

RNAi: a potential therapy for the dominantly inherited nucleotide repeat diseases

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Genetic diseases that are accompanied by central nervous system involvement are often fatal. Among these are the autosomal dominant neurogenetic diseases caused by nucleotide repeat expansion. For example, Huntington's disease (HD) and spinal cerebellar ataxia are caused by expansion of a tract of CAGs encoding glutamine. In HD and the other CAG-repeat expansion diseases, the expansion is in the coding region. Myotonic dystrophy is caused by repeat expansions of CUG or CCTG in noncoding regions, and the mutant RNA is disease causing. Treatments for these

disorders are limited to symptomatic intervention. RNA interference (RNAi), which is a method for inhibiting target gene expression, provides a unique tool for therapy by attacking the fundamental problem directly. In this review, we describe briefly several representative disorders and their respective molecular targets, and methods to accomplish therapeutic RNAi. Finally, we summarize studies performed to date.

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Introduction

RNA interference (RNAi) is a mechanism by which RNA can impart repressive activity on gene expression. It is now well established that interference of gene expression can occur in plant and mammalian cells either prior to transcription or post-transcriptionally.^{1–6} In many species, the inhibitory RNAs are processed from endogenously expressed larger precursors.⁷ In post-transcriptional gene silencing, endogenous targets whose expression is silenced have been bioinformatically predicted, and some experimentally validated.

The protein complexes involved in RNAi are being elucidated through molecular and biochemical studies (reviewed in Kim⁷). Fortunately, we can co-opt this naturally occurring system to accomplish RNAi against targets not normally silenced in a given population of cells. In our case, the target cells are neurons *in situ* and the mRNAs we wish to silence are encoded from genes that when mutant, cause disease. In general, investigators design short hairpin RNAs (shRNA) or duplex RNAs (small interfering RNA, (siRNA)) in which one strand of the stem (for shRNA) or duplex (for siRNA) is complementary to the target mRNA. siRNAs or expression vectors encoding the shRNAs are transfected into cells to assess the efficiency of target mRNA silencing. The choice of inhibitory sequences for silencing should incorporate the results of recent studies delineating base

pair preferences at the 5' and 3' ends of the guide strand (strand complimentary to target).^{8–11} As treatises of the machinery and rules for accomplishing RNAi are presented elsewhere in this review series, we focus our discussion on the application of RNAi for dominant neurogenetic diseases, and in particular, dominant neurogenetic diseases caused by nucleotide repeat expansion.

Dominantly inherited neurologic diseases induced by nucleotide repeat expansion

The list of diseases that result from the expansion of a region of reiterated nucleotides in genomic DNA is growing.^{12,13} Members of dominantly inherited nucleotide repeat (see Table 1) diseases include Huntington's disease (HD), spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7 and 17, dentarubral-pallidolusyan atrophy (DRPLA), spinobulbar muscular atrophy (SBMA), and myotonic dystrophy (DM1 and DM2).^{12–15} Each results in selective neuropathology, presumably because of regional differences in susceptibility to the disease-inducing RNA (DM1, DM2) or protein (SCA1,2,3,6,7 and 17 and HD).¹⁶ For the polyglutamine repeat diseases, such as HD, SCA, SBMA and DRPLA, it is noteworthy that the presence of polyglutamine tracts within a protein is not always deleterious. A number of nonpathogenic proteins, including transcription factors, have extended polyglutamine tracts. The development of the specific pathologies and symptoms of these disorders are therefore dependent on the expression of an expanded polyglutamine tract within the context of the specific mutant proteins. The mutant proteins exert cell-specific

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Table 1 RNAi therapy for nucleotide repeat diseases

Disease	Target transcript	Expansion
Huntington's disease	Huntingtin	CAG
Spinal cerebellar ataxia type 1 (SCA1) ^a	Ataxin-1	CAG
SCA2	Ataxin-2	CAG
SCA3	Ataxin-3	CAG
SCA7	Ataxin-7	CAG
SCA8	NA ^b	CTG
DRPLA	Atrophin-1	CAG
Myotonic dystrophy type I (DM1)	Dystrophin myotonia protein kinase (DMPK) ^c	CUG
DM2	Zinc-finger protein 9 ^d	CCTG

^aThis is only a partial listing of the dominant SCAs. For additional information see Schols *et al.*

^bNot assigned.

^cExpansion at 3' end of gene; RNA is pathogenic.

^dExpansion is in intron 1.

and time-dependent neuronal dysfunction followed, in some cell types, by neurodegeneration.

HD is characterized by progressive decline in motor function and cognition, and the development of psychiatric symptoms. HD is caused by the inheritance of a single copy of the *huntingtin* gene with a trinucleotide CAG repeat expansion within exon 1.¹⁷ Normally, the polymorphic *huntingtin* gene encodes a protein with 6–35 contiguous glutamines. HD results when *huntingtin* contains more than 36 CAG codons. The length of the expanded polyglutamine tract affects the progression of HD.^{15,18} Higher CAG repeat number correlates roughly with earlier age of symptom onset and increased rate of disease progression. Huntingtin, the protein product of *huntingtin*, is expressed throughout the brain and body during development and in adulthood.¹⁹ The broad cellular distribution of huntingtin and the fact that ablation of *huntingtin* is lethal during embryogenesis suggests that huntingtin plays an important role in normal cellular development. Huntingtin has been implicated in numerous cellular processes including trophic receptor signaling, intracellular transport and vesicular trafficking.²⁰ The mutant form of huntingtin, like normal huntingtin, is expressed in all cells of the body, however, the most pronounced pathological change in HD is the eventual loss of striatonigral and striatopallidal GABAergic medium spiny projection neurons of the caudate and putamen, leading to motor and cognitive deficits. Cell loss usually begins in the fourth to fifth decade of life, with disease manifestations appearing earlier in individuals with longer repeat lengths. Even before significant striatal cell loss, HD patients present with motor symptoms, cognitive decline and emotional disturbances. Neuronal dysfunction, therefore, plays a significant role in the development of HD symptoms and the dysfunction precedes selective cell death. No effective therapies are yet available for the treatment of HD although several pharmacological agents that modestly reduce symptoms have been tested in transgenic HD mice.^{21–27} The precise function of mutant huntingtin remains to be defined.

The SCAs are a genetically and clinically heterogeneous group of disorders. These disorders show anticipation, where the offspring of an affected individual may

have more rapid progression or severity of symptoms caused by the relative increase in the length of the CAG repeat during meiosis and the subsequent expression of proteins with longer polyglutamine tracts.²⁸ These diseases show a myriad of clinical effects including combinations of ocular dysfunction, pathology, pyramidal and extrapyramidal movement abnormalities, seizures, cognitive impairment and behavioral deficits. SCA1 is caused by the inheritance of a single copy of *ataxin-1* with a polyglutamine-encoding region that ranges from 44 to 82 CAG repeats.²⁹ Expression of mutant ataxin-1 leads to ataxia that progressively worsens over time, loss of Purkinje cells and brainstem neurons and cerebellar atrophy. Mutant ataxin-1, like the amino terminus of mutant huntingtin, translocates to the nucleus where it forms inclusion bodies. Transgenic mice that express ataxin-1 with an extended polyglutamine tract that lacks a functional nuclear localization signal do not develop Purkinje cell pathology or motor dysfunction.³⁰ Moreover, transgenic mice expressing ataxin-1 with an extended polyglutamine tract but missing the amino acids that participate in dimerization develop cellular pathology and motor dysfunction in the absence of nuclear aggregate formation.³⁰ This would imply that nuclear localization is essential for pathology; however, mutations in E6-AP ubiquitin ligase reduce nuclear inclusion frequency, but accelerate disease, in this transgenic model.³¹ These observations indicate that the soluble form of mutant ataxin-1 is pathogenic when the protein localizes to the nucleus but that nuclear aggregate formation is not essential for disease progression. Similar findings have been made in a culture model of CAG-repeat expansion disease.³² It has also been demonstrated that polyglutamine expansion prevents nuclear export of the amino terminus of mutant huntingtin and increases intranuclear concentrations³³ but that mutant huntingtin fragment-induced nuclear inclusion formation does not always precede cell death.^{32,34} The observations that transcription factors, like mutant huntingtin, have polar-zipper forming polyglutamine tracts, that some transcription factors can physically interact with mutant ataxin-1 and mutant huntingtin, that nuclear localization of these mutant proteins is necessary for disease progression and that gene expression is altered in SCA1 and HD support the hypothesis that these proteins negatively affect transcription.^{20,35} In SCA1 and HD, accumulation of mutant proteins may be due to inefficient clearance via the proteosomal pathways. Such protein accumulation may contribute to cellular dysfunction.

Unlike disorders caused by polyglutamine expansion, disorders such as Fragile X, myotonic dystrophy and Friedrich's ataxia are caused by trinucleotide repeat expansion in noncoding regions. In the case of myotonic dystrophy type 1 and type 2 (DM1 and DM2), the mutant RNA is pathogenic. In DM1 there is expansion of CTG at the 3' untranslated region (UTR) of the dystrophin myotonia protein kinase gene.³⁶ Expansion of CCTG in intron 1 of zinc-finger protein 9 (ZNF9) causes DM2.³⁷ The mutant RNAs induce dysregulation of RNA-binding proteins, which in turn alters splicing of various transcripts including the insulin receptor and chloride channel 1, explaining in part the development of insulin resistance and involuntary muscle contractions in DM1 and DM2 patients.¹³

Molecular targets for RNAi

There is considerable debate about how the proteins with expanded polyglutamine regions cause disease, what causes the selective degeneration of neurons by mutant proteins that are widely expressed and how the expression of particular mutant proteins (or RNA) causes the specific symptoms of each nucleotide repeat disease. However, there is no doubt that each of the CAG-induced diseases is caused by expression of the mutant gene. As each of these neurodegenerative disorders is caused by a defined genetic mutation and inherited in an autosomal dominant fashion, most patients at risk to develop these diseases can be identified prior to the occurrence of overt symptoms. In addition, because these diseases often progress slowly over decades, there is a considerable therapeutic window where intervention may slow or even prevent disease progression provided that mutant gene expression can be reduced. Post-transcriptional reduction of mRNAs, and the subsequent toxic proteins they encode in the case of SCA1, SCA3 and HD, is theoretically possible using strategies such as antisense oligonucleotides, ribozymes, DNA enzymes and siRNA. In the case of SCA1 and SCA3, the molecular target is the mutant ataxin-1 and mutant ataxin-3 mRNAs, respectively. In the case of HD, the molecular target is the mutant huntingtin mRNA.

The myotonic dystrophies present a unique problem for gene silencing since the target RNA is in the nucleus, and the disease is multisystemic. Unlike SCA1, SCA3 and HD, DM1 and DM2 induce cardiac abnormalities, muscular dystrophy, insulin resistance and serological changes. For SCA1 and HD, directed brain delivery for disease therapy benefits from a decade of work developing and optimizing gene and small molecule delivery. Systemic delivery to multiple organ systems remains a major hurdle. Secondary to that is the necessity to silence the disease-inducing mutant RNA in the nucleus. In recent work in HeLa cells, Robb *et al.*³⁸ showed that siRNA and miRNAs could mediate silencing of a targeted nuclear RNA, suggesting that an active RNA-induced silencing complex exists in HeLa cell nuclei. How RNAi could be restricted to the nuclear compartment to induce degradation of only the dominant, disease-inducing RNA leaving the cytoplasmic, protein-encoding RNAs intact, is unclear. Also, unknown is whether active RISC complexes exist in the nuclei of the terminally differentiated cells effected in DM1 and DM2.

As discussed above, HD and some of the other triplet repeat disorders result from a toxic gain of function. The most straightforward manner to inhibit disease onset or reduce disease severity is to inhibit expression of the mutant protein. As for the myotonic dystrophies, silencing of the disease allele specifically would be ideal. To do so requires taking advantage of nucleotide polymorphisms present in the coding sequence that segregates with the mutation. A thorough discussion of allele-specific silencing, and how it can be accomplished is provided by Paulson in this issue.³⁹

Can RNAi be therapeutic in the absence of allele-specific silencing? Some genes can be removed in mice with limited consequences, suggesting that targeting the disease allele for therapeutic purposes is unnecessary. For example, mice lacking *ataxin-1* are born and do not display ataxia, although they do possess learning and

memory deficits.⁴⁰ Ataxin-1 does not appear critical for cell survival. On the other hand, *huntington* knockout mice do not survive gestation,⁴¹ and when *huntington* is deleted postnatally in mice,⁴² there is evidence of neurodegeneration.

It is important to distinguish reduction in gene expression from RNAi from genetic knockouts. The latter ablates gene expression, while the former reduces gene expression. In our hands, the best gene silencing that can be accomplished, as measured by quantitative RT-PCR, is 85%.⁴³ What is the consequence of silencing an embryonically important protein in adult neurons? In the context of mouse models of HD, the answer is not known. It may be that huntingtin levels must remain above a certain level throughout adulthood for cell viability. Alternatively, adult neurons may tolerate a reduction of mutant and wild-type huntingtin. Experiments using RNAi-expressing vectors that target both the normal and mutant allele in knockin models harboring an expanded CAG-repeat could help answer this important question.^{44–46}

Accomplishing RNAi *in vivo*

The brain represents a unique target for the delivery of RNAi because it is not generally accessible to systemically delivered small molecules. Nonetheless, implanted pumps have been used to deliver chemically synthesized siRNAs for short-term knockdown of the pain-related cation channel P2X₃,⁴⁷ or the D2 receptor.⁴⁸ Direct delivery of viruses expressing shRNAs can also be used for inhibition of gene expression^{49,50} or disease targets.^{51–55} Both a benefit and a concern from viral-mediated delivery is that expression lasts from months to years. For a chronic neurogenetic disease, this would be ideal. However, if long-term expression of RNAi in brain causes adverse events, an 'off' switch would be desired. Although expression of shRNAs in rodent brain (from weeks to months) has not induced obvious pathology,^{43,50,51} methods for regulation of expression should continue to be investigated for improving RNAi as a human therapeutic.

siRNA formulations or vector expressing shRNAs may distribute broadly after introduction to brain, or remain confined to the injection site. A critical step prior to testing RNAi in disease models is ascertaining if the vector formulation or virus type will mediate RNAi in the cell type of interest. For example, a target structure for HD therapy is the striatum. Figure 1 shows extensive transduction of mouse striatum after injection of AAV vectors expressing the reporter gene hrGFP. While the distribution of vector often improves with increasing titer, some serotypes diffuse further from the injection site than others,^{56,57} or transduce nonneuronal cells.^{56,58}

Results to date

Cell and animal models of human disease states have been used to test the feasibility of RNAi as a therapy. With respect to human neurogenetic diseases, early *in vitro* studies demonstrated reduction of cell-associated aggregates in cellular models of CAG-repeat disease.^{59,60} *In vivo* knockdown of mutant ataxin-1 and mutant huntingtin has been achieved in transgenic mouse

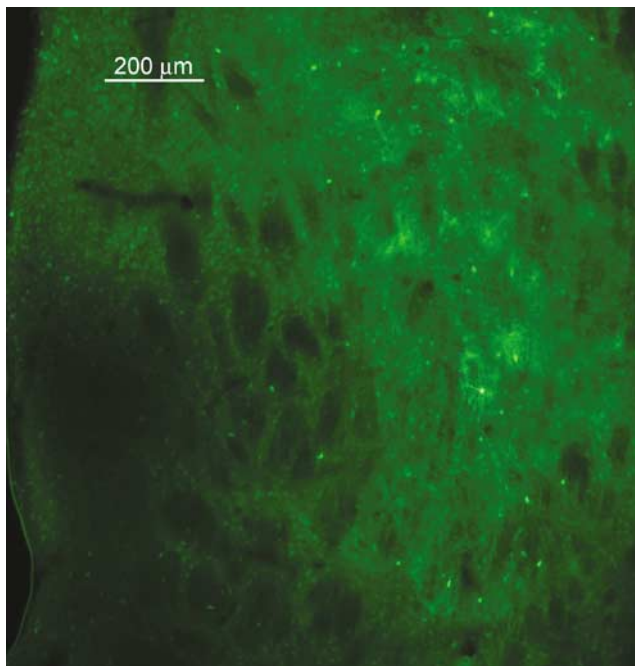


Figure 1 AAV-mediated transduction of the mouse striatum. The AAV vector expresses hrGFP for identification of transduced cells. The AAVhrGFP was instilled into the right hemisphere 3 weeks prior to brain harvest.

models of SCA1 and HD using recombinant adeno-associated viral (AAV) vectors^{43,51,55} Xia and colleagues used SCA182Q transgenic mice that express full-length human *ataxin-1* with 82 CAG repeats under the control of the PCP-2 promoter⁶¹ for their studies.⁵¹ These hemizygous mice have high levels of nuclear mutant ataxin-1 in cerebellar Purkinje cells and develop a SCA1-like phenotype. Following the selection of efficient anti-human ataxin-1 shRNAs, SCA1 mice received AAV serotype 1 viral vectors expressing shRNA under the control of the HI pol III promoter. The AAV1 serotype effectively transduced Purkinje cells and led to expression of the inhibitory RNAs within 10 days of viral delivery. SCA1 mice receiving cerebellar delivery of ataxin-1-specific shRNAs at 7 weeks of age showed significant improvements in motor function from 11 to 21 weeks of age compared to controls. Motor performance approached, but did not reach, that observed in wild-type littermates, probably due to the fact that injections were restricted to midline lobules rather than the entire cerebellum. Importantly, long-term expression of shRNA did not have any detrimental effect on motor performance or brain morphology in wild-type mice, and did not induce further disease in SCA1 mice. Overall organization of the cerebellar lobules that received the ataxin-1 targeting shRNA expression vector was improved, and levels of human ataxin-1 and nuclear inclusion staining was reduced to undetectable levels in antiataxin-1 shRNA transduced regions. This important study was the first to demonstrate that shRNA expression using AAV is a viable therapy for dominant CNS disorders in general, and trinucleotide repeat disorders in particular.

Efficacy following intrastriatal delivery of anti-huntingtin shRNAs has also been shown in two different transgenic HD mice lines.^{62,63} In one study, HD-N171-

82Q mice that express the amino terminus of human huntingtin with 82 CAG repeats, in addition to expressing a full complement of normal mouse huntingtin were treated with shRNAs specific to human huntingtin.⁴³ AAV1-encoded shRNAs against a sequence in exon 2 of human *huntingtin* under the control of the U6 promoter were widely expressed in neurons throughout the striatum. Expression of anti-huntingtin shRNAs reduced human huntingtin mRNA, protein and huntingtin immuno-reactive aggregates in transduced regions. In follow-up behavior studies, improvements in gait abnormalities and rotorod performance 14 weeks following delivery of viral vector was noted. In a second study, shRNA directed against a sequence in exon 1 upstream of the coding region of human huntingtin delivered in AAV5 vectors effectively reduced levels of mutant human huntingtin mRNA, protein and huntingtin-containing inclusions in R6/1 mice and modestly improved rotorod performance.⁵⁵ Anti-huntingtin shRNA also caused a modest increase in DARPP-32 and ppENK mRNA levels in transduced regions of R6/1 mice, suggesting that the function of mutant huntingtin protein that blocks transcription was partially ameliorated by reducing mutant huntingtin levels.

R6/1 mice show a more rapid progression and severity of phenotype compared to HD-N171-82Q mice, possibly due to higher intranuclear concentrations of truncated human mutant huntingtin encoded by exon 1 or the length of the polyglutamine repeat. In HD and SCA1 mice and their wild-type littermates, expression of shRNAs over several months had no obvious deleterious effects on striatal or cerebellar morphology or negative effects on phenotype. Together, these studies^{43,51,55} demonstrated that injections of AAV1 and 5 led to sustained expression of ataxin-1 and -huntingtin targeting shRNAs at levels sufficient to reduce target mRNA and protein levels. Importantly, the reduction of target mutant proteins partially ameliorated pathology and overt motor dysfunction in these models.

When and where to treat

Owing to the slow progressive nature of some nucleotide repeat diseases, some alterations in cell function and morphology could be secondary or tertiary responses to the primary effects of mutant proteins. In other words, following a period of expression of mutant protein, compensatory changes in the brain may have occurred that are not immediately reversible if mutant protein expression is blocked. Earlier intervention, therefore, may prevent such changes and dysfunction. It was observed that reversal of disease phenotype in an inducible model of SCA1 was more difficult if gene expression was prevented after a prolonged period of disease progression.⁶⁴ Moreover, although much attention has been focused on the particular neuronal populations that die in each disease, other regions of the brain may be negatively affected by expression of mutant proteins. For example, gene expression is altered in cortical neurons of HD mice although these neurons are not highly susceptible to mutant huntingtin-induced cell death.⁶⁵⁻⁶⁷ Lack of trophic support to the striatum by cortical projection neurons expressing mutant huntingtin may lead to cell death in the striatum. Other brain regions,

therefore, may need to be treated to achieve the maximum possible efficacy of shRNA molecules. In HD patients, there is clear cortical loss with disease progression, indicating that striatal and cortical therapy may be required.

HD patients often have difficulties maintaining weight. Similarly, transgenic N171-82Q and R6 HD mice fail to gain weight at the same rate as their wild-type littermates.^{62,63} The expression of shRNAs in the striatum did not positively affect weight gain in these strains^{43,55} suggesting that transduction of other brain regions, such as the hypothalamus which controls metabolism, or direct treatment of the periphery may improve loss of muscle mass and further improve motor behavior. A significant improvement in motor performance was observed when doxycycline was administered to block expression of mutant huntingtin in a conditional transgenic model of HD.⁶⁸ In this case, systemic administration of doxycycline completely blocked expression of the transgene in all cells suggesting that enhanced knock-down of mutant huntingtin, or treatment of other brain regions and/or peripheral organs, may increase shRNA efficacy. It is also possible that a combination of shRNA for huntingtin expression in brain and systemic administration of agents that improve motor performance and survival may have additive effects.

Summary

For the dominant, fatal neurogenetic diseases described above, RNAi can improve disease phenotypes in culture and in animal models of human disease. Thus, in addition to its application as a powerful research tool *in vitro* and *in vivo*, RNAi holds promise for human disease therapy. Although preliminary studies in disease and wild-type mice are promising, we do not yet know if co-opting the molecular machinery governing naturally occurring RNAi in cells to accomplish targeted gene silencing is safe for chronic brain diseases. Future studies in rodent and larger mammals will help clarify this important issue as we move to translate RNAi to the clinic.

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References

- 1 Mathieu O, Bender J. RNA-directed DNA methylation. *J Cell Sci* 2004; **117**: 4881–4888.
- 2 Matzke M, Aufsatz W, Kanno T, Daxinger L, Papp I, Mette MF *et al*. Genetic analysis of RNA-mediated transcriptional gene silencing. *Biochim Biophys Acta* 2004; **1677**: 129–141.
- 3 Morris KV, Chan SW, Jacobsen SE, Looney DJ. Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* 2004; **305**: 1289–1292.
- 4 Kawasaki H, Taira K. Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. *Nature* 2004; **431**: 211–217.

- 5 Ting AH, Schuebel KE, Herman JG, Baylin SB. Short double-stranded RNA induces transcriptional gene silencing in human cancer cells in the absence of DNA methylation. *Nat Genet* 2005; **37**: 906–910.
- 6 Tijsterman M, Plasterk RH. Dicers at RISC; the mechanism of RNAi. *Cell* 2004; **117**: 1–3.
- 7 Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. *Nat Rev Mol Cell Biol* 2005; **6**: 376–385.
- 8 Ma JB, Yuan YR, Meister G, Pei Y, Tuschl T, Patel DJ. Structural basis for 5'-end-specific recognition of guide RNA by the *A. fulgidus* Piwi protein. *Nature* 2005; **434**: 666–670.
- 9 Parker JS, Roe SM, Barford D. Structural insights into mRNA recognition from a PIWI domain-siRNA guide complex. *Nature* 2005; **434**: 663–666.
- 10 Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. *Cell* 2003; **115**: 199–208.
- 11 Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell* 2003; **115**: 505.
- 12 Klockgether T, Evert B. Genes involved in hereditary ataxias. *Trends Neurosci* 1998; **21**: 413–418.
- 13 Ranum LP, Day JW. Pathogenic RNA repeats: an expanding role in genetic disease. *Trends Genet* 2004; **20**: 506–512.
- 14 Schols L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. *Lancet Neurol* 2004; **3**: 291–304.
- 15 Gusella JF, MacDonald ME. Molecular genetics: unmasking polyglutamine triggers in neurodegenerative disease. *Nat Rev Neurosci* 2000; **1**: 109–115.
- 16 Ross CA. When more is less: pathogenesis of glutamine repeat neurodegenerative diseases. *Neuron* 1995; **15**: 493–496.
- 17 The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993; **72**: 971–983.
- 18 Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P *et al*. Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat Genet* 1993; **4**: 393–397.
- 19 Sharp AH, Loev SJ, Schilling G, Li SH, Li XJ, Bao J *et al*. Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron* 1995; **14**: 1065–1074.
- 20 Landles C, Bates GP. Huntingtin and the molecular pathogenesis of Huntington's disease. *EMBO Rep* 2004; **5**: 958–963.
- 21 Ona VO, Li M, Vonsattel JP, Andrews LJ, Khan SQ, Chung WM *et al*. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* 1999; **399**: 263–267.
- 22 Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK *et al*. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* 2000; **20**: 4389–4397.
- 23 Ferrante RJ, Andreassen OA, Dedeoglu A, Ferrante KL, Jenkins BG, Hersch SM *et al*. Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease. *J Neurosci* 2002; **22**: 1592–1599.
- 24 Ferrante RJ, Kubilus JK, Lee J, Ryu H, Beesen A, Zucker B *et al*. Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *J Neurosci* 2003; **23**: 9418–9427.
- 25 Ferrante RJ, Ryu H, Kubilus JK, D'Mello S, Sugars KL, Lee J *et al*. Chemotherapy for the brain: the antitumor antibiotic mithramycin prolongs survival in a mouse model of Huntington's disease. *J Neurosci* 2004; **24**: 10335–10342.
- 26 Dedeoglu A, Kubilus JK, Jeitner TM, Matson SA, Bogdanov M, Kowall NW *et al*. Therapeutic effects of cystamine in a murine model of Huntington's disease. *J Neurosci* 2002; **22**: 8942–8950.

- 27 Sanchez I, Mahlke C, Yuan J. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. *Nature* 2003; **421**: 373–379.
- 28 Chung MY, Ranum LP, Duvick LA, Servadio A, Zoghbi HY, Orr HT. Evidence for a mechanism predisposing to intergenerational CAG repeat instability in spinocerebellar ataxia type I. *Nat Genet* 1993; **5**: 254–258.
- 29 Banfi S, Servadio A, Chung MY, Kwiatkowski Jr TJ, McCall AE, Duvick LA *et al*. Identification and characterization of the gene causing type 1 spinocerebellar ataxia. *Nat Genet* 1994; **7**: 513–520.
- 30 Klement IA, Skinner PJ, Kaytor MD, Yi H, Hersch SM, Clark HB *et al*. Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell* 1998; **95**: 41–53.
- 31 Cummings CJ, Reinstein E, Sun Y, Antalffy B, Jian Y-H, Ciechanover A *et al*. Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. *Neuron* 1999; **24**: 879–892.
- 32 Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 2004; **431**: 805–810.
- 33 Cornett J, Cao F, Wang CE, Ross CA, Bates GP, Li SH *et al*. Polyglutamine expansion of huntingtin impairs its nuclear export. *Nat Genet* 2005; **37**: 198–204.
- 34 Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* 1998; **95**: 55–66.
- 35 Sugars KL, Rubinsztein DC. Transcriptional abnormalities in Huntington disease. *Trends Genet* 2003; **19**: 233–238.
- 36 Mankodi A, Logigian E, Callahan L, McClain C, White R, Henderson D *et al*. Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science* 2000; **289**: 1769–1773.
- 37 Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL *et al*. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* 2001; **293**: 864–867.
- 38 Robb GB, Brown KM, Khurana J, Rana TM. Specific and potent RNAi in the nucleus of human cells. *Nat Struct Mol Biol* 2005; **12**: 133–137.
- 39 Rodriguez-Lebron E, Paulson HL. Gene Therapy Review. *In Press* 2005.
- 40 Matilla A, Roberson ED, Banfi S, Morales J, Armstrong DL, Burrell EN *et al*. Mice lacking ataxin-1 display learning deficits and decreased hippocampal paired-pulse facilitation. *J Neurosci* 1998; **18**: 5508–5516.
- 41 Zeitlin S, Liu JP, Chapman DL, Papaioannou VE, Efstratiadis A. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat Genet* 1995; **11**: 155–163.
- 42 Dragatsis I, Levine MS, Zeitlin S. Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. *Nat Genet* 2000; **26**: 300–306.
- 43 Harper SQ, Staber PD, He X, Eliason SL, Martins I, Mao Q *et al*. RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc Natl Acad Sci USA* 2005; **102**: 5820–5825.
- 44 Wheeler VC, Gutekunst CA, Vrbanac V, Lebel LA, Schilling G, Hersch S *et al*. Early phenotypes that presage late-onset neurodegenerative disease allow testing of modifiers in Hdh CAG knock-in mice. *Hum Mol Genet* 2002; **11**: 633–640.
- 45 Menalled LB, Sison JD, Wu Y, Olivieri M, Li XJ, Li H *et al*. Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. *J Neurosci* 2002; **22**: 8266–8276.
- 46 Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. *J Comp Neurol* 2003; **465**: 11–26.
- 47 Hemmings-Mieszczak M, Dorn G, Natt FJ, Hall J, Wishart WL. Independent combinatorial effect of antisense oligonucleotides and RNAi-mediated specific inhibition of the recombinant rat P2X3 receptor. *Nucleic Acids Res* 2003; **31**: 2117–2126.
- 48 Thakker DR, Natt F, Husken D, Maier R, Muller M, van der Putten H *et al*. Neurochemical and behavioral consequences of widespread gene knockdown in the adult mouse brain by using nonviral RNA interference. *Proc Natl Acad Sci USA* 2004; **101**: 17270–17275.
- 49 Xia H, Mao Q, Paulson HL, Davidson BL. siRNA-mediated gene silencing *in vitro* and *in vivo*. *Nat Biotechnol* 2002; **20**: 1006–1010.
- 50 Hommel JD, Sears RM, Georgescu D, Simmons DL, DiLeone RJ. Local gene knockdown in the brain using viral-mediated RNA interference. *Nat Med* 2003; **9**: 1539–1544.
- 51 Xia H, Mao Q, Eliason SL, Harper SQ, Martins IH, Orr HT *et al*. RNAi suppresses polyglutamine-induced neurodegeneration in a mouse model of SCA1. *Nat Med* 2004; **10**: 816–820.
- 52 Raoul C, Abbas-Terki T, Bensadoun JC, Guillot S, Haase G, Szulc J *et al*. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nat Med* 2005; **11**: 423–428.
- 53 Ralph GS, Radcliffe PA, Day DM, Carthy JM, Leroux MA, Lee DC *et al*. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. *Nat Med* 2005; **11**: 429–433.
- 54 Miller TM, Kaspar BK, Kops GJ, Yamanaka K, Christian LJ, Gage FH *et al*. Virus-delivered small RNA silencing sustains strength in amyotrophic lateral sclerosis. *Ann Neurol* 2005; **57**: 773–776.
- 55 Rodriguez-Lebron E, Denovan-Wright EM, Nash K, Lewin AS, Mandel RJ. Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. *Mol Ther* 2005, July E-pub.
- 56 Davidson BL, Stein CS, Heth JA, Martins I, Kotin RM, Derksen TA *et al*. Recombinant adeno-associated type 2, 4 and 5 vectors: transduction of variant cell types and regions in the mammalian CNS. *Proc Natl Acad Sci USA* 2000; **97**: 3428–3432.
- 57 Passini MA, Watson DJ, Vite CH, Landsburg DJ, Feigenbaum AL, Wolfe JH. Intraventricular brain injection of adeno-associated virus type 1 (AAV1) in neonatal mice results in complementary patterns of neuronal transduction to AAV2 and total long-term correction of storage lesions in the brains of beta-glucuronidase-deficient mice. *J Virol* 2003; **77**: 7034–7040.
- 58 Liu G, Martins IH, Chiorini JA, Davidson BL. Adeno-associated virus type 4 (AAV4) targets ependyma and astrocytes in the subventricular zone and RMS. *Gene Therapy* 2005; **12** (20): 1503–1508.
- 59 Caplen NJ, Taylor JP, Statham VS, Tanaka F, Fire A, Morgan RA. Rescue of polyglutamine-mediated cytotoxicity by double-stranded RNA-mediated RNA interference. *Hum Mol Genet* 2002; **11** (2): 175–184.
- 60 Xia H, Mao Q, Davidson BL. The HIV tat protein transduction domain improves the biodistribution of β -glucuronidase expressed from recombinant viral vectors. *Nat Biotechnol* 2001; **19**: 640–644.
- 61 Burrell EN, Clark HB, Servadio A, Matilla T, Feddersen RM, Yunis WS *et al*. SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. *Cell* 1995; **82**: 937–948.
- 62 Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuc JA *et al*. Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum Mol Genet* 1999; **8** (3): 397–407.
- 63 Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C *et al*. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* 1996; **87** (3): 493–506.

- 64 Zu T, Duvick LA, Kaytor MD, Berlinger M, Zoghbi H, Clark HB *et al.* Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice. *J Neurosci* 2004; **24**: 8853–8861.
- 65 Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L *et al.* Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 2001; **293**: 493–498.
- 66 Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L *et al.* Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat Genet* 2003; **35**: 76–83.
- 67 Zuccato C, Liber D, Ramos C, Tarditi A, Rigamonti D, Tartari M *et al.* Progressive loss of BDNF in a mouse model of Huntington's disease and rescue by BDNF delivery. *Pharmacol Res* 2005; **52**: 133–139.
- 68 Yamamoto A, Lucas JJ, Hen R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* 2000; **101**: 57–66.