice overexpressing Atxn1L exhibited marked reduction in the time for the first touch and increased number of side touches in the 120 s interval, when compared to Atxn1Ldp/- mice (Figure 5D and 5E). Taken together, these behavioral data demonstrate that a 50% increase in the levels of Atxn1L is sufficient to partially rescue several behavioral deficits caused by loss of Atxn1 function. Furthermore, this rescue correlates with the molecular data, demonstrating that Atxn1L is a functional homolog of Atxn1 in vivo.

Discussion

Recently, we proposed the possibility that in addition to toxic gain-of-function due to polyglutamine-expanded ATXN1, a concomitant partial loss of ATXN1 function might contribute to SCA1 pathogenesis [23]. It is challenging to establish the extent of the contribution of a potential loss-of-function mechanism to SCA1 pathogenesis in models carrying the mutant protein, since the severe gain-of-function effects might mask any subtle loss-of-function component, thus confounding the interpretation of the results. In the present study, we sought to distinguish between gain- and loss-of-function mechanisms by focusing on transcriptional defects in Atxn1Ldp/+ mice, and comparing them to the knock-in model of SCA1, Atxn1514Q/+ mice. Using this approach, we identified several molecular changes that could be attributable to loss of ATXN1 function in SCA1.
**Figure 3.** *Atxn1L*<sup>dp</sup> stabilizes Cic protein levels in *Atxn1<sup>−/−</sup>* mice by enhancing *Atxn1L*-Cic complex formation. (A) Western blot analysis shows that overexpression of *Atxn1L* rescues the reduced Cic levels in *Atxn1<sup>−/−</sup>* mice (* p<0.05). (B) Co-immunoprecipitation of *Atxn1L* using Cic antibodies show that, despite the reduced levels of Cic protein in *Atxn1<sup>−/−</sup>* cerebella, the relative fraction of *Atxn1L* co-immunoprecipitated with Cic in *Atxn1<sup>−/−</sup>* protein extracts was greater than in wild-type cerebella. *Atxn1L* overexpression further increased the levels of *Atxn1L*-Cic co-immunoprecipitation in *Atxn1<sup>−/−</sup>*; *Atxn1L<sup>dp</sup>/+<sup>+</sup>* mice. Images show representative blots and the quantification of three independent experiments. Error bars in graphs represent +/− SEM. * p<0.05. (C) To determine if *Atxn1L*-Cic complexes are functional, we measured the transcriptional effect of Cic, Atxn1 and Atxn1L on the expression of a luciferase reporter construct containing a tandem array of Cic binding sites (CBS). Luciferase activity is expressed as fraction of the activity when the reporter is expressed alone (100%). Co-transfection of Cic and Atxn1[2Q]) results in synergistic co-repression of this luciferase reporter (Cic+Atxn1[2Q]). Interestingly, co-transfection of constructs expressing Cic and Atxn1L (Cic+Atxn1[2Q]) results in synergistic repression of the reporter similar to co-transfection of Cic and wild-type Atxn1. These results suggest that Atxn1L-Cic complexes are functional in Capicua-dependent repression. Assays were performed in duplicate in 5 independent experiments. Error bars in graph represent +/− SEM, *p<0.05,***p<0.005.

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We found that loss-of-function of Atxn1 in mice is sufficient to cause many transcriptional changes common to the Atxn1<sup>154Q/+</sup> knock-in mice, a model of SCA1 that faithfully replicates many features of the disease, and with SCA1<sup>92Q</sup>/transgenic mice. It has been reported that Atxn1 interacts with several factors involved in transcriptional regulation, including Cic, SMRTER, HDAC3, Gfi1 and RORx [15–18]. Therefore, these shared expression changes might be indicative of altered transcriptional functions of ATXN1 in SCA1 pathogenesis. Furthermore, we showed that a majority of the shared transcriptional changes go in the same direction in both Atxn1<sup>−/−</sup> and Atxn1<sup>154Q/+</sup> mice, strongly arguing that part of the transcriptional dysregulation in SCA1 might be explained by a partial loss-of-function of Atxn1. The up-regulation of direct targets of Atxn1-Cic provides evidence for this concept. Another important finding of this study is that there are many transcriptional changes that are unique to the Atxn1<sup>154Q/+</sup> model, which could potentially be related to toxicity of polyglutamine-expanded Atxn1. We propose that a combination of toxic gain-of-function and mild loss-of-function mechanisms contribute to SCA1 pathogenesis, with the partial loss-of-function of ATXN1 being sufficient to cause some transcriptional changes that are pathogenic in the cerebellum. Previous studies using microarray analysis reported on the down-regulation of the dopamine receptor D2 (Drd2) in Atxn1<sup>−/−</sup> mouse cerebella [35]. However, with the exception of a couple of genes (e.g., Pofbkh3, Sp1), we were unable to find extensive overlap between the changes reported by Goold et al. and the microarray analysis presented in this study. The differences in genetic background, microarray platform, and the age of the Atxn1<sup>−/−</sup> animals (5-week-old) in the Goold et al. studies [35], compared to 16 weeks in our studies, are likely to contribute to the minimal overlap in gene expression changes in the two studies.

Bioinformatic analyses of the genes commonly altered in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q/+</sup> cerebella show enrichment for categories associated with pathological pathways involved in neurodegeneration (Alzheimer’s disease), and also pathways previously implicated in pathogenesis both in knock-in and transgenic SCA1 mouse models, such as the phosphatidylinositol and calcium signaling pathways [26–28]. These results strongly suggest that Atxn1<sup>−/−</sup> mice have dysfunctional cerebella due to a loss of endogenous Atxn1 function. We also found that Atxn1<sup>−/−</sup> mice share significant overlap in cerebellar transcriptional profiles with staggerer mice, which have a spontaneous loss-of-function mutation in the gene encoding the transcription factor Rorx. Rorx-regulated genes involved in calcium signaling (Hpel and Calb1) and glutamatergic signaling (Gm1 and Slc1a6) are significantly down-regulated in Atxn1<sup>−/−</sup> cerebellum, as determined by microarray analysis and real-time qRT-PCR. It is noteworthy that loss-of-function mutations in several of these genes result in ataxic phenotypes (e.g., Tip61, Slc1a6, and Gm1) [36–38], raising the possibility that simultaneous down-regulation of several of these genes could contribute to the motor coordination impairments observed in Atxn1<sup>−/−</sup> mice. Rorx mRNA transcript and protein levels appear normal in Atxn1<sup>−/−</sup> cerebellum [20], ruling out that changes in Rorx targets are due to reduced Rorx protein levels in Atxn1<sup>−/−</sup> mice. Since ATXN1 and Rorx physically interact via Tip60, it is conceivable that loss of Atxn1 affects Rorx-dependent transcription directly [20]. Altogether, these data support two important conclusions: first, that Atxn1<sup>−/−</sup> cerebellum exhibits pathological molecular changes, even in the absence of progressive neurodegeneration, and second, that transcriptional changes in the loss-of-function model of Atxn1 could identify endogenous pathways that might also be altered by the expression of mutant Atxn1.

We previously described a reduction of Atxn1-Cic complexes in Atxn1<sup>154Q/+</sup> cerebella, with Atxn1<sup>154Q</sup> favoring the formation of an enhanced toxic gain-of-function complexes with RBM17 [23]. It is interesting that among the genes up-regulated both in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q/+</sup> cerebella, we identified two potential direct targets of Cic-dependent repression, Cnd1 and Ets5, the genes encoding for Cyclin D1 and Ets variant 5, respectively [29] and J. Fryer, unpublished data). We demonstrated that wild-type Atxn1 and Cic are bound to the promoter regions of Cnd1 and Ets5. Interestingly, we failed to detect mutant Atxn1<sup>154Q</sup> on these promoters in mice only expressing expanded Atxn1 [Atxn1<sup>154Q/+</sup>]. One interpretation of this result is that Atxn1<sup>154Q</sup> has diminished association to the promoters, resulting in reduced Atxn1-Cic dependent repression. Alternatively, it is possible that polyglutamine-induced conformational changes make the Atxn1<sup>154Q</sup>-Cic complexes less accessible for antibody recognition, resulting in reduced chromatin immunoprecipitation. Irrespective of the basis of the inability to detect Atxn1<sup>154Q</sup> binding on these promoters, the data strongly suggest that polyglutamine-expanded Atxn1 and Cic have reduced transcriptional repression function on these specific promoters in vivo. These results provide a mechanistic explanation on how diminished Atxn1-Cic function can contribute to transcriptional defects in SCA1.

Mild overexpression of the evolutionarily conserved gene Atxn1L partially suppresses the neuropathology caused by polyglutamine-expanded ATXN1 in flies and mice [19,24]. Increased Atxn1L levels induce the sequestration of polyglutamine-expanded Atxn1 into nuclear inclusions, leading to a proposed model in which Atxn1L suppresses toxicity by displacement of mutant Atxn1 from its major endogenous complexes that contain Cic [24]. In the present study, we show an additional mechanism contributing to this rescue, by demonstrating that mild overexpression of Atxn1L suppresses several molecular and behavioral phenotypes in Atxn1<sup>−/−</sup> mice, potentially by replacing Atxn1 in Cic-containing complexes (Figure 6). The motor coordination and learning deficits suppressed by Atxn1L are common to both Atxn1<sup>−/−</sup> and polyglutamine-expanded Atxn1<sup>154Q/+</sup> mouse models. Therefore, these data provide evidence for an additional mechanism in which Atxn1L can functionally compensate for a partial loss of Atxn1 function to suppress SCA1 pathogenesis. Although our studies demonstrate that Atxn1L is a functional homolog of Atxn1 in Cic-mediated transcriptional repression, we cannot rule out that Atxn1L overexpression can restore other yet to be determined Atxn1-related functions not addressed in these studies. In sum, based on our previous data and this study, we propose that partial loss of ATXN1 function actively contributes to SCA1 pathogenesis as part of a two-pronged mechanism, in which enhanced toxic gain-of-function of polyglutamine-expanded ATXN1 leads to neurodegeneration, while a simultaneous loss-of-function of another
Figure 5. Atxn1L<sup>dp</sup> suppresses the behavioral deficits in Atxn1<sup>−/−</sup> mice. We assessed the effects of mild overexpression of Atxn1L on Atxn1<sup>−/−</sup> phenotypes (A) Mice of the following genotypes: Atxn1<sup>++</sup> (n = 5), Atxn1<sup>++/−</sup> (n = 12), Atxn1<sup>−/−</sup> (n = 9), Atxn1<sup>++/dp</sup> (n = 10), Atxn1<sup>−/−/dp</sup> (n = 17), and Atxn1<sup>−/−/dp</sup>; Atxn1L<sup>dp</sup> (n = 12), were tested at 8 weeks for contextual conditioned fear. Atxn1<sup>−/−</sup> mice exhibited significantly reduced freezing behavior in the contextual fear-conditioning test compared to wild-type and Atxn1<sup>++/−</sup> littermates. However, Atxn1<sup>−/−</sup> mice carrying the Atxn1L duplication performed significantly better than Atxn1<sup>−/−</sup> littermates in this task (p<0.05). Wild-type and Atxn1<sup>++/−</sup> mice expressing the Atxn1L<sup>dp</sup> allele performed similarly to wild-type and Atxn1<sup>++/−</sup> mice without the Atxn1L duplication. (B–E) An independent cohort of mice was generated to test the effects of Atxn1L duplication on the motor deficits of Atxn1<sup>−/−</sup> mice. Atxn1<sup>++</sup> (n = 16), Atxn1<sup>++/−</sup>; Atxn1L<sup>dp</sup> (n = 23), Atxn1<sup>−/−</sup> (n = 14), and Atxn1<sup>−/−/dp</sup>; Atxn1L<sup>dp</sup> mice (n = 17) were tested on the dowel and wire hang paradigms at 8 weeks. The latency of Atxn1<sup>−/−</sup> mice to reach the side for the first time was increased compared to Atxn1<sup>++</sup> and Atxn1<sup>++/−</sup>; Atxn1L<sup>dp</sup> controls on the rod (B); they also walked off the rod fewer times in the 120 s interval (C). In contrast, Atxn1<sup>−/−</sup>; Atxn1L<sup>dp</sup> mice took less time to walk off the dowel (B), and they also crossed the dowel more times in 120 s than Atxn1<sup>−/−</sup> littermates (C). In the wire hang test, Atxn1<sup>−/−</sup> mice showed increased latency to reach the sides of the wire compared Atxn1<sup>++</sup> and Atxn1<sup>++/−</sup>; Atxn1L<sup>dp</sup> controls (D). Additionally, Atxn1<sup>−/−</sup> mice reached the sides fewer times than control littermates (E). In contrast, Atxn1<sup>−/−</sup> mice overexpressing Atxn1L exhibited marked reduction in the time for the first touch (D), and increased number of side touches in the 120 s interval, when compared to Atxn1<sup>−/−</sup> mice (E). Error bars in graphs represent +/- SEM, *p<0.05.

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stable endogenous protein complexes, Atxn1-Cic, contributes to the SCA1 phenotypes (Figure 6B).

It was previously reported that reduction of normal functions of genes involved in other polyglutamine diseases results in enhanced pathology, providing evidence for concomitant gain- and loss-of-function mechanisms in polyglutamine disorders [39–43]. In SBMA transgenic mouse models expressing polyglutamine-expanded androgen receptor (AR), loss of endogenous AR protein resulted in accelerated motor neuron degeneration [39]. These studies suggested two independent pathways contributing to SBMA pathogenesis: gain-of-function due to mutant AR nuclear toxicity and loss of AR trophic effects on motor neurons [39]. Conditional deletion of Htt in mouse forebrain leads to several features reminiscent of Huntington disease, including motor deficits, tremors and progressive degeneration of the striatum and cortex, hinting that loss of hit function could contribute to

Figure 6. Model of Atxn1L rescue through Cic stabilization in Atxn1-/- mice. (A) In wild-type mice, Atxn1-Cic and Atxn1L-Cic complexes bind the promoters of target genes and repress them effectively. (B) In mice expressing polyglutamine-expanded Atxn1, mutant Atxn1 associates preferentially with Rbm17, while the decrease in Atxn1-Cic complexes destabilizes Cic and reduces its levels at the promoters, thus leading to derepression of its target genes. (C) A similar Cic destabilization occurs in the absence of wild-type Atxn1 in Atxn1-/- mice, also resulting in increased expression of target genes. (D) When Atxn1L is moderately overexpressed in Atxn1-/- mice, it stabilizes Cic levels by forming functional Atxn1L-Cic complexes that can substitute for Atxn1-Cic at the promoters, thus rescuing target gene repression. We propose that this mechanism might also act to rescue loss-of-function of Atxn1 in Atxn1-154Q/; Atxn1Ldp/+ mice.

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Loss of Function of Ataxin-1 Contributes to SCA1

We used the Affymetrix Mouse Exon 1.0 ST microarray, which carries 1.2 million probe-sets covering one million exon clusters, with an average of 40 probes per gene. The exon array data were analyzed as previously described [52]. Briefly, raw data were processed in the R statistical programming environment using locally developed methods and the exonmap package. RNA normalization was applied, and linear models were calculated to analyze genotype effects for each gene. Genomic annotations were obtained from UCSC (http://genome.ucsc.edu). The normalized probe level data were then averaged within each exon to produce exon-level data for each gene for each animal. A two-way ANOVA with main effects for genotype and exonic region was calculated for each gene. The ANOVA model was fit using weighted least squares analysis where the weights were determined according to the probe counts within each exon. Since separate wild-type (WT) control littermates were used in the Atxn1154Q/+ and Atxn1154Q/+ experiments, a separate linear model was estimated for each gene in each model (one fit for each WT background). A linear contrast was calculated comparing the WT and mutant cross-exon means for each gene. The cut-off rule for determining genes was a fold change threshold of +/- 0.1 in both experiments, and a linear step up false discovery rate (FDR) of less than 0.01 value for the T-statistic corresponding to the linear contrast comparing each WT strain with its corresponding mutant. The gene set determined by this fold change and FDR multiplicity corrected cutoff, corresponds to a median raw marginal p-value of less than 0.00015 for the underlying T-statistics.

We performed Gene Ontology (GO) analysis on the obtained data using locally developed software and methods [52]. Briefly, the gene ontology vocabulary and current mouse annotations were obtained from the GO website (9/1/2007 build). The mouse exon array was mapped to Entrez identifiers, and these identifiers were mapped to the GO data structure using the available annotations. Using our local ontology analysis system (OntologyTraverser), we tabulated the genes annotated at or below each GO node for the entire exon array. We then used a hypergeometric sampling model to examine the statistical representation of each GO node for genes in our gene sets. In order to make comparisons between sets, we took differences between the standardized scores determined for each gene set. Because of the extreme overlapping structure of the GO, many GO nodes report duplicate or redundant information. To avoid this problem, we calculated the GO covariance structure and used this estimate to compute de-correlated GO scores. For the KEGG pathway analysis, the overlapping gene list containing the 197 most significant genes was uploaded and analyzed using the web-based Functional Annotation Bioinformatics Microarray Analysis DAVID 6.7 (National Institute of Allergy and Infectious Diseases NIH, david.abcc. nciifcr.gov/).

Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT–PCR)

For the independent validation of the exon array data, real-time qRT-PCR assays were performed on more than eight mice of each genotype. cDNA was synthesized from 1 μg of RNA using the RT2 First Strand Kit (SuperArray Bioscience Corporation, Frederick, MD). Quantitative real-time PCR reactions were performed on 10 ng of cDNA using RT2 SYBR Green/ROX PCR master mix and commercially available primers (SuperArray Bioscience Corporation, Frederick, MD). All RNA samples were analyzed in triplicate and normalized relative to Gapdh levels.

For validation of Ror targets, cDNA was synthesized from RNA isolated from seven Atxn1154Q/+ mice and control littermates, using SuperScript III (Invitrogen). Quantitative real-time PCR reactions were performed on 10 ng of cDNA using the SYBR Green PCR Master Mix (Applied Biosystems) using the following primer sets:

- **Brd1 5’- GGCCCAACAGCCTACAGATG-3’**
- **rv 5’- CTTCTTTTCTCACTGCTACAT-3’**
- **Grid2 5’- CCCCCAATTGAGTGCTC-3’**
value was calculated for each sample (Atxn1 between ChIP fractions, for every gene promoter studied, a precipitated DNA were determined based on the threshold cycle 

 realtime PCR experiments were performed in triplicate on followed by quantitative real-time PCR (SYBR green). Quantitative

Chromatin Immunoprecipitation-polymerase chain reaction analysis (ChIP-PCR)

ChIP was performed as previously described [52]. Cerebella were dissected from mice of each genotype at 7–8 weeks of age and incubated in 1% formaldehyde for 10 minutes at room temperature to cross-link DNA to associated proteins. Chromatin was treated with micrococcal nuclease and sheared by sonication to generate fragments with an average length of ~100–200 bps, as determined by agarose gel electrophoresis. For immunoprecipitation, 200 µl of chromatin was diluted 1:10 in ChIP dilution buffer (Millipore Corporation, Billerica, MA) and 1% of the diluted sample was saved as input. The sample was first precleared with protein A Dynabeads (Invitrogen Corporation, Carlsbad, CA), then incubated overnight with protein A Dynabeads that were pre-blocked with salmon sperm DNA and coupled to 20 µl of affinity purified rabbit anti-Atxn1 (11NQ) antibody, or 4 µl of guinea pig polyclonal anti-Cic antibody. Mock immunoprecipitation using non-specific preimmune sera for each antibody were included as negative controls. After immunoprecipitation, the beads were washed at room temperature with low salt buffer, followed by high salt buffer, LiCl buffer (Millipore Corporation, Billerica, MA), and TE buffer (10 mM Tris-HCl pH 8.0). Elution was performed twice in 250 µl of fresh elution buffer (1% SDS, 0.1 M NaHCO3) for 15 minutes at room temperature. The eluates were combined, the crosslinks were reversed, and DNA purified with Qiagen PCR cleanup kit (Qiagen, Valencia, CA) and recovered in 30 µl of 10 mM Tris-HCl pH 8.0. One or two µl of DNA were used for each PCR reaction using primer pairs for the promoter regions of the following genes:

Etk5 fw 5’-GGGGAACTTAGCTGATGAGTAA-3’
rv 5’-GTTTCTGTGTGTGGAACCGCA-AA-3’

Cond1 fw 5’-GGTTAAGCTGATGAGTAAATGTTT-3’
rv 5’-GGAATAGCTGATGAGTAAATGTTT-3’

Ccapicua binding to the promoters was also analyzed using ChIP followed by quantitative real-time PCR (SYBR green). Quantitative real-time PCR experiments were performed in triplicate on three independent sets of samples. Relative amounts of immunoprecipitated DNA were determined based on the threshold cycle (Ct) value for each PCR reaction. In order to control for variation between ChIP fractions, for every gene promoter studied, a ΔCt value was calculated for each sample (Atn1+/−, Atn1154Q/+), (Atn1−/−) by subtracting the Ct value for the input (Ctinput) from the Ct value for the immunoprecipitated sample (Ctantibody or Ctpreimmune). Since the input DNA fraction represents only 1% of the total material, the Ctinput value was first adjusted for this dilution factor by subtracting 6.644 cycles (Log2 of 100), then subtracted from the immunoprecipitated samples using the following formula:

\[
\Delta Ct_{\text{antibody}} \text{ or } Ct_{\text{preimmune}} = \left[ Ct_{\text{antibody or preimmune}} \right] - \left[ Ct_{\text{input}} - 6.644 \right].
\]

Differences between the specific immunoprecipitation and the preimmune serum background were then determined and plotted as fold enrichment over the preimmune serum (for each genotype sample: ΔCtantibody/ΔCtpreimmune).

Luciferase assays

Luciferase reporter assays for Cic-dependent repression were performed as previously described [18]. HEK293T cells in 24-well plates were co-transfected using Lipofectamine 2000 (Invitrogen) with 50 ng of the pGL3-Promoter (Promega) containing six copies of Cic binding sites (TGAATGAA or TGAATGGA), 10 ng of pRL-TK, and 10 ng of expression plasmids for Atxn1[2Q] (wild-type), Atxn1L and Cic-expressing plasmids as indicated. All constructs have also been previously described [18,24]. The total amount of DNA transfected was kept constant by adding pDNA3.1(-) (Invitrogen). Luciferase activities were measured using the dual luciferase reporter assay system (Promega).

Contextual fear conditioning

For this task, the mice were trained in a novel environment with a neutral stimulus (a tone) paired with a foot shock. The mice are placed in a different context with the same tone (cued test) or back in the same environment without a tone (contextual test) 24 h later. Mice of each genotype were placed in a Med Associates/Actimetrics chamber system where a 30 second tone was followed by a 2 second foot shock at 1.5 mA. The tone-foot shock were repeated at 2 min. Twenty-four hours later, the mice were tested for freezing in the same chamber with no tone to evaluate contextual fear. Scoring for freezing was automated in this system. Analysis was performed using ANOVA and t-test analysis.

Dowel and wire hang paradigms

Mice were placed on the center of a 0.9 cm wooden dowel suspended between two platforms. If the mouse walked off of the
dowel onto the platform, the mouse was placed back in the center. The test lasted 2 min beginning when the mouse was placed on the dowel. Data were analyzed using ANOVA and Student’s t-test.

Supporting Information

Figure S1 Cellular component gene ontology for genes commonly up-regulated in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella. Gene ontology categories shown were significantly enriched (positive z score, green) or depleted (negative z score, green) with the de-correlated z score for enrichment plotted in the x-axis. Only gene ontology categories with more than one gene represented and a z score>|+/-2| are represented.

Found at: doi:10.1371/journal.pgen.1001021.s001 (0.24 MB TIF)

Figure S2 Biological process gene ontology for genes commonly up-regulated in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella. Gene ontology categories shown were significantly enriched (positive z score, green) or depleted (negative z score, green) with the de-correlated z score for enrichment plotted in the x-axis. Only gene ontology categories with more than one gene represented and a z score>|+/-2| are represented.

Found at: doi:10.1371/journal.pgen.1001021.s002 (0.53 MB TIF)

Figure S3 Molecular function gene ontology for genes commonly up-regulated in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella. Gene ontology categories shown were significantly enriched (positive z score, green) or depleted (negative z score, green) with the de-correlated z score for enrichment plotted in the x-axis. Only gene ontology categories with more than one gene represented and a z score>|+/-2| are represented.

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Figure S4 Cellular component gene ontology for genes commonly down-regulated in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella. Gene ontology categories shown were significantly enriched (positive z score, green) or depleted (negative z score, green) with the de-correlated z score for enrichment plotted in the x-axis. Only gene ontology categories with more than one gene represented and a z score>|+/-2| are represented.

Found at: doi:10.1371/journal.pgen.1001021.s004 (0.38 MB TIF)

Figure S5 Biological process gene ontology for genes commonly down-regulated in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella. Gene ontology categories shown were significantly enriched (positive z score, green) or depleted (negative z score, green) with the de-correlated z score for enrichment plotted in the x-axis. Only gene ontology categories with more than one gene represented and a z score>|+/-2| are represented.

Found at: doi:10.1371/journal.pgen.1001021.s005 (0.74 MB TIF)

Figure S6 Molecular function gene ontology for genes commonly down-regulated in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella. Gene ontology categories shown were significantly enriched (positive z score, green) or depleted (negative z score, green) with the de-correlated z score for enrichment plotted in the x-axis. Only gene ontology categories with more than one gene represented and a z score>|+/-2| are represented.

Found at: doi:10.1371/journal.pgen.1001021.s006 (0.73 MB TIF)

Figure S7 Real time qRT-PCR validation of Rorα targets in Atxn1<sup>−/−</sup> cerebella. Four out of six tested genes were significantly down-regulated in Atxn1<sup>−/−</sup> cerebella. Error bars in graph represent +/- SEM  p<0.05.

Found at: doi:10.1371/journal.pgen.1001021.s007 (0.30 MB TIF)

Figure S8 Open Field Analysis to measure activity in pure C57Bl/6J Atxn1<sup>−/−</sup> mice. (A) Atxn1<sup>−/−</sup> mice at 10–11 weeks of age spend more time in the center of the field compared to wild-type littermates, suggesting they are less anxious. (B) Atxn1<sup>−/−</sup> and wild-type littermates traveled similar total distances. (C) Atxn1<sup>−/−</sup> mice had decreased rearing, as measured by vertical activity in the open field. Since Atxn1<sup>−/−</sup> mice show increased center/total, their reduced vertical activity probably reflects motor defects affecting rearing, and not increased anxiety. Error bars represent +/- SEM, ***p<0.005.

Found at: doi:10.1371/journal.pgen.1001021.s008 (0.31 MB TIF)

Figure S9 Light/Dark box and Elevated Plus maze to test for anxiety in pure C57Bl/6J Atxn1<sup>−/−</sup> mice. Atxn1<sup>−/−</sup> mice (n = 10) and wild-type controls (n = 9) were tested at 12 weeks of age. (A) Atxn1<sup>−/−</sup> mice had a small trend to spend more time in the light side than wild-type controls, although this trend does not reach significance (p = 0.26). (B) Both wild type and Atxn1<sup>−/−</sup> mice make the same number of transitions between the light and dark side of the box. (C) In the elevated plus maze, Atxn1<sup>−/−</sup> mice spent more time in the open arms than controls, and (D) also made more arm entries than controls. This shows that pure C57Bl/6J Atxn1<sup>−/−</sup> mice are not hypoactive, as reported before for Atxn<sup>154Q</sup> mice in a mixed C57Bl/6J/129svEv background. Error bars represent +/- SEM, ***p<0.0001.

Found at: doi:10.1371/journal.pgen.1001021.s009 (0.44 MB TIF)

Figure S10 Conditioned Fear Analysis to measure Pavlovian learning in Atxn1<sup>−/−</sup> mice. Seven mice of each genotype were tested at 12 weeks of age. (A) In the contextual conditioned fear test, mice were exposed to a tone paired to a foot shock and 24 hrs later, placed in the same chamber and the amount of freezing behavior is recorded. (B) In the cued test, only the tone is administered in a different chamber and freezing is recorded. Atxn1<sup>−/−</sup> mice show less freezing both in the contextual and cued test (A and B), indicating amygdala and hippocampal deficits in learning and memory. Error bars represent +/- SEM, *p<0.05,**p<0.01.

Found at: doi:10.1371/journal.pgen.1001021.s010 (0.27 MB TIF)

Figure S11 Dowel and wire hang analysis to measure gross motor ability in Atxn1<sup>−/−</sup> mice. In the Dowel test for motor coordination, mice are placed on a 0.9 cm rod and the time that it takes to reach the side and number of side touches in 2 minutes is recorded. Atxn1<sup>−/−</sup> mice (n = 11) and wild-type littermates (n = 9) were tested at 12 weeks. (A) Atxn1<sup>−/−</sup> mice performed poorly in the dowel, with increased latency for reaching the side for the first time. (B) Atxn1<sup>−/−</sup> mice also made less number of side touches in 2 min. (C and D) The wire hang test is similar to the dowel, except a wire is used instead of a rod. Atxn1<sup>−/−</sup> mice had a trend to reach the sides fewer times in 2 minutes than the controls (D), albeit not significant (p<0.06) Error bars +/- SEM, *p<0.05,**p<0.01.

Found at: doi:10.1371/journal.pgen.1001021.s011 (0.52 MB TIF)

Table S1 List of 197 commonly altered genes in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella. (p<0.01 Fold Change |+/−|0.1| log2 scale).

Found at: doi:10.1371/journal.pgen.1001021.s012 (0.37 MB DOC)

Table S2 KEGG pathway analysis of the 197 commonly altered genes in Atxn1<sup>−/−</sup> and Atxn1<sup>154Q</sup> cerebella.

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Author Contributions

Conceived and designed the experiments: JCB, JDF, HYZ. Performed the experiments: JCB, JDF. Analyzed the data: JCB, JDF, CAS, HTO, HYZ. Conceived and designed the experiments: JCB, JDF, HYZ. Performed the experiments: JCB, JDF, CAS, HTO, HYZ. Contributed reagents/materials/analysis tools: CAS, HTO. Wrote the paper: JCB. Provided substantial input on the manuscript: HYZ, JDF, HTO.
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