Cross-talking noncoding RNAs contribute to cell-specific neurodegeneration in SCA7

Jennifer Y Tan1,2,11, Keith W Vance1,2,12, Miguel A Varela2,12, Tamara Sirey1,2, Lauren M Watson2,3, Helen J Curtis2, Martina Marinello4–7, Sandro Alves4–7, Bruno R Steinkraus8, Sarah Cooper9, Tatyana Nesterova9, Neil Brockdorff9, Tudor A Fulga8, Alexis Brice4–7,10, Annie Sittler4–7, Peter I Oliver1,2, Matthew J Wood2,3, Chris P Ponting1,2,13 & Ana C Marques1,2,11,13

What causes the tissue-specific pathology of diseases resulting from mutations in housekeeping genes? Specifically, in spinocerebellar ataxia type 7 (SCA7), a neurodegenerative disorder caused by a CAG-repeat expansion in ATXN7 (which encodes the most abundantly expressed miRNA in the CNS14, because targeted deletion of this miRNA in mice partially mimicked Dicer knockout and resulted in increased degeneration of retinal and neuronal cells15; the two cell types with the highest miR-124 expression.

Similarly to miRNAs, long (>200-nucleotide) noncoding RNAs (lncRNAs), whose expression is often spatially and temporally restricted16,17, have also been previously associated with neurodegeneration. Spinocerebellar ataxia type 8 (SCA8)19, for instance, is caused by the toxicity of a CUG trinucleotide–repeat expansion in the lncRNA antisense to ATXN8 as well as by the loss of function.
of the polyQ-expanded mutant ATXN8 protein. As in SCA8, ribonuclear inclusions have been found in other polyQ disorders, such as myotonic dystrophy and Huntington’s disease—like type 2 (ref. 21), thus suggesting that repeat-expanded RNA may be pathogenic. IncRNAs can also contribute to disease by regulating transcript abundance of their overlapping disease-causing genes, as found for BACE1-AS, in Alzheimer’s disease22, and for SCAANT1, a noncoding transcript overlapping ATXN7 (ref. 23). Several intergenic IncRNAs have been found to be dysregulated in patients with Huntington’s23 or Alzheimer’s25 diseases, and an intergenic IncRNA was recently found to be correlated in expression with a strong intergenic risk allele for Parkinson’s disease. However, whether such associations reflect causal contributions by intergenic IncRNAs or are consequences of the disease pathologies remains unknown.

We sought to establish the origin of the tissue-specific pathology of SCA7 by investigating the regulation of ATXN7, a ubiquitously expressed gene, by tissue-specifically expressed noncoding RNAs. Our results demonstrate that the regulation of the abundance of mutant ATXN7 through cross-talking noncoding RNAs that are highly specific to the retina and the cerebellum contributes to the selective neurodegeneration observed in SCA7.

RESULTS

Inc-SCA7 is a post-transcriptional regulator of Atxn7

We identified a retropseudogene, Inc-SCA7 (official symbol ATXN7L3B) that is unusually conserved across placental mammals and whose expression is significantly correlated with that of ATXN7 across human and mouse adult tissues and postnatal central nervous system (CNS) regions (Supplementary Fig. 1a and Supplementary Fig. 1). In mice, this correlation is stronger in CNS regions (Pearson’s $R^2 = 0.94$, $P < 0.001$, two-tailed correlation test; $n = 10$ CNS regions and 10 non-CNS regions) than elsewhere (Pearson’s $R^2 = 0.69$, $P < 0.05$; Supplementary Fig. 1b), thus suggesting that an additional layer of regulation could control the relative abundance of both transcripts in these regions.

Inc-SCA7 arose from retrotransposition of the gene encoding ataxin-7–like protein 3 (Atxn7L3), a distant paralog of Atxn7, in the common ancestor of placental mammals approximately 100 million years ago. We found no homology between the 1-kb genomic regions upstream of Atxn7L3 and of Inc-SCA7, results indicating that they are unlikely to have homologous promoters. Inc-SCA7 inserted downstream of a preexisting CpG island, and its duplication accumulated frame-shifting deletions that resulted in premature stop codons and a truncated open reading frame (ORF) (Supplementary Fig. 1c). The small putative polypeptide (97 amino acids) encoded by Inc-SCA7 lacks the two annotated functional ATXN7L3 protein domains (Supplementary Fig. 1d). Despite both transcripts being expressed at similarly high levels in mouse neuroblastoma cells (N2A; Supplementary Fig. 1e), a custom antibody raised against the N-terminal protein sequence conserved between Atxn7L3 and the putative Inc-SCA7 protein (Supplementary Fig. 1c) detected translation of ATXN7L3 (predicted size 39 kDa) but not of a polypeptide of the size expected for Inc-SCA7 protein (11 kDa; Supplementary Fig. 1f and Supplementary Data Set 1). The transcript originating from this gene is thus unlikely to be translated into a stable protein product in these cells.

The coordinated expression between Inc-SCA7 and Atxn7 in both mice and humans prompted us to explore whether this long noncoding RNA regulates Atxn7’s transcript abundance. In mouse N2A cells, Inc-SCA7 depletion (up to 20% of control) by multiple target-specific short hairpin RNA (shRNA) constructs (Supplementary Fig. 2a) significantly reduced Atxn7 transcript levels (up to 60% of control; Fig. 1b and Supplementary Fig. 2b) and Atxn7 protein levels (by approximately 60% of control; Fig. 1c and Supplementary Data Set 1). Furthermore, in N2A cells, overexpression (6.8-fold) of the region downstream of Inc-SCA7’s putative stop codon (nucleotides 599–3607, hereafter termed Inc-SCA7-WT) significantly increased levels of Atxn7 transcript (2.3-fold; Fig. 1d) and Atxn7 protein (Fig. 1e and Supplementary Data Set 1). We observed comparable increases in Atxn7 transcript levels upon overexpression of either full-length Inc-SCA7 sequence (Inc-SCA7-full) or a recombinant mutant with a premature stop codon (Inc-SCA7-stop; Fig. 1d and Online Methods).

We conclude that, in mice, Inc-SCA7 modulates the expression of Atxn7 via a transcript-dependent mechanism that does not rely on the translation of its putative ORF. Given its cytoplasmic localization (Supplementary Fig. 2c), we hypothesized that, similarly to other IncRNAs27, Inc-SCA7 modulates Atxn7 expression post-transcriptionally by competing for the binding of shared miRNAs.
miR-124 mediates interaction of Inc-SCA7 and Atxn7 transcripts

To test this hypothesis, we took advantage of a mouse embryonic stem (ES) cell line that is conditionally deficient for Dicer (Dcr\(^{-/\alpha}\)), an essential component of the miRNA biogenesis pathway in mammals\(^{28}\). We found that, as observed in N2A cells, Inc-SCA7 knockdown (76% relative to control) in wild-type ES cells significantly reduced the expression of Atxn7 (82% of control; Fig. 2a). In contrast, in Dcr-deficient ES cells a similar level of Inc-SCA7 knockdown had no significant effect on Atxn7 expression (Fig. 2b). This is consistent with the regulation of Atxn7 expression by Inc-SCA7 being miRNA-dependent.

Of all brain-expressed miRNAs, only two, miR-16 and miR-124, have corresponding predicted miRNA-response elements (MREs) within the 3′ untranslated regions (UTRs) of both Atxn7 and Inc-SCA7 that are conserved (between mice and humans) (Fig. 2c, Supplementary Fig. 3a,b and Supplementary Table 1). In contrast to miR-16, which has no known role in the brain, miR-124 is the most abundantly expressed miRNA in the CNS\(^{14}\) and has well-established roles in neuronal development\(^ {29}\).

Transfection of N2A cells with miR-124 mimics reduced Inc-SCA7 and Atxn7 expression levels relative to those after transfection of a nonspecific miRNA negative control (32% and 19% of control, respectively; Fig. 2d), whereas reduction of endogenous levels of miR-124 (62% of control) led to significant increases in expression of both Inc-SCA7 and Atxn7 (by 1.8- and 2.5-fold, respectively; Fig. 2e).

In contrast, miR-16 mimics failed to significantly alter the levels of these transcripts (Supplementary Fig. 3c). These findings, together with miR-16’s considerably lower abundance (Supplementary Fig. 3d), indicated that miR-124 but not miR-16 is likely to mediate cross-talk between Inc-SCA7 and Atxn7 transcripts in mouse neurons.

This conclusion was further supported by the dependence of the reduction in reporter activity after cotransfection of miR-124 mimics and recombinant Inc-SCA7 or Atxn7 luciferase-reporter constructs (23% and 42% of control, respectively; Fig. 2f) on the presence of the predicted miR-124 MREs in these transcripts. More specifically, transfected constructs bearing inverted seed sequences of all miR-124 MREs predicted within Inc-SCA7 (six MREs) and Atxn7 (two MREs) (hereafter referred to as Inc-SCA7-mut and Atxn7-mut, respectively) abolished the effect of miR-124 on reporter activity (Fig. 2f).

As expected, neither Inc-SCA7-mut nor Atxn7-mut overexpression (7.7- and 9.7-fold, respectively) had a significant impact on transcript levels of Atxn7 (Fig. 2g) or Inc-SCA7 (Fig. 2h), consistently with the ability of Inc-SCA7 and Atxn7 transcripts to modulate each other’s abundance in a miR-124-dependent manner.

A feedback loop involving ATXN7 mRNA and miR-124

Reduction in Inc-SCA7 levels surprisingly led to depletion of both mature (23% of control; Fig. 3a) and precursor–miR-124 levels (47% of control; Supplementary Fig. 4a). Decreased levels (63% of control) of Inc-SCA7 in human neuroblastoma cells (SH-SY5) were associated...
Figure 3 Transcription of miR-124 precursors is STAGA dependent. (a) Effect of lnc-SCA7 (blue) knockdown on mature miR-124 levels (gray) in N2A cells over a 72-h time course. (b) Fold enrichment in Gcn5 binding, relative to IgG control, in the promoter regions of pri-miR-124–encoding genes (gray) with negative control (NC) in white), measured by chromatin immunoprecipitation and qPCR (ChiP-qPCR). Labels on y axis refer to regions within the control or pri-miRNA promoter, as depicted in Supplementary Fig. 4d-f. (c) Fold difference in normalized luciferase activity after cotransfection of empty pcDNA3.1(+) control (white), Inc-SCA7-WT (dark gray) or Inc-SCA7-mut (light gray) with all three miR-124–promoter-luciferase (miR-124–prom-luc) reporter constructs. Error bars, s.e.m. (n = 3 cell cultures per condition). **P < 0.01; ***P < 0.001; not significant (NS), P > 0.05 by two-tailed Student’s t test.

with decreased expression levels of both ATXN7 (84% of control) and primary (pri)-miR-124a-1 (87% of control), consistently with the conservation of regulatory interactions in humans (Supplementary Fig. 4b). Furthermore, and as seen in mouse neuroblastoma cells, decreased levels of endogenous miR-124 were associated with increased lnc-SCA7 and ATXN7 transcription abundance in these cells (Supplementary Fig. 4c), consistently with the evolutionary conservation of cross-talk between these transcripts. Changes in miR-124 levels do not reflect a general effect of Inc-SCA7 on miRNA expression or processing because genome-wide analysis of miRNA abundance revealed no significant differences in N2A miRNA repertoires after either knockdown or overexpression of Inc-SCA7 (Supplementary Table 2 and Online Methods).

This suggested that Inc-SCA7 abundance correlates with the rate of transcription of precursor–miR-124 loci and that STAGA might be required for miR-124 transcriptional initiation. To test this hypothesis, we first identified the putative promoters of the three pri-miR-124–encoding genes (Mir124a-1, Mir124a-2 and Mir124a-3) as their nearest upstream DNase I-hypersensitivity region that was marked with acetylated histone H3 K27 (H3K27ac) in mouse cerebellum (Supplementary Fig. 4d–f). STAGA is required for the transcriptional activation of miR-124 loci: each of these promoters exhibited reporter activity at least 8.5-fold higher than that for the control antisense sequence (Supplementary Note) and was bound by Gcn5, STAGA’s histone acetyltransferase, at 2.0- to 3.5-fold greater levels than for IgG control (Fig. 3b). Furthermore, although overexpression of Inc-SCA7-WT increased luciferase activity for all three miR-124 promoters (1.5- to 1.8-fold), we detected no significant changes in activity after overexpression of Inc-SCA7-mut (Fig. 3c). Accordingly, Inc-SCA7 knockdown decreased Gcn5 binding (Supplementary Fig. 4g) and luciferase activity for each of the three miR-124 promoters (Supplementary Fig. 4i).

In summary, Atxn7 transcripts promote miR-124 transcription initiation; in turn, miR-124 is key to the post-transcriptional cross-talk between Inc-SCA7, Atxn7 transcripts and other STAGA mRNAs in the CNS (Supplementary Note), predominantly in the tissues in which miR-124 is more highly expressed, namely the retina and the cerebellum (Supplementary Fig. 5).

Noncoding RNAs mediate SCA7’s tissue-specific pathology

In SCA7, the polyQ-expanded ATXN7 protein is associated with decreased STAGA chromatin-modification activity30,31 and with reduced levels of transcripts from loci relying on STAGA transcriptional initiation32. The regulatory feedback loop (Fig. 4a) revealed by our in vitro analysis predicts that a decrease in STAGA activity in SCA7 would result in: (i) diminished pri-miR-124 transcriptional initiation and, as a consequence, (ii) decreased mature miR-124 and (iii) increased Inc-SCA7 levels (Fig. 4b).

We first validated these predictions in a human model of SCA7 by comparing the levels of ATXN7, Inc-SCA7 and miR-124 in fibroblasts derived from three patients with SCA7, who carry 42, 49 or 55 polyQ-repeat expansions in ATXN7, against the levels in control fibroblasts (with ten polyQ-repeats). Expression levels of miR-124 (Supplementary Note) was bound by Gcn5, STAGA’s histone acetyltransferase, at 2.0- to 3.5-fold greater levels than for IgG control (Fig. 3b). Furthermore, although overexpression of Inc-SCA7-WT increased luciferase activity for all three miR-124 promoters (1.5- to 1.8-fold), we detected no significant changes in activity after overexpression of Inc-SCA7-mut (Fig. 3c). Accordingly, Inc-SCA7 knockdown decreased Gcn5 binding (Supplementary Fig. 4g) and luciferase activity for each of the three miR-124 promoters (Supplementary Fig. 4i).

In summary, Atxn7 transcripts promote miR-124 transcription initiation; in turn, miR-124 is key to the post-transcriptional cross-talk between Inc-SCA7, Atxn7 transcripts and other STAGA mRNAs in the CNS (Supplementary Note), predominantly in the tissues in which miR-124 is more highly expressed, namely the retina and the cerebellum (Supplementary Fig. 5).

Noncoding RNAs mediate SCA7’s tissue-specific pathology

In SCA7, the polyQ-expanded ATXN7 protein is associated with decreased STAGA chromatin-modification activity30,31 and with reduced levels of transcripts from loci relying on STAGA transcriptional initiation32. The regulatory feedback loop (Fig. 4a) revealed by our in vitro analysis predicts that a decrease in STAGA activity in SCA7 would result in: (i) diminished pri-miR-124 transcriptional initiation and, as a consequence, (ii) decreased mature miR-124 and (iii) increased Inc-SCA7 levels (Fig. 4b).

We first validated these predictions in a human model of SCA7 by comparing the levels of ATXN7, Inc-SCA7 and miR-124 in fibroblasts derived from three patients with SCA7, who carry 42, 49 or 55 polyQ-repeat expansions in ATXN7, against the levels in control fibroblasts (with ten polyQ-repeats). Expression levels of miR-124 (Supplementary Note) was bound by Gcn5, STAGA’s histone acetyltransferase, at 2.0- to 3.5-fold greater levels than for IgG control (Fig. 3b). Furthermore, although overexpression of Inc-SCA7-WT increased luciferase activity for all three miR-124 promoters (1.5- to 1.8-fold), we detected no significant changes in activity after overexpression of Inc-SCA7-mut (Fig. 3c). Accordingly, Inc-SCA7 knockdown decreased Gcn5 binding (Supplementary Fig. 4g) and luciferase activity for each of the three miR-124 promoters (Supplementary Fig. 4i).

In summary, Atxn7 transcripts promote miR-124 transcription initiation; in turn, miR-124 is key to the post-transcriptional cross-talk between Inc-SCA7, Atxn7 transcripts and other STAGA mRNAs in the CNS (Supplementary Note), predominantly in the tissues in which miR-124 is more highly expressed, namely the retina and the cerebellum (Supplementary Fig. 5).

Noncoding RNAs mediate SCA7’s tissue-specific pathology

In SCA7, the polyQ-expanded ATXN7 protein is associated with decreased STAGA chromatin-modification activity30,31 and with reduced levels of transcripts from loci relying on STAGA transcriptional initiation32. The regulatory feedback loop (Fig. 4a) revealed by our in vitro analysis predicts that a decrease in STAGA activity in SCA7 would result in: (i) diminished pri-miR-124 transcriptional initiation and, as a consequence, (ii) decreased mature miR-124 and (iii) increased Inc-SCA7 levels (Fig. 4b).

We first validated these predictions in a human model of SCA7 by comparing the levels of ATXN7, Inc-SCA7 and miR-124 in fibroblasts derived from three patients with SCA7, who carry 42, 49 or 55 polyQ-repeat expansions in ATXN7, against the levels in control fibroblasts (with ten polyQ-repeats). Expression levels of miR-124 (Supplementary Note) was bound by Gcn5, STAGA’s histone acetyltransferase, at 2.0- to 3.5-fold greater levels than for IgG control (Fig. 3b). Furthermore, although overexpression of Inc-SCA7-WT increased luciferase activity for all three miR-124 promoters (1.5- to 1.8-fold), we detected no significant changes in activity after overexpression of Inc-SCA7-mut (Fig. 3c). Accordingly, Inc-SCA7 knockdown decreased Gcn5 binding (Supplementary Fig. 4g) and luciferase activity for each of the three miR-124 promoters (Supplementary Fig. 4i).

In summary, Atxn7 transcripts promote miR-124 transcription initiation; in turn, miR-124 is key to the post-transcriptional cross-talk between Inc-SCA7, Atxn7 transcripts and other STAGA mRNAs in the CNS (Supplementary Note), predominantly in the tissues in which miR-124 is more highly expressed, namely the retina and the cerebellum (Supplementary Fig. 5).
were reduced by more than two-fold (45% of control), whereas transcript abundance of Inc-SCA7 and ATXN7 increased substantially (by up to 1.8-fold and 5.2-fold, respectively) in these patients’ cells (Fig. 5a). Furthermore, decreased levels of endogenous miR-124 (62% of control) in human fibroblasts were associated with increased expression of both Inc-SCA7 and ATXN7 (y axis, blue) and Atxn7 (y axis, red) with miR-124 (x axis) in SCA7100Q/100Q mice, as measured with qRT-PCR, relative to SCA75Q/5Q matched controls. (d,e) RNA in situ hybridization of miR-124 and Atxn7 RNA in the retina and cerebellum of SCA7266Q/5Q mice and lnc-SCA75Q/5Q controls in the retina (d; ganglion cell layer (GCL); inner nuclear layer (INL); outer nuclear layer (ONL)) and cerebellum (e; granule cell layer (GCL); Purkinje cell layer (PCL)). Error bars, s.e.m. for 3 cell cultures per condition (a) and cerebellum tissues derived from 3 individual mice per condition (b). *P < 0.05; **P < 0.01; ***P < 0.001; not significant (NS), P > 0.05 by two-tailed Student’s t test.

Fig. 5 Contribution of noncoding RNAs to the tissue-specific pathology of SCA7. (a) Fold difference in expression of mature miR-124 (dark gray), Inc-SCA7 (dark blue) and ATXN7 (red) in fibroblasts from patients with SCA7 with 42, 49 or 55 expanded ATXN7 polyQ repeats relative to unaffected control (white). (b) ChIP-qPCR showing significantly decreased enrichment, relative to IgG negative control, in GCN5 binding at three miR-124 promoters in SCA7100Q/100Q mice (dark gray) and control mice (white). (c) Correlation between the fold difference in expression levels between Inc-SCA7 (y axis, blue) and ATXN7 (y axis, red) with miR-124 (x axis) in SCA7100Q/100Q mice, as measured with qRT-PCR, relative to SCA75Q/5Q matched controls. (d,e) RNA in situ hybridization of miR-124 and Atxn7 RNA in the retina and cerebellum of SCA7266Q/5Q mice and lnc-SCA75Q/5Q controls in the retina (d; ganglion cell layer (GCL); inner nuclear layer (INL); outer nuclear layer (ONL)) and cerebellum (e; granule cell layer (GCL); Purkinje cell layer (PCL)). Error bars, s.e.m. for 3 cell cultures per condition (a) and cerebellum tissues derived from 3 individual mice per condition (b). *P < 0.05; **P < 0.01; ***P < 0.001; not significant (NS), P > 0.05 by two-tailed Student’s t test.
neurons observed in patients with SCA7 has thus far remained unexplained. Our in vitro and in vivo analyses in mice suggest that post-transcriptional regulation by two cross-talking noncoding RNAs, Inc-SCA7 and miR-124, mediates the tissue specificity of SCA7 pathology. Using two SCA7 mouse models, we showed that levels of miR-124 decrease, owing to reduced transcriptional activation activity of the STAGA complex as a result of ATXN7 mutation; thereafter, lower miR-124 levels contribute to the increased abundance of ATXN7 transcripts. These molecular interactions are expected to be more prevalent in the retina and cerebellum—the two tissues in which miR-124 is most highly expressed and exerts the strongest control on ATXN7 transcript abundance—mirroring the cell-specific pathology of SCA7. Post-transcriptional cross-talk between ATXN7 and lnc-SCA7 transcripts is likely to amplify the cellular impact of aberrant miR-124 levels. Nuclear aggregates of mutant ATXN7 protein are often found in the retina and cerebellum—the two tissues in which lnc-SCA7 locus may underlie the associated ataxia-like symptoms.

Our results provide much-needed insight into the contributions to human disease of noncoding RNAs, specifically those that confer the cell-specific disease pathology caused by mutations in ubiquitously expressed genes. Identifying additional noncoding RNAs that contribute to the tissue specificity in other diseases should further improve understanding of how RNA cross-talk modulates disease phenotypes.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank E. Becker (University of Oxford) for vectors, helpful discussions and comments on the manuscript; A. Barnard, M. McClements and R. MacLaren (all at John Radcliffe Hospital, University of Oxford) for WERI cells; members of the A.C.M. and C.P.P. laboratories for insightful comments and suggestions; I. Baumgarten for valuable discussions and establishing patient fibroblast cultures; and H.Y. Zoghbi for the SCA7 KO mice. This work was supported by funding from the Medical Research Council (to C.P.P and Weatherall Institute of Molecular Medicine (WIMM) Strategic Award, MRC G0902418, to B.R.S. and T.A.F.), a Marie Curie Intra-European Career Development Award (to A.C.M.), the University of Oxford (to A.C.M.), the Royal Society (to A.C.M.), a European Research Council Advanced Grant (to C.P.P., A.C.M., K.W.V., and T.S.), the French National Research Foundation (to S.A.), the South African National Research Foundation (to L.M.W.), the Medical Research Council (to S.A. and L.M.W.), the University of Cape Town (to L.M.W.), the Harry Crossley Foundation (to L.M.W.), the Commonwealth Scholarship Commission (to L.M.W.), the Clarendon Fund (to J.Y.T.), the Natural Sciences Engineering Research Council of Canada (to J.Y.T.), the Wellcome Trust (WT081385 to S.C., T.N. and N.B.), a European Research Council Starting Grant (to P.L.O.), Ataxia UK (to H.J.C. and M.A.V.), the French Association against Myopathies (AFM) (to A.B. and long-term fellowship to S.A.), the Association Connaître les Syndrômes Cérébelleux (to A.S. and S.A.) and a French Ministry of Research fellowship (to M.M.).

AUTHOR CONTRIBUTIONS

A.C.M. conceived the study; J.Y.T. performed experiments and analyzed results with contributions from K.W.V., M.A.V., T.S., L.M.W., H.J.C., M.M., M.A.V., B.R.S., S.C., T.N. and P.L.O.; N.B., T.A.F., A.B., A.S., M.J.W., C.P.P. and A.C.M. supervised the analysis; C.P.P. and A.C.M. supervised the study; J.Y.T., C.P.P. and A.C.M. wrote the manuscript. All authors read, contributed to and agreed with the final version of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Gouw, L.G. et al. Analysis of the dynamic mutation in the SCA7 gene shows marked parental effects on CAG repeat transmission. Hum. Mol. Genet. 7, 525–532 (1998).
2. David, G. et al. Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. Nat. Genet. 17, 65–70 (1997).
3. Gouw, L.G., Digre, K.B., Harris, C.P., Haines, J.H. & Ptcacek, L.J., Autosomal dominant cerebellar ataxia with retinal degeneration: clinical, neuropathologic, and genetic analysis of a large kindred. Neurology 44, 1441–1447 (1994).
4. Holmberg, M. et al. Spinocerebellar ataxia type 7 (SCA7): a neurodegenerative disorder with neuronal intranuclear inclusions. Hum. Mol. Genet. 7, 913–918 (1998).
5. Heinlinger, D. et al. Ataxon-7 is a subunit of GCN5 histone acetyltransferase-containing complexes. Hum. Mol. Genet. 13, 1257–1265 (2004).
6. Cancel, G. et al. Distribution of ataxon-7 in normal human brain and retina. Brain 123, 2519–2530 (2000).
7. Mattick, J.S. The genetic signatures of noncoding RNAs. PLoS Genet. 5, e1000459 (2009).
8. Sayer, D. & Abdelatif, M. MicroRNAs in development and disease. Physiol. Rev. 91, 827–887 (2011).
9. Packer, A.N., Xing, Y., Harper, S.Q., Jones, L. & Davidson, B.L. The bifunctional microRNA miR-8/miR-9+ regulates REST and GoREST and is downregulated in Huntington’s disease. J. Neurosci. 28, 14341–14346 (2008).
10. Johnson, R. & Buckley, N.J. Gene dysregulation in Huntington’s disease: REST, microRNAs and beyond. Neuronovol. Med. 11, 183–199 (2009).
11. Lee, Y. et al. miR-19, miR-101 and miR-130 co-regulate ATXN1 levels to potentially modulate SCA1 pathogenesis. Nat. Neurosci. 11, 1137–1139 (2008).
12. Damiani, D. et al. Dicer inactivation leads to progressive functional and structural degeneration of the mouse retina. J. Neuroscience 28, 4878–4887 (2008).
13. Schaefer, A. et al. Cerebellar neurodegeneration in the absence of microRNAs. J. Exp. Med. 204, 1553–1558 (2007).
14. Lagos-Quintana, M. et al. Identification of tissue-specific microRNAs from mouse. Curr. Biol. 12, 735–739 (2002).
15. Sanuki, R. et al. mir-124a is required for hippocampal axogenesis and retinal cone survival through Lin2x suppression. Nat. Neurosci. 14, 1125–1134 (2011).
16. Derrien, T. et al. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 22, 1775–1789 (2012).
17. Cabili, M.N. et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev. 25, 1915–1927 (2011).
18. Qureshi, I.A., Mattick, J.S. & Mehler, M.F. Long non-coding RNA expression. Genes Dev. 21, 247–258 (2010).
19. Faghihi, M.A. et al. Evidence for natural antisense transcript-mediated inhibition of microRNA function. Genome Biol. 11 (2010).
20. Sophier, B.L. et al. CTCF regulates ataxin-7 expression through promotion of a convergently transcribed, antisense noncoding RNA. Neuron 70, 1071–1084 (2011).
21. Bithell, A., Johnson, R. & Buckley, N.J. Transcriptional dysregulation of coding and non-coding genes in cellular models of Huntington’s disease. Biochem. Soc. Trans. 37, 1270–1275 (2009).
22. Mui, E., Hof, P.R. & Tiedge, H. Dendritic BC200 RNA in aging and in Alzheimer’s disease. Proc. Natl. Acad. Sci. USA 104, 10679–10684 (2007).
23. Kumar, V. et al. Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. PLoS Genet. 9, e1003201 (2013).
24. Tay, Y., Rinn, J. & Pandolfi, P.P. The multilayered complexity of ceRNA crosstalk and competition. Nature 505, 344–352 (2014).
25. Nesterova, T.B. et al. Dicer regulates Xist promoter methylation in ES cells indirectly through transcriptional control of Dnmt3a. Epigenetics Chromatin 1, 2 (2008).
26. Visvanathan, J., Lee, S., Lee, B., Lee, J.W. & Lee, S.K. The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. Genes Dev. 21, 744–749 (2007).
27. McMahon, S.J., Pray-Grant, M.G., Schieltz, D., Yates, J.R. & Grant, P.A. Polyglutamine-expanded spinocerebellar ataxia-7 protein disrupts normal SAGA and SLIK histone acetyltransferase activity. Proc. Natl. Acad. Sci. USA 102, 8478–8482 (2005).
28. Palhan, V.B. et al. Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration. Proc. Natl. Acad. Sci. USA 102, 8472–8477 (2005).
29. McCullough, S.D. et al. Reelin is a target of polyglutamine expanded ataxin-7 in human spinocerebellar ataxia type 7 (SCA7) astrocytes. Proc. Natl. Acad. Sci. USA 109, 21319–21324 (2012).
30. Chen, Y.C. et al. GenR loss-of-function accelerates cerebellar and retinal degeneration in a SCA7 mouse model. Hum. Mol. Genet. 21, 394–405 (2012).
31. Yoo, S.Y., et al. SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant ataxin-7 in neurons and abnormalities in short-term plasticity. Neuron 37, 383–401 (2003).
32. Karginov, F.V. et al. A biochemical approach to identifying microRNA targets. Proc. Natl. Acad. Sci. USA 104, 19291–19296 (2007).
33. Agire, X. et al. Epigenetic silencing of the tumor suppressor microRNA Hsa-miR-124a regulates CKD6 expression and confers a poor prognosis in acute lymphoblastic leukemia. Cancer Res. 69, 4443–4453 (2009).
34. Yao, A.S., Staalhi, B.T., Chen, L. & Crabtree, G.R. MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 460, 642–646 (2009).
35. Makeev, E.V., Zhang, J., Carrasco, M.A. & Maniatis, T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol. Cell 27, 435–448 (2007).
36. Liu, X.S. et al. MicroRNA profiling in subventricular zone after stroke: MiR-124a regulates proliferation of neural progenitor cells through Notch signaling pathway. PLoS ONE 6 (2011).
37. Shi, X.B. et al. Tumor suppressive miR-124 targets androgen receptor and inhibits proliferation of prostate cancer cells. Oncogene 32, 4130–4138 (2013).
38. Xia, H. et al. Loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells. J. Biol. Chem. 287, 9962–9971 (2012).
39. Hendrickson, D.G., Hogan, D.J., Herschlag, D., Ferrell, J.E. & Brown, P.O. Systematic identification of miRNAs recruited to argonaute 2 by specific microRNAs and corresponding changes in transcript abundance. PLoS ONE 3, e2126 (2008).
40. Fang, M. et al. The miR-124 regulates the expression of BACE1/β-secretase correlated with cell death in Alzheimer’s disease. Toxicol. Lett. 209, 94–105 (2012).
41. Nakamachi, Y. et al. MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis Rheum. 60, 1294–1304 (2009).
42. Zander, C. et al. Similarities between spinocerebellar ataxia type 7 (SCA7) cell models and human brain: proteins recruited in inclusions and activation of caspase-3. Hum. Mol. Genet. 10, 2569–2579 (2001).
43. Bandiera, S. et al. Genetic variations creating microRNA target sites in the FXN 3′-UTR affect frataxin expression in Friedreich ataxia. PLoS ONE 8, e54791 (2013).
44. Chou, A.H. et al. Polyglutamine-expanded ataxin-7 causes cerebellar dysfunction by inducing transcriptional dysregulation. Neurochem. Int. 56, 329–339 (2010).
45. Abou-Sleymane, G. et al. Polyglutamine expansion causes neurodegeneration by altering the neuronal differentiation program. Hum. Mol. Genet. 15, 691–703 (2006).
46. Rajakulendran, S. et al. Deletion of chromosome 12q21 affecting KCNC2 and ATXN7L3 in a family with neurodevelopmental delay and ataxia. J. Neurol. Neurosurg. Psychiatry 84, 1225–1257 (2013).
ONLINE METHODS

Human and mouse gene expression profiling. Microarray gene expression data for ATXN7 and lnc-SCA7, also known as ATXN7L3B, were obtained from Gene Expression Atlas (GNA) through BioGPS (http://biogps.org/) for humans58, and their correlation coefficient (Pearson’s correlation, R2) was computed across all 59 available tissues or cells where both loci were expressed (AD > 20, Pearson’s R2 = 0.24, P < 0.05; data not shown).

Total RNA from 20 human normal adult tissues (adipose, bladder, brain, cervix, colon, esophagus, heart, kidney, liver, lung, ovary, placenta, prostate, skeletal muscle, small intestine, spleen, testes, thymus, thyroid and trachea) and 11 mouse normal adult tissues (bladder, brain, colon, heart, kidney, liver, lung, pancreas, skeletal muscle, small intestine and stomach) were included. (First)Choice Human Total RNA Survey Panel from Invitrogen, and mouse panel from Ambion. Total RNA from mouse retina and eight brain tissues (cerebellum, cortex, entorhinal cortex, hippocampus, hypothalamus, medulla, olfactory bulb and striatum) was extracted with TRIzol (Invitrogen). Tissues were pooled from seven postnatal day 5 mice before extraction. RNA from mouse retina was extracted from wild-type (WT) C57BL/6 mice (pooled from two animals), and human retinal RNA was extracted from human WERI retinaloblastoma cells with the RNeasy kit (Qiagen).

RNA was reverse transcribed into cDNA with a QuantiTect Reverse Transcription kit (Qiagen) according to the manufacturer’s instructions. Expression levels were estimated by real-time quantitative PCR (qRT-PCR) on a StepOne Real-Time PCR thermocycler (ABI) with SYBR green PCR Master Mix (ABI) and loci-specific primers (Supplementary Table 4) in triplicate. Non-reverse-transcribed RNA was used as a negative amplification control.

Western blotting. N2A whole cell extracts were lysed with RIPA buffer (Sigma), and the protein concentration was determined with the BCA assay kit ( Pierce). 100 µg of protein was loaded onto a 4–20% Tris-glycine gel (Invitrogen) and separated by gel electrophoresis, and then transferred onto a PVDF membrane in 1 × transfer buffer (48 mM Tris, 39 mM glycine, and 20% methanol) at 40 V for 2 h. The membrane was stained with Ponceau S (Sigma P7170-1L) according to the manufacturer’s guidelines and destained with water.

The membrane was blocked with (5% skimmed milk) for 1 h and incubated overnight in TBS-T buffer (0.9% NaCl, 100 mM Tris, and 1% Tween20) with the primary antibodies anti-Atrx (sc-21110, Santa Cruz Biotechnology, working dilution 1:200) and custom anti-lnc-SCA7/Atxn7l3 (Amsbio, working dilution 1:100) according to the manufacturer's guidelines and destained with water.

The membrane was washed three times for 30 min in TBS-T and incubated with biotinylated secondary antibodies (ab6884 and ab7089, Abcam) for Atrx7 and putative lnc-SCA7/Atxn7l3 (Ambio, working dilution 1:100) at 4 °C. The membrane was washed four times for 30 min in TBS-T and incubated with horseradish peroxidase-conjugated streptavidin (ab7403; Abcam, 1:10,000) for an additional hour. Enhanced chemiluminescence detection was performed as recommended by the manufacturer (Amersham). The membrane was incubated twice for 10 min in stripping buffer, (25 mM glycine, 1% SDS, and 0.1% Tween 20); this was followed by two washes in PBS for 10 min and two washes in TBS-T for 5 min. The membrane was then blocked and reprobed with a positive-control antibody, anti–α-tubulin (ab7291, Abcam, working dilution 1:5,000); this was followed by the detection procedure described above (secondary antibody, ab64255). Commercial antibodies were validated by manufacturer as stated on their websites. Original images of blots used in this study can be found in Supplementary Data Set 1.

Knockdown and overexpression constructs. Three small interfering RNA (siRNA) constructs were designed with the siRNA selection program from the Whitehead Institute59 to specifically target Inc-lnc-SCA7 (Supplementary Fig. 2a and Supplementary Table 4). For a control, we used an oligonucleotide with no significant sequence similarity to miRNAs in the mouse genome and with similar nucleotide composition (scrambled control; Supplementary Table 4). siRNAs and scrambled control were used to create short hairpin RNAs (shRNAs) by linking the two arms of the hairpin (loop sequence TTCAAGAGA). HPLC-purified custom-made oligonucleotides (10 µM, Sigma-Aldrich) were annealed in annealing buffer (10 mM Tris, pH 8, and 50 mM NaCl) at 95 °C for 5 min. After being cooled to room temperature, oligonucleotides were phosphorylated with T4 Polynucleotide Kinase (NEB) and cloned downstream of a U6 promoter from a modified pl3.7 vector (courtesy of E. Becker). The shRNA construct that had the greatest impact on Inc-lnc-SCA7 levels (Supplementary Fig. 2b) was used in subsequent experiments. Three stable Inc-lnc-SCA7–knockdown and control N2A polyclonal cell lines were independently derived by cotransfection of the Inc-lnc-SCA7–knockdown and scrambled shRNA constructs with pTKit-Hyg vector (courtesy of K. Vance). Cells were grown in hygromycin-containing medium (Invitrogen, 200 µg/ml) until high confluence was reached (after approximately 10 d). Medium was changed every 48 h.

Alex7-3 UTR (nucleotides 2876–6882, ENSMUST00000022527, ENSEMBL Build 70, Alex7-7WT) and the putative 3′ noncoding untranslated sequences of mouse Inc-lnc-SCA7 (nucleotides 599–3607, Inc-lnc-SCA7-WT), the full-length mouse Inc-lnc-SCA7 (Inc-lnc-SCA7-full) as well as Inc-lnc-SCA7-stop were cloned downstream of a CMV promoter on the pcDNA3.1(+) vector. Inc-lnc-SCA7-stop was generated by direct mutagenesis of position 14 of Inc-lnc-SCA7-full from C to A.

To disrupt miR-124–binding sites within Inc-lnc-SCA7-WT and Alex7-7WT, all miR-124 MRE regions complementary to the miRNA seed, 5′-TGCGCCTT-3′, within these constructs were mutated by reversing the sequence, with direct mutagenesis, to 5′-TTCCGT-3′. Empty pcDNA3.1(+) vector was used as transfection control in overexpression experiments.

Tissue culture. Mouse retinaloblastoma (N2A) cells were grown at 37 °C in a humidified atmosphere supplemented with 5% CO2 in Dulbecco’s Modified Eagle Medium (DMEM) containing antibiotic penicillin/streptomycin, supplemented with 10% fetal calf serum (FCS). Mouse DTCM23/49 XY embryonic stem (ES) cell lines were grown as described previously60. Deletion of Der’s RNase III domain was induced by culturing the cells in the presence of 800 nM (Z)-4-hydroxytamoxifen (+-OH, Sigma); noninduced cells were treated with 0.1% ethanol and used as control. Both conditional and Der-deficient ES cells were routinely maintained on a feeder layer of mitomycin-inactivated mouse primary embryonic fibroblasts. Prior to the analyses, feeder cells were depleted from the cultures by preplating trypsinized cells for 25 min and transferring ES-enriched cell suspension to a new gelatin-coated plate in a culturing medium supplemented with leukemia inhibitory factor (Invitrogen) and 2i inhibitors (CHIR99021 at 3 µM and PD0325901 at 1 µM, Stemgent). After removal of the RNase III domain of Der, cells were kept for no longer than six passages.

Fibroblast cell lines derived from patients with SCA7 (SCA77Q10Q, SCA79Q10Q, and SCA74Q10Q) were incubated at 37 °C in 5% CO2 in DMEM Glutamax (Gibco-BRL) supplemented with 10% FBS and antibiotics.

1 d before transfection, N2A or ES cells (1.0 × 105 cells/ml) were seeded in six-well dishes. Knockdown and overexpression constructs and their respective control constructs (1 µg) were transfected with FuGENE 6 Transfection Reagent (Roche) according to the manufacturer’s guidelines. After transfection, cells were grown under standard conditions before harvesting. A miRNAs mimic (50 nM/well) (miRNA, ABI) and its negative control (cat. no. 446058, ABI), or an siRNA (5 nM/well) (FlexiTube GS382423, Qiagen) and its negative control (cat. no. 1027280, Qiagen), were transfected with Lipofectamine RNAiMAX Reagent (Invitrogen). Cells were harvested 48 h after transfection.

RNA extraction and quantification. Total cellular RNA was extracted with the RNeasy kit (Qiagen) according to the manufacturer’s instructions. To quantify levels of mature miRNAs, total RNA was extracted with the miRNeasy kit (Qiagen). Genomic DNA was removed with the DNA-free kit (Ambion). RNA was reverse transcribed, and cDNA was used to quantify gene expression changes, relative to Gapdh, with sequence-specific primers (Supplementary Table 4), as described above.

For miRNA quantification, RNA was reverse transcribed with the TaqMan MicroRNA Reverse Transcription kit (Invitrogen). For comparison of miRNA expression between tissues, RNA was reverse transcribed with the NCCode VLO miRNA cDNA Synthesis kit (Invitrogen). miRNA abundance was measured by qRT-PCR (SYBR green) with miRNA-specific TaqMan MicroRNA Assays (ABI) according to the manufacturer’s instructions. The expression levels of miRNAs were normalized to that of 18S rRNA.

Mutagenesis. Directed mutagenesis of constructs was generated by PCR with 2 µl of reverse-transcribed cDNA with 300 nM of primers (Supplementary Table 4). 1 U Expand High Fidelity DNA polymerase (Roche), 1.5 mM MgCl2, 0.2 mM dNTPs, 5% DMSO and 10× buffer in 50 µl total volume. PCR reactions were carried out in a Veriti 96-well (Applied Biosystems) thermocycler as follows: 94 °C for 2 min; 5 cycles with 15 s at 94 °C, 15 s at a temperature gradient of 58–68 °C and 2 min at 94 °C; 15 cycles with 15 s at 94 °C, 30 s at a temperature...
gradient of 55–65 °C and 2 min at 72 °C, with a 5-s extension added after each cycle; and a terminal step at 72 °C for 7 min. All constructs were verified by Sanger sequencing.

**Subcellular fractionation.** Subcellular fractionation of N2A cells was carried out with the PARIS kit (Invitrogen) according to the manufacturer’s instructions. After isolation of the nuclear and cytoplasmic fractions from total cell lysates, RNA from each subcellular compartment was extracted, reverse transcribed, and quantified as described above. With qRT-PCR, expression levels of all genes were measured independently in the cytoplasmic and nuclear fractions. Fold enrichment between the distinct compartments (expression level measured in the cytoplasm/expression level measured in the nucleus) was reported after normalization to Gapdh.

**Luciferase assays.** lnc-SCA7 and Atxn7 3’ UTRs were cloned downstream of the luciferase reporter gene in the pGL3-promoter (pGL3-pro) vector (Promega), luc–lnc-SCA7–WT and luc–Atxn7–WT, respectively. luc–lnc-SCA7–mut and luc–Atxn7–mut were generated by site-directed mutagenesis of all miR-124–binding sites. Each luciferase construct (2 µg) was cotransfected with 10 ng of pRL Renilla luciferase control vector (Promega) and 50 nM mirVana miR-124 or negative-control miRNA mimics (mir-NC) (Invitrogen) with the FuGENE 6 Transfection Reagent (Roche).

Putative promoter elements of the three pri-miR-124s (miR-124-1, chr 14: 65205705–65207200; miR-124-2, chr 3: 17694143–17695600; miR-124-3, chr 2: 18062743–180628900; mm9) and negative control (NC, chr 14: 65183839–65185271, mm9) were cloned upstream of the luciferase reporter gene in the pGL3-enhancer (pGL3-eh) vector (Promega): miR-124-1–prom-luc, miR-124-2–prom-luc, miR-124-3–prom-luc and NC–prom-luc. As controls, the same sequences were cloned into the same locations in the reverse orientation. Constructs were transfected at the same concentration and with the same conditions as described above.

After transfection, N2A cells were grown under standard conditions for 48 h before harvesting. Dual luciferase activity was measured with the Dual-luciferase Reporter Assay System (Promega) according to the manufacturer’s guidelines on a FLUOstar OPTIMA (BMG Labtech) fluorescence plate reader. Luciferase activity was normalized against measured Renilla luciferase activity (transfection control) according to the manufacturer’s instructions.

**Prediction of miRNA-response elements.** miRNA-response elements (MREs) for the human lnc-SCA7 (ATXN7L3B), ATXN7 and STAGA subunit—encoding mRNAs (obtained from nXIProt.com35) were downloaded from http://www. mircorn.org/ (all mirSVR scores)34. The observed percentage of shared MREs between lnc-SCA7 and STAGA subunit—encoding mRNAs was compared to the fraction of shared MREs found across 10,000 randomly selected sets of 23 brain-expressed transcripts for genes that are not part of the STAGA complex (all mirSVR scores, http://www.mircorn.org/). An empirical P value was calculated by comparing the number of MREs shared between lnc-SCA7 and the STAGA mRNAs with those shared between lnc-SCA7 and the randomizations of sets of 23 brain-expressed transcripts. MREs predicted to be shared between lnc-SCA7 and STAGA-encoding mRNAs or randomly selected mRNAs were represented with Circos plots35 (http://circos.ca/).

**Genome-wide analysis of miRNA abundance.** N2A cells were transfected with lnc-SCA7–targeting or scrambled control shRNAs (1 µg) and lnc-SCA7–WT or lnc-SCA7–mut overexpression constructs or pcDNA3.1(+) empty vector control (1 µg). Cells were harvested, and total RNA was extracted with the mirNeasy kit (Qiagen) 48 h after transfection. Four biological replicates (independent cell cultures) were used for each experimental condition. A total of 611 mouse and murine virus–targeting or scrambled control shRNAs were directly cross-linked for 10 min at 37 °C by addition of 1% formaldehyde to the tissue-culture medium. For mouse cerebellar tissue, dissected samples were homogenized in PBS with a Dounce homogenizer to generate a single-cell suspension. A 1% final concentration of formaldehyde was added, and the samples were incubated for 10 min at room temperature with rotation. Cross-linking reactions were quenched with 0.125 M glycine. Nuclei were isolated and chromatin sheared to approximately 500 bp with a Bioruptor (Diagenode). Samples with 1-mg cross-links were immunoprecipitated with 5 µg anti-rabbit GCNS (Santa Cruz H-75) or anti-rabbit IgG control (Millipore) antibodies overnight at 4 °C (antibody availability validated on manufacturers’ websites). Complexes were collected with Protein A–coated magnetic beads (Pierce), washed and eluted, and the cross-links were reversed at 65 °C overnight. DNA was precipitated, treated with Proteinase K (Roche) and purified with a PCR Purification Kit (Qiagen).

We tested STAGA binding across five consecutive regions (250 bp in length) upstream of each transcription start site (TSS) of precursor–miR-124 transcripts (pri-miR-124) annotated by the ENCODE consortium (UCSC browser)38 as sensitive to DNase I treatment and enriched in H3K27ac marks in the cerebellum within chr 14: 65205705–65207200; chr 3: 17694143–17695600; and chr 2: 18062743–180628900 for pre-miR-124-1, pre-miR-124-2 and pre-miR-124-3, respectively (Supplementary Fig. 4f–d). The control region (chr 14: 65183839–65185271) was selected on the basis of its lack of DNase I sensitivity and H3K27ac marks, and its proximity to the predicted STAGA-bond regions (Supplementary Fig. 4d). Primes used to detect all regions were designed with a similar nucleotide composition (Supplementary Table 4). Specific enrichment of Gcn5 relative to IgG was determined from three independent ChIP assays by qPCR.

**SCA7 knock-in mouse models.** SCA7100Q/5Q knock-in mice carrying 100 CAG repeats on the pathological allele in the mouse SCA7 locus were kindly provided by H.Y. Zoghbi39. Heterozygous SCA7100Q/5Q males were mated with SCA7100Q/5Q females. Genotyping was as described previously40. Homozygous SCA7100Q/100Q male mice (aged 28 weeks, n = 4) were used for subsequent RNA quantification by qRT-PCR and by in situ hybridization. The experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals41. European Directive no. 86/609 and the guidelines of the local institutional animal care and use committee. The study was approved (26 June 2010) by the local Institutional Review Board (Direction Générale pour la Recherche et l’Innovation). SCA7266Q/5Q mice42 were obtained from Jackson Laboratories (stock number 008682) and maintained by crossing heterozygous SCA7266Q/5Q with wild-type 5Q/5Q animals. Genotyping was performed as previously described40. Heterozygous SCA7266Q/32Q male animals (aged 5 weeks, n = 3) were used for subsequent RNA quantification by qRT-PCR and by in situ hybridization. Experiments were carried out according to United Kingdom Home Office Animals (Scientific Procedures) Act 1986 and with local ethical approval from the University of Oxford. In both cases, the mice were maintained on a 12-h light/dark cycle with access ad libitum to food and water. Only regions with the highest Gcn5 binding found in N2As were tested in the cerebellum of SCA7100Q/100Q mice—namely 1a, 2b, 3c and NA for mir-124–1, mir-124–2, mir-124–3 and negative-control region, respectively. No statistical method was used to predetermine sample size. The experiments were not randomized and were not performed blind to the conditions of the experiments.

**Tissue preparation for RNA analyses.** Tissues of homozygous SCA7100Q/100Q mice at the late stage of disease (28 weeks of age, n = 4) and wild-type age- and sex-matched controls (n = 2) were used to perform qRT-PCR expression
analyses. *In situ* hybridization and qRT-PCR were also carried out from heterozygous SCA7<sup>266Q/352Q</sup> mice (5 weeks of age, *n* = 3 for both experiments) and wild-type age- and sex-matched littermate controls (SCA7<sup>266Q/352Q</sup>, *n* = 3 for each experiment). Biopsies from neuronal tissues (retina, cerebellum, cortex, striatum, olfactory bulb and spinal cord) were collected at 4 °C with an adult mouse brain matrix slicer. Non-neuronal tissues (liver, lung and muscle) were dissected at 4 °C. After dissection, biopsies were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Tissues were homogenized in RLT buffer with 1% β-mercaptoethanol (RNaseasy kit, Qiagen) and prepared for RNA extraction as described previously. Reverse transcription to cDNA was performed as described previously and was followed by gene expression detection by qRT-PCR.

**In situ hybridization.** Target sequences were generated by PCR or qRT-PCR and cloned into pCR4-TOPO (Invitrogen), and digoxigenin (DIG)-labeled riboprobes were synthesized from linearized plasmid DNA. Tissue samples were snap frozen in OCT (VWR), and 14-μm sections were cut with a cryostat (Leica) and mounted onto Superfrost Plus slides (VWR). For ATXN7<sup>+</sup> probe hybridization, washing and signal detection with an alkaline phosphatase–conjugated anti-DIG antibody was carried out as previously described<sup>52</sup>. Sense-strand probes were also tested to obtain a negative-control signal (data not shown). Primer sequences for riboprobe cloning of lnc-SCA7 and ATXN7<sup>+</sup> can be found in [Supplementary Table 4](#). For miR-124, a DIG-labeled LNA probe (Exiqon) was hybridized as above with some slight modifications<sup>63</sup>. To ensure that signals obtained before analysis were at subsaturation levels, the ATXN7 probe was hybridized for 16 h, whereas the miR-124 probe was hybridized for 4 h.

**SCA7 human fibroblasts.** Transcript abundance of SCA7 fibroblast cell lines against that of a control fibroblast cell line (10Q/10Q) was quantified by qRT-PCR with SYBR green PCR Master Mix (Invitrogen) and target-specific primers in combination with a TaqMan (Invitrogen) probe (GAPDH, cat. no. 4331182, Invitrogen; custom-made Inc-SCA7 probe ([Supplementary Table 4](#))). Results illustrated in Figure 5a were measured with sensitive TaqMan-based qRT-PCR. Ethics approval for the establishment of patient fibroblast cultures was granted by the University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 380/2009 and 434/2011) and was renewed annually. Informed consent was obtained from all human subjects. No statistical method was used to predetermine sample size. The experiments were not randomized and were not performed blind to the conditions of the experiments.

**Statistics.** All expression correlation comparisons were determined with the Pearson’s correlation test, and all differential expression comparisons were determined with Student’s *t* test. Asterisks indicate significance in the level of the comparison between the expression of target transcripts (*P* < 0.05; **P** < 0.01; ***P** < 0.001; not significant (NS), *P* > 0.05). For each experimental analysis, statistical values were calculated with data collected from three independent experiments.

---

50. Su, A. I. et al. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc. Natl. Acad. Sci. USA* **101**, 6062–6067 (2004).
51. Yuan, B., Latek, R., Hossbach, M., Tuschi, T. & Lewitter, F. siRNA Selection Server: an automated siRNA oligonucleotide prediction server. *Nucleic Acids Res.* **32**, W130–W134 (2004).
52. Nesterova, T.B. et al. Dicer regulates Xist promoter methylation in ES cells indirectly through transcriptional control of Dnmt3a. *Epigenetics Chromatin* **1** (2008).
53. Lane, L. et al. neXtProt: a knowledge platform for human proteins. *Nucleic Acids Res.* **40**, D76–D83 (2012).
54. Betel, D., Wilson, M., Gabow, A., Marks, D.S. & Sander, C. The microRNA.org resource: targets and expression. *Nucleic Acids Res.* **36**, D149–D153 (2008).
55. Krzywinski, M. et al. Circos: an information aesthetic for comparative genomics. *Genome Res.* **19**, 1639–1645 (2009).
56. Geiss, G.K. et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat. Biotechnol.* **26**, 317–325 (2008).
57. Brumbaugh, C.D., Kim, H.J., Giovacchini, M. & Pourmand, N. NanoStriDE: normalization and differential expression analysis of NanoString nCounter data. *BMC Bioinformatics* **12**, 479 (2011).
58. Myers, R.M. et al. A user’s guide to the Encyclopedia of DNA elements (ENCODE). *PLoS Biol.* **9** (2011).
59. Chen, Y.C. et al. Gcn5 loss-of-function accelerates cerebellar and retinal degeneration in a SCA7 mouse model. *Hum. Mol. Genet.* **21**, 394–405 (2012).
60. Yoo, S.Y. et al. SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant ataxin-7 in neurons and abnormalities in short-term plasticity. *Neuron* **37**, 383–401 (2003).
61. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996).
62. Chodoroff, R.A. et al. Long noncoding RNA genes: conservation of sequence and plasticity. *Proc. Natl. Acad. Sci. USA* **101**, 6062–6067 (2004).
63. Dee, M., Yu, J.Y., Chung, K.H., Tippens, M. & Turner, D.L. Detection of mammalian microRNA expression by *in situ* hybridization with RNA oligonucleotides. *Dev. Dyn.* **235**, 2538–2548 (2006).
Corrigendum: Cross-talking noncoding RNAs contribute to cell-specific neurodegeneration in SCA7

Jennifer Y Tan, Keith W Vance, Miguel A Varela, Tamara Sirey, Lauren M Watson, Helen J Curtis, Martina Marinello, Sandro Alves, Bruno R Steinkraus, Sarah Cooper, Tatyana Nesterova, Neil Brockdorff, Tudor A Fulga, Alexis Brice, Annie Sittler, Peter L Oliver, Matthew J Wood, Chris P Ponting & Ana C Marques

Nat. Struct. Mol. Biol. 21, 955–961 (2014); published online 12 October 2014; corrected after print 18 February 2015

In the version of this article initially published, Supplementary Figure 4k showed levels of mature miRNA-124 instead of miR-124 precursor. The error has been corrected in the Supplementary Text and Figures file and in the HTML and PDF versions of the article.