Partial loss of Tip60 slows mid-stage neurodegeneration in a spinocerebellar ataxia type 1 (SCA1) mouse model

Kristin M. Gehrking1,2, J. Michael Andresen1,3, Lisa Duvick1, John Lough4, Huda Y. Zoghbi5,6 and Harry T. Orr1,2,3,∗

1Institute of Human Genetics and Institute of Translational Neuroscience, 2Department of Biochemistry, Biophysics and Molecular Biology and 3Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455, USA, 4Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, 5Department of Molecular and 6Department of Human Genetics, Pediatrics, and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA

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Spinocerebellar ataxia type 1 (SCA1) is one of nine dominantly inherited neurodegenerative diseases caused by polyglutamine tract expansion. In SCA1, the expanded polyglutamine tract is in the ataxin-1 (ATXN1) protein. ATXN1 is part of an in vivo complex with retinoid acid receptor-related orphan receptor alpha (Rora) and the acetyltransferase tat-interactive protein 60 kDa (Tip60). ATXN1 and Tip60 interact directly via the ATXN1 and HMG-box protein 1 (AXH) domain of ATXN1. Moreover, the phospho-mimicking Asp amino acid at position 776, previously shown to enhance pathogenesis, increases the ability of ATXN1 to interact with Tip60. Using a genetic approach, the biological relevance of the ATXN1/Tip60 interaction was assessed by crossing ATXN1[82Q] mice with Tip601/2 animals. Partial Tip60 loss increased Rora and Rora-mediated gene expression and delayed ATXN1[82]-mediated cerebellar degeneration during mid-stage disease progression. These results suggested a specific, temporal role for Tip60 during disease progression. We also showed that genetic background modulated ATXN1[82Q]-induced phenotypes. Of interest, these latter studies showed that some phenotypes are enhanced on a mixed background while others are suppressed.

INTRODUCTION

Spinocerebellar ataxia type 1 (SCA1) is one of nine inherited polyglutamine diseases that cause neurodegeneration (1,2). In these diseases, the mutant gene encodes an expanded glutamine tract, which results in a polyglutamine expansion within the protein. In SCA1, the mutant ATXN1 gene encodes the protein ataxin-1 (ATXN1), which is widely expressed (3,4); however, neurodegeneration is limited to cerebellar Purkinje cells, brainstem and spinal cord (5). As with most autosomal dominant ataxias, symptoms are characterized by a progressive loss of motor coordination, neuropathies, slurred speech, cognitive impairment and loss of other functional abilities arising from deep cerebellar nuclei (6).

Evidence indicates that a normal function of ATXN1 is to regulate gene expression. For example, ATXN1 interacts with a variety of transcription factors, including the zinc-finger transcription factors Drosophila Senseless and its mammalian homolog growth factor-independent 1 (7), the transcription corepressor silencing mediator of retinoid and thyroid hormone receptors (8), the human homolog of the Drosophila transcription repressor Capicua (9), the transcription factor Sp1 (10), the retinoid acid receptor-related orphan receptor alpha/tat-interactive protein 60 kDa (Rora/Tip60) complex (11) and the RNA-binding protein RBM17 (12). Moreover, ATXN1 contains a nuclear localization sequence (13). Wild-type (WT) ATXN1 shuttles between the nucleus and cytoplasm (14). Importantly, mutant ATXN1 must enter the nucleus to cause

∗To whom correspondence should be addressed. Email: orrxx002@umn.edu

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disease (13) and its nuclear export is greatly reduced (14). Within ATXN1 is the ATXN1 and HMG-box protein 1 (AXH) domain, a 120 amino acid stretch found in many proteins (15). The ATXN1 AXH domain crystal structure reveals an oligonucleotide-binding fold (16), which is likely the site of (15). The ATXN1 AXH domain crystal structure reveals an oligonucleotide-binding fold (16), which is likely the site of

Some of these ATXN1-interacting factors, in a dosage-dependent fashion, modify pathogenesis of SCA1 in mouse and fly models (7–9,11). Notably, Rora haploinsufficiency results in enhanced pathogenesis in SCA1 transgenic mice (11). Previous work demonstrates that in the murine cerebellum ATXN1, Tip60 and Rora exist in an endogenous complex. The ability of GST–ATXN1 bind to in vitro transcribed and translated Tip60 indicates that these proteins interact directly (11). To examine the biological import of the ATXN1/Tip60 interaction in vivo, we utilized a genetic approach to investigate the impact of Tip60 haploinsufficiency in an SCA1 mouse model.

RESULTS

Mapping the Tip60-binding region of ATXN1

Previously using co-immunoprecipitations from WT cerebellar extracts, we showed that ATXN1, Rora and the coregulator Tip60 exist in a complex in which ATXN1 interacts directly with Tip60 (11). To examine the Tip60/ATXN1 interaction in more detail, a series of ATXN1 deletion constructs were generated and used in a GST-pull-down assay to assess their ability to interact with Tip60 (Fig. 1A). The results showed that an ATXN1 deletion construct that contained the AXH domain alone was sufficient to promote interaction with Tip60 (Fig. 1B, lane 9). In addition, all of the ATXN1 constructs containing the AXH domain (full length, full length without the self-association region (SAR) (18), fragment IV and fragment V) bound to Tip60 (Fig. 1B, lanes 3, 4, 6 and 7), while those lacking the AXH domain (fragment I and the SAR domain alone) did not bind Tip60 (Fig. 1B, lanes 5 and 8). Thus, we conclude that Tip60 binds to ATXN1 via the AXH domain.

Several lines of evidence indicate that phosphorylation of Ser776 is critical for the induction of neuronal dysfunction in SCA1 (12,19–21). Among these are data that a S776D substitution mimics phosphorylation and enhances disease severity (12,21). To examine whether D776 might also affect the interaction of ATXN1 with Tip60, GST pull-downs were performed using ATXN1–fragment V with either WT phosphorylatable Ser, phospho-resistant Ala or phospho-mimicking Asp at position 776. While substituting an Ala for the Ser at position 776 had no effect on the ability of ATXN1–fragment V to interact with Tip60, a phospho-mimicking Asp at residue 776 increased the ability of ATXN1–fragment V to interact with Tip60 (Fig. 1C), suggesting that the ATXN1/Tip60 interaction is modulated by S776 phosphorylation.

ATXN1[82Q] mice with haploinsufficiency of Tip60

As a means to examine the biological relevance of the ATXN1/Tip60 complex, we used a genetic strategy to determine whether gene dosage impacts the SCA1 phenotype. The approach involved crossing Tip60+/− animals with ATXN1[82Q] mice from the B05 line in which an expanded ATXN1 transgene is driven by the Purkinje cell protein 2 (Pcp2/L7) promoter (22). Heterozygous Tip60 mice were generated using homologous recombination to replace exons 1–9 with a neomycin-targeting vector (23). Heterozygous mice with a null Htatip allele (Tip60+/−) are viable and phenotypically normal. However, complete Tip60 loss of function (Tip60−/−) causes embryonic lethality near the blastocyst stage (23). The Tip60 heterozygous mice were on the SV-129;C57BL/6 genetic background, and the ATXN1[82Q] mice were on the FVB background. Thus, offspring of this cross had genotypes of WT, ATXN1[82Q];Tip60+/−, ATXN1[82Q];Tip60+/+ and Tip60−/− all on a mixed FVB;SV-129;C57BL/6 background (Supplementary Material, Fig. S1A).

First, two crucial control analyses were performed. To confirm that Tip60−/− mice had a partial loss of function, we measured Tip60 mRNA levels in cerebellar lysates. Heterozygous Tip60 null mice expressed approximately half of WT Tip60 levels (Supplementary Material, Fig. S1B). To assess whether partial Tip60 loss affected ATXN1[82Q] transgene expression, we compared transgene expression between ATXN1[82Q] and ATXN1[82Q];Tip60+/− mice. Since the expanded ATXN1[82Q] protein cannot be quantitatively solubilized from Purkinje cells of SCA1 transgenic mice (22), we used RNA levels to quantify transgene expression. By quantitative-PCR, ATXN1[82Q];Tip60−/− transgene expression was found to be similar regardless of Tip60 gene dosage (Supplementary Material, Fig. S1C). In addition, ATXN1 immunostaining revealed no detectable difference in amount or deposition of ATXN1 in Purkinje cells between ATXN1[82Q] and ATXN1[82Q];Tip60+/− mice (Supplementary Material, Figs S1D and S1E).

Effect of genetic background on SCA1 phenotypes

Morphological and neurological assessments were used to examine the extent to which SCA1 phenotypes varied on the FVB and FVB;SV-129;C57BL/6 mixed backgrounds. Progressive thinning of the cerebellar molecular layer, reflecting Purkinje cell degeneration and dendritic tree atrophy, is a quantitative hallmark of pathology in the Pcp2-SCA1 mice (21,24). To begin with, we determined the molecular layer thickness in WT-FVB and WT-FVB;SV-129;C57BL/6 mice and whether it varied with age. WT-FVB cerebella had a relatively stable molecular layer thickness between 5 and 20 weeks of age. On the other hand, WT-FVB;SV-129;C57BL/6 mice had a slightly thinner molecular layer that decreased somewhat with age (Fig. 2A). Consistent with previous data (22,23,25), ATXN1[82Q] on an FVB background induced a slow, progressive Purkinje cell degeneration from 5 to 20 weeks of age. In contrast, the ATXN1[82Q]-FVB;SV-129;C57BL/6 mice had a slightly thinner molecular layer that decreased rapidly by 12 weeks of age and remained at 115 μm from 16 to 20 weeks of age (Fig. 2B).

Next, we assessed the motor phenotype using the accelerating rotarod paradigm. ATXN1[82Q] mice on the FVB genetic background showed a rotarod deficit as early as 5 weeks of age and had a more pronounced phenotype at 12 weeks (22,25). In contrast, ATXN1[82Q] mice on the FVB;SV-129;C57BL/6 genetic background (ATXN1[82Q]-mix) did not show a rotarod deficit until 30 weeks of age (Fig. 2C). Thus, while
SCA1 molecular layer pathology was more pronounced on the mixed background, development of the neurological phenotype as determined by the rotarod was delayed in ATXN1[82Q]-mix mice.

Partial loss of Tip60 slows mid-stage ATXN1[82Q]-induced Purkinje cell pathology

To determine whether a partial loss of Tip60 affected the SCA1 phenotype, several parameters were compared in WT, ATXN1[82Q] and ATXN1[82Q]:Tip60+/− mice all on the mixed FVB;SV-129;C57BL/6 background. We first examined the effect of Tip60+/− on SCA1 pathology in vivo by calbindin immunostaining and measurement of the molecular layer thickness (Fig. 3A). As quantified in Figure 3B, partial Tip60 loss slowed neurodegeneration in ATXN1[82Q]:Tip60+/−-mix mice relative to ATXN1[82Q]-mix mice during the mid-stage of disease. While the molecular layer was reduced in both ATXN1[82Q]-mix and ATXN1[82Q]:Tip60+/−-mix mice at 5 weeks of age, at 12 and 16 weeks ATXN1[82Q]:Tip60+/−-mix mice...
Genetic background affects SCA1 phenotypes in ATXN1[82Q] transgenic mice. (A) Cerebellar molecular layer thickness in aging WT-FVB and FVB;SV-129;C57BL/6 (mix) mice (n = 3–7 mice/genotype/age). (B) Cerebellar molecular layer thickness in aging FVB and FVB;SV-129;C57BL/6 (mix) mice expressing the ATXN1[82Q] transgene (n = 3–7 mice/genotype/age). (C) Motor performance using the accelerating rotarod paradigm in aging FVB;SV-129;C57BL/6 (mix) mice expressing the ATXN1[82Q] transgene (n = 6–12 mice/genotype).

Previously, we showed that the extension of climbing fiber terminals along the Purkinje cell dendritic tree is compromised in ATXN1[82Q]-mix mice (21). Using calbindin immunofluorescence to visualize Purkinje cell dendrites and VGluT2 immunofluorescence to visualize climbing fiber terminals, the extent of climbing fiber terminal extension along the Purkinje cell dendrites was measured in ATXN1[82Q]:Tip60<sup>+-</sup>-mix cerebella (Fig. 3C). At all ages, WT mice had a significantly greater climbing fiber extension along Purkinje cell dendrites compared with ATXN1[82Q]-mix expressing mice (Fig. 3D). However, at 12 and 16 weeks, partial loss of Tip60 resulted in a reduction in the extent to which climbing fiber terminal extension was compromised by ATXN1[82Q]. At 12 and 16 weeks, ATXN1[82Q]:Tip60<sup>+-</sup>-mix mice had a significantly greater climbing fiber extension along Purkinje cell dendrites than their ATXN1[82Q]-mix littersmates. By 20 weeks of age, extension of climbing fiber terminals in ATXN1[82Q]:Tip60<sup>+-</sup>-mix mice was as compromised as in ATXN1[82Q]-mix animals (Fig. 3D). Thus, by a second measure of ATXN1[82Q]-induced pathology, partial loss of Tip60 afforded a protective period during the mid-stage, 12–16 weeks of age, of disease.

Restoration of ATXN1[82Q]-induced changes in Rora, and Rora-mediated gene expression by a partial loss of Tip60

In ATXN1[82Q]-mix mice, mutant ATXN1 depletes Rora from cerebellar Purkinje cells and Rora haploinsufficiency results in enhanced pathogenesis in SCA1 transgenic mice (11). For the following set of experiments, we focused on a group of genes downregulated in both SCA1 and staggerer mice. Within this group, a subset of genes is known to bind Rora at the promoter (11–27–30). We sought to determine whether the protective effects on ATXN1[82Q]-induced pathology by Tip60<sup>+-</sup> correlated with restoration of Rora and Rora-mediated gene expression.

Western blot analysis revealed that partial Tip60 loss increased Rora protein expression relative to ATXN1[82Q]-mix littersmates (Fig. 4A, lanes 3 versus 4). Next, we characterized the effect of partial Tip60 loss on those Rora-mediated genes whose expression was previously found decreased in SCA1 and staggerer mouse cerebella (11). These include Pcp2 (Purkinje cell protein 2), Pcp4 (Purkinje cell protein 4), Slc1a6 (solute carrier family 1-high affinity aspartate/glutamate transporter 6) and Itp1 (inositol 1,4,5-triphosphate receptor, type 1). Gene expression was assessed at three ages: 8 weeks, an early stage with intermediate Purkinje cell atrophy; 12 weeks, similar to the same advanced level. At all ages, WT-mix and Tip60<sup>+</sup>-mix gave the same results (data not shown).

In 8-week-old mice, there were no significant differences between WT-mix, ATXN1[82Q]-mix and ATXN1[82Q]:Tip60<sup>+</sup>-mix expression of Slc1a6, Pcp4, Pcp2 or Itp1.

Figure 2. Genetic background affects SCA1 phenotypes in ATXN1[82Q] transgenic mice. (A) Cerebellar molecular layer thickness in aging WT-FVB and FVB;SV-129;C57BL/6 (mix) mice (n = 3–7 mice/genotype/age). (B) Cerebellar molecular layer thickness in aging FVB and FVB;SV-129;C57BL/6 (mix) mice expressing the ATXN1[82Q] transgene (n = 3–7 mice/genotype/age). (C) Motor performance using the accelerating rotarod paradigm in aging FVB;SV-129;C57BL/6 (mix) mice expressing the ATXN1[82Q] transgene (n = 6–12 mice/genotype).

Figure 3. Tip60 haploinsufficiency rescues SCA1 cerebellar pathology during the mid-stage of disease. (A) Calbindin immunofluorescence of Purkinje cells in aging WT, ATXN1[82Q] and ATXN1[82Q]:Tip60<sup>+</sup>- FVB;SV-129;C57BL/6 (mix) mice. (B) Quantitative analysis of the molecular thickness in aging WT, ATXN1[82Q] and ATXN1[82Q]:Tip60<sup>+</sup>- FVB;SV-129;C57BL/6 (mix) mice (n = 3–7 mice/genotype/age). (C) Immunofluorescence of calbindin (red) and VGluT2 (green) in WT, ATXN1[82Q] and ATXN1[82Q]:Tip60<sup>+</sup>- FVB;SV-129;C57BL/6 (mix) mice. (D) Quantitative analysis of the climbing fiber extension along Purkinje cell dendrites in aging WT, ATXN1[82Q] and ATXN1[82Q]:Tip60<sup>+</sup>- FVB;SV-129;C57BL/6 (mix) mice (n = 3–5 mice/genotype/age).
Tip60 to the promoter complex (29). In each case, expression of at least some Rora-mediated genes become abnormally elevated in the presence of ATXN1[82Q] and with increasing age, the expression was significantly higher than in WT-mix cerebella. Thus, in ATXN1/Tip60 animals, the protective effect of reduced Tip60 levels was associated with increased Rora protein and Rora-mediated gene expression. These results indicate that Tip60-mediated pathways contribute to SCA1.

By two measures of pathology, we found Purkinje cell degeneration was slowed in ATXN1[82Q]-Tip60+/−-mix compared with ATXN1[82Q]-mix animals. The protective effect of a partial loss of Tip60 slowed the progression of ATXN1[82Q]-induced Purkinje cell atrophy. The protective effect of reduced Tip60 levels was associated with increased Rora protein and Rora-mediated gene expression. These results indicate that Tip60-mediated pathways contribute to SCA1.

DISCUSSION

Previously, we showed that ATXN1, Rora and the coregulator Tip60 exist in a complex in which ATXN1 interacts directly with Tip60 (11). In the present study, we further examined the interaction of ATXN1 and Tip60, showing that this interaction is both dependent on ATXN1’s AXH domain and enhanced by a phospho-mimicking Asp at position 776. Importantly, the AXH domain is required for mutant, polyglutamine-containing ATXN1 to induce neurodegeneration (7). Recently, we showed that a phospho-mimicking Asp at residue 776 enhances ATXN1-induced pathogenesis (21). Thus, Tip60 interacts with a region of ATXN1 crucial for disease, and this interaction is promoted by an amino acid substitution that enhances pathogenesis. Moreover, the finding that D776 promotes the ATXN1/Tip60 interaction strengthens the concept that the conformational change induced by S776 phosphorylation includes the AXH domain. Based on these features, we hypothesized that the ATXN1/Tip60 interaction has biological relevance.

As a basis for examining the ATXN1/Tip60 interaction in vivo, we reasoned that reducing Tip60 levels might modify ATXN1[82Q]-induced disease. We found that a partial loss of Tip60 slowed the progression of ATXN1[82Q]-induced Purkinje cell atrophy. The protective effect of reduced Tip60 levels was associated with increased Rora protein and Rora-mediated gene expression. These results indicate that Tip60-mediated pathways contribute to SCA1.
to separate disease initiation and neuronal dysfunction from subsequent Purkinje cell death (21), suggesting that perhaps the pathways critical for progression to neuronal death overlap with those that limit the extent of the Tip60\(^{+/−}\) protective effect.

The pattern of the Tip60\(^{+/−}\) effect on Rora-mediated gene expression illustrates the complex role of this system in SCA1. Both Rora and Rora-mediated gene expression are decreased in ATXN1\([82Q]\) mutant mice (11). Consistent with Rora’s role in SCA1 pathogenesis, the ATXN1\([82Q]\)-induced loss of Rora was reversed at 12 weeks of age in ATXN1\([82Q]\):Tip60\(^{+/−}\)-mix animals. Moreover, Tip60\(^{+/−}\) was also associated with a restoration of certain Rora-mediated genes at 12 weeks of age. Interestingly, not all Rora-mediated gene expression levels were restored by partial Tip60 loss at 12 weeks of age. However, by 20 weeks of age, expression of all four Rora-mediated genes examined was at or above the WT level. Thus, the restoration of some Rora-mediated genes (e.g., Slc1a6 and Pcp4) occurred early in the Tip60\(^{+/−}\) protective window, while the restoration of others was delayed (e.g., Pcp2 and Itprl). This suggests that only a subset of Rora-mediated genes may contribute to the Tip60\(^{+/−}\) protective effect. It is important to note that Pcp4 and Slc1a6 are not only genes with Rora-mediated expression, but are also genes with Rora-dependent recruitment of Tip60 to the promoter complex (29). Conversely, the expression of three genes examined that did not show significant changes in ATXN1\([82Q]\):Tip60\(^{+/−}\) mice (Calb1, Grm1 and Cals) are genes where Rora does not recruit Tip60 to the transcription complex (29). Interestingly, expression of all of the four Rora-mediated genes that were elevated in ATXN1\([82Q]\):Tip60\(^{+/−}\) mice remained elevated at 20 weeks of age when Tip60\(^{+/−}\) was no longer protective. Perhaps in these mice, overexpression of certain Rora-mediated genes, e.g. Slc1a6, contributes to toxicity.

Whether partial Tip60 loss enhances Rora-mediated gene expression by relieving ATXN1-induced Rora depletion or perhaps alters specific posttranslational modifications that impact Rora-mediated transcription remains an open question. Tip60 was shown to be a co-activator for the androgen nuclear receptor (32). Moreover, androgen receptor activation by Tip60 acetylation requires direct interaction via Tip60’s LXXL motif (33). It is intriguing to speculate that perhaps the acetyltransferase activity of Tip60 is in some way connected to the decrease in cerebellar Rora induced by ATXN1\([82Q]\). If this were to be the case, this would support a model where the function of Tip60, coactivator versus corepressor, varies depending on the nuclear receptor and other proteins with which it associates.

Our results also showed that the time course and severity of SCA1 symptoms in the mouse varied when the same mutant ATXN1\([82Q]\) transgene was expressed on two different genetic backgrounds. Purkinje cell degeneration was more rapid when ATXN1\([82Q]\) was expressed on the mixed genetic background versus the FVB background. Moreover, development of motor performance deficit (ataxia) was delayed on the mixed background compared with the FVB background. This strongly suggests the presence of genetic modifiers regulating the severity of SCA1 phenotypes in mice. Disease presentation in SCA1 patients is also greatly influenced by non-polyglutamine factors, which may include genetic modifiers (34,35). Identifying the genetic modifiers that influence SCA1 phenotypes in mice should illuminate additional pathways that influence neurodegeneration.

In summary, we conclude the ATXN1/Tip60 interaction contributes to SCA1 pathogenesis with a partial loss of Tip60 delaying cerebellar degeneration in an SCA1 mouse model, specifically during mid-stage disease. These findings, with the observation that ATXN1\([82Q]\)-mediated pathology and ATXN1\([82Q]\)-mediated motor deficits are differentially influenced by genetic background, indicate that not only is the relationship between the molecular pathways that underlie disease initiation and progression complex, so is the relationship between pathology and neuronal dysfunction.

MATERIALS AND METHODS

In vitro transcription/translation GST pull-down assay

ATXN1 cDNAs were cloned into pGEX vectors (Amersham Biosciences) and expressed in E. coli BL21(DE3) cells. Human Tip60 was cloned into pCDNA3.1/His expression vector (Invitrogen). In vitro transcription/translation and GST pull-down were done as described (11). Samples were run on NuPage 4–12% Bis–Tris polyacrylamide gels (Invitrogen), stained with SimplyBlue SafeStain colloidal Coomassie (Invitrogen) and dried on Whatman 3MM paper. Amount of ATXN1 was measured by densitometry of the Coomassie staining, and bound radioactive Tip60 was determined by autoradiography followed by band excision and scintillation counting.

Mouse genotyping

Animal breeding. WT, Tip60\(^{+/−}\), ATXN1\([82Q]\) and ATXN1\([82Q]\):Tip60\(^{+/−}\) mice were the F1 progeny (1:1:1:1) resulting from breeding ATXN1\([82Q]\) and Tip60\(^{+/−}\) mice. ATXN1\([82Q]\) mice were maintained on the FVB background and the Tip60\(^{+/−}\) mice on a SV-129;C57BL/6 background.

Genotyping of mice. PCR was used to identify Tip60\(^{+/−}\) and ATXN1\([82Q]\) transgenic animals as described by Hu et al. (23) and Burright et al. (22), respectively.

Immunostaining and quantitative measurements

Animals were perfused with 10% formalin and 50 micron vibratome sections cut as described (21). Floating cerebellar slices were incubated with the following primary antibodies in blocking buffer [2% donkey serum, 0.3% Triton X-100 in 1 x phosphate buffered saline (PBS)] at dilutions indicated: goat calbindin (SC-7691, Santa Cruz Biotechnology) at 1:500, rabbit 11750/ataxin-1 at 1:2500 and mouse VGLUT2 (MAB5504, Millipore) at 1:1000. Secondary antibodies used were: donkey anti-goat Cy3 (#705-165-147, Jackson Immunoresearch) at 1:500, donkey anti-rabbit Cy2 (#711-225-152, Jackson Immunoresearch) at 1:500 and donkey anti-mouse Cy5 (#115-175-146, Jackson Immunoresearch) at 1:500. Sections were washed, mounted onto microscope slides with glycerol–gelatin containing 4 mg/ml n-propyl gallate (Sigma).
and visualized on a FluoView inverted confocal, laser-scanning microscope (#FV1000 IX2, Olympus). Molecular layer thickness was measured with FluoView software.

For molecular layer measurements, calbindin-stained Purkinje cells were analyzed from two sagittal sections that were analyzed per mouse from at least five mice per genotype. Using confocal laser-scanning microscopy, six measurements were taken at the primary fissure and averaged to determine molecular layer thickness (21,24). For climbing fiber–Purkinje cell measurements, climbing fibers were similarly measured with VGluT2 antibody staining from six measurements at the primary fissures of two sagittal sections per mouse in at least three mice per genotype. Purkinje cell dendrite lengths were obtained as stated above, and the climbing fiber:Purkinje cell ratio was calculated using averaged measurements. Data are expressed as the mean ± SEM. The P-value was calculated using Student’s t-test (two-tailed equal variance).

**Gait analysis**

Accelerating Rotarod (Model 7650 Ugo Basile) analysis was performed at 9, 12, 16, 20 and 30 weeks of age as described (25). Student’s t-test was used to assess statistical significance.

**Western blot analysis**

Mouse cerebella were homogenized in brain extraction buffer: 0.25 M Tris–HCl pH 7.5 with phosphatase inhibitors cocktails I and II (P2850, P5726 Sigma) and protease inhibitors (1183617001, Roche). Forty milligrams of protein were denatured, run on 4–12% Bis–Tris Gel (NP0321BOX, Invitrogen) and blotted on nitrocellulose membrane (Protran BA 85, Whatman/GE Healthcare). Membranes were blocked overnight at 4°C with 10% bovine serum albumin in 1 × Tris-buffered saline with 0.1% v/v Tween-20. Blocked membranes were incubated with RORα antibody (H-65 #sc-28612, Santa Cruz Biotechnology) for 1 h at 22°C, washed three times with 0.1% PBS + 0.1% Tween and incubated with anti-goat horse radish peroxidase secondary antibody for 45 min. Samples were probed with mouse anti-GAPDH (#MAB374, Chemicon) as a loading control. Densitometry was used to quantify protein levels.

**Quantitative RT–PCR**

Total RNA was isolated from the cerebella of ATXN1[82Q], ATXN1[82Q]:Tip60+/− and WT littermates at 8, 12 and 20 weeks using the TRIzol method (Invitrogen). A minimum of three mice was used per genotype. Fifty nanograms of total RNA were used per reaction in triplicate with the TaqMan one-step reverse transcriptase (RT)–PCR kit (#4309169, Applied Biosystems). The following probes were assayed by Applied Biosystems: Pcp2 (Mm00435514_m1), Pcp4 (Mm00500973_m1), Slc1a6 (Mm00436591_m1) and Itpr1 (Mm01183049_m1). Samples were normalized to GAPDH. Reactions were run on a Real-time Quantitative PCR System ABI PRISM 7500.


