Disrupted Calcium Signaling in Animal Models of Human Spinocerebellar Ataxia (SCA)

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Abstract: Spinocerebellar ataxias (SCAs) constitute a heterogeneous group of more than 40 autosomal-dominant genetic and neurodegenerative diseases characterized by loss of balance and motor coordination due to dysfunction of the cerebellum and its efferent connections. Despite a well-described clinical and pathological phenotype, the molecular and cellular events that underlie neurodegeneration are still poorly understood. Emerging research suggests that mutations in SCA genes cause disruptions in multiple cellular pathways but the characteristic SCA pathogenesis does not begin until calcium signaling pathways are disrupted in cerebellar Purkinje cells. Ca2+ signaling in Purkinje cells is important for normal cellular function as these neurons express a variety of Ca2+ channels, Ca2+-dependent kinases and phosphatases, and Ca2+-binding proteins to tightly maintain Ca2+ homeostasis and regulate physiological Ca2+-dependent processes. Abnormal Ca2+ levels can activate toxic cascades leading to characteristic death of Purkinje cells, cerebellar atrophy, and ataxia that occur in many SCAs. The output of the cerebellar cortex is conveyed to the deep cerebellar nuclei (DCN) by Purkinje cells via inhibitory signals; thus, Purkinje cell dysfunction or degeneration would partially or completely impair the cerebellar output in SCAs. In the absence of the inhibitory signal emanating from Purkinje cells, DCN will become more excitable, thereby affecting the motor areas receiving DCN input and resulting in uncoordinated movements. An outstanding advantage in studying the pathogenesis of SCAs is represented by the availability of a large number of animal models which mimic the phenotype observed in humans. By mainly focusing on mouse models displaying mutations or deletions in genes which encode for Ca2+ signaling-related proteins, in this review we will discuss the several pathogenic mechanisms related to deranged Ca2+ homeostasis that leads to significant Purkinje cell degeneration and dysfunction.

Keywords: spinocerebellar ataxias; Purkinje cells; Ca2+ signaling

1. Introduction

The cerebellum, the second largest area exceeded only by the cerebral cortex, contains more neurons than the rest of the brain. For most of the 20th century, it has long been thought that the cerebellum was involved in motor learning and coordination [1,2]. More recently, it has been recognized to play a role in cognitive and emotional processes [3]. Thus, cerebellar dysfunctions can cause motor and/or non-motor deficits. Following lesions confined to cerebellum, Schmahmann and Sherman first described a novel clinical condition characterized by an impairment of (1) executive functions, (2) spatial cognition, and (3) language [4]. This constellation of symptoms, known in humans as cerebellar cognitive-affective syndrome (CCAS), was ascribed to the functional disruption of cerebellar connections to the association cortices and limbic system [4]. On the other hand, lesions
to the cerebellum can cause abnormal and uncoordinated body movements, particularly of the gait (ataxia), tremor and loss of muscle tone [5,6]. There can be a myriad of causes for cerebellar damage, including cerebellar agenesis, dysplasia and hypoplasia, cerebellar stroke, tumors, inflammation, trauma, viral infections, toxin exposure, and neurodegenerative diseases, such as hereditary ataxias [7]. The hereditary ataxias are a group of genetically and clinically defined neurological disorders phenotypically characterized by gait ataxia, often accompanied by lack of coordination of hands, speech, and eye movements. Furthermore, atrophy of the cerebellum has frequently been detected [8,9]. Hereditary ataxia can be subdivided by mode of inheritance (i.e., autosomal dominant, autosomal recessive, X-linked, and mitochondrial), the mutated gene or chromosomal locus. Currently, more than 40 autosomal dominant types, named spinocerebellar ataxia (SCA) based upon the temporal order of discovery of the defective gene, are known, although the number is raising (Table 1) [10–12].

Table 1. SCA: Spinocerebellar ataxias.

| Location | SCA | OMIM Number | Distinguishing Clinical Manifestations | Gene | Type of Mutation |
|----------|-----|-------------|--------------------------------------|------|------------------|
| 6p22.3   | SCA1 | 164400      | Pyramidal signs, peripheral neuropathy, and ophthalmpaeresis | ATXN1 | (CAG)n |
| 12q24.12 | SCA2 | 183090      | Hyporeflexia, tremor and slow eye movements | ATXN2 | (CAG)n |
| 14q32.12 | SCA3 | 109150      | Motor neuron involvement and Parkinsonian features | ATXN3 | (CAG)n |
| 16q22.1  | SCA4 | 600222      | Sensory peripheral neuropathy | Unknown | Unknown |
| 11q13.2  | SCA5 | 600224      | Early onset and very slow disease progression. | SPTBN2 | Point mutations |
| 19p13.13 | SCA6 | 183086      | Late-onset, very slow disease progression, and nystagmus. | CACNA1A | (CAG)n |
| 3p14.1   | SCA7 | 164500      | Visual loss | ATXN7 | (CAG)n |
| 13q21    | SCA8 | 608768      | Cognitive dysfunction, pyramidal and sensory signs | ATXN8 | (CTG*CAG)n |
| 22q13.31 | SCA10| 603516      | Occasional epilepsy | ATXN10 | (ATTCT)n |
| 15q15.2  | SCA11| 604432      | Pyramidal signs. | TTBK2 | Point mutations |
| 5q32     | SCA12| 604326      | Tremor, Parkinsonian features and dementia | PPP2R2B | (CAG)n |
| 19q13.33 | SCA13| 605259      | Delayed motor and cognitive development | KCNC3 | Point mutations |
| 19q13.42 | SCA14| 605361      | Dystonia and myoclonus. | PRKCG | Point mutations |
| 3p26.1   | SCA15/16 | 606658 | Tremor and cognitive impairment. | ITPR1 | Point mutations |
| 6q27     | SCA17| 607136      | Dementia and Parkinsonian features | TBP | (CAG)n |
| 7q22–q32 | SCA18| 607458      | Sensory and motor neuropathy | IFRD1 | Point mutations |
| 1p13.2   | SCA19/2 | 607346 | Cognitive impairment and myoclonus | KCND3 | Point mutations |
| 11q12    | SCA20| 608687      | Cerebellar dysarthria | Unknown | Genomic duplication |
| 1p36.33  | SCA21| 607454      | Mild cognitive impairment, and Parkinsonian features | TMEM240 | Unknown |
| 2p13     | SCA22| 610245      | Pyramidal signs | PDYN | Point mutations |
| 2p21–p13 | SCA25| 608703      | Peripheral neuropathy, unknown | Unknown | Unknown |
| 19p13.3  | SCA26| 609306      | Eye movement abnormalities. | EEF2 | Point mutations |
| 13q33.1  | SCA27| 609307      | Tremor and dystonia | FGF14 | Point mutations |
| 18p11.21 | SCA28| 610246      | Spastic ataxia | AFG3L2 | Point mutations |
| 3p26.1   | SCA29| 117360      | Intellectual disability. | ITPR1 | Point mutations |
| Location | SCA   | OMIM Number | Distinguishing Clinical Manifestations (1) | Gene      | Type of Mutation |
|----------|-------|-------------|-------------------------------------------|-----------|------------------|
| 4q34.3–q35.1 | SCA30 | 613371      | Pure ataxia.                              | ODZ3      | Unknown          |
| 16q21    | SCA31 | 117210      | Abnormal sensation                        | BEAN1     | (TGGAA)n         |
| 6q14.1   | SCA34 | 133190      | Hyperkeratosis                            | ELOVL4    | Unknown          |
| 20p13    | SCA35 | 613908      | Ocular dysmetria, tremor and hyperreflexia | TGM6      | Point mutations  |
| 14q32.11– q32.12 | SCA36 | 614153      | Motor neuron involvement                  | NOP56     | (GGCCTG)n        |
| 1p32.2   | SCA37 | 615945      | Altered vertical eye movements.           | DAB1      | (GGCCTG)n        |
| 6p12.1   | SCA38 | 615957      | Nystagmus and dysarthria                  | ELOVL5    | Point mutations  |
| 4q27     | SCA41 | 616410      | Ocular dysmetria and tremor               | CCDC88C   | Point mutations  |
| 17q21.33 | SCA42 | 618087      | Gait instability, dysarthria and nystagmus| CACNA1G   | Point mutations  |
| 3q25.2   | SCA43 | 617018      | Peripheral neuropathy                     | MME       | Point mutations  |
| 6q24.3   | SCA44 | 617691      | Dysarthria, dysphagia and dysmetria       | GRM1      | Point mutations  |
| 5q33.1   | SCA45 | 617769      | Nystagmus, and dysarthria.                | FAT2      | Point mutations  |
| 19q13.2  | SCA46 | 617770      | Sensory ataxic neuropathy                 | PLD3      | Point mutations  |
| 12p13.31 | DRLPA | 125370      | Involuntary movements, mental and emotional problems | ATN1       | (CAG)n          |
| 4q22.1–q22.2 | GRID2-related spinocerebellar ataxia | 616204 | Motor, speech and cognitive delay and eye movement abnormalities | GRID2 | Rarely AD inheritance | Point mutations |

(1) from https://www.orpha.net/consor/cgi-bin/index.php?lng=EN or https://www.omim.org/.

The most common forms of SCAs are caused by an abnormal expansion of the CAG (or CGT)-repeat sequence that leads to lengthening of polyglutamine (polyQ) tract in appropriate proteins [13–16]. In contrast, other forms of SCAs are characterized by conventional point mutations, including insertions and deletions [17–19]. Disruption of intracellular calcium (Ca²⁺) signaling in Purkinje cells is thought to be a key mechanism in the pathogenesis of SCAs [12,20–22]. Ca²⁺ signaling in Purkinje cells is important for normal cellular function as these neurons express a variety of Ca²⁺ channels, Ca²⁺-dependent kinases and phosphatases, and Ca²⁺-binding proteins to tightly maintain Ca²⁺ homeostasis and regulate physiological Ca²⁺-dependent processes. Abnormal Ca²⁺ levels can activate toxic cascades leading to cell death. The output of the cerebellar cortex is conveyed to the deep cerebellar nuclei (DCN) by Purkinje cells via inhibitory signals; thus, Purkinje cell dysfunction or degeneration would partially or completely impair the cerebellar output in SCAs. In the absence of the inhibitory signal emanating from Purkinje cells, DCN will become more excitable, thereby affecting the motor areas receiving DCN input and resulting in uncoordinated movements [22–24]. An outstanding advantage in studying the pathogenesis of SCAs is represented by the availability of a large number of animal models which mimic the phenotype observed in humans [6,25,26]. By mainly focusing on mouse models displaying mutations or deletions in genes which encode for Ca²⁺ signaling-related proteins, in this review we will discuss the hypothesis that the pathogenesis of diverse SCAs is related to deranged Ca²⁺ homeostasis that leads to significant Purkinje cell degeneration and dysfunction.

2. Mutations in the CACNA1A Gene

Different mutations in the CACNA1A gene, encoding for the pore-forming, voltage-sensing α1A-subunit of voltage-dependent Ca²⁺ Cav2.1 type channels (P/Q-type), are known to result in
neurological disorders, such as episodic ataxia type 2 (EA2), familial hemiplegic migraine type 1 (FHM1) and SCA type 6 (SCA6; Table 1) [27–29]. Each disorder is associated with different mutations in the CACNA1A gene that have differential effects on Cav.2.1 function and, therefore, either decrease or increase neuronal Ca\(^{2+}\) influx. SCA6 is associated with small CAG repeat expansions expressed as a polyglutamine (poliQ) sequence at protein level [28]. Voltage-dependent Ca\(^{2+}\) channels (VDCCs) mediate Ca\(^{2+}\) influx into neurons in response to membrane depolarization, thereby modulating cellular excitability and triggering a variety of Ca\(^{2+}\)-dependent cellular processes, such as neurotransmitter release, synaptic plasticity, gene transcription, cell division and cell death [30,31].

P/Q-type VDCCs are highly expressed in the cerebellum, in particular in Purkinje cells where they account for more than 90% of Ca\(^{2+}\) currents [32–35]. P/Q-type channels play key roles in regulating spike firing properties and contributing to Ca\(^{2+}\) transient/complex spikes that result from climbing fiber activity [36]. Moreover, they regulate heterosynaptic competition between climbing fibers and parallel fibers and also drive homosynaptic competition among multiple climbing fibers [37]. In contrast to human CACNA1A dominant mutations, the first animal model to be characterized showed predominantly recessive mutation in the CACNA1A gene (tottering mice) [38,39]. The tottering (tg) mice display mildly ataxic behavior during locomotion [40–42]. In tg Purkinje cells, the P/Q-type current density is decreased by ~40% [43] and spike firing patterns show enhanced irregularities with periods of pauses and bursts [41]. Consistent with a reduced functional role of P/Q-type channels, parallel fiber–Purkinje cell synapses are impaired in tg mutants [44]. Additionally, electron microscopic and Golgi-staining procedures have revealed shrunken Purkinje cells with a reduced size in the soma, abnormal Purkinje cell connectivity and diffuse axonal swellings [45–47]. Two additional recessive tg mutations have been identified showing different but overlapping features (leaner and rolling Nagoya mice). As opposed to tg, leaner (tg\(^{le}\)) and rolling Nagoya (tg\(^{rn}\)) mice are severely ataxic [39,48,49]. In addition to causing a reduction in P/Q-type current density by ~60%, the tg\(^{le}\) mutation affects Ca\(^{2+}\) channel gating kinetics [43]. In detail, Ca\(^{2+}\) channel currents in tg\(^{le}\) Purkinje cells show a distinct change in the voltage dependence of activation and inactivation. Furthermore, these mice exhibit Purkinje cell degeneration whose parasagittal striped pattern is similar to the pattern of zebrin expression [39,50]. Likewise, electrophysiological studies have demonstrated that also the tg\(^{rn}\) mutation in Purkinje cells results in the reduced voltage sensitivity (i.e. mutated channels are less sensitive to voltage stimuli) and diminished activity of P/Q-type channels (~40%) [51,52]. Overall, morphological investigations have revealed characteristic synaptic alteration between parallel fiber varicosity and Purkinje cell dendritic spines in all the three mutant mouse models of SCA. Multiple Purkinje cell dendritic spines synapse with single parallel fiber varicosity [47]. A recently described ataxic model in rats (GRY; groggy) is also featured by a recessive mutation [53]. The phenotype of the GRY rat resembles that of the tg mouse rather than that of the other two mutant mice. In 2007, however, Xie and colleagues reported the first dominant ataxic mouse model of CACNA1A mutation, called Wobbly (wb) on the basis of its peculiar gait [54]. Heterozygotes show severe ataxia and reduced locomotor activity with associated degeneration of Purkinje cells [54,55]. Purkinje cells display altered passive and active membrane properties and an altered synaptic excitation/inhibition balance in wb mice. Specifically, wb Purkinje cells are less excitable showing increased resting membrane potential and action potential threshold. Parallel fiber stimulation fails to evoke excitatory synaptic currents in more than 50% of Purkinje cells, while evoked synaptic inhibition is shown to be stronger [55]. Later, another dominant mutation, known as Tg-5J, in CACNA1A gene, resembling the wobbly and many human mutations, was described by Miki and coworkers [56]. Heterozygotes Tg-5J mice are extremely ataxic and homozygotes rarely survive. The Tg-5J mutation determines a negative shift in the P/Q-type channel activation curve despite of no significant changes in the Ca\(^{2+}\) current density [56]. Moreover, the dendritic arbor is significantly reduced in complexity and overall mass in Tg-5J Purkinje cells [56]. Since SCA6 share features in common with other polyglutamine (poliQ) diseases, it is reasonable to expect that the poliQ expansions would affect the P/Q-type channel function. Different studies, performed on several lines of knockin (KI) mice carrying expanded or hyperexpanded CAG repeat tracts in the CACNA1A gene, have achieved highly variable, often conflicting results. Matsuyama and colleagues showed that a
P/Q-type channel with 24 polyglutamines (SCA624Q) exhibited normal gating properties whereas 40 expanded polyglutamines (SCA640Q) caused an hyperpolarizing shift in the voltage dependence of inactivation [57], suggesting an overall reduction of Ca2+ influx. On the contrary, another study revealed that showed the voltage dependence of inactivation of P/Q-type channels was negatively shifted in SCA624Q mice [58]. Recently, KI mice were generated by introducing hyperexpanded polyglutamines (SCA684Q) [59–62]. These mice develop progressive motor impairment in adult age. Extracellular recordings of cerebellar slices from SCA684Q mice revealed a decrease of Purkinje cell firing (<100 Hz) [63] accompanied by a reduction of the spike timing precision in spite of unchanged P/Q-type channel kinetics and current density, suggesting that expanded CAG repeat per se does not affect the intrinsic properties of the channels. In addition, surplus climbing fiber inputs on developing SCA684Q Purkinje cells were observed [63]. The aforementioned mouse models with intrinsic properties affected in P/Q-type channels, such as pore conductance, gating and inactivation kinetics, can provide further opportunities to understand how perturbations in Ca2+ signaling regulate neuronal excitation and its underlying relation with human SCAs.

3. Mutations in the CACNA1G Gene

SCA42 is a rare, autosomal dominant neurological disorder characterized by pure and slowly progressive cerebellar defects, including incoordination of gait, dysarthria, nystagmus, and saccadic eye movements (Table 1). Post-mortem histological examination revealed cerebellar atrophy in association with a prominent Purkinje cell loss [64]. A heterozygous single point mutation in the CACNA1G gene which causes an arginine-to-histidine (p.Arg1715His) change in the S4 voltage-sensing region of the T-type voltage-gated Ca2+ channel protein Ca(v)3.1 has concurrently been associated to SCA42 in ten families [64–67]. T-type Ca2+ channels are widely expressed in the brain, including the thalamus, hippocampus, neocortex, and cerebellum [68]. In situ hybridization showed that Ca(v)3.1 is the major isoform of T-type Ca2+ channels expressed in cerebellar motor circuit, including Purkinje cells, DCN and inferior olivary (IO) neurons, a result that is also supported by immunocytochemical localization [68–71]. The unique electrophysiological properties of T-type Ca2+ channels (especially the low voltage-activated Ca2+ current) are well suited to regulating neuronal excitability and oscillatory behavior near the resting membrane potential [72]. Specifically, T-type Ca2+ channels have a significant role in dendritic Ca2+ spikes and the resultant spontaneous bursts in Purkinje cells [73,74], whereas they mediate a rebound burst firing after hyperpolarization in DCN neurons [71] and underlie resonance and subthreshold membrane potential oscillations in IO neurons [75,76]. Recent findings on SCA42 using whole-exosome sequencing in HEK293T cells found that arginine-to-histidine change in CACNA1G gene was able to determine a positive shift of T-type Ca2+ channel voltage-dependence [65]. In order to elucidate whether this point mutation is implicated in the SCA42 phenotype, a new transgenic (Cacna1g-Arg1723His) mouse model, harboring the same mutation identified in the SCA42 families, has been generated [77]. Cacna1g-Arg1723His mice showed an adult-onset ataxic phenotype concomitant with cerebellar atrophy and Purkinje loss, as reported in human SCA42. Furthermore, Cacna1g-Arg1723His Purkinje cells displayed a change in the voltage-dependence of T-type Ca2+ channels which strongly impact on their excitability [77]. Interestingly, Hashiguchi’s study also described that Arg1723His mutation altered the resonance properties of IO neurons, a result that has also been reported in Ca.3.1 KO mice [78,79]. Since Ca.3.1 KO mice are reported to have normal growth, normal brain weight and structure, and no significant defects in motor learning and motor coordination were observed [80,81], the CACNA1G gene mutation most likely causes ataxic symptoms through a process different from a classic loss-of-function mechanism.

4. Mutations in the GRID2 Gene

Recently, GRID2 point mutations with putative gain-of-function mechanisms were reported in several patients diagnosed with dominant inheritance of cerebellar ataxia [82]. They affect the same amino acid as that previously described by Phillips in Lurcher mouse model [83]. GRID2 gene encodes for the δ2 glutamate (GluD2) receptor, which is mostly expressed in Purkinje cells, where it is co-
localized with AMPA receptors at the postsynaptic density (PSD) of parallel fiber synapses [84,85]. Although GluD2 receptors belong to the ionotropic glutamate receptor family [86], they are not gated by L-glutamate but selectively bind to D-serine and glycine [87]. GluD2 receptors are required for proper development and function of the cerebellum [88,89]. They play a crucial role in Purkinje cell synapse formation by interacting with presynaptic proteins [87,90]. Actually, several studies have demonstrated motor incoordination related to ataxia in GluD2 receptor KO mice. In addition, the number of contacts between parallel fiber and Purkinje cell is reduced while the innervation by climbing fibers into the distal part of the Purkinje cell dendritic tree is extended, thus invading the parallel fiber territory [89,91–93]. Furthermore, LTD impairment was observed at the parallel fiber–Purkinje cell synapse [89]. Homozygous Lurcher mice die at birth [94], whereas heterozygotes exhibit prominent ataxic gait and motor coordination deficit as a result of selective cell-autonomous apoptosis of Purkinje cells during the third postnatal week, when about 90% of the Purkinje cells have disappeared [95–97]. Lurcher Purkinje cells have a very high membrane conductance and a depolarized resting potential due to a large inward current resulting from continuous Na⁺ and Ca²⁺ influx through constitutively open GluD2 receptors in the absence of ligand binding [6,97–99]. The mechanism by which continuous ion flow through mutated receptors is capable of inducing Purkinje cell death was proposed by [100] (Figure 1).

**Figure 1.** Lurcher δ2 glutamate (GluD2)-induced cell death. *Lurcher* GluD2 receptors show constitutive and continuous influx of Na⁺ and Ca²⁺. Intracellular ATP levels are decreased probably by overactivation of the Na⁺/K⁺-ATPase. Compromised ionic homeostasis together with decreased ATP levels can lead to cell swelling and subsequent cell death. Secondary Ca²⁺ influx through voltage-gated Ca²⁺ channels (VGCCs) could activate a variety of Ca²⁺-dependent enzymes, such as calpains, and potentially contribute to Purkinje cell death through different pathways. Modified from [100].

In response to the constitutive ion influx through *Lurcher* GluD2 receptors, intracellular ATP levels decrease probably by overactivation of the Na⁺/K⁺-ATPase. Indeed, in *Lurcher* Purkinje cell dendrites, the mitochondrial oxidative respiration is increased in order to balance the energy demands of removing excess Na⁺ and Ca²⁺ entering the cells in response to the constitutive GluD2 current [96]. Compromised ionic homeostasis together with decreased ATP levels can lead to cell swelling and subsequent cell death. Furthermore, Ca²⁺ influx through VGCCs could activate a variety of Ca²⁺-dependent enzymes, such as the cysteine protease calpain, and potentially contribute to Purkinje cell death through different pathways. Studies to assess the contribution of intracellular Ca²⁺ levels in triggering downstream pathways mediating Purkinje cell death in *Lurcher* mice will be directly relevant for our understanding the mechanisms of neurodegenerative diseases, such as SCAs. In conclusion, *Lurcher* mice will continue to be a valuable model for the development of disease-targeted therapeutic approaches.
5. Mutations in the PRKCG Gene

Spinocerebellar ataxia type 14 (SCA14; Table 1), characterized by slowly progressive cerebellar dysfunction, dysarthria and abnormal eye movements, is caused by almost 40 different mutations in PRKCG gene, encoding the protein kinase C gamma (PKCγ) [19,101]. PKCγ is a member of the classical PKC subfamily which is activated by Ca\(^{2+}\) and the second messenger diacylglycerol (DAG), which is synthesized upon phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) hydrolysis by phospholipase C (PLC) [102]. Whereas an increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) is required to stimulate the cytosolic PKCγ to translocate towards the plasma membrane, subsequent DAG binding is mandatory for enzyme activation. PKCγ is expressed solely in the brain and spinal cord and its localization is restricted to neurons [103]. In the cerebellum, PKCγ is most abundant in Purkinje cell soma and dendritic processes where it plays a role in normal development of the climbing fiber input from the inferior olive [104–107]. In both PKCγ KO mice and mice expressing a transgenic PKCγ inhibitor, multiple climbing fiber innervation of Purkinje cells persists into adulthood [106,108,109]. Nonetheless, the PKCγ KO mice only show mild ataxia and no gross morphological abnormalities in Purkinje cells [106]. In contrast, cerebellar atrophy and loss of Purkinje cells were described at post-mortem in SCA14 patients [110,111]. These studies show the importance of PKCγ activity in Purkinje cell development and neuronal connectivity and suggest the possibility that a gain of toxic function rather a loss of function of PKCγ underlies the pathogenic mechanism of SCA14. A previous report showed that two SCA14 mutations led to increased PKCγ intrinsic activity. The mutant kinase displays enhanced Ca\(^{2+}\)-induced membrane translocation, which links Purkinje cell loss to a potential gain of PKCγ-mediated signal transduction [112]. More recently, Adachi and co-workers [113] have studied the PKCγ activity in 20 spontaneous mutations found in PRKCG gene of SCA14 patients. Nineteen of the 20 mutations showed an increased PKCγ activity although they were unable to bind DAG and their residency time within the plasma membrane was significantly shorter. As a consequence, the mutant PKCγ could not phosphorylate and inhibit extracellular Ca\(^{2+}\) entry through Transient Receptor Potential Canonical 3 (TRPC3). Of note, TRPC3 provides a second messenger-operated Ca\(^{2+}\)-entry pathway which is gated by DAG upon stimulation of metabotropic receptors [114]. As a consequence, [Ca\(^{2+}\)]\(_i\) is remarkably increased in the presence of mutant PKCγ, thereby leading to aberrant intracellular signaling. These results indicate that an alteration in Ca\(^{2+}\) homeostasis and Ca\(^{2+}\)-mediated signaling in Purkinje cells may be responsible for the neurodegeneration characteristic of SCA14. However, another report showed that SCA14 mutant PKCγ activity is reduced in living cells causing an inefficient activation of downstream mitogen-activated protein kinase (MAPK) signaling. These events might therefore lead to reduced expression of target genes and affect neural development and survival [115,116]. To date, only two transgenic mouse models of SCA14 have been generated: PKCγ-H101Y and PKCγ-S361G mice, respectively [117,118]. Although the PKCγ-H101Y transgenic mouse express H101Y mutation under a universal promoter, PKCγ-H101Y is peculiarly expressed in Purkinje cell soma and dendrites. The authors report an altered morphology and loss of Purkinje cells at the age of four weeks combined with an ataxic phenotype which is more severe than PKCγ KO mice [117]. In the most recent transgenic mouse model of SCA14, by using the L7 promoter, the S361G mutation of the PKCγ was specifically expressed in Purkinje cells [118]. Purkinje cells from PKCγ-S361G transgenic mice show severe inhibition of dendritic development which is identical to Purkinje cells treated with a PKCγ agonist, motor deficits typical for cerebellar ataxias and Purkinje cell degeneration and loss in a localized area (lobule 7). These findings support the notion that the increased activity of PKCγ results in the functional cerebellar deficit usually recognized in SCA14 patients. Interestingly, point mutations inducing other forms of SCA often affect signaling proteins which are involved in the PKCγ pathways, such as TRPC channels and inositol-1,4,5-trisphosphosphate receptors (IP\(_3\)Rs). Since PKCγ is likely to play a crucial role for proper Purkinje cell function, it might be an appealing drug target for the treatment of SCAs [118].

6. Mutations in the TRPC3 Gene
The Moonwalker mouse (Mkw) is a recently described model of dominantly inherited cerebellar ataxia that displays impaired motor and coordination control associated with a slow but progressive loss of Purkinje cells [119,120]. The phenotype is caused by a gain of function mutation (T365A) of the TRPC3 gene encoding for the Ca\textsuperscript{2+}-permeable, non-selective cation channel TRPC3 (Transient Receptor Potential Canonical 3) [119–121]. TRPC3 mediates extracellular Ca\textsuperscript{2+} entry in response to G\textsubscript{q/11}-Protein Coupled Receptor (GPCRs) or Tyrosine Kinase Receptors (TKRs), thereby modulating a host of several physiological functions, including synaptic transmission, arterial tone regulation and angiogenesis [122–127]. TRPC3 channels are abundantly expressed in the cerebellum, particularly in Purkinje cells and unipolar brush cells (UBCs), a small glutamatergic neurons mainly found in the granular layer of vestibulocerebellum [119,128,129]. During Purkinje cell postnatal development, TRPC3 channel expression is upregulated promoting a rapid dendritic growth and synapse formation [130]. Electrophysiological recordings from Trpc3 KO Purkinje cell have established that TRPC3 channels mediate slow postsynaptic currents (sEPSCs) in response to stimulation of subtype I metabotropic glutamate receptors (mGluR1) [123,129]. When stimulated, mGluR1 activates PLC\textbeta to hydrolyze PIP\textsubscript{2} into IP\textsubscript{3} and DAG [131]. As a consequence of IP\textsubscript{3}-induced endoplasmic reticulum (ER)-Ca\textsuperscript{2+} store depletion, the ER-Ca\textsuperscript{2+} sensor Stromal Interaction Molecule 1 (STIM1) translocates to peripheral ER cisternae, where it aggregates within sub-membranal clusters and gates Orai1 [132] and/or TRPC3 channels [133–136]. In addition to mediating sEPSCs (Hartmann et al., 2008), the functional coupling between mGluR1 and TRPC3 channels was found to be critical for the induction of long-term depression (LTD) at the parallel fiber–Purkinje cell synapse [124,125]. Finally, a recent study has revealed that the difference in intrinsic firing activity of zebrin II-positive and zebrin II-negative Purkinje cells is generated by differential activity of TRPC3 channels [137]. In the Mkw mutation, threonine 365 is converted into alanine in the highly conserved S4/S5 linker region of the TRPC3 protein leading to abnormal channel gating [119,128]. Accordingly, TRPC3 was activated following low mGluR1 stimulation in Mkw Purkinje cells (Becker et al., 2009). In addition, these cells are reduced in size and show a lower complexity of dendritic arborization which is characterized by few and short branches. Concurrent with dendritic abnormalities, a significant decrease in climbing fiber synapses along dendrites is observed [119,120]. UBCs are also severely reduced contributing to pronounced balance impairment of Mkw mice [128]. As Ca\textsuperscript{2+} signaling plays a fundamental role in regulating dendritogenesis and connectivity in the developing brain [138] and Mkw phenotype derives from a gain-of-function in the Ca\textsuperscript{2+}-permeable TRPC3 channel, an abnormal Ca\textsuperscript{2+} influx during the critical period of development has been suggested to be the cause of limited dendritic arborization in Mkw Purkinje cells. Based upon the interaction between PKC\gamma and TRPC3 described above, Becker and colleagues [119,121,125] have proposed a model in which mGluR1-TRPC3-PKC\gamma signaling at parallel fiber–Purkinje cell synapse is essential in controlling developmental and functional processes [129,130,139,140] (Figure 2).
TRPC3 channels are present in the same protein complex with mGluR1 and both receptors localize to Purkinje cell soma and dendrites [119,130,140]. Moreover, TRPC3 channels and PKCγ primarily respond to PLC-coupled receptors, such as mGluR1. Finally, TRPC3 channel activity has been shown to be negatively regulated through phosphorylation by PKCγ at the distinct site of threonine 365, as also discussed above [113,119,141,142]. Thus, the absence of the PKCγ-mediated negative feedback on TRPC3 channels, which could occur upon mutations in the PRKCG gene, might determine a prolonged activation, resulting in sustained high levels of intracellular Ca2+ into Purkinje cells. Of note, besides modulating TRPC3 activity via PKCγ, DAG is also able to gate TRPC3 in a non-PKC-dependent manner [122]. Thus PKC is a powerful inhibitor of TRPC3 channels while DAG is a dual regulator [136]. In addition, TRPC3-mediated sEPSCs may be also induced following mGluR1-dependent activation of phospholipase D (PLD) through the small GTP-binding protein Rho [143]. In addition, recent studies have demonstrated that mGluR1 activation may be sustained by the physical association with the GluD2 receptors. GluD2 receptors share remarkable sequence homology with ionotropic glutamate receptors, although they neither bind to glutamate nor conduct ion currents [144]. A recent investigation demonstrated that mGluR1 may physically associate with GluD2, PKCγ, and TRPC3 in mouse cerebellar Purkinje neurons, thereby favoring the cell surface expression of the protein complex and boosting the efficacy of mGluR1-mediated synaptic transmission [145,146]. Perturbed TRPC3 signaling is likely to be a common pathological mechanisms in other genetic forms of cerebellar ataxia. Indeed, TRPC3 gene was recently identified as a central player in mouse models of SCA1 [113,129], SCA1 [147,148] and SCA2 [149] (see paragraphs above). SCA1 and SCA2 are caused by an abnormally expanded CAG-repeat sequence in the Ataxin-1 (ATXN1) and Ataxin-2 (ATXN2) genes, respectively. In transgenic mice expressing the human ATXN1 and ATXN2 genes with an expanded CAG tract under the Purkinje cell-specific Pcp2 promoter, TRPC3 gene was
downregulated at an early stage in pathogenesis [148,149]. Intriguingly, the first functionally pathogenic variant in the human TRPC3 gene was identified in a patient with adult-onset cerebellar ataxia (SCA41; Table 1) [150,151]. Collectively, these findings point to a crucial role of TRPC3 signaling in hereditary forms of human cerebellar ataxia, making the TRPC3 gene a promising candidate for screening ataxic patients with unknown genetic etiology [152].

7. Mutations in the Itpr1 Gene

Inositol 1,4,5-trisphosphate receptors (IP3Rs) are intracellular IP3-gated channels that release Ca2+ from the endoplasmic reticulum (ER). IP3 is generated from PIP2 hydrolysis by PLCγ and PLCβ in response to various extracellular stimuli acting on GPCRs and TKRs [153]. In mammals, including humans, the IP3R family consists of three isoforms (types 1–3) encoded by distinct genes (Itpr1, Itpr2, and Itpr3). Although human IP3Rs share around 70% sequence homology, they exhibit a different sensitivity to IP3 and tissue-/cell specific distribution [154–157]. IP3 receptor type 1 (IP3R1) is primarily expressed in the cerebellum, especially in Purkinje cells, but it is also localized in other brain areas, including the cerebral cortex, basal ganglia, thalamus, and hippocampus [154,158–160]. Several reports suggest that abnormal IP3-mediated Ca2+ signaling is involved in the pathogenesis of spinocerebellar ataxia types 15/16 (SCA F; Table 1) and 29 (SCA29; Table 1), as well as other neurodegenerative disorders, such as Alzheimer’s disease (AD) and Huntington’s disease [161,162]. Van de Leemput and colleagues first described a heterozygous deletion of Itpr1 gene, encompassing exons 1–10, 1–40, and 1–44 in Australian and British families of patients for SCA15, which is characterized by the adult-onset of very slowly progressive gait and limb ataxia with episodic dystonic symptoms [163–167]. Soon after, a complete deletion of the gene was found in a Japanese patient with SCA15 [168]. A subsequent study reported only a heterozygous deletion limited to exons 1–48 of the Itpr1 gene in a patient with SCA16 [169], indicating that haploinsufficiency of Itpr1 gene was the cause of both SCA15 and SCA16. Gardner proposed to designate SCA16 as a “vacant SCA”; however, the term SCA15/16 is still widely employed [170]. At least six different heterozygous point mutations have been identified in SCA29 which is distinguished by infantile-onset (congenital) motor development delay followed by non-progressive ataxia and mild cognitive impairment [171,172]. One of this mutation is located in the allosteric inhibitor of IP3R1, the carbonic anhydrase VIII-binding protein (Car8; [173]). There are two spontaneous mutant mice that naturally possess an Itpr1 gene mutation, ITPR1opt/opt mice and ITPR1Δ18/Δ18 mice. Both the mouse models show a decrease in the normally high level of IP3R1 expression in the cerebellum [164,174]. Their phenotype start to show ataxic and convulsive phenotypes around 10 days after birth and die within 4 weeks [164,175]. In the regulatory domain of the Itpr1 gene, ITPR1opt/opt and ITPR1Δ18/Δ18 mice have a homologous in-frame deletion of exons 43/44 and exon 36, respectively [164]. As a result of this deletion, several putative modulatory sites are removed [154,175,176]. Interestingly, the opt-IP3R1 seems to be more subject to proteolysis than the wild-type IP3-R1 isoforms. Additionally, the single-channel conductance of the opt-IP3R1 is reduced by 20% and sensitivity to potentiation by ATP is reduced 20-fold [177]. Surprisingly, Street and colleagues [175] reported that, despite markedly decreased IP3R1 protein level, repetitive mGluR stimulation elicited a supranormal Ca2+ release from ER in ITPR1opt/opt Purkinje cells. These data suggest that the phenotype observed in ITPR1opt/opt mice may be caused by the physiological misregulation of a functional IP3R1. Currently, Ca2+ measurements and membrane electrophysiology would be necessary in order to ascertain whether ITPR1Δ18/Δ18 mice match the physiology of SCA15/16. Lastly, IP3R1 KO mice show a postnatal phenotype similar to those of ITPR1opt/opt and ITPR1Δ18/Δ18 mice [178,179]. Purkinje cells from IP3R1 KO mice exhibit abnormal dendritic arborization and enlarged parallel fiber terminals characterized by an accumulation of synaptic vesicles (Hisatsune et al., 2006). Hisatsune and colleagues suggested that BDNF production through IP3R1-mediated Ca2+ signaling in cerebellar granule cells intercellularly controls the dendritic outgrowth of Purkinje cells, resulting in altered parallel fiber–Purkinje cell synaptic efficacy. Indeed, electrophysiological experiments in IP3R1 KO mice demonstrated that long-term depression (LTD) at the parallel fiber–Purkinje cell synapse was impaired [180]. Furthermore, recordings in freely behaving conditional cerebellum/brainstem IP3R1 KO mice revealed distinct patterns of Purkinje cell
firing linked to the dystonic symptoms which could be rescued by the deletion of Purkinje cells or by pharmacological inactivation of the inferior olive [181]. Taken together, the spectrum of ITPRI-associated phenotypes is intriguing. The heterozygous IlprI gene deletions in late onset SCA15 suggest haploinsufficiency as a disease mechanism. Conversely, cases with a congenital or infantile onset of SCA29 appear to be caused exclusively by IlprI gene point mutation that alter the structure of IP3R1, presumably through a dominant negative effect [182].

8. Mutations in the ATXN2/ATXN3 Genes

SCA2 and SCA3 are two of the more prevalent polyQ diseases caused by CAG repeat expansion in the genes encoding ataxin-2 (Atxn2) and ataxin-3 (Atxn3) proteins which are widely expressed in neuronal tissue [183–189]. Typically, mutant proteins have an increased tendency to aggregate forming ubiquitinated microscopically visible intranuclear inclusions [189]. SCA2 is characterized by progressive cerebellar ataxia, dysarthria, and oculomotor deficits [190,191] whereas SCA3, also known as Machado–Joseph disease (MJD), is a multi-systemic disorder affecting various different regions of the brain and spinal cord. Although the cerebellar cortex and olivary nuclei are relatively spared, high-resolution magnetic resonance imaging shows cerebellar atrophy. SCA3 is characterized by a broad spectrum of clinical signs involving cerebellar, pyramidal, extrapyramidal and spinal motor functions [192,193]. The role of Ca2+ signaling in the pathogenesis of SCA2 is supported by the genetic inverse correlation between CAG-repeat length in the CACNA1A gene and the age of disease onset [194] and further reinforced by finding that mutant Atxn2 has been shown to interact with the cytosolic COOH-terminal of IP3R1 by increasing the sensitivity of the receptor to its ligand [20]. Transgenic animals expressing 58 glutamine repeats in the Atxn2 gene under the control of the L7 promoter (SCA2-58Q) exhibit Purkinje cell loss or marked changes in the Purkinje cell dendritic arborization, without nuclear localization or a detectable increase in ubiquitin-conjugated protein complexes [189]. These features are typical for SCA2 patient pathology. Moreover, specific behavioral assays quantified by rotarod and beam-walk analysis reveal age-dependent motor coordination deficits [20]. These reports are consistent with a gain-of-function or toxic gain-of-function of mutant Atxn2. In addition, mice deficient in Atxn2 do not show any evidence of ataxia or major morphological abnormalities in the cerebellar cortex [195]. This evidence further supports the notion that CAG repeat expansion in Atxn2 does not cause a loss of function nor does it have a dominant negative properties. Recent analyses of Purkinje cell pacemaking activity recorded from SCA2-58Q transgenic mice using extracellular single-unit in vivo recordings demonstrated an age-dependent bursting and irregular firing patterns [196,197]. Furthermore, Ca2+ imaging recordings showed a significant increase in Ca2+ release from ER via IP3R1 upon mGluR stimulation resulting in significantly more cell death [20]. Chronic suppression of IP3R-mediated Ca2+ signaling by the over-expression of IP3 5-phosphatase (SPP) using adeno-associated virus in aging SCA2-58Q transgenic mice has been shown to prevent Purkinje cell death, normalize their firing pattern, and attenuate motor coordination deficits [197]. The mechanism by which the IP3R1-mutant Atxn2 interaction results in increased IP3R1 activity leading to deranged neuronal Ca2+ signaling was proposed by Kasumu and colleagues [22]. Aberrant IP3R1-dependent ER Ca2+ release results in the mitochondrial Ca2+ overload, release of cytochrome C and, consequently, induction of Purkinje cell death via dark cell degeneration (Figure 3), which represents a peculiar mode of excitotoxic cell death.
Interestingly, the positive modulation of small conductance Ca\(^{2+}\)-gated K\(^+\) channels type 2 (SK2), which are known to play an important role in the regulation of Purkinje cell spiking activity [198,199], restores regular firing pattern of 58-Q Purkinje cells [200]. Furthermore, long-term feeding of aging SCA2-58Q transgenic mice with a novel selective positive modulator of SK2 channels (NS13001) improves motor performance [200]. This result identifies NS13001 as a potential therapeutic drug for treatment of SCA2. It is conventionally presumed that, except for Atxn2 which has an exclusively cytoplasmic localization in normal and SCA2 human brain [201], nuclear expression of CAG-expanded proteins is required for the pathogenesis of polyQ diseases [11,202,203]. Recently, genetic experiments, using transgenic mice expressing CAG-expanded Atxn3-79Q (SCA3-79Q), which display various symptoms of motor dysfunction, have confirmed the nuclear toxicity of mutant Atxn3 (Bichelmeier et al., 2007). In addition to forming toxic aggregates, single-channel recordings in reconstituted lipid bilayer membranes revealed that mutant Atxn3 may increase IP\(_3\)R activation by IP\(_3\), thus destabilizing neuronal Ca\(^{2+}\) signaling [161,204]. However, blocking the Ca\(^{2+}\)-dependent protease, calpain, abrogated the formation of inclusions in cells expressing the mutant Atxn3 [205]. Moreover, in SCA3-79Q transgenic mice, pharmacological inhibition of Ca\(^{2+}\)-induced Ca\(^{2+}\) release through ryanodine receptors (RyRs) was shown to counterbalance the increased activity of IP\(_3\)R, thereby resulting in an improvement of their performance in motor coordination assays [204]. These results strongly support the role of deranged Ca\(^{2+}\) signaling in the pathogenesis of SCA3. Finally, in SCA3-79Q transgenic mice, microarrays analysis and RT-PCR assays indicated downregulated expression of cerebellar genes involved in signal trasduction, neurotransmission or synaptic plasticity, including those encoding IP\(_3\)R1, PLC\(_{\gamma}\) and calcineurin [206]. Whole-cell patch-clamp recordings provided the evidence that mutant Atxn3-induced transcriptional repression impaired LTD induction at the parallel fiber–Purkinje cell synapse in SCA3-79Q mice [206]. These findings are at odds with the reported gain of function of IP\(_3\)R1 following binding to the mutant Atxn3 [161,204]. These contradictory findings suggest that it could not be the reduced or increased activity of IP\(_3\)R1 but rather the loss of a precise regulation of its activity that triggers the disease.
9. Mutations in the ATXN1 Gene

Expansion of CAG repeats within the ATXN1 gene encoded the cytosol/nuclear protein ataxin-1 (Atxn1) causes the adult-onset neurodegenerative disease SCA1 characterized by progressive ataxia, oculomotor deficits, pyramidal/extrapyramidal signs [207–211]. Atxn1 is widely expressed in the normal human brain and peripheral tissues. Immunohistochemical studies have revealed that Atxn1 is predominantly nuclear in neuronal cell types although Purkinje cells also present a minor cytoplasmic component [212]. The primary neurodegeneration in SCA1-affected individuals results in Purkinje cell loss and atrophy of specific brainstem neurons [208,213–216]. Moreover, nuclear inclusions consisting of the mutant Atxn1 have been described in neurons of SCA1 patients [217,218]. Interestingly, by using RNA in situ hybridization, high levels of Atxn1 expression in murine Purkinje cells were detected during postnatal maturation when the growth of the dendritic tree is being completed and synaptic connections are being completed and becoming functional. This finding suggests that Atxn1 might play a role in Purkinje cell development and function [219,220]. Because the normal form of Atxn1 as well as Atxn1 with the expanded CAG repeat are transcribed and translated in the tissues of affected SCA1 patients, and the absence of Atxn1 in the mouse does not lead to any ataxic symptoms or major motor coordination abnormalities [221], the toxic-gain of function exerted by mutant Atxn1 has been suggested. In order to test this hypothesis, different SCA1 transgenic mice have been generated. The first transgenic mouse model of SCA1 employed the Purkinje cell-specific promoter L7 to precisely target the expression of the human ATXN1 gene [222]. Two lines were created with various repeat lengths; a control line with over-expression of the normal allele containing 30 CAG repeats (30Q), while the expansion construct contained 82 CAG repeats (82Q). ATXN1(30Q) mice exhibit a normal phenotype with no signs of neurological disorders. Conversely, ATXN1(82Q) mice reveal severe ataxia and progressive Purkinje cell loss [222–224]. ATXN1(82Q) Purkinje cells develop two pathological features described in cerebellar tissue from patients with SCA1: atrophic dendritic arborization and nuclear aggregates of mutant Atxn1 [224]. Using Ca2+-imaging and electrophysiological approaches in ATXN1(82Q) mice, a recent study reported a significant reduction in the responsiveness of Purkinje cells to climbing fiber activation at early-disease stage (~6 weeks) while parallel fiber–Purkinje cell transmission alteration occurred during SCA1 progression (28–40 weeks) [225]. Furthermore, immunofluorescent labeling of climbing fiber terminals revealed a reduction of climbing fiber synapses in the distal segment of the Purkinje cell dendritic tree resulting in incomplete innervation territory [225]. Finally, several specific genes involved in Ca2+ homeostasis have been shown to be sequentially downregulated in ATXN1(82Q) Purkinje cells, with IP-R1 and the Sarco-Endoplasmic Reticulum Ca2+-ATPase 2 (SERCA2) affected first, followed by TRPC3 channels [148]. A similar downregulation has also been found in SCA1 human cerebellum. Moreover, changes in the dendritic expression of the Ca2+-binding proteins parvalbumin (PV) and calbindin D-28k (CaB) were observed [223,226]. Lastly, in order to assess the role of PV and CaB in the pathogenesis of SCA1, double mutant animals were generated by crossing CaB KO with ATXN1(82Q) mice, leading to an increased disease phenotype [227]. Although indirectly, these findings strengthen the suggestion that the downregulation of proteins implicated in Ca2+ homeostasis and Ca2+ signaling in Purkinje cells can be an important component in the etiology of SCA1. Further understanding of Ca2+ signaling mechanisms could offer clues to the design of novel and effective therapeutic strategies for treatment of spinocerebellar ataxias.

10. Mutations in the GMRI Gene

mGluR1, encoded by GRM1 gene, is one of the most abundant of its group of receptors in the mammalian central nervous system and is expressed at particularly high levels in Purkinje cells. Disease caused by mutations in GRM1 gene are extremely rare [228]. To date, the only GRM1 mutations identified have been found to cause an autosomal recessive spinocerebellar ataxia (SCAR13; [229]). Recently, Watson and colleagues reported heterozygous dominant mutations in GRM1 gene that are associated with distinct disease phenotypes: gain-of-function point mutations that lead to enhanced receptor activity causing an adult-onset cerebellar ataxia and a truncation
mutation that result in a dominant-negative effect causing a juvenile-onset cerebellar ataxia characterized by cognitive impairment (SCA44; Table 1) [230]. mGluR1 loss of function has been revealed in some animal models of human cerebellar ataxia, such as SCA1 and SCA3 transgenic mice. In ATXN1(82Q) mice, it has been shown that the progressive loss of mGluR1 function is restored by the GABAs receptor agonist baclofen leading to rescue the motor coordination [223,231–233]. In SCA3 Purkinje cells, mGluR1 have been found to be mislocalized to non-synaptic site resulting in a disruption of mGluR1 signaling [234]. On other hand, mutations that causes a mGluR1 gain of function have been associated with SCA. For example, in SCA2-127Q transgenic mice, the significant increase in Ca²⁺ release from ER via IP3R1 caused by an amplification of mGluR1 signaling is prevented by buffering basal Ca²⁺ concentration at normal resting levels [20,235,236]. In conclusion, functional alteration of mGluR1 activity has been documented in different SCA mouse model, while, in human patients, several forms of cerebellar ataxia result from mutations in genes in this pathway, which suggests that disruption of mGluR1 signaling and downstream Ca²⁺ homeostasis form a common pathological mechanism underlying SCAs [21].

11. Prospects for Therapeutic Development

Currently, there is no preventive or curative treatment for SCAs, but over the last few years, several drugs and therapeutic strategies that potentially slow the disease progression are being tested. The therapeutical approaches can be divided into pharmacological and gene therapies that target the toxic downstream effect and stem cell replacement (neurotransplantation). Since disrupted Purkinje cell Ca²⁺ signaling has been identified as a common underlying mechanism in most of SCAs (Table 2), one pharmacological approach has been to propose that Ca²⁺ blockers and stabilizers have a potential utility for patients diagnosed with SCAs.

| SCA     | Gene     | Protein                                      | Effect on Ca²⁺ Signaling |
|---------|----------|----------------------------------------------|--------------------------|
| SCA1    | ATXN1    | Ataxin-1                                     | Decrease                 |
| SCA2    | ATXN2    | Ataxin-2                                     | Increase                 |
| SCA3    | ATXN3    | Ataxin-3                                     | Increase                 |
| SCA6    | CACNA1A  | Ca²⁺-voltage-gated channel subunit α1A       | Decrease                 |
| SCA14   | PRKCG    | PKCγ                                         | Increase/Decrease        |
| SCA15/16| ITTPR1   | IP₃ receptor                                 | Increase/Decrease        |
| SCA29   | ITTPR1   | IP₃ receptor                                 | Decrease                 |
| SCA41   | TRPC3    | TRPC3 channel                                | Increase                 |
| SCA42   | CACNA1G  | Ca²⁺ voltage-gated channel subunit α1G       | Decrease                 |
| SCA44   | GMR1     | mGlu receptor 1                              | Increase                 |

Long-term feeding with intracellular dantrolene (Ca²⁺ stabilizer) resulted in improved motor performance and decreased Purkinje cell loss in mouse models of SCA2 and SCA3 [20,204,237]. Similar effects were demonstrated for a novel Itpr1 inhibitor T-558 [238]. Another interesting pharmacological approach, with some preclinical success, has been the use of caffeine. In a mouse model of SCA3 administration of caffeine through the drinking water alleviated motor deficits [239]. Finally, the treatment with glutamate release inhibitor, riluzole, in a randomized placebo-controlled clinical trial in 40 SCA patients for 12 months (SCA1, 2, SCA6), improved ataxic symptoms [240].
Gene therapy has made great progress over the past decade, especially in the cases of the polyglutamine (polyQ)-repeat SCAs. Currently, the most encouraging and innovative are RNA-targeting therapies including RNA interference (RNAi) or antisense oligonucleotides (AONs), which have been validated in different mouse models [241]. RNAi is an evolutionarily process of post-transcriptional gene suppression initiated by double-stranded RNA sequences to reduce mRNA expression. RNAi can be induced by microRNAs (miRNA) or small hairpin RNA (shRNA). By causing a severe degradation of mRNA, miRNAs and shRNA are considered potent tools to achieve gene silencing. A recent study found that miRNA expression in the human cerebellum was downregulated in aging brain resulting in an increase of ATXN1 gene expression. Thus, the activation of miRNA in SCA1 brain could serve to reduce the cytotoxic effect of CAG expanded ATXN1 gene [242]. The potential use of shRNA as a therapeutic candidate in a mouse model of SCA1 was published in 2004 [243]. Intracerebellar stereotoxic injections of recombinant AAV expressing shRNA improved motor function, rescued cerebellar morphology and decreased Atxn1 inclusions in Purkinje cells. Finally, AONs are relatively short, single-stranded DNA/RNA sequences that can bind the target mRNA through Watson–Crick base pairing. This binding arrests translation and decreases synthesis of the encoded protein. SCA2 mice treated with intrathecal AONs injections displayed a reduced cerebellar ATXN2 expression associated with improvements in motor functions. Interestingly, Purkinje cell firing frequency returned to normal levels [244]. AONs have also been administered to SCA3 mice and were shown to sustain reduction of mutated Atxn3 and to rescue motor impairment. A correlation was also found with a recovery of defects in Purkinje cell firing frequency [245]. In recent years, stem cell-based clinical trials have taken the emerging field offering significant potential to deliver new treatments for SCAs. The first preclinical experiment into SCA1 mouse model has been published by Kaemmerer and Low (1999). They found that transplantation of embryonic cerebellar cells led to improvement in motor skills and enhancement in Purkinje cell survival [246]. Ten years later Chintawar and his colleagues [247] transplanted neural precursor cells derived from adult mice into SCA1 mouse cerebellum. These animals functioned better on behavioral tests and displayed more surviving Purkinje cells. Another study demonstrated that cerebellar neural stem cells transplanted into cerebellum of adult SCA3 mice alleviated motor impairments [248]. In SCA2 mouse model, the intravenous injection of human mesenchymal stem cells ameliorated the survival of Purkinje cells and motor performance and delayed the onset of disease [249]. Somatic cells that can be reprogrammed into iPSCs represent the last frontier within the stem cell-based therapy [250,251]. Currently, patient-derived SCA iPSCs are tested for drug screening and disease modelling [243,252,253]. First stem cell-based clinical trials in humans with cerebellar degeneration have already tested [254,255]. The findings support cerebellar transplantation as a promising therapy but intensive preclinical research is still necessary.

12. Conclusions

A wide variety of mutations causing ataxia reveals the central role of cerebellar Purkinje cells in the pathogenesis of motor dysfunctions in SCAs. Almost all ionic channels or receptors expressed by Purkinje cells involved in Ca²⁺ signaling or homeostasis, when mutated, cause ataxic symptoms but, currently, how their functions are altered in disease remain to be determined. Accumulating lines of evidence suggest that multiple cellular pathways are disrupted by majority of these mutated proteins in Purkinje cells [256], but the distinctive SCA pathogenesis does not begin until Ca²⁺ signaling pathways are deranged either as a result of an toxic increase or a suppression of compensatory mechanisms likely leading to the characteristic Purkinje cells loss, cerebellar atrophy, and ataxia. Additionally, alterations of any synaptic input to the Purkinje cells also cause ataxic symptoms. In the case of the parallel fiber synapse, the reduced number of contacts between parallel fiber and Purkinje cell, the extended innervation of the climbing fiber invading the parallel fiber territory and the LTD impairment are sufficient to cause motor symptoms related to ataxia, as in GluD2 mutant mice. In contrast, even a slight maturation disturbance of the climbing fiber–Purkinje cell synapse can cause acute motor deficits suggesting that the refinement of the CF projection causes more severe
ataxia than the loss of cerebellar cortex output. Notably, extracellular Ca\(^{2+}\) influx through Ca(v)2.1 is involved in development, functional differentiation and maturation of climbing fiber synapses. Studies on ataxic mutant mice have also resulted in the identification of other molecules involved in the phase of climbing fiber synapse elimination, such as PKC\(\gamma\) and its downstream signal transduction pathway, as well as A\(\text{tau}\)1. These mechanisms mentioned above could act independently or, more likely, interact and synergize with each other, triggering the accumulation of Purkinje cell damage that eventually leads to cerebellar dysfunction. This suggests that simultaneous targeting of several pathways might be therapeutically necessary to prevent neurodegeneration and preserve neuronal function [256].

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