

# Precision medicine in spinocerebellar ataxias: treatment based on common mechanisms of disease

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**Abstract:** Spinocerebellar ataxias (SCAs) are a heterogeneous group of dominantly inherited neurodegenerative disorders affecting the cerebellum and its associated pathways. There are no available symptomatic or disease-modifying therapies available for any of the over 30 known causes of SCA. In order to develop precise treatments for SCAs, two strategies can be employed: (I) the use of gene-targeting strategies to silence disease-causing mutant protein expression, and (II) the identification and targeting of convergent mechanisms of disease across SCAs as a basis for treatment. Gene targeting strategies include RNA interference and antisense oligonucleotides designed to silence mutant genes in order to prevent mutant protein expression. These therapies can be precise, but delivery is difficult and many disease-causing mutations remain unknown. Emerging evidence suggests that several common disease mechanisms may exist across SCAs. Disrupted protein homeostasis, RNA toxicity, abnormal synaptic signaling, altered intracellular calcium handling, and altered Purkinje neuron membrane excitability are all disease mechanisms which are seen in multiple etiologies of SCA and could potentially be targeted for treatment. Clinical trials with drugs such as riluzole, a potassium channel activator, show promise for multiple SCAs and suggest that convergent disease mechanisms do exist and can be targeted. Precise treatment of SCAs may be best achieved through pharmacologic agents targeting specific disrupted pathways.

**Keywords:** Ataxia; calcium; neurodegeneration; neuronal excitability; protein misfolding; Purkinje neuron; RNA toxicity; spinocerebellar ataxias (SCAs)

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## Introduction

Spinocerebellar ataxias (SCAs) are a heterogeneous group of dominantly inherited neurological disorders associated with degeneration of neurons in the cerebellum and its associated pathways. Although SCAs are generally characterized by symptoms of cerebellar dysfunction, such as unsteady gait, uncoordinated limb movements, and slurred speech, individual SCAs have variable involvement of extra-cerebellar areas of the nervous system. There is also great variability in the age of onset and rate of disease progression in the individual SCAs. Additionally, symptoms vary even

in different individuals harboring the same disease-causing SCA mutation. This phenomenon can be most clearly seen in the polyglutamine ataxias (SCAs 1, 2, 3, 6, 7, and 17), diseases caused by an expanded CAG repeat sequence, which encodes glutamine, in the disease-causing gene (1). In these cases, individuals with similar repeat sizes can have significantly different ages of disease onset, rate of disease progression, and involvement of the cerebellum and other areas of the nervous system (2). This suggests that modifiers of disease exist and play a substantial role in disease onset and progression.

One obstacle to developing precise treatments for

SCAs is the diversity of causes of the condition. Mutations in more than 30 genes can result in SCA and, while the affected genes are known for many etiologies, the overlap in clinical symptoms makes accurate early diagnosis difficult (1). When broadened to include all forms of cerebellar ataxia, there are likely hundreds of disease-causing mutations. Despite the numerous causes for cerebellar ataxia, no effective symptomatic or disease-modifying therapies currently exist. While improvements in diagnostic strategies including whole exome sequencing (3,4) will increase the likelihood of identifying specific disease-causing mutations, the development of precise therapies for SCAs is challenging given the diverse genetic causes of SCA and variability in clinical features even in a defined genetic cause. Two approaches are possible for the development of these treatments: (I) the use of gene-targeting strategies for specific disease-causing mutations, and (II) the identification of convergent mechanisms of dysfunction as the basis for treatment. This review will highlight current progress on these two therapeutic approaches. The identification and targeting of common mechanisms of neuronal dysfunction is likely to be a more effective and realistic therapeutic strategy in the short term. We identify mechanisms of dysfunction including aberrant protein homeostatic pathways, RNA toxicity, altered Purkinje neuron membrane physiology, and intracellular calcium handling as potential pathogenic mechanisms that are shared across the different SCAs, and how precisely targeting these pathways may treat different etiologies of SCA.

### **Approach I: treating SCA through gene suppression strategies**

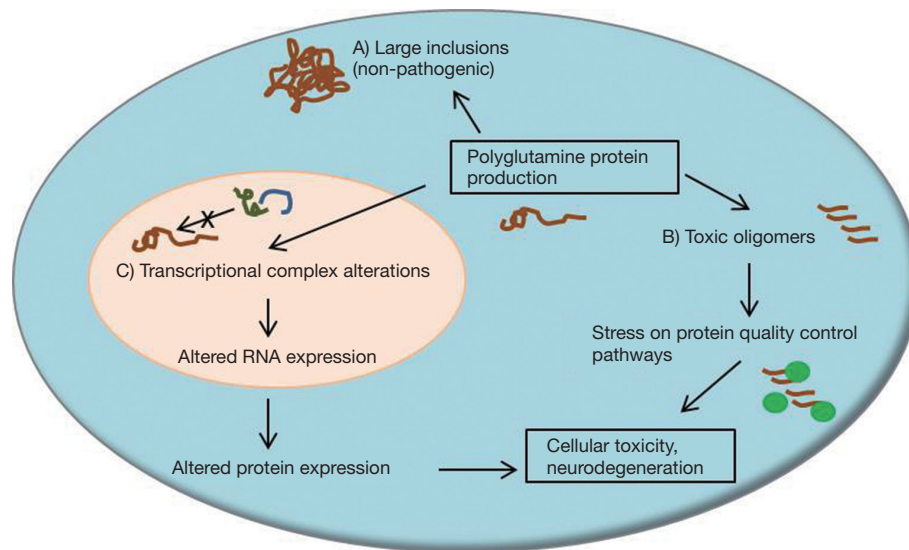
Many SCAs, including the polyglutamine ataxias, are caused by autosomal-dominant gain-of-function mutations. Gene suppression strategies, such as RNA interference and antisense oligonucleotides, are an attractive option for the treatment of gain-of-function mutations (5-7).

Strategies which target disease-causing alleles have been effective in multiple SCA models. Adeno-associated viral delivery of short hairpin RNAs (shRNAs) against the mutant human ataxin-1 gene improved Purkinje neuron morphology and motor function in a mouse model of SCA1 (8), while both AAV-mediated overexpression of wild-type ataxin-1 and miRNA against ataxin-1 have recently been shown to be effective (9). Furthermore, shRNA against mutant ataxin-1 has been effectively delivered to deep cerebellar nuclei in order to transduce

Purkinje neurons, resulting in improved cerebellar Purkinje neuron morphology and motor function (10). Recently, small interfering RNA (siRNA) designed to non-specifically silence both mutant and wild-type ataxin-7 was delivered in AAV2/1 shuttle vectors in a mouse model of SCA7. This strategy reduced nuclear inclusions and improved Purkinje neuron molecular layer thickness, demonstrating the potential efficacy of knocking down overall levels of ataxin-7 at improving the disease phenotype (11). Allele-specific silencing of mutant ataxin-7 was previously shown in patient derived fibroblasts (12). However, a similar strategy of injecting an AAV which encodes a microRNA-like molecule directed against mutant *ATXN3* did not improve motor function in SCA3 mice, although the treatment was effective at reducing mutant ataxin-3 protein levels (13). Interestingly, antisense oligonucleotides which are designed to promote skipping of exon 9 (the polyglutamine-containing exon) of the *ATXN3* gene can effectively limit the presence of this exon in control mice (14). Taken together, these studies indicate that both RNA interference-based and antisense oligonucleotide-based strategies to silence specific alleles may have relevance for the treatment of polyglutamine SCAs.

It may be possible to use RNA interference strategies to target multiple polyglutamine expansion disorders through a single construct. Antisense oligonucleotides which have been modified to specifically target expanded polyglutamine sequences can effectively reduce mRNA and protein expression of mutant huntingtin, mutant ataxin-1, and mutant ataxin-3 in patient-derived fibroblasts (15). This study corroborates prior work indicating that antisense oligonucleotides designed to target expanded CAG repeats are effective in specifically silencing mutant ataxin-3 and mutant huntingtin alleles in cultured cells (16). These studies indicate that expanded CAG repeat regions are potential targets for gene-suppression strategies in polyglutamine ataxias.

Although gene-targeting strategies are attractive for the precise treatment of SCAs which result from gain-of-function mutations, the development of targeted treatments for each and every etiology of SCA is not currently feasible. Over 30 known mutations result in SCA (1), and many other disease-causing mutations likely remain undiscovered. Gene-targeting strategies and, particularly, delivery options are likely not economically viable in the short term. Identifying common, treatable mechanisms of neuronal dysfunction which contribute to disease are likely to be more effective for the treatment of diverse causes of SCA.



**Figure 1** Altered protein homeostasis in SCAs. Translated polyglutamine proteins may contribute to neurodegeneration in SCAs. (A) The accumulation of large protein aggregates may be a consequence of expanded polyglutamine sequences. Recent evidence suggests that these aggregates are non-pathogenic and may be a protective cellular response. (B) Expanded polyglutamine proteins may form small oligomers in the cell. These oligomers may promote cellular toxicity by recruiting a large number of protein quality-control pathways, which prevents these pathways from functioning normally in the cell. (C) In some SCAs, polyglutamine-expanded proteins may no longer incorporate into transcriptional complexes as normal (e.g., SCA1, 7, and 17). As a result, gene transcription may be altered and may promote cellular toxicity leading to neurodegeneration. SCA, spinocerebellar ataxias.

## Approach II: search for convergent mechanisms of disease for the treatment of SCAs

### Altered protein homeostasis

In several of the SCAs, aberrant protein folding likely plays a significant role in disease pathogenesis. This phenomenon of misfolding is particularly relevant for the polyglutamine ataxias, since expanded glutamine repeat sequences are particularly prone to misfolding events and seem to have toxic effects in certain neuronal populations (17,18). Proteins harboring expanded glutamine repeat sequences form large neuronal inclusions and smaller oligomeric protein products found throughout the cell. Evidence suggests that large inclusions, once thought to be central to neuronal toxicity in polyglutamine diseases, may in fact be a normal byproduct of protein quality control mechanisms and are possibly representative of a protective cellular response (19-21). Alternatively, small polyglutamine protein oligomers, and the cellular processes which are altered upon their formation, may be a larger cause of neuronal toxicity in polyglutamine ataxia (17,22,23). These features of disease are outlined in *Figure 1*.

Molecular chaperone pathways play a large role in protein

homeostasis and are altered in several neurodegenerative protein misfolding diseases. These pathways include the heat shock proteins (Hsp), which are likely involved in the pathogenesis of neurodegenerative disorders (17,24). Molecular chaperone pathways seem to have relevance for the pathogenesis of multiple etiologies of SCA. SCA7 patient-derived lymphoblastoid cells show reduced expression of Hsp70 and Hsp27 with no change in Hsp90 levels (25), and SCA3 patient-derived fibroblasts display a reduction in Hsp40 levels which correlates with age-of-onset (26). In cell-based assays, expression of mutant PKC gamma, which results in SCA14, is associated with an increase in Hsp70, which may be a protective response to reduce elevated cell stress due to the presence of mutant protein oligomers. Knockdown of Hsp70 increased protein aggregation and decreased survival in this assay, suggesting that the Hsp70 pathway may be important for protein quality control in SCAs (27).

Since neurodegeneration improves upon Hsp70 activation or overexpression in some models of protein folding disease, Hsp70 activators may be therapeutic options for the treatment of human SCAs (28,29). Recently, Hsp70 levels have been shown to influence neuronal survival by

facilitating transcription of mesencephalic astrocyte-derived neurotrophic factor (MANF) in a knock-in mouse model of SCA17 (30). Hsp70 expression levels are progressively decreased in this model and correlate with the onset and severity of motor impairment and neurodegeneration. In cell-based assays, this was shown to be due in part to a decreased interaction of TATA-box-binding protein and XBP1s, a transcription factor, resulting in decreased MANF expression. Increasing Hsp70 levels improved the interaction between TATA-box-binding protein and XBP1s, which improved MANF transcription, and independently increasing MANF expression *in vivo* decreased neurodegeneration (30). Levels of several other chaperones, including Hsp27 and the co-chaperone sarsin, are reduced in other SCA animal models, further indicating that molecular chaperones are targets for pharmacologic intervention in SCA (31-33).

Overall, restoring balance to protein homeostatic pathways may have great benefits for the protein folding disorders such as the polyglutamine ataxias. One proposed treatment strategy for polyglutamine-induced neurodegeneration is the use of Hsp90 inhibitors to force a greater proportion of misfolded proteins to the proteasome for substrate degradation (34,35). Hsp70 activators may promote clearance and degradation of polyglutamine proteins (35). Additionally, Hsp27 induction was associated with gene transcription in cells transfected with mutant TATA-box-binding protein, the mutation which causes SCA17 (36). Hsp27 promoted transcription of the tyrosine kinase receptor TrkA, which is important for nerve growth factor (NGF) signaling and promotes neuronal survival. Therefore, Hsp27 activation may also have neuroprotective benefits in SCAs (36). Modulators of Hsp function, and other strategies which limit the stress placed on protein homeostatic pathways by polyglutamine expanded proteins, may have great benefits for the treatment polyglutamine ataxias and, potentially, other SCA etiologies which involve mutations which result in the accumulation of misfolded proteins.

### ***Transcriptional dysregulation***

Several SCAs are associated with altered gene transcription through the disruption of transcriptional complexes (*Figure 1*). For example, SCA17 is a result of a polyglutamine expansion in TATA-box-binding protein (TBP) (37,38). Disruption of normal TBP function reduces the interaction with the transcription factor TFIIB, thereby reducing the

expression of genes under normal TBP control (39,40). One such gene is TrkA, a tyrosine kinase receptor for NGF and undergoes reduced expression in the presence of mutant TBP (41). Similarly, SCA7 results from a polyglutamine expansion of the *ATXN7* gene, which encodes the ataxin-7 protein, a member of the STAGA complex which is a transcriptional regulator possessing both acetyltransferase and deubiquitinase activity. Although it is still unclear what the exact role polyglutamine expansion has on STAGA function, the complex may disassociate, become rendered inactive, or experience increased deubiquitinase or acetyltransferase activity (42). At least one mouse model has illustrated that polyglutamine expansion in the ataxin-7 protein has profound effects on gene transcription (43). Finally, evidence also suggests that SCA1 disease pathogenesis is at least partially due to altered protein complex formation, as ataxin-1 plays an important role in both an RNA binding complexes containing RBM17 or U2AF65 (44,45) and a transcriptional repression complex containing capicua (46,47).

Direct alterations in transcriptional complex formation can influence the transcription of downstream gene targets. There is evidence that altered histone deacetylase (HDAC) activity accompanies pathology in several polyglutamine SCAs. In a mouse model of SCA3, histones H3 and H4 were found to be hypo-acetylated, suggesting that HDAC overactivity may be present. Indeed, the HDAC inhibitor sodium butyrate improved histone acetylation and increased the expression of genes suppressed in SCA3 mouse cerebellum. Additionally, sodium butyrate improved motor function and survival in SCA3 mice (48). Ataxin-7 has been shown to interact directly with HDAC3 in transfected HEK293T cells, possibly promoting toxicity through altered deacetylation in a polyglutamine-dependent manner. Indeed, HDAC3 protein levels were increased in the cerebellum of SCA7 mice, suggesting that HDAC3 inhibition may be a therapeutic target (49). However, one recent study suggests that a cautious approach should be taken to HDAC inhibition as a therapy for SCAs. SCA1 mice were crossed with Purkinje-neuron specific HDAC3-null mice in order to determine whether HDAC inhibition may improve motor impairment. Surprisingly, while haploinsufficiency of HDAC3 did not improve disease progression, full knockout of HDAC3 worsened motor impairment and neurodegeneration in SCA1 mice (50). This suggests that HDAC3 function may be essential for normal Purkinje neuron function and therefore be a poor choice for therapeutic targeting.

Taken together, even though HDAC inhibitors may have therapeutic promise for the treatment of some forms of SCA, particularly those which display repressed gene transcription, further studies are needed to determine the specific classes of HDAC which contribute most significantly in ataxia and whether their inhibition will be a safe therapeutic option.

### **RNA toxicity**

While polyglutamine protein toxicity and disrupted protein homeostasis is thought to play a major causative role in the progression of SCAs, transcribed RNA products of expanded CAG repeat sequences may play an independent role in cellular toxicity. Structurally, repeat sequences within transcribed RNA regions, including CAG repeat sequences, tend to form hairpins of varying size and stability (51). These hairpin structures likely play specific roles in the cellular processing of RNA strands, such as recruiting certain RNA-binding proteins to influence translation or degradation, but the roles of polyglutamine repeat regions within RNA transcripts are not fully known. However, it is plausible that expanded polyglutamine repeat sequences can alter mRNA homeostasis in a manner similar to the disruption of protein homeostasis discussed previously. It has been proposed that expanded CAG repeat sequences can recruit high levels of RNA splicing factors and transcription factors, leading to an abnormal expression of rare splice variants of other mRNAs (52). The resultant altered gene expression may lead to neuronal dysfunction and influence neurodegeneration.

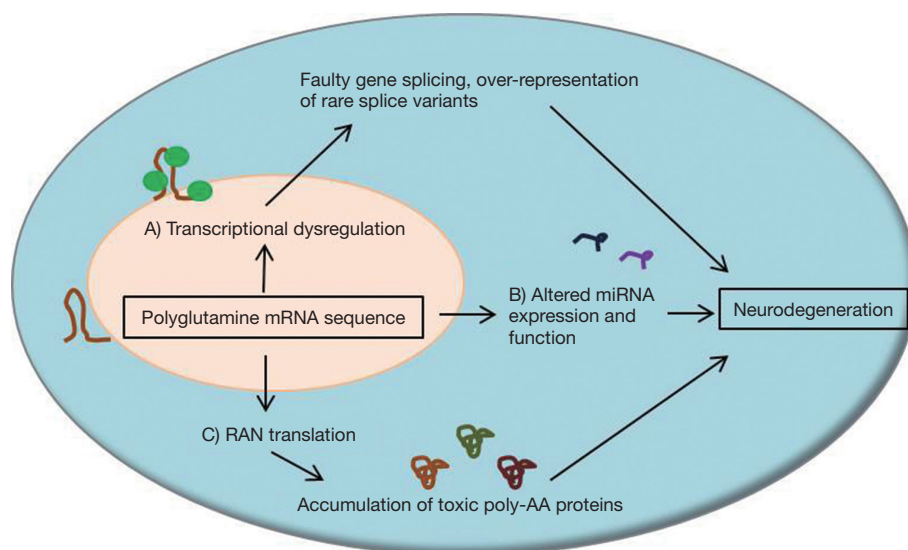
Disease models and human mutations support a toxic RNA hypothesis of neurodegeneration in SCAs. In a model of SCA3, transcribed polyglutamine RNA sequences were sufficient to induce neurodegeneration even in the absence of polyglutamine protein (53). In humans, SCA10 results from a large pentanucleotide sequence of AAUCU, localized to intron 9 of the *ATXN10* gene. Even though this expanded pentanucleotide repeat sequence is non-coding, RNA aggregates were seen in affected cells and were associated with toxicity (54). Large microsatellite repeat regions localized to introns of the *TK2* gene, which encodes thymidine kinase 2, or *BEAN* (brain expressed, associated with Nedd4) cause SCA31. Expanded pentanucleotide repeats of TGGAA are associated with SCA31 and, since they are localized to non-coding regions of DNA, may also alter splicing or result in toxic mRNA production by forming RNA aggregates in nuclei (55). This suggests

that RNA toxicity alone, even in the absence of translated protein, can result in neurodegeneration in SCAs.

A novel repeat-associated non-AUG (RAN) translation has recently been described for repeat containing RNA. In RAN translation, repeat-containing RNA strands are translated in multiple reading frames, thereby producing up to three different protein products when occurring within a polyglutamine repeat sequence (56,57). In SCA8, it has been reported that transcriptional initiation of the *ATXN8/ATXN8os* gene can occur in all three reading frames, resulting in not only poly-glutamine proteins but also poly-alanine and poly-serine proteins (56,57). These protein products occur even though the expanded polyglutamine sequence is located in a non-coding region of the *ATXN8/ATXN8os* gene (58), suggesting a toxic RNA gain-of-function mutation (59,60). Interestingly, all three of these transcribed protein products were shown to be present in the same cell, suggesting that these processes can be active simultaneously, and that these protein products are present in cerebella from both mouse models of SCA8 and human SCA8 patients (57). RAN products may play unknown roles in other SCAs, so they should be considered potential therapeutic targets.

Recently, altered microRNA (miRNA) function has been noted in multiple models of SCA. Aberrant miRNA expression has been shown to influence disease throughout the body, including the brain (61). Knockout of *Dicer1*, a gene which is necessary for the normal production and expression of miRNAs, causes neurodegeneration in mouse cerebellum (62,63). Several miRNA variants are likely important in neurological disease. miR-19, miR-101, and miR-130 have been shown to regulate *ATXN1* levels and likely influence disease in a mouse model of SCA1 (64). A recent study found that aberrant cross-regulation between miR-124 and long non-coding RNA associated with the *ATXN7* gene may directly impact gene transcription and neurodegeneration in both cerebellum and retina of SCA7 mice (65). Additionally, the ataxin-2 protein is involved in miRNA pathways in a *Drosophila* model (66). Future studies will need to determine the prevalence of miRNA dysfunction in other SCA etiologies in order to consider their potential as therapeutic targets.

Overall, RNA toxicity likely plays a role in the disease progression of SCAs and may directly influence neuronal dysfunction and neurodegeneration (*Figure 2*). Therapies which limit splicing factor and transcription factor recruitment to expanded CAG repeat sequences, along with agents which limit RAN translation or aberrant miRNA



**Figure 2** RNA toxicity in SCAs. Transcribed polyglutamine RNA sequences may have direct effects on cellular toxicity in SCAs. (A) Transcriptional dysregulation, which includes the faulty recruitment of transcriptional machinery and splicing factors to expanded glutamate repeat structures, may result in altered splicing of other genes and induce differential expression of rare splice variants. Altered gene expression may then result in cellular stress and neurodegeneration. (B) Both altered miRNA expression and impaired regulation between miRNAs and polyglutamine-expanded RNA sequences may lead to neurodegeneration. The mechanisms which lead to neurodegeneration are not yet well understood, but may include aberrant transcriptional control and abnormal protein expression. (C) RAN translation of non-coding RNA sequences can result in the production of poly-amino acid proteins made up of glutamine, alanine, and serine. These proteins may directly contribute to neuronal toxicity or may influence cellular function in an unknown manner, leading to neurodegeneration. SCA, spinocerebellar ataxias.

expression, may have a role in the treatment of SCAs.

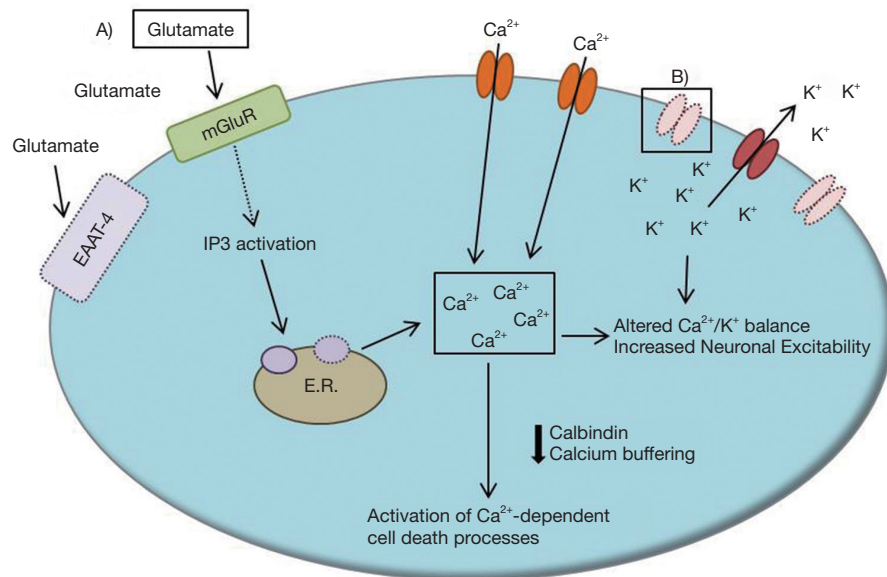
### ***Aberrant intracellular calcium handling***

Calcium homeostasis is central to proper neuronal function. As a mediator of many cellular processes, cytosolic calcium levels are tightly regulated in neurons by a number of calcium buffers and transporters. For example, the calcium buffers parvalbumin and calbindin are particularly enriched in Purkinje neurons, perhaps to account for the large levels of calcium influx associated with spontaneous, repetitive action potential firing and increased metabolic load in these neurons. Neuronal calcium transients can come from a variety of sources, including intracellular calcium release stores upon synaptic metabotropic glutamate receptor (mGluR) activation and through voltage-gated calcium channels throughout the Purkinje neuron membrane during cycles of depolarization.

Alterations in calcium entry and intracellular calcium regulation are known causes of human ataxia. *ITPR1* mutations cause SCA15 and likely contribute to imprecise

synaptic signaling (67,68). The mutation in *ITPR1*, the inositol 1,4,5-trisphosphate receptor, may result in neurodegeneration by preventing the normal intracellular calcium release from occurring upon excitatory synaptic input (67). Additionally, SCA14 results from a mutation in protein kinase C gamma, which has been proposed to lead to alterations in intracellular calcium homeostasis and impaired Purkinje neuron responses to synaptic input (69,70). Finally, *CACNA1A* mutations result in both SCA6 and episodic ataxia type 2, suggesting that improper calcium entry through voltage-gated calcium channels can influence Purkinje neuron function and induce neurodegeneration (71,72).

Altered Purkinje neuron calcium handling is a prominent feature in animal models of SCA (73). In a mouse model of SCA1, the intracellular calcium-release channels *ITPR1* and *SERCA3* are expressed at lower levels in the cerebellum at the onset of motor impairment, a feature which was also noted in human patient tissue samples (74). The down-regulation of these channels may be in response to an impaired ability of the Purkinje neuron



**Figure 3** Altered membrane excitability and calcium homeostasis in SCAs. (A) Binding of glutamate to mGluR results in downstream activation of IP3. Reduced expression of glutamate transporters such as EAAT4, denoted by a dashed line, may result in prolonged excitatory synaptic transmission and glutamate toxicity. Prolonged mGluR activation may also over-activate IP3, leading to larger intracellular calcium release events from stores such as the endoplasmic reticulum. IP3 receptor expression may be reduced in response to over-activation of IP3 and subsequent elevated intracellular calcium concentrations. (B) Reduced expression or function of membrane potassium channels can directly lead to increased neuronal excitability and altered neuronal firing properties. This results in increased calcium entry through voltage-gated calcium channels, also contributing to increased intracellular calcium levels. Elevated intracellular calcium concentrations, along with a reduced ability of the neuron to buffer intracellular calcium, may cause neurodegeneration through the activation of calcium-dependent cell death processes. SCA, spinocerebellar ataxias; mGluR, metabotropic glutamate receptor; EAAT-4, excitatory amino acid transporter type 4; ER, endoplasmic reticulum. IP3, inositol 1,4,5-trisphosphate.

to control intracellular calcium levels, since calbindin D-28K is also reduced in SCA1 mouse cerebellum (75). More evidence for this hypothesis comes from a recent study which showed that suppression of calbindin D-28K worsens motor performance in SCA1 mice, indicating that impaired calcium buffering capacity may be an important mechanism of neuronal dysfunction in Purkinje neurons (76). Additionally, knockout of the acid-sensing ion channel isoform 1a (ASIC1a), a channel which passes inward calcium current upon activation, also improves motor performance in SCA1 mice and improves dendritic morphology, calbindin staining intensity, and parvalbumin staining intensity (77). Together, these studies indicate that SCA1 Purkinje neurons are particularly sensitive to perturbations in intracellular calcium levels, and that intracellular calcium handling capacity is likely impaired due to altered expression of calbindin D-28K protein. Reduced calbindin levels have also been noted in mouse models of SCA2 and SCA7, suggesting that this may be a

common feature of SCAs (33,78).

In a series of studies, polyglutamine-expanded ataxin-2 and polyglutamine-expanded ataxin-3 proteins have been shown to interact directly with the inositol 1,4,5-trisphosphate receptor, thereby increasing sensitivity to inositol 1,4,5-trisphosphate and facilitating intracellular calcium release (79,80). In SCA2 mice, suppression of intracellular calcium release was achieved by expression of inositol 1,4,5-phosphatase, an enzyme which reduces the levels of intracellular inositol 1,4,5-trisphosphate. This strategy improved motor performance and, surprisingly, improved Purkinje neuron firing properties (81).

Taken together, it is likely that aberrant intracellular calcium handling is present in multiple SCAs (Figure 3). Treatments which increase expression of calcium binding proteins, such as calbindin, or inhibit intracellular calcium release stores may be one strategy to improve neuronal health and limit calcium-mediated neuronal toxicity in SCAs.

### *Synaptic alterations*

Purkinje neurons undergo plasticity at the parallel fiber synapse in response to excitatory synaptic input. Classically, long-term depression (LTD) at the parallel fiber synapse has been associated with cerebellar learning. In particular, calcium entry upon mGluR activation and TRPC3 channel opening, along with subsequent activation of inositol 1,4,5-trisphosphate, allows LTD to occur at the parallel fiber synapse (82), while glutamate re-uptake at the Purkinje neuron presynaptic cleft is extremely important for precise signaling and is controlled by the glutamate transporters EAAT-4, GLT-1, and GLAST (83). Altered glutamatergic input strength and precision could have profound effects on Purkinje neuron physiology and contribute to the generation of ataxia. For example, SCA5 is caused by a mutation in the *SPTBN2* gene, which encodes the beta-III spectrin protein (84). Beta-III spectrin stabilizes EAAT-4 at the Purkinje neuron membrane, indicating that loss-of-function mutations in SCA5 likely contribute to excess synaptic glutamate load and subsequent postsynaptic glutamate toxicity.

In mouse models of SCA1, several synaptic proteins show reduced expression by mid-stage disease and likely play a role in altered synaptic function. Through comparative microarrays, one study identified transcriptional changes in the presence of mutant ataxin-1 protein. Results from this analysis point to synaptic dysregulation as a likely source of altered Purkinje neuron physiology (75). In addition to reduced expression of the calcium-binding proteins mentioned previously, EAAT-4 reduction is seen in SCA1 mouse cerebellum early in disease (74,75). While synaptic calcium transients were shown to be unaffected in SCA1 mice upon glutamate stimulation, secondary intracellular calcium release was prolonged (85). This suggests that an interaction between synaptic calcium entry and altered intracellular calcium handling may be present and important for cellular excitability in SCA1 mice.

Glutamate toxicity has been proposed to play a role in several other mouse models of SCA. Non-cell autonomous Purkinje neuron degeneration has been demonstrated in multiple models of SCA7 (86,87). Impaired Bergmann glial glutamate transport appears to play a role in disease progression in at least one of these models, suggesting that synaptic dysregulation contributes to Purkinje neuron dysfunction in SCA7 (86). Additionally, application of the mGluR1/5 agonist DHPG induces larger intracellular calcium transients in both cultured rat medium spiny

neurons transfected with polyglutamine ataxin 3 and cultured Purkinje neurons from SCA2 mice (79,80). Direct glutamate application was also shown to cause increased neuronal death in cultured SCA2 Purkinje neurons, suggesting that synaptic glutamate toxicity may be a source of calcium-induced neuronal death in SCAs (79). Furthermore, inhibiting downstream calcium release with the IP<sub>3</sub> receptor inhibitor dantrolene improved motor performance in both SCA2 and SCA3 mice, and molecular layer thickness in SCA2 mice (79,80). This suggests that synaptic dysregulation and intracellular calcium levels are likely closely tied in SCAs.

Overall, excess synaptic glutamate load, combined with a reduced ability of the Purkinje neuron to buffer intracellular calcium, increases neuronal excitability and likely influences excitotoxic cell death in multiple SCAs (*Figure 3*). Drugs which improve glutamate uptake or reduce intracellular calcium release will likely improve synaptic physiology in these SCAs. Dantrolene and glutamate transport activators may have therapeutic potential in this capacity.

### *Altered Purkinje neuron membrane excitability*

Altered neuronal physiology within the olivo-cerebellar circuit can result in ataxia (88). In humans, mutations in the *KCNC3* gene lead to Purkinje neuron degeneration in SCA13 through a loss in voltage-gated potassium channel function and, presumably, neuronal hyperexcitability (89,90). SCA19/22 is a result of a mutation in the *KCND3* gene, which encodes for the voltage-gated potassium channel K<sub>v</sub>4.3 (91,92). SCA6 and episodic ataxia type 2 both result from mutations in *CACNA1A*, which likely influences both neuronal calcium entry and firing properties (71,72).

Mouse models have outlined how ion channel dysfunction, either through mutations or altered expression levels, are sufficient to induce ataxia through disrupted Purkinje neuron firing. In mouse models of episodic ataxia type 2, irregular Purkinje neuron pacemaking results from mutations in the *CACNA1A* gene, which encodes a P/Q-type voltage-gated calcium channel, and results in motor impairment (93-96). Activators of small-conductance calcium-activated potassium (SK) channels, 1-EBIO and chlorzoxazone, both improve the regularity of Purkinje neuron firing and improve motor impairment in these mice (93,97). These studies highlight the importance of regular Purkinje neuron pacemaking for motor function, and that correcting Purkinje neuron electrophysiologic dysfunction can have beneficial effects on motor performance.



In several mouse models of SCA, Purkinje neuron electrophysiologic dysfunction correlates with the onset of motor impairment and early stages of neurodegeneration. The findings from these studies suggest that electrophysiologic dysfunction may contribute to pathogenesis early in disease, and that correcting this dysfunction may be beneficial for prolonging normal motor function and slowing neurodegeneration. In a mouse model of SCA1, progressively reduced Purkinje neuron firing frequency was noted alongside an increased density of surface Kv4.3 channel expression. Use of 4-aminopyridine (4-AP), a compound which blocks voltage-gated potassium channels, increased simple spike firing frequency and improved motor performance, suggesting that the SCA1 phenotype may be partially explained by a reduced Purkinje neuron firing rate (98). This result agrees with a previous study, which illustrates that Purkinje neurons from SCA1 mice have a delayed onset to the initial spike in a depolarizing current injection protocol, and this effect is suppressed by either 4-AP administration or a hyperpolarizing prepulse (85).

Recently, in the same mouse model of SCA1, altered Purkinje neuron membrane properties were shown to result from a reduction in expression and function of two potassium channels, the large-conductance calcium-activated potassium (BK) and G-protein coupled inwardly-rectifying potassium type 1 (GIRK1) channels, which resulted in membrane depolarization. BK and GIRK1 channels showed a progressive reduction over the course of disease, and increasing BK channel expression through an adeno-associated virus improved motor performance and reduced dendritic degeneration (99). These findings suggest that altered Purkinje neuron excitability in SCA1 mice can be attributed to an imbalance of depolarizing and hyperpolarizing currents due to altered potassium channel function.

Altered membrane physiology has been noted in several other models of SCA. In SCA6 mice, polyglutamine expansion of the *CACNA1A* gene results in decreased current density of P/Q-type calcium channels in dissociated Purkinje neurons (100). Additionally, in mice lacking beta-III spectrin, the gene which results in SCA5 in humans, Purkinje neurons have a reduced firing rate which may be due in part to a reduction in voltage-gated sodium channel current (101). These findings indicate that a subset of SCAs may contain mutations which decrease Purkinje neuron excitability. It will be important to determine how neuronal excitability is altered in different etiologies of SCA, in order to develop pharmacologic strategies which target altered neuronal firing.

SK channel activators are particularly intriguing candidate compounds, as activating SK channels has been shown to restore membrane physiology and reduce neurodegeneration in several animal models of SCA. In a model of SCA2, a progressive reduction in firing frequency was noted in parallel with increased severity of neurodegeneration and increasing levels of motor impairment (78). No alterations in ion channel expression were found to accompany this change in physiology, but an exhaustive analysis of ion channels was not performed. In another SCA2 mouse model, treatment with the SK channel activators CyPPA, NS309, and NS13001 improved Purkinje neuron firing regularity and improved motor performance (102). SK channel activating compounds were also shown to be effective in a mouse model of SCA3, where alterations in voltage-gated potassium channel activation and deactivation contribute to altered Purkinje neuron firing patterns and increased neuronal excitability (103). Finally, SK channel activators also improve pacemaking regularity and normalize neuronal excitability in mouse models of ataxia due to *CACNA1A* mutations, suggesting that compounds which activate SK channels may have profound effects on improving Purkinje neuron function in SCA etiologies which involve altered Purkinje neuron pacemaking (97,104).

Among the compounds likely to effectively treat multiple etiologies of SCA, SK channel activators seem to have particular promise. Recently, a clinical trial for the drug riluzole was shown to be effective for the symptomatic treatment of several etiologies of autosomal dominant SCA and Friedrich's ataxia (105,106). The trial suggests that common mechanisms of dysfunction may exist across different etiologies of human SCA. Although the mechanism of action of riluzole in this study is unclear, among its other actions, riluzole is an SK channel activator (107). Agents which have greater specificity for SK channels than riluzole have been developed and may be more effective for the treatment of SCAs (108).

Together, these studies suggest that altered Purkinje neuron excitability, possibly due to altered potassium and calcium channel function, may be a common mechanism of neuronal dysfunction in the SCAs (Figure 3). Potassium channel modulators, and particularly SK channel activators, appear to be good candidates for treating neuronal dysfunction across different causes of SCA.

### Strategy for intervention

SCAs are relentlessly progressive, debilitating diseases. In

order to design treatment strategies which are optimally effective for SCAs, it will be necessary to improve diagnosis during presymptomatic stages. Presymptomatic diagnosis will maximize the benefit of treatments by allowing early treatments to slow or halt disease progression before extensive neurodegeneration has occurred. This will improve the possibility that connectivity between neuronal populations in the olivo-cerebellar circuit can be preserved, thereby helping SCA patients maintain motor function. Trials like RISCA, which is focused on the identification and proper diagnosis of cerebellar ataxia in presymptomatic cerebellar ataxia patients, are essential for the treatment of these diseases (109). Additionally, presymptomatic diagnoses will improve the ability of clinical trials to determine the efficacy of therapeutics by offering the earliest possible stage of intervention.

## Conclusions

While many ataxia-causing mutations are currently known, there are many others which remain unidentified. Targeting convergent mechanisms of neuronal dysfunction in ataxia appears to be the most effective therapeutic intervention in the near future. Toxic protein oligomers can potentially be reduced through the activation of Hsp family members, while reducing RNA aggregation and transcriptional dysregulation may also have beneficial effects on disease progression. However, compounds which effectively target these pathways are not well characterized or remain unknown. Alternatively, compounds which limit Purkinje neuron excitability and reduce intracellular calcium release have proven beneficial in multiple models of SCA. These compounds, such as SK channel activating compounds and dantrolene, could improve Purkinje neuron function by normalizing pacemaker firing and could reduce the activation of calcium-dependent cell death processes leading to neurodegeneration. The success of riluzole in clinical trials indicates that SK channel activation may have particular benefits for the treatment of multiple etiologies of SCA.

In the long term, gene targeting strategies may be best for the specific targeting of dominant mutations which cause cerebellar ataxias. However, gene delivery options are currently cumbersome and are associated with significant risk. With the wide range of disease-causing mutations in SCAs, common mechanisms of Purkinje neuron dysfunction are the most attractive target for widespread treatment of SCAs. More studies are necessary to determine

whether shared mechanisms of disease truly exist across all ataxias, and whether the selective vulnerability of cerebellar neurons may be due to their unique metabolic and electrophysiologic properties. Targeting aberrant neuronal physiology, faulty protein and mRNA homeostasis, and calcium dysregulation is the most accessible form of treatment available for cerebellar ataxia patients in the near future, and efforts should be taken to design and test widely applicable therapies for SCA which are designed to target these features of disease.

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## Footnote

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