Published in final edited form as:

Drugs Future. 2009 December ; 34(12): . doi:10.1358/dof.2009.034.12.1443434.

Therapeutic prospects for spinocerebellar ataxia type 2 and 3

Ilya Bezprozvanny¹ and Thomas Klockgether²

¹Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390, USA

²Department of Neurology, University Hospital Bonn, Bonn, Germany

Abstract

Spinocerebellar ataxia type 2 (SCA2) and type 3 (SCA3) are autosomal-dominant neurodegenerative disorders. SCA2 primarily affects cerebellar Purkinje neurons. SCA3 primarily affects dentate and pontine nuclei and substantia nigra. Both disorders belong to a class of polyglutamine (polyQ) expansion disorders. SCA2 is caused by a polyQ expansion in the amino-terminal region of a cytosolic protein ataxin-2 (Atxn2). SCA3 is caused by a polyQ expansion in the carboxy-terminal portion of a cytosolic protein ataxin-3 (Atxn3). Both disorders are found worldwide, but SCA2 is common among people of Cuban decent and SCA3 is common among people of Portuguese decent. No effective treatment exist for SCA2, SCA3 or any other polyQ-expansion disorder. Based on anecdotal evidence, a number of small scale clinical trials have been attempted previously for SCA2 and SCA3. These trials were underpowered and did not yield any promising results so far. A number of pathogenic mechanisms have been proposed to explain neuronal dysfunction and degeneration in SCA2 and SCA3. Knockdown of mutant Atxn2 and Atxn3 protein by RNAi or similar approach is most promising avenue of therapeutic development in the long term, but translation of this approach to clinic faces very serious technical challenges. Recent preclinical studies in SCA2 and SCA3 genetic mouse model suggested that abnormal neuronal calcium (Ca^{2+}) signaling may play an important role in SCA2 and SCA3 pathology. These studies also suggested that dantrolene and other Ca^{2+} signaling inhibitors and stabilizers may have a therapeutic value for treatment of SCA2 and SCA3. Controlled clinical evaluation of dantrolene, memantine, riluzole, dihydropyridines, CoQ10, creatine or other Ca^{2+} blockers and stabilizers in SCA2 and SCA3 patients is necessary to test clinical importance of these ideas. The EUROSCA consortium provides a potential framework for such clinical evaluation.

Keywords

ataxia; ataxin-2; ataxin-3; neurodegeneration; polyglutamine expansion; apoptosis; mitochondria; calcium signaling; dantrolene; memantine; RNAi

Spinocerebellar ataxias (SCAs) – clinical overview

The SCAs are a genetically and clinically heterogeneous group of autosomal dominantly inherited progressive ataxia diseases. Up to now, almost 30 different gene loci have been

found [1]. Of the mutations that have been identified, SCA1,2,3,6,7,17 are translated CAG repeat expansions that code for an elongated polyglutamine tract within the respective proteins [2-9]. These diseases belong to a larger group of polyglutamine disorders that also include Huntington's disease, dentatorubro-pallidoluyisian atrophy and spinobulbar muscular atrophy [10,11]. Three SCAs, SCA8,10,12 are caused by untranslated repeat expansions in non-coding regions of the respective genes [12-14]. In SCA5 (beta-III spectrin, SPTBN2) [15], SCA11 (tau tubulin kinase-2, TTBK2) [16], SCA13 (voltage-gated potassium channel, KCNC3) [17], SCA14 (protein kinase C γ , PKC γ) [18], SCA15 (inositol 1,4,5-triphosphate receptor type 1, ITPR1) [19], SCA27 (fibroblast growth factor 14, FGF14) [20], SCA28 (AFG3L2) [21] and 16q22-linked ADCA (puratrophin) [22] non-repeat mutations have been found in the respective genes. In all other SCAs, the affected genes and mutations have not yet been identified. There are only few prevalence studies of SCAs. According to these studies, the prevalence ranges between 3.0 and 4.0 for 100,000 inhabitants [23,24]. Due to founder effects, the prevalence may be much higher in certain regions. For example, the prevalence of SCA2 is 43 for 100,000 in the Cuban province Holguin [25]. The worldwide most frequent SCA is SCA3 [26].

Spinocerebellar ataxia type 2 (SCA2)

Autopsy studies of SCA2 patients consistently show olivopontocerebellar atrophy with marked reduction of Purkinje cells, degeneration of the inferior olives, pontine nuclei, and pontocerebellar fibers [27,28]. Atrophy of the cerebellum and brainstem can be nicely visualized in vivo by MRI [29]. In most cases, there is additional degeneration of posterior columns and spinocerebellar pathways, and cell loss in the substantia nigra. Ubiquitinated nuclear inclusions have not been observed in brains of SCA2 patients. SCA2 patients suffer from a progressive cerebellar syndrome with ataxia of gait and stance, ataxia of limb movements, and dysarthria [1,28,30]. Saccade slowing is a highly characteristic feature that is observed in the majority of SCA2 patients. About half of the patients have vertical or horizontal gaze palsy. Cerebellar oculomotor abnormalities are rarely found in SCA2. Typically, tendon reflexes are absent or decreased. Pyramidal tract signs are present in less than 20% of the patients. Vibration sense is decreased in most patients, while sensation is otherwise normal [29,31,32]. Atypical SCA2 phenotypes with prominent dementia, an ALS-like presentation and levodopa-responsive parkinsonism are also encountered. Disease onset in SCA2 varies between childhood and adulthood with an average around the age of 30 years. As in other polyglutamine disorders, there is an inverse correlation between CAG repeat length and age of onset. Anticipation is present in many SCA2 families, in particular if the disease is inherited from the father. Median latency to become wheelchair-bound after disease onset is 15 years, median survival after onset of symptoms 21 years, and median age at death 68 years [28,33].

Spinocerebellar ataxia type 3 (SCA3)

SCA3 is a multi systemic disorder characterized by degeneration of spinocerebellar tracts, dentate nucleus, pontine and other brainstem nuclei, substantia nigra and pallidum [34]. In contrast to most other SCA, the cerebellar cortex and the inferior olives are widely spared. MRIs show cerebellar atrophy which is less pronounced than in SCA2 in combination with brainstem atrophy. Nuclear inclusions containing expanded ataxin-3 have been found in

neurons of affected brain regions. The clinical picture of SCA3 is characterized by a wide range of clinical manifestations, the precise nature of which partly depends on repeat length. All SCA3 patients suffer from a progressive syndrome with ataxia of gait and stance, ataxia of limb movements, and dysarthria [35]. Vertical or horizontal gaze palsy are frequent additional findings which occur independently of age of onset. At least 40% of SCA3 patients have a levodopa-responsive restless legs syndrome. Saccade velocity is usually normal. Patients with very long repeats, usually of more than 74 have an early disease onset, and clinical features of pyramidal tract and basal ganglia involvement. Most of these patients have increased tendon reflexes, extensor plantar responses, spasticity, and dystonia. Patients with an intermediate repeat length have a disease onset in middle age and show mainly ataxia and gaze palsy. In patients with shorter repeats and a later disease onset, signs of peripheral neuropathy with loss of tendon reflexes, amyotrophy, and decreased vibration sense are often prominent. However, the boundaries between these clinical syndromes are vague, and the clinical phenotype of an individual may change with progression of the disease [32,36]. The onset in SCA3 varies between adolescence and late adulthood with an average around the age of 42 years. As in other polyglutamine disorders, there is an inverse correlation between CAG repeat length and age of onset. Median latency to become wheelchair-bound after disease onset is 15 years, median survival after onset of symptoms 25 years, and median age at death 72 years [33,34].

2. Spinocerebellar ataxias (SCAs) – molecular genetics and pathogenic mechanisms

It is generally assumed that many polyQ-expansion disorders share a common pathogenic mechanism [11,37,38]. Huntington's disease (HD) is the most extensively studied among these disorders. As a result of these studies it was concluded that polyQ expanded Huntingtin protein (Htt^{exp}) acquires a “toxic gain of function” [39]. A number of toxic functions have been assigned to Htt^{exp}, including formation of toxic aggregates, effect on gene transcription, induction of apoptosis, and disruption of key neuronal functions such as proteasomal function, ubiquitination, axonal transport, endocytosis, synaptic transmission and Ca²⁺ signaling [40-46]. Many of the proposed mechanisms suggest that the mutant Htt^{exp} is involved in pathological interactions with other signaling proteins in cells, leading to neuronal dysfunction and death. As discussed in the following sections, similar ideas have been expanded from HD to other polyglutamine expansion disorders including SCA2 and SCA3.

Spinocerebellar ataxia type 2 (SCA2)

At the molecular level, the cause of SCA2 is the expansion of an unstable trinucleotide CAG repeat in the *ATXN2* gene, which encodes a polyglutamine (polyQ) track in the ataxin-2 protein (Atxn2). In normal individuals, *ATXN2* alleles contain between 14 and 31 CAG repeats, with the 22 CAG repeat allele predominant in the normal population [3-5]. The pathological alleles of *ATXN2* found in SCA2 patients contain 32 CAG repeats [3-5,47]. The Atxn2 is a cytosolic ubiquitously expressed protein composed of 1,312 amino acid residues with a molecular mass of approximately 140 kDa [48] (Fig 1A). Atxn2 contains an RNA-binding Lsm domain [49,50] (Fig 1A) and has been implicated in RNA translation and

splicing [49,51,52]. The role in endocytosis [53], and actin-cytoskeleton organization [54] were also postulated for Atxn2. Genetic knockouts of *ATXN2* orthologs in fly and worm resulted in embryonic lethality [54,55]. Atxn2 knockout mice were viable but displayed a late-onset obesity phenotype [56]. Mice deficient in Atxn2 did not show Purkinje cell loss or marked changes in the Purkinje cell dendritic tree [56]. Non-essential role of Atxn2 in rodents is most likely related to the presence of orthologs and redundancy in its function [56].

The connection between polyQ-expansion in Atxn2 and neuronal degeneration is poorly understood. The SCA2 mouse model was developed which express cDNA for a human Atxn2-58Q protein under control of Purkinje cell-specific promoter [57]. These mice displayed age-dependent progressive phenotype in motor coordination assays and approximately 15% loss of cerebellar Purkinje cells by 12 months of age [57,58]. Analysis of these mice revealed that Atxn2-58 remained cytosolic in Purkinje neurons and did not form nuclear inclusions or was heavily ubiquitinated [57]. More recently SCA2 mouse model expressing human Atxn2-75Q gene under control of self promoter was established [59]. These mice also demonstrated rotarod deficit and Purkinje cell degeneration when compared with the wild type mice [59]. Genetic analysis of age of onset modifiers in SCA2 families revealed a connection with polymorphisms in *CACNA1A* voltage-gated Ca^{2+} channel gene [47]. The connection between SCA2 and neuronal Ca^{2+} signaling was further strengthened by discovery that polyQ-expanded Atxn2 but not wild type Atxn2 directly binds to and activates type 1 inositol (1,4,5)-trisphosphate receptor ($\text{InsP}_3\text{R1}$), an intracellular Ca^{2+} release channel [58]. In the same study it was demonstrated that dantrolene, a clinically relevant inhibitor of intracellular Ca^{2+} release, protects Purkinje cells from SCA2-58Q mice model from cell death both *in vitro* and *in vivo* [58]. These results provided strong experimental support to the “ Ca^{2+} hypothesis of SCA2 pathogenesis” [58] (Fig 2).

Spinocerebellar ataxia type 3 (SCA3)

At molecular level the cause of SCA3 is a polyQ expansion in the carboxy-terminal of ataxin-3 (Atxn3) protein [60,61]. The ataxin-3 is a 43 kDa cytosolic protein of 316 amino acids which contains amino-terminal Josephin domain and 3 ubiquitin-interactions motifs (UIMs) [48,62] (Fig 1B). The solution structure of Atxn3 Josephin domain was solved by NMR [63]. Consistent with the presence of Josephin domain Atxn3 functions as deubiquitinating enzyme (DUB) [64]. Atxn3 binds both K48- and K63-linked ubiquitin chains, yet preferentially cleaves K63-linked Ub chains *in vitro* [65]. Therefore, Atxn3 appear to function as a novel DUB that edits topologically complex short, mixed-linkage ubiquitin chains. The polyQ-expansion has no effect on DUB activity of Atxn3 [64,65], so it is not likely that neurodegeneration in SCA3 develops due to impaired normal function of Atxn3. Ataxin-3 has been shown to act as a potent transcriptional repressor, a function that is probably related to its DUB activity [66]. Knockout of the *ATXN3* gene in mice increases protein ubiquitination but does not result in an obvious behavioral phenotype [67].

The initial pathogenic mechanism suggested for SCA3 was focused on nuclear aggregates formed by mutant Atxn3 [68,69] and on impairment in the ubiquitin-proteasome pathway

[70]. Further support for these ideas was provided by studies of transgenic flies expressing mutant human Atxn3 [71] and by recent findings that genetic reduction or elimination of cochaperone and ubiquitin ligase CHIP (C-terminus of Hsp70-interacting protein) accelerated progression of the phenotype in transgenic mice expressing mutant human Atxn3 [72]. Nuclear localisation of ataxin-3 is essential for the pathogenesis of SCA3, and the phosphorylation state critically determines the intracellular localisation and toxicity of ataxin-3 [73]. All these results suggested that aggregates of expanded Atxn3 exert pathological influence on neurons, most likely by causing impairment of ubiquitin-proteasome system function and by affecting gene transcription.

Transgenic invertebrate models of SCA3 have been developed by expressing an expanded human Atxn3 protein in *Caenorhabditis elegans* [74] and in *Drosophila melanogaster* [75]. Analysis of these models supported an important role played by ubiquitination and proteasomal systems in SCA3 pathogenesis [71,74-76]. In addition, potential importance of Atxn3 cleavage by caspases [77] and RNA toxicity mechanisms [78] have been implicated in SCA3 pathogenesis based on the analysis of fly model of SCA3.

Several transgenic mouse models of SCA3 have been developed. In most of these models expanded human Atxn3 protein has been expressed under control of a mouse prion promoter (PrP) [79]. [80,81] These mice develop severe phenotype that includes progressive postural instability, gait and limb ataxia, weight loss and premature death [79] [80,81]. In another mice human Atxn3-84Q expressed in the context of endogenous gene regulatory elements using yeast artificial chromosome (YAC) technology [82]. These mice develop much more subtle and delayed motor phenotype [82], which is more closely resembles disease manifestation in SCA3 patients. Ageing SCA3-YAC-84Q mice display impaired performance in beam walk and gait walk assays and stereological analysis revealed significant neuronal loss in pontine nuclei (Pn) and substantia nigra (SN) regions of these mice at 14 months of age [83]. Recently a mouse expressing human Atxn3-148 transgenic construct under control of huntingtin promoter (HDProm) have been developed and characterized [84]. These mice developed late-onset (12 months) motor phenotype without prominent intranuclear aggregate formation [84]

Abnormal neuronal Ca^{2+} signaling has also been implicated in SCA3 pathogenesis. It was discovered that inhibition of Ca^{2+} -dependent protease calpain suppressed aggregation of polyglutamine-expanded Atxn3 in transfected cells [85]. Similar to mutant Htt and mutant Atxn2, mutant Atxn3 but not wild type Atxn3 binds to and activates InsP₃R1, an intracellular Ca^{2+} release channel [83]. Moreover, long-term feeding of SCA3-YAC-84Q transgenic mouse with Ca^{2+} stabilizer dantrolene alleviated age-dependent motor coordination deficits in this mice and prevented neuronal loss in SNc and PN [83]. These results provided strong experimental support to the “ Ca^{2+} hypothesis of SCA3 pathogenesis” [83] (Fig 2).

3. Previous clinical trials in SCA2 and SCA3 patients

There have been several attempts to pharmacologically improve ataxia with centrally acting drugs such as physostigmine, L-5-hydroxytryptophan, buspirone, thyrotropin releasing

hormone and others [86,87]. The trials in which these compounds were tested were performed in mixed groups of ataxia patients with variable diagnoses, and the antiataxic efficacy of the tested drugs was not proven. To date, there are only a limited number of clinical trials in SCA3 and no trials in SCA2. The trials that have been completed in SCA3 were performed in small number of patients and often had an inadequate study design. A major critique of all published studies is the short study period often not exceeding a few weeks. In none of the studies, there was a strong scientific rationale for selecting the respective study compound. Based on the observation that treatment of an SCA3 patient with the antibiotic sulfamethoxazole/trimethoprim improved ataxia, Correia et al. (1995) tested sulfamethoxazole/ trimethoprim in a double-blind, placebo-controlled crossover trial in 8 SCA3 patients using timed tests as an outcome parameter. There was a modest improvement of undetermined clinical relevance [88]. Sakai et al. (1995) used a very similar design and also found a small improvement which the authors attributed to an increase of brain bipterins caused by the treatment [89]. These effects were not confirmed in a 26 week, double-blind, placebo-controlled crossover trial in 22 SCA3 patients [90]. In another double-blind, placebo-controlled crossover trial, Sakai et al. (1996) tested tetrahydobioppterin and found a weak short-term effect [91]. More recent studies, which were all performed in an open-label design, tested taltireline hydrate, fluoxetine, tandospirone and lamotrigine [92-95]. Positive results were reported for all compounds except fluoxetine, but due to the lack of control, the significance of these findings is limited.

4. Perspectives for clinical intervention in SCA2 and SCA3 patients

There are limited targets for therapeutic intervention available for developing SCA2 and SCA3 treatment. The most obvious targets are mutant Atxn2 and Atxn3 proteins themselves. Expression of mutant Htt and mutant ataxin-1 (Atxn1) was reduced by injection of RNAi-encoding viruses, resulting in beneficial effects in mouse models of HD and SCA1 [96,97]. Similar RNAi knockdown approach could also be expanded to SCA2 and SCA3. Selective knockdown of mRNA encoding mutant Htt and mutant Atxn3 proteins was recently demonstrated using peptide nucleic acid (PNA) and locked nucleic acid (LNA) antisense oligomers targeting expanded CAG repeats [98]. These are very attractive approaches, but their utility at the moment is limited by the absence of RNAi or antisense brain delivery system that can be used in humans.

In addition to reducing expression of polyQ-expanded proteins at the mRNA level, another potential therapeutic strategy is based on developing agents that selectively bind to mutant forms of these proteins. A polyQ-containing protein can exist in multiple conformations, some of which are nontoxic and some of which are aggregation-prone and toxic [99]. One possibility to reduce the amount of toxic conformations of Atxn2 and Atxn3 proteins is to increase the levels of chaperons which control protein folding. Although these approaches are being developed for polyQ-expansion disorders [100-102], they have not produced clinical leads yet. Several small molecules were isolated in screens as inhibitors of polyQ aggregation. Some of these molecules were able to reduce polyQ aggregation and toxicity in cellular and animal models [103-108]. A specific polyQ binding peptide (QBP1) was isolated in the phage library screen [109]. It has been reported that QBP1 peptide prevented toxic conformational transition within polyQ-expanded proteins [109] and exerted

neuroprotective effects in cellular and animal models of polyQ toxicity [110-112]. Despite excellent scientific rationals, so far none of these candidates has successfully advanced into clinical trials due to problems related to finding an appropriate mode of delivery, poor pharmacokinetics and low efficacy *in vivo*. Recent solution of the crystal structure of Htt exon 1 containing polyQ region [113] opens perspective for rational design of novel polyQ-binding therapeutic agents.

There is very limited number of candidates that can be considered for SCA2 and SCA3 clinical trials. Treatment with lithium carbonate resulted in beneficial effects in SCA1 mouse model, apparently due to modulation of glycogen synthase kinase 3 (GSK3) activity [114]. Although lithium has not been directly tested in SCA2 and SCA3 mouse models, it is likely that lithium can produce beneficial effects in these models as well. Lithium is widely used for treatment of bipolar disease and has a long history of use in humans. Another potential candidate is Ca^{2+} stabilizer dantrolene, which has been evaluated with beneficial effects in both SCA2 and SCA3 mouse models [58,83] (Fig 2). Dantrolene also has a long history of human use for treatment of malignant hyperthermia and for neurological indications. In considering design of the clinical trial potential side effects of long term exposure to lithium or dantrolene must be taken into consideration.

Although never directly tested in the context of SCA models, additional therapeutic interventions can be potentially considered for treatment of SCA2 and SCA3 on the basis of proposed “ Ca^{2+} hypothesis” (Fig 2). NMDAR inhibitor memantine and anti-glutamate agent riluzole were neuroprotective in experiments with primary MSN cultures from YAC128 HD mouse model, with memantine being more effective [115]. Memantine was also effective in 3-NP model of HD [116] and in YAC128 genetic HD model [117]. Memantine demonstrated some beneficial effects in small scale pilot evaluation in HD patients [118] and is currently being tested soon in phase IV HD clinical trial. Riluzole was tested in phase III HD clinical trial, but it was not successful [119] (Table 2). It is possible that memantine and riluzole may have a beneficial effect in SCA2 and SCA3 patients (Fig 2). In addition to NMDAR, voltage-gated Ca^{2+} channels constitute another potential target for SCA treatment. In recent studies L-type Ca^{2+} channel inhibitor isradipine significantly protected SNc neurons in animal models of Parkinson's disease [120]. Moreover, a recent retrospective epidemiological study has found that treatment of hypertension with Ca^{2+} channel antagonists significantly diminished the risk of developing PD [121]. It is possible that isradipine and other dihydropyridines (DHP) may also be useful for treatment of SCA2 and SCA3 patients (Fig 2). More conservative potential strategy involves use of mitochondrial stabilizers and energizers, such as creatine, CoQ10, and MitoQ (Fig 2). In previous neurodegenerative trials these compounds resulted in modest benefit [122] and it is likely that similar result will be obtained for SCA2 and SCA3 patients as well. Recent clinical research of the EUROSCA consortium demonstrated the feasibility of large-scale clinical trials in SCAs. The EUROSCA consortium devised and validated a novel rating scale for ataxias, the Scale for the Assessment and Rating of Ataxia (SARA) [123] that is now considered standard in clinical ataxia studies. A European SCA registry was created that currently contains entries of approximately 4000 SCA patients. The registry is connected to a web-based electronic data capture system that is designed to run and monitor

observational and interventional trials in SCAs. A large cross-sectional study of more than 500 SCA1, SCA2, SCA3 and SCA6 patients led to the identification of factors that determine disease severity and extracerebellar involvement in these disorders [32]. Longitudinal observation of this cohort allowed to calculate the required sample sizes for future interventional trials [124]. The EUROSCA provides a potential framework for controlled clinical evaluation of lithium, dantrolene, memantine, riluzole, dihydropyridines, creatine, CoQ10 or other potential therapeutic agents in ataxia patients in the near future.

Acknowledgments

IB is a holder of Carla Cocke Francis Professorship in Alzheimer's Research and supported by the McKnight Neuroscience of Brain Disorders Award. The work on SCA2 and SCA3 was supported by the National Organization for Rare Disorders, National Ataxia Foundation, Ataxia MJD Research Project, and the National Institutes of Health grants R01NS38082 and R01NS056224. TK receives grants from the European Union (EUROSCA/LSHM-CT-2004-503304), German Ministry of Education and Research (RISCA) and Deutsche Forschungsgemeinschaft (DFG).

References

- 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. [PubMed: 15099544]
- Orr HT, Chung MY, Banfi S, Kwiatkowski TJ Jr, Servadio A, Beaudet AL, McCall AE, Duvick LA, Ranum LP, Zoghbi HY. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat Genet.* 1993; 4:221–6. [PubMed: 8358429]
- Pulst SM, Nechiporuk A, Nechiporuk T, Gispert S, Chen XN, Lopes-Cendes I, Pearlman S, Starkman S, Orozco-Diaz G, Lunkes A, DeJong P, Rouleau GA, Auburger G, Korenberg JR, Figueroa C, Sahba S. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. *Nat Genet.* 1996; 14:269–76. [PubMed: 8896555]
- Imbert G, Saudou F, Yvert G, Devys D, Trottier Y, Garnier JM, Weber C, Mandel JL, Cancel G, Abbas N, Durr A, Didierjean O, Stevanin G, Agid Y, Brice A. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat Genet.* 1996; 14:285–91. [PubMed: 8896557]
- Sanpei K, Takano H, Igarashi S, Sato T, Oyake M, Sasaki H, Wakisaka A, Tashiro K, Ishida Y, Ikeuchi T, Koide R, Saito M, Sato A, Tanaka T, Hanyu S, Takiyama Y, Nishizawa M, Shimizu N, Nomura Y, Segawa M, Iwabuchi K, Eguchi I, Tanaka H, Takahashi H, Tsuji S. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat Genet.* 1996; 14:277–84. [PubMed: 8896556]
- Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat Genet.* 1997; 15:62–9. [PubMed: 8988170]
- Riess O, Schols L, Bottger H, Nolte D, Vieira-Saecker AM, Schimming C, Kreuz F, Macek M Jr, Krebsova A, Macek MS, Klockgether T, Zuhlke C, Laccone FA. SCA6 is caused by moderate CAG expansion in the alpha1A-voltage-dependent calcium channel gene. *Hum Mol Genet.* 1997; 6:1289–93. [PubMed: 9259275]
- David G, Abbas N, Stevanin G, Durr A, Yvert G, Cancel G, Weber C, Imbert G, Saudou F, Antoniou E, Drabkin H, Gemmill R, Giunti P, Benomar A, Wood N, Ruberg M, Agid Y, Mandel JL, Brice A. Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nat Genet.* 1997; 17:65–70. [PubMed: 9288099]
- Nakamura K, Jeong SY, Uchihara T, Anno M, Nagashima K, Nagashima T, Ikeda S, Tsuji S, Kanazawa I. SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. *Hum Mol Genet.* 2001; 10:1441–8. [PubMed: 11448935]
- Orr HT, Zoghbi HY. Trinucleotide repeat disorders. *Annu Rev Neurosci.* 2007; 30:575–621. [PubMed: 17417937]

11. Gusella JF, MacDonald ME. Molecular genetics: unmasking polyglutamine triggers in neurodegenerative disease. *Nat Rev Neurosci.* 2000; 1:109–15. [PubMed: 11252773]
12. Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW, Ranum LP. An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nat Genet.* 1999; 21:379–84. [PubMed: 10192387]
13. Matsuura T, Yamagata T, Burgess DL, Rasmussen A, Grewal RP, Watase K, Khajavi M, McCall AE, Davis CF, Zu L, Achari M, Pulst SM, Alonso E, Noebels JL, Nelson DL, Zoghbi HY, Ashizawa T. Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. *Nat Genet.* 2000; 26:191–4. [PubMed: 11017075]
14. Holmes SE, O'Hearn EE, McInnis MG, Gorelick-Feldman DA, Kleiderlein JJ, Callahan C, Kwak NG, Ingersoll-Ashworth RG, Sherr M, Sumner AJ, Sharp AH, Ananth U, Seltzer WK, Boss MA, Viera-Saecker AM, Epplen JT, Riess O, Ross CA, Margolis RL. Expansion of a novel CAG trinucleotide repeat in the 5' region of PPP2R2B is associated with SCA12. *Nat Genet.* 1999; 23:391–2. [PubMed: 10581021]
15. Ikeda Y, Dick KA, Weatherspoon MR, Gincel D, Armbrust KR, Dalton JC, Stevanin G, Durr A, Zuhlke C, Burk K, Clark HB, Brice A, Rothstein JD, Schut LJ, Day JW, Ranum LP. Spectrin mutations cause spinocerebellar ataxia type 5. *Nat Genet.* 2006; 38:184–90. [PubMed: 16429157]
16. Houlden H, Johnson J, Gardner-Thorpe C, Lashley T, Hernandez D, Worth P, Singleton AB, Hilton DA, Holton J, Revesz T, Davis MB, Giunti P, Wood NW. Mutations in TTBK2, encoding a kinase implicated in tau phosphorylation, segregate with spinocerebellar ataxia type 11. *Nat Genet.* 2007; 39:1434–6. [PubMed: 18037885]
17. Waters MF, Minassian NA, Stevanin G, Figueroa KP, Bannister JP, Nolte D, Mock AF, Evidente VG, Fee DB, Muller U, Durr A, Brice A, Papazian DM, Pulst SM. Mutations in voltage-gated potassium channel KCNC3 cause degenerative and developmental central nervous system phenotypes. *Nat Genet.* 2006; 38:447–51. [PubMed: 16501573]
18. Chen DH, Brkanac Z, Verlinde CL, Tan XJ, Bylenok L, Nochlin D, Matsushita M, Lipe H, Wolff J, Fernandez M, Cimino PJ, Bird TD, Raskind WH. Missense mutations in the regulatory domain of PKC gamma: a new mechanism for dominant nonepisodic cerebellar ataxia. *Am J Hum Genet.* 2003; 72:839–49. [PubMed: 12644968]
19. van de Leemput J, Chandran J, Knight MA, Holtzclaw LA, Scholz S, Cookson MR, Houlden H, Gwinn-Hardy K, Fung HC, Lin X, Hernandez D, Simon-Sanchez J, Wood NW, Giunti P, Rafferty I, Hardy J, Storey E, Gardner RJ, Forrest SM, Fisher EM, Russell JT, Cai H, Singleton AB. Deletion at ITPR1 underlies ataxia in mice and spinocerebellar ataxia 15 in humans. *PLoS Genet.* 2007; 3:e108. [PubMed: 17590087]
20. van Swieten JC, Brusse E, de Graaf BM, Krieger E, van de Graaf R, de Koning I, Maat-Kievit A, Leegwater P, Dooijes D, Oostra BA, Heutink P. A mutation in the fibroblast growth factor 14 gene is associated with autosomal dominant cerebellar ataxia [corrected]. *Am J Hum Genet.* 2003; 72:191–9. [PubMed: 12489043]
21. Maltecca F, Magnoni R, Cerri F, Cox GA, Quattrini A, Casari G. Haploinsufficiency of AFG3L2, the gene responsible for spinocerebellar ataxia type 28, causes mitochondria-mediated Purkinje cell dark degeneration. *J Neurosci.* 2009; 29:9244–54. [PubMed: 19625515]
22. Ishikawa K, Toru S, Tsunemi T, Li M, Kobayashi K, Yokota T, Amino T, Owada K, Fujigasaki H, Sakamoto M, Tomimitsu H, Takashima M, Kumagai J, Noguchi Y, Kawashima Y, Ohkoshi N, Ishida G, Gomyoda M, Yoshida M, Hashizume Y, Saito Y, Murayama S, Yamanouchi H, Mizutani T, Kondo I, Toda T, Mizusawa H. An autosomal dominant cerebellar ataxia linked to chromosome 16q22.1 is associated with a single-nucleotide substitution in the 5' untranslated region of the gene encoding a protein with spectrin repeat and Rho guanine-nucleotide exchange-factor domains. *Am J Hum Genet.* 2005; 77:280–96. [PubMed: 16001362]
23. van de Warrenburg BP, Sinke RJ, Verschuuren-Bemelmans CC, Scheffer H, Brunt ER, Ippel PF, Maat-Kievit JA, Dooijes D, Notermans NC, Lindhout D, Knoers NV, Kremer HP. Spinocerebellar ataxias in the Netherlands: prevalence and age at onset variance analysis. *Neurology.* 2002; 58:702–8. [PubMed: 11889231]
24. Erichsen AK, Koht J, Stray-Pedersen A, Abdelnoor M, Tallaksen CM. Prevalence of hereditary ataxia and spastic paraplegia in southeast Norway: a population-based study. *Brain.* 2009; 132:1577–88. [PubMed: 19339254]

25. Velazquez-Perez L, Santos FN, Garcia R, Paneque HM, Hechavarria PR. Epidemiology of Cuban hereditary ataxia. *Rev Neurol*. 2001; 32:606–11. [PubMed: 11391485]
26. Moseley ML, Benzow KA, Schut LJ, Bird TD, Gomez CM, Barkhaus PE, Blindauer KA, Labuda M, Pandolfo M, Koob MD, Ranum LP. Incidence of dominant spinocerebellar and Friedreich triplet repeats among 361 ataxia families. *Neurology*. 1998; 51:1666–71. [PubMed: 9855520]
27. Geschwind DH, Perlman S, Figueroa CP, Treiman LJ, Pulst SM. The prevalence and wide clinical spectrum of the spinocerebellar ataxia type 2 trinucleotide repeat in patients with autosomal dominant cerebellar ataxia. *Am J Hum Genet*. 1997; 60:842–50. [PubMed: 9106530]
28. Lastres-Becker I, Rub U, Auburger G. Spinocerebellar ataxia 2 (SCA2). *Cerebellum*. 2008; 7:115–24. [PubMed: 18418684]
29. Burk K, Abele M, Fetter M, Dichgans J, Skalej M, Laccone F, Didierjean O, Brice A, Klockgether T. Autosomal dominant cerebellar ataxia type I clinical features and MRI in families with SCA1, SCA2 and SCA3. *Brain*. 1996; 119(Pt 5):1497–505. [PubMed: 8931575]
30. Filla A, De Michele G, Santoro L, Calabrese O, Castaldo I, Giuffrida S, Restivo D, Serlenga L, Condorelli DF, Bonuccelli U, Scala R, Coppola G, Caruso G, Coccozza S. Spinocerebellar ataxia type 2 in southern Italy: a clinical and molecular study of 30 families. *J Neurol*. 1999; 246:467–71. [PubMed: 10431773]
31. Schols L, Gispert S, Vorgerd M, Menezes Vieira-Saecker AM, Blanke P, Auburger G, Amoiridis G, Meves S, Epplen JT, Przuntek H, Pulst SM, Riess O. Spinocerebellar ataxia type 2. Genotype and phenotype in German kindreds. *Arch Neurol*. 1997; 54:1073–80. [PubMed: 9311350]
32. Schmitz-Hubsch T, Coudert M, Bauer P, Giunti P, Globas C, Baliko L, Filla A, Mariotti C, Rakowicz M, Charles P, Ribai P, Szymanski S, Infante J, van de Warrenburg BP, Durr A, Timmann D, Boesch S, Fancellu R, Rola R, Depondt C, Schols L, Zdienicka E, Kang JS, Dohlinger S, Kremer B, Stephenson DA, Melegh B, Pandolfo M, di Donato S, du Montcel ST, Klockgether T. Spinocerebellar ataxia types 1, 2, 3, and 6: disease severity and nonataxia symptoms. *Neurology*. 2008; 71:982–9. [PubMed: 18685131]
33. Klockgether T, Ludtke R, Kramer B, Abele M, Burk K, Schols L, Riess O, Laccone F, Boesch S, Lopes-Cendes I, Brice A, Inzelberg R, Zilber N, Dichgans J. The natural history of degenerative ataxia: a retrospective study in 466 patients. *Brain*. 1998; 121(Pt 4):589–600. [PubMed: 9577387]
34. Stevanin G, Durr A, Brice A. Clinical and molecular advances in autosomal dominant cerebellar ataxias: from genotype to phenotype and pathophysiology. *Eur J Hum Genet*. 2000; 8:4–18. [PubMed: 10713882]
35. Coutinho P, Andrade C. Autosomal dominant system degeneration in Portuguese families of the Azores Islands. A new genetic disorder involving cerebellar, pyramidal, extrapyramidal and spinal cord motor functions. *Neurology*. 1978; 28:703–9. [PubMed: 566869]
36. Maciel P, Gaspar C, DeStefano AL, Silveira I, Coutinho P, Radvany J, Dawson DM, Sudarsky L, Guimaraes J, Loureiro JE, et al. Correlation between CAG repeat length and clinical features in Machado-Joseph disease. *Am J Hum Genet*. 1995; 57:54–61. [PubMed: 7611296]
37. Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration. *Annu Rev Neurosci*. 2000; 23:217–47. [PubMed: 10845064]
38. Cummings CJ, Zoghbi HY. Trinucleotide repeats: mechanisms and pathophysiology. *Annu Rev Genomics Hum Genet*. 2000; 1:281–328. [PubMed: 11701632]
39. Tobin AJ, Signer ER. Huntington's disease: the challenge for cell biologists. *Trends Cell Biol*. 2000; 10:531–6. [PubMed: 11121745]
40. Rubinsztein DC. Lessons from animal models of Huntington's disease. *Trends Genet*. 2002; 18:202–9. [PubMed: 11932021]
41. Ross CA. Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. *Neuron*. 2002; 35:819–22. [PubMed: 12372277]
42. Li SH, Li XJ. Huntingtin-protein interactions and the pathogenesis of Huntington's disease. *Trends Genet*. 2004; 20:146–54. [PubMed: 15036808]
43. Bezprozvanny I. Calcium signaling and neurodegenerative diseases. *Trends Mol Med*. 2009; 15:89–100. [PubMed: 19230774]
44. Cha JH. Transcriptional signatures in Huntington's disease. *Prog Neurobiol*. 2007; 83:228–48. [PubMed: 17467140]

45. Truant R, Atwal RS, Desmond C, Munsie L, Tran T. Huntington's disease: revisiting the aggregation hypothesis in polyglutamine neurodegenerative diseases. *FEBS J.* 2008; 275:4252–62. [PubMed: 18637947]
46. Sugars KL, Rubinsztein DC. Transcriptional abnormalities in Huntington disease. *Trends Genet.* 2003; 19:233–8. [PubMed: 12711212]
47. Pulst SM, Santos N, Wang D, Yang H, Huynh D, Velazquez L, Figueroa KP. Spinocerebellar ataxia type 2: polyQ repeat variation in the CACNA1A calcium channel modifies age of onset. *Brain.* 2005; 128:2297–303. [PubMed: 16000334]
48. Albrecht M, Golatta M, Wullner U, Lengauer T. Structural and functional analysis of ataxin-2 and ataxin-3. *Eur J Biochem.* 2004; 271:3155–70. [PubMed: 15265035]
49. Ralser M, Albrecht M, Nonhoff U, Lengauer T, Lehrach H, Krobitsch S. An integrative approach to gain insights into the cellular function of human ataxin-2. *J Mol Biol.* 2005; 346:203–14. [PubMed: 15663938]
50. Kozlov G, Trempe JF, Khaleghpour K, Kahvejian A, Ekiel I, Gehring K. Structure and function of the C-terminal PABC domain of human poly(A)-binding protein. *Proc Natl Acad Sci U S A.* 2001; 98:4409–13. [PubMed: 11287632]
51. Shibata H, Huynh DP, Pulst SM. A novel protein with RNA-binding motifs interacts with ataxin-2. *Hum Mol Genet.* 2000; 9:1303–13. [PubMed: 10814712]
52. Satterfield TF, Pallanck LJ. Ataxin-2 and its Drosophila homolog, ATX2, physically assemble with polyribosomes. *Hum Mol Genet.* 2006; 15:2523–32. [PubMed: 16835262]
53. Ralser M, Nonhoff U, Albrecht M, Lengauer T, Wanker EE, Lehrach H, Krobitsch S. Ataxin-2 and huntingtin interact with endophilin-A complexes to function in plastin-associated pathways. *Hum Mol Genet.* 2005; 14:2893–909. [PubMed: 16115810]
54. Satterfield TF, Jackson SM, Pallanck LJ. A Drosophila homolog of the polyglutamine disease gene SCA2 is a dosage-sensitive regulator of actin filament formation. *Genetics.* 2002; 162:1687–702. [PubMed: 12524342]
55. Kiehl TR, Shibata H, Pulst SM. The ortholog of human ataxin-2 is essential for early embryonic patterning in *C. elegans*. *J Mol Neurosci.* 2000; 15:231–41. [PubMed: 11303786]
56. Kiehl TR, Nechiporuk A, Figueroa KP, Keating MT, Huynh DP, Pulst SM. Generation and characterization of Sca2 (ataxin-2) knockout mice. *Biochem Biophys Res Commun.* 2006; 339:17–24. [PubMed: 16293225]
57. Huynh DP, Figueroa K, Hoang N, Pulst SM. Nuclear localization or inclusion body formation of ataxin-2 are not necessary for SCA2 pathogenesis in mouse or human. *Nat Genet.* 2000; 26:44–50. [PubMed: 10973246]
58. Liu J, Tang TS, Tu H, Nelson O, Herndon E, Huynh DP, Pulst SM, Bezprozvanny I. Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 2. *J Neurosci.* 2009; 29:9148–62. [PubMed: 19625506]
59. Aguiar J, Fernandez J, Aguilar A, Mendoza Y, Vazquez M, Suarez J, Berlanga J, Cruz S, Guillen G, Herrera L, Velazquez L, Santos N, Merino N. Ubiquitous expression of human SCA2 gene under the regulation of the SCA2 self promoter cause specific Purkinje cell degeneration in transgenic mice. *Neurosci Lett.* 2006; 392:202–6. [PubMed: 16203087]
60. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I, et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat Genet.* 1994; 8:221–8. [PubMed: 7874163]
61. Paulson HL, Das SS, Crino PB, Perez MK, Patel SC, Gotsdiner D, Fischbeck KH, Pittman RN. Machado-Joseph disease gene product is a cytoplasmic protein widely expressed in brain. *Ann Neurol.* 1997; 41:453–62. [PubMed: 9124802]
62. Scheel H, Tomiuk S, Hofmann K. Elucidation of ataxin-3 and ataxin-7 function by integrative bioinformatics. *Hum Mol Genet.* 2003; 12:2845–52. [PubMed: 12944423]
63. Nicastro G, Menon RP, Masino L, Knowles PP, McDonald NQ, Pastore A. The solution structure of the Josephin domain of ataxin-3: Structural determinants for molecular recognition. *Proc Natl Acad Sci U S A.* 2005; 102:10493–8. [PubMed: 16020535]

64. Burnett B, Li F, Pittman RN. The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. *Hum Mol Genet.* 2003; 12:3195–205. [PubMed: 14559776]
65. Winborn BJ, Travis SM, Todi SV, Scaglione KM, Xu P, Williams AJ, Cohen RE, Peng J, Paulson HL. The deubiquitinating enzyme ataxin-3, a polyglutamine disease protein, edits Lys63 linkages in mixed linkage ubiquitin chains. *J Biol Chem.* 2008; 283:26436–43. [PubMed: 18599482]
66. Evert BO, Araujo J, Vieira-Saecker AM, de Vos RA, Harendza S, Klockgether T, Wullner U. Ataxin-3 represses transcription via chromatin binding, interaction with histone deacetylase 3, and histone deacetylation. *J Neurosci.* 2006; 26:11474–86. [PubMed: 17079677]
67. Schmitt I, Linden M, Khazneh H, Evert BO, Breuer P, Klockgether T, Wuellner U. Inactivation of the mouse *Atxn3* (ataxin-3) gene increases protein ubiquitination. *Biochem Biophys Res Commun.* 2007; 362:734–9. [PubMed: 17764659]
68. Paulson HL, Perez MK, Trotter Y, Trojanowski JQ, Subramony SH, Das SS, Vig P, Mandel JL, Fischbeck KH, Pittman RN. Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron.* 1997; 19:333–44. [PubMed: 9292723]
69. Perez MK, Paulson HL, Pittman RN. Ataxin-3 with an altered conformation that exposes the polyglutamine domain is associated with the nuclear matrix. *Hum Mol Genet.* 1999; 8:2377–85. [PubMed: 10556285]
70. Chai Y, Koppenhafer SL, Shoesmith SJ, Perez MK, Paulson HL. Evidence for proteasome involvement in polyglutamine disease: localization to nuclear inclusions in SCA3/MJD and suppression of polyglutamine aggregation in vitro. *Hum Mol Genet.* 1999; 8:673–82. [PubMed: 10072437]
71. Warrick JM, Morabito LM, Bilen J, Gordesky-Gold B, Faust LZ, Paulson HL, Bonini NM. Ataxin-3 suppresses polyglutamine neurodegeneration in *Drosophila* by a ubiquitin-associated mechanism. *Mol Cell.* 2005; 18:37–48. [PubMed: 15808507]
72. Williams AJ, Knutson TM, Colomer Gould VF, Paulson HL. In vivo suppression of polyglutamine neurotoxicity by C-terminus of Hsp70-interacting protein (CHIP) supports an aggregation model of pathogenesis. *Neurobiol Dis.* 2009; 33:342–53. [PubMed: 19084066]
73. Mueller T, Breuer P, Schmitt I, Walter J, Evert BO, Wullner U. CK2-dependent phosphorylation determines cellular localization and stability of ataxin-3. *Hum Mol Genet.* 2009; 18:3334–43. [PubMed: 19542537]
74. Khan LA, Bauer PO, Miyazaki H, Lindenberg KS, Landwehrmeyer BG, Nukina N. Expanded polyglutamines impair synaptic transmission and ubiquitin-proteasome system in *Caenorhabditis elegans*. *J Neurochem.* 2006; 98:576–87. [PubMed: 16805848]
75. Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, Pittman RN, Bonini NM. Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. *Cell.* 1998; 93:939–49. [PubMed: 9635424]
76. Bilen J, Bonini NM. Genome-wide screen for modifiers of ataxin-3 neurodegeneration in *Drosophila*. *PLoS Genet.* 2007; 3:1950–64. [PubMed: 17953484]
77. Jung J, Xu K, Lessing D, Bonini NM. Preventing Ataxin-3 protein cleavage mitigates degeneration in a *Drosophila* model of SCA3. *Hum Mol Genet.* 2009; 18:4843–52. [PubMed: 19783548]
78. Li LB, Yu Z, Teng X, Bonini NM. RNA toxicity is a component of ataxin-3 degeneration in *Drosophila*. *Nature.* 2008; 453:1107–11. [PubMed: 18449188]
79. Goti D, Katzen SM, Mez J, Kurtis N, Kiluk J, Ben-Haiem L, Jenkins NA, Copeland NG, Kakizuka A, Sharp AH, Ross CA, Mouton PR, Colomer V. A mutant ataxin-3 putative-cleavage fragment in brains of Machado-Joseph disease patients and transgenic mice is cytotoxic above a critical concentration. *J Neurosci.* 2004; 24:10266–79. [PubMed: 15537899]
80. Bichelmeier U, Schmidt T, Hubener J, Boy J, Ruttiger L, Habig K, Poths S, Bonin M, Knipper M, Schmidt WJ, Wilbertz J, Wolburg H, Laccone F, Riess O. Nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3: in vivo evidence. *J Neurosci.* 2007; 27:7418–28. [PubMed: 17626202]
81. Chou AH, Yeh TH, Ouyang P, Chen YL, Chen SY, Wang HL. Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. *Neurobiol Dis.* 2008; 31:89–101. [PubMed: 18502140]

82. Cemal CK, Carroll CJ, Lawrence L, Lowrie MB, Ruddle P, Al-Mahdawi S, King RH, Pook MA, Huxley C, Chamberlain S. YAC transgenic mice carrying pathological alleles of the MJD1 locus exhibit a mild and slowly progressive cerebellar deficit. *Hum Mol Genet.* 2002; 11:1075–94. [PubMed: 11978767]
83. Chen X, Tang TS, Tu H, Nelson O, Pook MA, Hammer RE, Nukina N, Bezprozvanny I. Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. *J Neuroscience.* 2008; 28:12713–12724.
84. Boy J, Schmidt T, Schumann U, Grasshoff U, Unser S, Holzmann C, Schmitt I, Karl T, Laccone F, Wolburg H, Ibrahim S, Riess O. A transgenic mouse model of spinocerebellar ataxia type 3 resembling late disease onset and gender-specific instability of CAG repeats. *Neurobiol Dis.* 2009
85. Haacke A, Hartl FU, Breuer P. Calpain inhibition is sufficient to suppress aggregation of polyglutamine-expanded ataxin-3. *J Biol Chem.* 2007; 282:18851–6. [PubMed: 17488727]
86. Ogawa M. Pharmacological treatments of cerebellar ataxia. *Cerebellum.* 2004; 3:107–11. [PubMed: 15233578]
87. Trujillo-Martin MM, Serrano-Aguilar P, Monton-Alvarez F, Carrillo-Fumero R. Effectiveness and safety of treatments for degenerative ataxias: a systematic review. *Mov Disord.* 2009; 24:1111–24. [PubMed: 19412936]
88. Correia M, Coutinho P, Silva MC, Guimaraes J, Amado J, Matos E. Evaluation of the effect of sulphamethoxazole and trimethoprim in patients with Machado-Joseph disease. *Rev Neurol.* 1995; 23:632–4. [PubMed: 8597984]
89. Sakai T, Matsuishi T, Yamada S, Komori H, Iwashita H. Sulfamethoxazole-trimethoprim double-blind, placebo-controlled, crossover trial in Machado-Joseph disease: sulfamethoxazole-trimethoprim increases cerebrospinal fluid level of biopterin. *J Neural Transm Gen Sect.* 1995; 102:159–72. [PubMed: 8748680]
90. Schulte T, Mattern R, Berger K, Szymanski S, Klotz P, Kraus PH, Przuntek H, Schols L. Double-blind crossover trial of trimethoprim-sulfamethoxazole in spinocerebellar ataxia type 3/Machado-Joseph disease. *Arch Neurol.* 2001; 58:1451–7. [PubMed: 11559318]
91. Sakai T, Antoku Y, Matsuishi T, Iwashita H. Tetrahydrobiopterin double-blind, crossover trial in Machado-Joseph disease. *J Neurol Sci.* 1996; 136:71–2. [PubMed: 8815181]
92. Shirasaki H, Ishida C, Nakajima T, Kamei H, Koide T, Fukuhara N. A quantitative evaluation of spinocerebellar degeneration by an acoustic analysis--the effect of taltirelin hydrate on patients with Machado-Joseph disease. *Rinsho Shinkeigaku.* 2003; 43:143–8. [PubMed: 12884823]
93. Monte TL, Rieder CR, Tort AB, Rockenback I, Pereira ML, Silveira I, Ferro A, Sequeiros J, Jardim LB. Use of fluoxetine for treatment of Machado-Joseph disease: an open-label study. *Acta Neurol Scand.* 2003; 107:207–10. [PubMed: 12614314]
94. Takei A, Fukazawa T, Hamada T, Sohma H, Yabe I, Sasaki H, Tashiro K. Effects of tandospirone on “5-HT1A receptor-associated symptoms” in patients with Machado-Joseph disease: an open-label study. *Clin Neuropharmacol.* 2004; 27:9–13. [PubMed: 15090930]
95. Liu CS, Hsu HM, Cheng WL, Hsieh M. Clinical and molecular events in patients with Machado-Joseph disease under lamotrigine therapy. *Acta Neurol Scand.* 2005; 111:385–90. [PubMed: 15876340]
96. Harper SQ, Staber PD, He X, Eliason SL, Martins IH, Mao Q, Yang L, Kotin RM, Paulson HL, Davidson BL. RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc Natl Acad Sci U S A.* 2005; 102:5820–5. [PubMed: 15811941]
97. Xia H, Mao Q, Eliason SL, Harper SQ, Martins IH, Orr HT, Paulson HL, Yang L, Kotin RM, Davidson BL. RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. *Nat Med.* 2004; 10:816–20. [PubMed: 15235598]
98. Hu J, Matsui M, Gagnon KT, Schwartz JC, Gabillet S, Arar K, Wu J, Bezprozvanny I, Corey DR. Allele-specific silencing of mutant huntingtin and ataxin-3 genes by targeting expanded CAG repeats in mRNAs. *Nat Biotechnol.* 2009; 27:478–484. [PubMed: 19412185]
99. Shao J, Diamond MI. Polyglutamine diseases: emerging concepts in pathogenesis and therapy. *Hum Mol Genet.* 2007; 16:R115–23. Spec No. 2. [PubMed: 17911155]

100. Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. *Nat Genet.* 1999; 23:425–8. [PubMed: 10581028]
101. Wyttenbach A, Sauvageot O, Carmichael J, Diaz-Latoud C, Arrigo AP, Rubinsztein DC. Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. *Hum Mol Genet.* 2002; 11:1137–51. [PubMed: 11978772]
102. Wacker JL, Zareie MH, Fong H, Sarikaya M, Muchowski PJ. Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. *Nat Struct Mol Biol.* 2004; 11:1215–22. [PubMed: 15543156]
103. Heiser V, Engemann S, Bocker W, Dunkel I, Boeddrich A, Waelter S, Nordhoff E, Lurz R, Schugardt N, Rautenberg S, Herhaus C, Barnickel G, Bottcher H, Lehrach H, Wanker EE. Identification of benzothiazoles as potential polyglutamine aggregation inhibitors of Huntington's disease by using an automated filter retardation assay. *Proc Natl Acad Sci U S A.* 2002; 99(Suppl 4):16400–6. [PubMed: 12200548]
104. Sanchez I, Mahlke C, Yuan J. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. *Nature.* 2003; 421:373–9. [PubMed: 12540902]
105. Zhang X, Smith DL, Meriin AB, Engemann S, Russel DE, Roark M, Washington SL, Maxwell MM, Marsh JL, Thompson LM, Wanker EE, Young AB, Housman DE, Bates GP, Sherman MY, Kazantsev AG. A potent small molecule inhibits polyglutamine aggregation in Huntington's disease neurons and suppresses neurodegeneration in vivo. *Proc Natl Acad Sci U S A.* 2005; 102:892–7. [PubMed: 15642944]
106. Wang J, Gines S, MacDonald ME, Gusella JF. Reversal of a full-length mutant huntingtin neuronal cell phenotype by chemical inhibitors of polyglutamine-mediated aggregation. *BMC Neurosci.* 2005; 6:1. [PubMed: 15649316]
107. Ehnhoefter DE, Duennwald M, Markovic P, Wacker JL, Engemann S, Roark M, Legleiter J, Marsh JL, Thompson LM, Lindquist S, Muchowski PJ, Wanker EE. Green tea (-)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum Mol Genet.* 2006; 15:2743–51. [PubMed: 16893904]
108. Chopra V, Fox JH, Lieberman G, Dorsey K, Matson W, Waldmeier P, Housman DE, Kazantsev A, Young AB, Hersch S. A small-molecule therapeutic lead for Huntington's disease: preclinical pharmacology and efficacy of C2-8 in the R6/2 transgenic mouse. *Proc Natl Acad Sci U S A.* 2007; 104:16685–9. [PubMed: 17925440]
109. Nagai Y, Tucker T, Ren H, Kenan DJ, Henderson BS, Keene JD, Strittmatter WJ, Burke JR. Inhibition of polyglutamine protein aggregation and cell death by novel peptides identified by phage display screening. *J Biol Chem.* 2000; 275:10437–42. [PubMed: 10744733]
110. Nagai Y, Fujikake N, Ohno K, Higashiyama H, Popiel HA, Rahadian J, Yamaguchi M, Strittmatter WJ, Burke JR, Toda T. Prevention of polyglutamine oligomerization and neurodegeneration by the peptide inhibitor QBP1 in *Drosophila*. *Hum Mol Genet.* 2003; 12:1253–9. [PubMed: 12761040]
111. Popiel HA, Nagai Y, Fujikake N, Toda T. Protein transduction domain-mediated delivery of QBP1 suppresses polyglutamine-induced neurodegeneration in vivo. *Mol Ther.* 2007; 15:303–9. [PubMed: 17235308]
112. Popiel HA, Nagai Y, Fujikake N, Toda T. Delivery of the aggregate inhibitor peptide QBP1 into the mouse brain using PTDs and its therapeutic effect on polyglutamine disease mice. *Neurosci Lett.* 2009; 449:87–92. [PubMed: 18603372]
113. Kim MW, Chelliah Y, Kim SW, Otwinowski Z, Bezprozvanny I. Secondary structure of Huntingtin amino-terminal region. *Structure.* 2009; 17:1205–12. [PubMed: 19748341]
114. Watase K, Gatchel JR, Sun Y, Emamian E, Atkinson R, Richman R, Mizusawa H, Orr HT, Shaw C, Zoghbi HY. Lithium therapy improves neurological function and hippocampal dendritic arborization in a spinocerebellar ataxia type 1 mouse model. *PLoS Med.* 2007; 4:e182. [PubMed: 17535104]
115. Wu J, Tang TS, Bezprozvanny I. Evaluation of clinically-relevant glutamate pathway inhibitors in in vitro model of Huntington's disease. *Neurosci Lett.* 2006; 407:219–223. [PubMed: 16959411]

116. Lee ST, Chu K, Park JE, Kang L, Ko SY, Jung KH, Kim M. Memantine reduces striatal cell death with decreasing calpain level in 3-nitropropionic model of Huntington's disease. *Brain Res.* 2006; 1118:199–207. [PubMed: 16959224]
117. Okamoto SI, Pouladi MA, Talantova M, Yao D, Xia P, Ehrnhoefer DE, Zaidi R, Clemente A, Kaul M, Graham RK, Zhang D, Vincent Chen HS, Tong G, Hayden MR, Lipton SA. Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nat Med.* 2009
118. Ondo WG, Mejia NI, Hunter CB. A pilot study of the clinical efficacy and safety of memantine for Huntington's disease. *Parkinsonism Relat Disord.* 2007; 13:453–4. [PubMed: 17046312]
119. Landwehrmeyer GB, Dubois B, de Yebenes JG, Kremer B, Gaus W, Kraus PH, Przuntek H, Dib M, Doble A, Fischer W, Ludolph AC. Riluzole in Huntington's disease: a 3-year, randomized controlled study. *Ann Neurol.* 2007; 62:262–72. [PubMed: 17702031]
120. Chan CS, Guzman JN, Ilijic E, Mercer JN, Rick C, Tkatch T, Meredith GE, Surmeier DJ. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature.* 2007; 447:1081–6. [PubMed: 17558391]
121. Becker C, Jick SS, Meier CR. Use of antihypertensives and the risk of Parkinson disease. *Neurology.* 2008; 70:1438–44. [PubMed: 18256367]
122. Chaturvedi RK, Beal MF. Mitochondrial approaches for neuroprotection. *Ann N Y Acad Sci.* 2008; 1147:395–412. [PubMed: 19076459]
123. Schmitz-Hubsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, Giunti P, Globas C, Infante J, Kang JS, Kremer B, Mariotti C, Melegh B, Pandolfo M, Rakowicz M, Ribai P, Rola R, Schols L, Szymanski S, van de Warrenburg BP, Durr A, Klockgether T, Fancellu R. Scale for the assessment and rating of ataxia: development of a new clinical scale. *Neurology.* 2006; 66:1717–20. [PubMed: 16769946]
124. Schmitz-Hubsch T, Fimmers R, Rakowicz M, Rola R, Zdzienicka E, Klockgether T. SCA Responsiveness of different rating instruments in spinocerebellar ataxia patients. *Neurology.* 2009 in press.

Fig 1. Domain structure of ataxin-2 and ataxin-3

Based on [48]

A. Ataxin-2 is a 1312 aa protein. The polyQ-expansion region (Q) is in the amino-terminal portion of the protein (aa 166-187). Two globular domains in the context of ataxin-2 are Lsm domain (Like Sm, aa 254-345) and LsmAD domain (Lsm-associated domain, aa 353-475) of unknown function. The rest of the protein is unstructured but contain PAM2 motif (aa 908-925) which binds to polyA-binding protein.

B. Ataxin – 3 is 376 aa protein. The polyQ-expansion region (Q) is in the carboxy-terminal portion of the protein (aa 296-317). Ataxin-3 consists of amino-terminal Josephin domain (aa 1 – 170) which has deubiquitinating activity. The rest of the protein is unstructured but contains 3 ubiquitin-interacting motifs (UIMs).

Fig 2. Ca^{2+} hypothesis of SCA2 and SCA3

Based on [58,83].

Pathological interactions between polyQ-expanded Atxn2 or Atxn3 (Atx^{exp}) and $\text{InsP}_3\text{R1}$ carboxy-terminal result in supranormal Ca^{2+} release from the endoplasmic reticulum (ER) in response to stimulation of mGluR receptors and InsP_3 generation. Resulting excessive Ca^{2+} release causes activation of calpains and promotes mitochondrial Ca^{2+} overload and mitochondrial permeability pore (MPTP) opening, induction of apoptosis and apoptotic cell death. Potential points of therapeutic intervention include partial inhibition of Ca^{2+} release from the ER by dantrolene, block of NMDAR-mediated Ca^{2+} influx by memantine (MMT), block of Ca^{2+} influx mediated by voltage-gated Ca^{2+} channels by dihydropyridines (DHP), inhibition of glutamate signaling by riluzole and stabilization of mitochondrial function by CoQ10 and creatine.