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Role of Inositol 1,4,5-Trishosphate Receptors in Pathogenesis of Huntington's Disease and Spinocerebellar Ataxias

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Abstract

Huntington's disease (HD) and spinocerebellar ataxias (SCAs) are autosomal-dominant neurodegenerative disorders. HD is caused by polyglutamine (polyQ) expansion in the amino-terminal region of a protein huntingtin (Htt) and primarily affects medium spiny striatal neurons (MSN). Many SCAs are caused by polyQ-expansion in ataxin proteins and primarily affect cerebellar Purkinje cells. The reasons for neuronal dysfunction and death in HD and SCAs remain poorly understood and no cure is available for the patients. Our laboratory discovered that mutant huntingtin, ataxin-2 and ataxin-3 proteins specifically bind to the carboxy-terminal region of the type 1 inositol 1,4,5-trisphosphate receptor (IP₃R1), an intracellular Ca²⁺ release channel. Moreover, we found that association of mutant huntingtin or ataxins with IP₃R1 causes sensitization of IP₃R1 to activation by IP₃ in planar lipid bilayers and in neuronal cells. These results suggested that deranged neuronal Ca²⁺ signaling might play an important role in pathogenesis of HD, SCA2 and SCA3. In support of this idea, we demonstrated a connection between abnormal Ca²⁺ signaling and neuronal cell death in experiments with HD, SCA2 and SCA3 transgenic mouse models. Additional data in the literature indicate that abnormal neuronal Ca²⁺ signaling may also play an important role in pathogenesis of SCA1, SCA5, SCA6, SCA14 and SCA15/16. Based on these results I propose that IP₃R and other Ca²⁺ signaling proteins should be considered as potential therapeutic targets for treatment of HD and SCAs.

Keywords

Calcium signaling; Huntingtin; Neurodegeneration; Polyglutamine expansion; Inositol 1,4,,5-trisphosphate; Ataxin-2; Ataxin-3; Spinocerebellar ataxias; Transgenic mice; NMDA receptor; Apoptosis; Mitochondria; Memantine

Huntington Disease (HD) and Spinocerebellar Ataxias (SCA)

Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder with the age of onset between 35 and 50 years and inexorable progression to death 15–20 years after onset. The symptoms include motor abnormalities including chorea and psychiatric disturbance with gradual dementia [1]. Neuropathological analysis reveals selective and progressive neuronal loss in the striatum (caudate nucleus, putamen and globus pallidus) [1,2]. GABAergic medium spiny striatal neurons (MSN) are the most sensitive to neuronal degeneration in HD [1,2]. Positional cloning efforts demonstrated that at the molecular level the cause of HD is polyglutamine (polyQ) expansion in the amino-terminal of a 350 kDa

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evolutionary conserved cytosolic protein called huntingtin (Htt) [3]. Clinical signs of HD develop if the length of polyQ track in Htt exceeds a pathological threshold of 35Q. The CAG repeat length is inversely correlated with age of onset [4]. Htt is widely expressed in the brain and in non-neuronal tissues and not particularly enriched in the striatum [5–7]. Htt plays an essential function in development, as deletion of the Htt gene in mice is embryonic lethal [8,9]. Analysis of Htt primary sequence suggests that Htt is likely to function as a signaling scaffold [10], but the precise function of Htt in cells is not known. In order to elucidate the pathogenesis of HD, a number of transgenic HD mouse models have been generated [11]. Early symptoms of HD appear to be related to corticostriatal network dysfunction [12–14]. At the early stage HD symptoms in many patients can be controlled by dopamine modulators, such as tetrabenazine, but eventually neuronal loss becomes too severe for effective symptomatic treatments [12,14].

Spinocerebellar ataxias (SCAs) constitute a heterogeneous group of autosomal-dominant genetic and neurodegenerative disorders. SCAs are generally characterized by cerebellar atrophy and a progressive uncoordination of movement known as ataxia [15–17]. A total of 29 SCAs have been identified and named in the chronological order of their discovery from SCA1 to SCA30. SCA15 and SCA16 are allelic [18,19]. Although all SCA patients present with the phenotypic overlap of cerebellar atrophy and ataxia, other brain regions are differentially affected in each SCA. Seventeen genes have been associated with these SCAs and it is not understood how mutations in those SCA-associated genes lead to the SCA pathogenesis [20]. A significant fraction of SCAs, but not all SCAs, is caused by polyglutamine (polyQ) expansion mutations. The pathogenesis of SCAs is not fully understood, however, several different pathogenic mechanisms have been studied in SCAs such as dysregulation of transcription and gene expression, alterations in calcium homeostasis and synaptic neurotransmission, mitochondrial stress and apoptosis (reviewed in [21–24]). Currently, therapy for SCA patients is mainly supportive and directed at treating individual symptoms in each patient using methods such as deep brain stimulation and levodopa supplements [23–25]. No disease modifying therapy exists for any of the SCAs. In order to develop a successful treatment of SCAs, it will be important that a valid therapeutic target and the pathogenic pathways are identified.

Here I discuss recently emerging results that support the concept that excessive activity of inositol (1,4,5)-trisphosphate receptors and abnormal Ca^{2+} signaling is playing a major role in pathogenesis of HD and SCAs.

Mutant Huntingtin Specifically Sensitizes $\text{IP}_3\text{R1}$ to IP_3

The inositol (1,4,5)-trisphosphate receptor (IP_3R) is an intracellular calcium (Ca^{2+}) release channel that plays an important role in neuronal Ca^{2+} signaling [26]. Three isoforms of IP_3R have been identified [27]. The type 1 receptor ($\text{IP}_3\text{R1}$) is the predominant neuronal isoform. Mice lacking $\text{IP}_3\text{R1}$ display severe ataxic behavior [28], and mice with a spontaneous mutation in the *IP3R1* gene experience convulsions and ataxia [29], suggesting a major role of $\text{IP}_3\text{R1}$ in neuronal function. Our laboratory has been heavily involved in elucidating the interaction between mHtt and $\text{IP}_3\text{R1}$, as well as other Ca^{2+} -related mechanisms relevant to HD (for reviews see [14,30,31]). We first discovered that mHtt binds directly and specifically to the C-terminal region of the type 1 IP_3 receptor ($\text{IP}_3\text{R1}$) [32]. Recently, unbiased high-throughput screening assays confirmed mHtt binding to $\text{IP}_3\text{R1}$ [33]. Interestingly the affinity of mHtt to $\text{IP}_3\text{R1}$ increases when mHtt is associated with HAP1a [32]. Moreover, mHtt, but not normal Htt, augmented $\text{IP}_3\text{R1}$ activity in planar lipid bilayers [32]. Similarly, application of subthreshold concentrations of DHPG, an mGluR1/5 agonist, sensitized Ca^{2+} release in YAC128 primary MSN cultures [32]. This is consistent with the fact that glutamate-induced apoptosis of MSNs from YAC128 mice is mediated by

mGluR1/5 and NR2B receptors [34]. In fact, specific blockade of IP₃R1 with 2-APB and Enoxaparin is neuroprotective in the same model [34]. In a follow-up study, we found disturbed Ca²⁺ signaling, enhanced glutamate-induced apoptosis, and augmented NR2B activity in cultured MSNs from YAC128 mice, which express full-length human Htt, but these effects were completely absent in shortstop mice that express an amino-terminal fragment of mHtt (exons 1 and 2) and does not show HD pathology [35]. In more recent experiments, it was demonstrated that viral delivery of a peptide that disrupts mHtt association with IP₃R1 protected YAC128 MSNs in vitro and in vivo [36]. Augmented IP₃R1 activity further implicates mGluR5 receptor signaling in HD pathology. Importantly, inhibitors of IP₃R1 may impede intracellular Ca²⁺ overload early in the disease state and protect MSNs from glutamate-induced excitotoxicity. MSNs are highly enriched for mGluR5, a member of the group I mGluRs [37–41]. Stimulation of group I mGluR in MSNs leads to the generation of IP₃ and release of Ca²⁺. The alterations in ER enzymes that have been observed in HD postmortem brains [42] are consistent with malfunction of ER Ca²⁺ handling in HD MSN neurons. Interestingly, recent findings from Dr Mikoshiba's laboratory directly implicated ER stress in HD pathogenesis. It was discovered in these studies that InsP₃R1 association of ER stress chaperone protein GRP78 is impaired in Huntington's disease R6/2 model mice, resulting in misregulation of InsP₃R1 gating [43].

Mutant Huntingtin Activates NR2B-Containing NMDA Receptors

MSNs abundantly express NR2B subtype of NMDA receptors [44–46]. In contrast to NMDA receptors containing NR2A subtype, NR2B-containing NMDA receptors have significant permeability for Ca²⁺ and activation of these receptors may have a dramatic effect on intracellular Ca²⁺ signals in MSNs. Importantly, studies from Lynn Raymond's and Michael Hayden's laboratories suggested that expression of mutant Htt^{exp} protein facilitates activity of NR2B subtype of NMDAR receptors in a heterologous HEK293 cells expression system [47]. Interestingly, the potentiating effect of Htt^{exp} was specific for the NR1/NR2B NMDAR subtype and not for the NR1/NR2A NMDAR subtype. Using the same HEK293 cells expression system it was also demonstrated that cells co-transfected with NMDAR and Htt-138Q plasmids were more sensitive to NMDA-induced apoptosis than the cells co-transfected with NMDAR and Htt-15Q or GFP (control) plasmids [48]. Similar to effects on NMDAR currents, potentiating effects of Htt-138Q on excitotoxic cell death were more pronounced in the presence of the NR1/NR2B NMDAR subunit combination than in the presence of the NR1/NR2A subunit combination [48]. These initial findings were corroborated in the YAC mouse, whereby facilitation of NMDA-evoked current amplitude in MSNs from YAC72 and YAC128 mice compared to WT was induced by the NR1/NR2B receptor [35,49]. Similarly, cultured MSNs from YAC72 mice showed enhanced NMDA-mediated cell death relative to WTs. Furthermore, treatment with ifenprodil, an NR2B-type selective antagonist, blocked most NMDA-mediated cell death in both YAC72 and WT mice [49]. The neuroprotective effect of ifenprodil was later recapitulated in YAC128 MSN cultures [34]. Findings from dissociated MSNs from presymptomatic R6/2 mice have revealed that NR2A, and not NR1 or NR2B, mRNA levels are reduced, which is concomitant with larger NR2B-mediated NMDAR currents compared to WTs [50].

The studies that indicated NR2A-type NMDARs are targeted primarily to the synapse and NR2B-type receptors are preferentially expressed at extrasynaptic sites (outside the synapse) are intriguing [51–53]. In fact, dual roles for synaptic and extrasynaptic NMDARs have been proposed (for details see [54,55]). Whereas synaptic NMDARs activate pro-survival pathways (e.g., CREB phosphorylation, BDNF transcription, ERK activation, antioxidant mechanisms), extrasynaptic NMDARs activate cell death pathways (e.g., loss of mitochondrial membrane potential, inhibition of ERK and BDNF, dendritic blebbing) [56–58]. A recent study by Milnerwood and colleagues has shown that, indeed, mHtt results in

increased extrasynaptic NMDAR expression and currents [59] (also see [60]). Examination of extrasynaptic activation, by mimicking glutamate spillover into the synaptic cleft with either strong evoked excitatory postsynaptic currents (EPSCs) or paired-pulse stimuli, revealed increased EPSC charge and elevated NMDA peak current and charge in presymptomatic (1 month) YAC128 MSNs relative to YAC18 [59]. Accordingly, application of TBOA, a GLT1 transport inhibitor that enhances glutamate spillover to extrasynaptic receptors, resulted in slowing of NMDAR EPSC kinetics in WT and YAC18 mice, but the slowing was consistently more augmented in YAC72 and YAC128. Moreover, in the presence of TBOA, NMDA currents and peak charge were larger in YAC128 compared to WT and YAC18, while YAC72 exhibited intermediate values. Altered TBOA-induced NMDA currents in YAC128 mice were ameliorated upon application of ifenprodil, which blocks NR2B extrasynaptic signaling. Spontaneous NMDA activity was not altered by mHtt, further suggesting involvement of NR2B-type and not NR2A-type NMDARs in altered MSN function. Surprisingly, treatment with low dose memantine, which is thought to preferentially antagonize extrasynaptic over synaptic NMDARs, in 2–4 month YAC128 mice resulted in increased CREB activation and improved motor learning (number of falls when learning the rotarod) relative to WT [59]. Direct in vivo evidence for NR2B-mediated excitotoxicity in HD comes from recent findings demonstrating exacerbated striatal neurodegeneration in double-mutant mice that express 150Q and overexpress NR2B subunits [61].

How does mHtt augment NR2B activity? Numerous studies have investigated mRNA and protein expression of NMDARs in human postmortem brain and in HD mouse models, yet no conclusive correlation exists between CAG repeat number and receptor expression levels, nor receptor sensitivity (for discussion see [62]). Some studies suggested that Htt^{exp} affects NMDAR function via PSD95-dependent mechanism [63,64]. Indeed, treatment of YAC72 and YAC128 MSN cultures with the Tat-NR2B9C peptide (200 nM), which blocks binding of NR2B with PSD95 and reduces surface expression of NMDARs, resulted in a marked reduction in NMDA-induced apoptosis [65]. Knockdown of PSD95 with siRNA resulted in the same effect in YAC128 MSNs [65]. Another possibility is related to changes in NMDAR trafficking to the plasma membrane in the presence of Htt^{exp} mutation [66]. Future experiments will be needed to clarify exact molecular mechanisms responsible for increase in NMDAR activity in HD MSNs.

From above discussion is clear that hyperfunction of extrasynaptic NMDARs (NR2B) is a major problem in HD and these receptors warrant targeting. The NR2B-specific inhibitors (e.g., ifenprodil, memantine) are likely candidates. Indeed, low dose memantine is neuroprotective in vitro [67,68] and in vivo [59,67]. Memantine demonstrated some beneficial effects in small scale pilot evaluation in HD patients [69] and large phase IV clinical trial of memantine in HD patients is currently in progress. Furthermore, a continued search for compounds that positively regulate synaptic NMDARs (NR2A) and/or negatively regulate extrasynaptic NMDARs (NR2B) is warranted.

Deranged Ca²⁺ Signaling and Apoptosis of HD MSN

Several lines of evidence indicate that glutamate-mediated excitotoxicity plays a role in neurodegeneration of HD MSN. Striatal injection of kainic acid induced death of MSN and yielded one of the first animal models of HD [70,71]. Importantly, effects of kainate required presence of corticostriatal neurons [72], suggesting that glutamate release is required for kainate-induced MSN cell death. More direct evidence for an involvement of NMDAR was obtained when HD-like lesions were observed following striatal injection of the NMDAR agonist quinolinic acid [73–75]. Consistent with the excitotoxicity hypothesis, striatal neurons from YAC72 mouse were sensitized to neuronal death induced by quinolinic

acid and NMDA [49]. Moreover, excitotoxic cell death of YAC72 MSN was blocked by ifenprodil [49], supporting a direct involvement of NR1/NR2B NMDAR subtypes in HD.

Based on the results described above (Sects. “Mutant Huntingtin Specifically Sensitizes IP₃R1 to IP₃” and “Mutant Huntingtin Activates NR2B-Containing NMDA Receptors”) we previously suggested that overactivation of IP₃R1-mediated Ca²⁺ release and NR2B-mediated Ca²⁺ influx in HD MSN may lead to Ca²⁺ overload and apoptosis of these neurons [30,31]. To test this “Ca²⁺ hypothesis of HD” my laboratory used TUNEL assay to compare glutamate-induced apoptosis of MSN cultured from wild type mice and mice expressing mutant human Htt-128Q gene (YAC128 mouse [76]). The mice expressing normal human Htt-18Q gene (YAC18 [77]) was used as a control in these experiments. At 14 DIV all 3 groups of MSN were challenged by an 8 h application of glutamate (from 0 to 250 μ M) to mimic physiological stimulation. Following exposure to glutamate, MSN were fixed, permeabilized and scored for apoptotic cell death using TUNEL staining. We determined that in basal conditions (no glutamate added) approximately 10% of MSN in all 3 experimental groups were apoptotic (TUNEL-positive). Addition of 25 μ M or 50 μ M glutamate increased the number of apoptotic cells to 15–20% in all 3 experimental groups. Addition of 100 μ M or 250 μ M glutamate increased apoptotic death to 60–70% for YAC128 MSN, but only to 25–30% for wild type and YAC18 MSN. Thus we reasoned that exposure to glutamate concentrations in 100–250 μ M range leads to selective apoptosis of YAC128 MSN [34].

This “in vitro HD” model enabled us to test a connection between abnormal Ca²⁺ signaling and apoptosis of HD MSN. We found that inhibition of mGluR1/5 receptors (by a mixture of MPEP and CPCCOEt) reduced the glutamate-induced apoptosis of YAC128 MSN to WT MSN levels. NMDAR-inhibitor (+) MK801 or NR2B-specific antagonist ifenprodil had similar neuroprotective effects. Consistent with direct involvement of IP₃R1, preincubation of the MSN cultures with a membrane-permeable IP₃R blocker 2-APB [78] protected YAC128 MSN from glutamate-induced apoptosis [34]. All these results supported an idea that glutamate-induced Ca²⁺ overload play a key role in induction of apoptotic cell death of HD MSN.

IP₃R and Abnormal Ca²⁺ Signaling in SCA2 Neurons

SCA2 patients suffer from a progressive cerebellar syndrome with ataxia of gait and stance, ataxia of limb movements, and dysarthria [15–17]. Similar to HD, the SCA2 is caused by an expansion and translation of unstable CAG repeats in the gene encoding ataxin-2 from the normal 22 to more than 31 extra glutamine repeats [79–81] (Table 1). The pathogenesis of SCA2 is currently not understood. Similar to wild type ataxin-2, polyglutamine-expanded ataxin-2 protein is ubiquitously expressed in cells without severe aggregation and formation of inclusion bodies [82], but the cerebellar PCs in SCA2 patients are mostly affected with a loss of over 75% [16]. Genetic knockouts of ATXN2 orthologs in fly and worm resulted in embryonic lethality [83,84]. Atxn2 knockout mice were viable but displayed a late-onset obesity phenotype [85]. Mice deficient in Atxn2 did not show Purkinje cell loss or marked changes in the Purkinje cell dendritic tree [85]. Non-essential role of Atxn2 in rodents is most likely related to the presence of orthologs and redundancy in its function [85]. Thus, the polyglutamine expansion in ataxin-2 likely does not cause a loss of function nor a dominant negative effect but a gain of toxic function [85]. The role of calcium signaling in the pathogenesis of SCA2 is supported by the genetic association between polymorphisms in the CACNA1A gene and the age of disease onset in patients diagnosed with SCA2. The CACNA1A gene encodes the pore-forming α 1A subunit of Ca_v2.1, a P/Q-type voltage-gated calcium channel. Patients with a prematurely early age of onset of SCA2 tended to

have a longer CAG-repeat length in the CACNA1A gene [86]. Longer CAG-repeat lengths in this gene have been genetically linked to SCA6.

The role of aberrant neuronal calcium signaling in SCA2 pathogenesis was strengthened further by a finding in our laboratory that ATX2^{exp} but not wild type ATX2 (ATX2^{wt}) specifically binds IP₃R1 [87]. This suggested that its association would result in either a sensitization or desensitization of IP₃R1 to activation by inositol 1,4,5-triphosphate (IP₃) during glutamate signaling in PCs. PCs express extremely high levels of intracellular IP₃R1 [88,89], which are present on endoplasmic reticulum (ER) membranes. ATX2^{exp} and ATX2^{wt} were found to localize and associate with ER membranes [90]. In a lipid bilayer reconstitution experiment, we examined the effect of ATX2^{exp} expression on IP₃R1 activation in single channel recordings of IP₃R1 co-expressed with ATX2^{exp}. We found the presence of ATX2^{exp} substantially sensitized IP₃R1 to activation by inositol 1,4,5 triphosphate (IP₃) [87].

To test the importance of Ca²⁺ signaling in SCA2 pathogenesis we performed a series of experiments with SCA2 transgenic mouse model (SCA2-58Q, generated by [82]) expressing 58 glutamine repeats (ATX2^{exp}) in the ataxin-2 gene under the control of the L7/Pcp2 PC-specific promoter. These mice exhibit behavioral deficits, loss of Purkinje cell dendritic arborization and Purkinje cell death, which is progressive and akin to SCA2 patient pathology [82]. Consistent with involvement of excitotoxic mechanism, we discovered that PC cells in SCA2 mouse undergo dark cell degeneration (DCD) mode of cell death [24]. We found that there was a significant increase in calcium release from ER stores via ITPR1 in calcium imaging experiments in primary PCs cultured from SCA2-58Q transgenic mice, which was not observed in PCs cultured from wild type littermates [87]. When ryanodine or dantrolene were added to block ryanodine receptors and ER calcium release in PC cultures, the effect of ATX2^{exp} expression was immediately reversed as ER calcium release returned to wild type levels [87]. In a TUNEL assay of PC death, we found that the addition of dantrolene to block the excessive ER calcium release caused by ATX2^{exp} expression attenuated exogenous glutamate-induced PC death [87]. Furthermore, long-term feeding of SCA2-58Q mice with a calcium stabilizer dantrolene alleviated the age-dependent motor coordination deficits in these mice quantified by rotarod and beam-walk behavioral assays [87]. Stereological counting of PCs showed a rescue of PC death in 12-month old SCA2-58Q mice fed with dantrolene [87]. The above lines of evidence supported the hypothesis that deranged calcium signaling plays a role in SCA2 pathogenesis [23,24,87] (Table 1).

IP₃R and Abnormal Ca²⁺ Signaling in SCA3 Neurons

SCA3 is a multi systemic disorder characterized by degeneration of spinocerebellar tracts, dentate nucleus, pontine and other brainstem nuclei, substantia nigra and pallidum [91]. 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 [92]. The cause of SCA3 is a polyQ expansion in the carboxy-terminal of ataxin-3 (Atxn3) protein [93] (Table 1). 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) [94,95]. Consistent with the presence of Josephin domain Atxn3 functions as deubiquitinating enzyme (DUB) [96]. The polyQ-expansion has no effect on DUB activity of Atxn3 [96,97], 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 [98]. Knockout of the *ATXN3* gene in mice increases protein ubiquitination but does not result in an obvious behavioural phenotype [99].

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 [100]. Similar to mutant Htt and mutant Atxn2, mutant Atxn3 but not wild type Atxn3 binds to and activates $\text{IP}_3\text{R1}$, an intracellular Ca^{2+} release channel [101]. 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 [101]. These results provided strong experimental support to the “ Ca^{2+} hypothesis of SCA3 pathogenesis” [23,24,101] (Table 1).

Abnormal Neuronal Ca^{2+} Signaling in Other SCAs

The Ca^{2+} hypothesis is not only relevant for SCA2 and SCA3 but can be expanded to other SCAs [23,24,30,102]. Deranged calcium signaling likely plays a role in the pathogenesis of SCA1. Similar to SCA2, SCA1 is caused by the translation of polyglutamine expansions, in the gene encoding ataxin-1 from the normal 30 (ATXN1^{wt}) to over 40 expanded CAG repeats ($\text{ATXN1}^{\text{exp}}$) (Table 1). $\text{ATXN1}^{\text{exp}}$ is widely expressed and selectively toxic in Purkinje cells [103]. Though its aggregation is not required to cause SCA1 pathology, $\text{ATXN1}^{\text{exp}}$ has to translocate to PC nuclei to cause cell death and ataxia [104]. In the nucleus, $\text{ATXN1}^{\text{exp}}$ alters transcription by destabilizing the $\text{ROR}\alpha$ and SP-1 transcription factors [105,106]. Microarray analyses of transgenic mouse models have shown that SCA1 mice may have altered calcium signaling. Early in SCA1 pathogenesis, SCA1 mice express significantly reduced levels of the calcium buffers calbindin and parvalbumin, ITPR1 , type 1 inositol phosphate 5-phosphatase, ER calcium transporter SERCA, glutamate transporter EAAT4, EAAT4-stabilizer β -spectrin III, T-type voltage gated calcium channels and transient receptor potential type 3 (TRPC3) calcium channels [105,107–109]. Since ITPR1 and SERCA2 have contradictory roles on ER calcium storage, it is possible that $\text{ATXN1}^{\text{exp}}$ downregulates the expression of only one of the two. One mechanism may be that by blocking ER loading, SERCA2 causes higher cytoplasmic calcium levels and the PC counteracts this effect by downregulating ITPR1 expression, which decreases ER calcium release and reduce cytoplasmic calcium levels [110]. We suggest that decreased expression of glutamate transporters in SCA1 could result in excitotoxic glutamate signaling that is upstream of calcium signaling leading to cytosolic calcium overload. Similar hypothesis has been previously proposed to explain SCA1 phenotype [109]. Reduced expression of calcium buffers could also decrease the ability of PCs to handle high calcium levels in the cytoplasm. Furthermore, SCA1 mice crossed with calbindin-knockout mice presented with an accelerated SCA1 phenotype [108], which supports the role of calcium buffers in working to relieve SCA1 PCs from excitotoxic calcium levels and the role of deranged calcium homeostasis in the progression of SCA pathogenesis. We have also found that mutant $\text{ATXN1}^{\text{exp}}$ specifically interacts with ITPR1 (X. Chen and I. Bezprozvanny, unpublished observations). As we have seen in SCA2, this suggests that excessive calcium release from ER stores via ITPR1 s may also contribute to pathogenesis of SCA1. Therefore it would be necessary to study SCA1 mouse models further for any other change in calcium signaling besides the already reported differences in gene expression of calcium signaling proteins [105,107,108].

SCA6 is caused by a polyglutamine expansion in the C-terminus of a P/Q-type calcium channel, $\text{Ca}_v2.1$ (Table 1). It has been reported that this mutation enhances P/Q-type Ca^{2+} channels activity [111]. However, recent analysis of SCA6 knock-in mouse model indicated

that pathology may be related to aggregation of mutant CaV2.1 subunits and reduction in the density of dendritic P/Q-type Ca²⁺ currents [112]. Animals expressing polyQ expanded Ca_v2.1 subunit exhibit age-dependent motor deficits and lower expression of the Ca_v2.1 channels but no difference in calbindin staining of slices when compared to WT [112]. This further supports our hypothesis of ataxia preceding cell death as these animals are ataxic in the absence of significant cell death. Future functional studies will be needed to understand alterations in Ca²⁺ signaling in PC cells from SCA6 mice.

Abnormal neuronal calcium signaling is not restricted to polyQ-expansion ataxias [102]. In SCA5, mutant β -III spectrin (SPTBN2) is unable to stabilize the glutamate transporter, excitatory amino acid transporter (EAAT4) on the PC membrane (Table 1). This would allow extended glutamate activation in the parallel fiber-PC synapse and lead to excitotoxicity in SCA5 [113]. Recent genetic evidence indicated that SCA14 is caused by mutations in protein kinase C γ (PKC γ). PKC γ is highly expressed in PCs [114] (Table 1). Eighteen of the 22 identified mutations in PKC γ decrease the ability of PKC γ to phosphorylate and inactivate TRPC3 channels, which allow sustained calcium influx into the cell and results in elevated cytosolic calcium levels [115]. These calcium elevations, if left uncontrolled, could become toxic and result in PC loss via excitotoxic cascades described above. The rest of the mutations suppress calcium influx likely by hyperphosphorylating and over-inactivating TRPC3 channels [115]. Suppression of calcium signaling has been shown to be a cause of apoptotic cell death [116]. Therefore, even though we have described excitotoxicity as one possible effect of SCA-associated gene mutations on calcium signaling and cell survival, suppression of calcium signaling could be as detrimental as increasing it.

Unlike most of the SCAs we have discussed above, SCA15 and SCA16 are a result not of excitotoxicity but of suppressed cytosolic calcium signaling (Table 1). SCA15 is genetically linked to 5' deletions, total deletions or missense mutation (P1059L) in the gene encoding ITPR1 and located in 3p26.1–p25.3 chromosomal region [18,117]. Although initial studies of SCA16 family suggested linkage to chromosome 8q22.1–24.1 [118], additional studies of the same family showed linkage to chromosome 3pter-p26.2 [119]. Initially point mutation in the 3' untranslated region of the contactin 4 gene (CNTN4) located in the same region was implicated [119,120], but more recent analysis demonstrated that the real cause of SCA16 is haploinsufficiency of ITPR1 expression due to heterozygous deletion of exons 1 to 48 of the ITPR1 gene [19]. Thus, SCA15 and SCA16 are the same disorder (SCA15/16), due to haploinsufficiency of ITPR1 (Table 1). Although, SCA15/16 is not polyQ disorder, aberrant calcium signaling is likely to play a role in pathogenesis given the high expression of ITPR1 in PCs [88], and their importance for stimulating BDNF production for PC dendritic morphology [121], and cerebellar plasticity [122]. There are at least two possible physiological roles of these alterations on ITPR1 function and calcium homeostasis in PC. A gain of function of ITPR1 could occur if the mutation or deletion causes constitutive activity or increased sensitivity to activation of the mutated protein. A loss of function could also occur if the mutation or deletion suppresses ITPR1 activation and signaling. Affected patients of SCA15 and mice with spontaneous mutations in ITPR1 express less ITPR1 protein [117]. As we await studies on the functional roles of the reported ITPR1 mutation and deletions in the pathogenesis of SCA15/16, we can only speculate that suppressed activity of ITPR1 s in SCA15/16 may uncouple plasticity mechanisms in PCs. Indeed, ITPR1 knockout mice do not survive past postnatal day 20–23 and completely lack cerebellar LTD, although hippocampal LTD is unaffected and hippocampal LTP is partially intact [121–124]. Thus, disruption of ITPR1 expression or function may result in cerebellar-specific phenotypes, as observed in SCA15/16 patients.

Ca²⁺ Blockers and Perspectives for Clinical Intervention in HD and SCA Patients

Despite the fact that huntingtin and ataxins have been cloned more than 15 years ago, there is still no cure for these disorders. The most obvious targets are mutant huntingtin and ataxin 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 [125,126]. Similar RNAi knockdown approach could also be expanded to other polyglutamine expansion SCAs. 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 [127]. 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 [128]. One possibility to reduce the amount of toxic conformations of Atxn2 and Atxn3 proteins by increasing the levels of chaperons, which control protein folding. Although these approaches are being developed for polyQ-expansion disorders [129–131], 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 [132–137]. A specific polyQ binding peptide (QBP1) was isolated in the phage library screen [138]. It has been reported that QBP1 peptide prevented toxic conformational transition within polyQ-expanded proteins [138] and exerted neuroprotective effects in cellular and animal models of polyQ toxicity [139–141]. Despite excellent scientific rationale, 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 [142] opens perspective for rational design of novel polyQ-binding therapeutic agents.

Our laboratory demonstrated beneficial effects of Ca²⁺ stabilizer dantrolene in both SCA2 and SCA3 mouse models [87,101]. 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 dantrolene must be taken into consideration. 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 [68]. Memantine was also effective in 3-NP model of HD [143] and in YAC128 genetic HD model [67]. Memantine demonstrated some beneficial effects in small scale pilot evaluation in HD patients [69] 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 [144]. In addition to NMDAR, voltage-gated Ca²⁺ channels constitute another potential target for SCA treatment. In recent studies L-type Ca²⁺ channel inhibitor isradipine significantly protected SNc neurons in animal models of Parkinson's disease [145]. Moreover, a recent retrospective epidemiological study has found that treatment of hypertension with Ca²⁺ channel antagonists significantly diminished the risk of developing PD [146]. It is possible that isradipine and other dihydropyridines (DHP) may also be useful for treatment of HD and SCA patients. More conservative potential strategy involves use of mitochondrial stabilizers and energizers, such as creatine, CoQ10, and MitoQ. In previous neurodegenerative trials these compounds resulted in modest benefit [147] and it is likely that similar result will be obtained for SCA patients as well. Controlled

clinical evaluation of Ca^{2+} inhibitors in HD and SCA patients will provide an ultimate test to the proposed “ Ca^{2+} hypothesis” of these disorders.

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Table 1
Features of Spinocerebellar ataxias linked with abnormal neuronal Ca^{2+} signaling

Disease subtype	Locus	Protein	Mutation	Normal repeats	SCA patient repeats	Pathology	Effect on calcium signaling
SCA1	6p	Ataxin 1	PolyQ exp	6–39	39–82	>75% PC loss	Increase
SCA2	12q	Ataxin 2	PolyQ exp	14–31	33–64	>75% PC loss	Increase
SCA3	14q	Ataxin 3	PolyQ exp	12–42	52–86	<25% PC loss	Increase
SCA5	11q	β -III spectrin	Non-repeat mutations & deletions	N/A	N/A	No data	Increase
SCA6	19p	CACNA1A	PolyQ exp	4–18	19–30	>75% PC loss	Increase/decrease
SCA14	19q	PKC γ	Non-repeat mutations	N/A	N/A	No data	Increase/decrease
SCA15/16	3p	ITPR1	Non-repeat deletions or mutations	N/A	N/A	No data	Decrease

Adapted from [24]. Number of repeats adapted from [20]. SCA pathology adapted from [16]

PolyQ exp Polyglutamine expansion, *N/A* Not applicable