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New Approaches in Studies of the Molecular Pathogenesis of Type 2 Spinocerebellar Ataxia

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Type 2 spinocerebellar ataxia (SCA2) is an inherited progressive disease whose cause at the genetic level is an expansion of the polyglutamine tract in ataxin-2 protein. Effective treatment and disease-modifying therapy remain unavailable to patients with SCA2. Patients are currently given only symptomatic treatment, along with palliative medical care. With the aim of seeking new therapeutic targets for treatment of SCA2, many scientific groups have tried to study the physiological, molecular, and biochemical changes to cerebellar neurons in patients with SCA2 and in various model systems. State-of-the-art approaches to studies of the pathogenesis of SCA2 have yielded new data on the molecular mechanisms of the disease and have suggested possible strategies for the potential treatment of this disease. The present review summarizes current data on the genetic basis of SCA2, describes the known properties and functions of ataxin-2 protein, considers the mechanisms of degeneration of cerebellar cortex cells, impairments to their physiological function, and associated damage to the conducting pathways of the cerebellum, and presents data on contemporary model systems used for studies of the basis of SCA2; we also present information on novel approaches to studies of the molecular mechanisms underlying the pathology of SCA2 such as aggregation, oxidative stress, and damage to cell signaling and calcium signaling, and consider the role of autophagy and the microglia in the molecular pathogenesis of SCA2.

Keywords: type 2 spinocerebellar ataxia, polyglutamine tract diseases, cerebellum, calcium signaling, aggregation.

Type 2 spinocerebellar ataxia (SCA2) is a genetic disease with autosomal dominant inheritance and is due to expansion of CAG triplets in the ubiquitously expressed gene *ATXN2*, which encodes ataxin-2 protein with an extended polyglutamine tract [1–5]. The pathophysiological signs of SCA2 include progressive cerebellar atrophy and subsequent gradually increasing ataxia [2]. There is as yet no disease-modifying therapy for SCA2, such that SCA2 patients are supported exclusively by symptomatic therapy and palliative medical approaches. Ataxin-2 protein, the protein produced by the gene mutated in SCA2, has various functions within cells, including an involvement in RNA metabolism [6]. A number of model systems in cells, yeasts, worms, flies, mice, and neurons from induced pluripotential stem cells (iPSC) from SCA2 patients have been developed to study the bases of the pathology of SCA2. In SCA2 in mice and patients, the primary damage is to Purkinje cells (PC) in the cerebellar cortex and the conducting tracts of the cerebellum. Studies in these model systems have demonstrated damage to the morphology, biochemistry, and neurophysiology of PC and the conducting tracts of the cerebellum [7, 8], which has helped produce new conclusions on possible molecular mechanisms of the disease. Experimental data indicate that the molecular pathogenesis of SCA2 involves such different mechanisms as aggregation, oxidative stress, impairments to cell signaling, dysregulation of calcium homeostasis, anomalous autophagy, and incorrect DNA processing [9–16]. Current approaches to studies of the pathogenesis of SCA2 have led to the development of various potential therapeutic strategies, which will probably be used in future for the effective treatment of SCA2 patients.

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Promising data have already been obtained in preclinical studies of antisense therapy [17, 18] and early clinical trials of iPSC [19] and riluzole [20]. Disease-modifying therapy using calcium stabilizers has yielded promising results in preclinical studies on transgenic SCA2 mice [21–23].

Mutations in the *ATXN2* Gene. Genetically, SCA2 is due to expansion of CAG triplets in the coding region of the *ATXN2* gene, which is located in chromosome 12q24.1 [24]. The pathology of SCA2 is apparent in patients with 33 or more CAG repeats [25]. With dominant inheritance, the expansion of CAG repeats acts as a function-acquisition mutation [26]. Overall, the instability of the SCA2 mutation in the *ATXN2* locus between generations depends on the length of the expansion of CAG repeats, sex, and the age of the mutation-carrying parent at conception [27, 28].

Ataxin-2 is a cytoplasmic protein of molecular weight 140 kDa produced by the *ATXN2* gene. The protein is located in the rough endoplasmic reticulum (EPR) [29] and also on other cell components, including the Golgi apparatus, stress granules, inclusion bodies, and the cytoplasm itself. Ataxin-2 insufficiency leads to obesity, impairments to lipid metabolism, insulin resistance [30, 31], and also to impairments to the circadian system in mice [32] and fruit flies [33, 34]. Studies of ataxin-2 with an extended polyglutamine tract using staining for cytoplasmic aggregates and analysis of the frequency of neuronal inclusions in the brainstem of SCA2 patients demonstrated aggregation of mutant ataxin-2 in the tissues of these patients [35]. The protein structure of ataxin-2 contains a PAM2 motif, which takes part in the association with poly(A)-binding protein (PABP) [36], which probably leads to association of ataxin-2 with polyribosomes [6]. Ataxin-2 also contains two globular Lsm domains and one Lsm-associated domain (Lsm-AD), which have been shown to interact directly with RNA [6], such that ataxin-2 is most likely involved in in RNA metabolism. In fact, in vitro studies on neurons and mouse embryo fibroblasts have shown increases in ataxin-2 expression in response to lack of nutrients, depending on mammalian target of rapamycin (mTOR) signaling. Lack of ataxin-2 led to adaptive changes in phosphoinositide-3-kinase (PI3K)/mTOR-mediated phosphorylation of ribosomal protein S6 and protein 1, which binds eukaryotic translation initiation factor 4E (4E-BP1) as regulatory components of the 48S mRNA translation initiation complex [37].

Another study showed that the intracellular ataxin-2 level affects the formation of stress granules (SG) and P bodies, thus regulating mRNA metabolism, including translation, stability, and degradation [38]. The Lsm/Lsm-AD domain of ataxin-2 has been shown to interact with DEAD/H-Box RNA helicase DDX6, which is involved in the formation of SG and P bodies. Decreases in ataxin-2 levels led to anomalous assembly of SG and increases in PABP levels, PABP being another component of SG [38]. Recent studies showed that disordered domains in ataxin-2 lead to formation of granules containing mRNA and RNA-binding proteins, promote impairments to long-term synaptic plasticity, and produce cytotoxicity [39].

Recent investigations have shown that Pbp1 protein, the yeast ortholog of human ataxin-2, regulates retrotransposon activity, as changes in its expression suppressed retrotransposon TY1 by defined mechanisms [40]. Whole-transcriptome analysis of blood from SCA2 patients showed that ataxin-2 is a cytosolic RNA processing factor and identified its role in regulating transcription on formation of the response to mitochondrial stress, including mitochondrial quality control transcription factor PINK1 [41]. These physiological functions of ataxin-2 are probably impaired in SCA2 pathology, such that increases in the concentration of ataxin-2 with the normal number of CAG repeats might have potential therapeutic effects in SCA2.

Studies on post mortem brain tissues from SCA2 patients, SCA2 fibroblasts, SCA2 PSC, and in mouse models of SCA2 have shown that the *ATXN2* locus is transcribed bidirectionally, the antisense transcript *ATXN2-AS* with expansion of CUG repeats displaying neurotoxic activity in the cellular model of SCA2 and also forming RNA aggregates in SCA2 PC [42]. Thus, the *ATXN2-AS* antisense transcript may be involved in a supplementary mechanism of pathogenesis of SCA2 and can be regarded as a novel therapeutic target in SCA2.

The question of whether the ataxin-2 mutation in SCA2 is a mutation of acquired function or a mutation of loss of function is controversial. Many studies have shown that loss of ataxin-2 leads to impairments to mRNA metabolism [38], indirectly provoking derangement to the pathways of ribosome biogenesis, translation initiation, EPR secretion, and lipid metabolism [43], producing pathological changes to the processes of nutrient assimilation and metabolism [44], and having negative actions on the expression of the genes regulating calcium balance [45]. Thus, physiological decreases in ataxin-2 levels due to mutations in the *ATXN2* gene and subsequent processing of the mutant protein with impaired functions can in fact be regarded as mutations leading to loss of function. However, it should be noted that the appearance of functions of mutant ataxin-2 as an acquisition-of-function mutant is also significant. The detection of aggregates of mutant ataxin-2 in cerebellar tissue from SCA2 patients [35], along with results from experiments on planar lipid bilayers showing that ataxin-2 with an increased number of CAG repeats binds the inositol-1,4,5-triphosphate receptor (IP3R) and significantly increases its activation by IP3 molecules [21], is evidence that the mutation responsible for SCA2 leads to acquisition of a novel pathological function. It can also be suggested that the truth of the type of mutation is somewhere in the middle: while SCA2 shows loss of the normal physiological function of ataxin-2, mutant ataxin-2 with an increased number of CAG repeats has cytotoxic properties.

Molecular Changes Leading to Loss of PC. At the cellular level, patients with SCA2 primarily display damage

to PC. Post mortem studies of SCA2 patients contain heterotopic PC whose cell bodies are displaced towards the molecular layer of the cerebellum [46].

A recent analysis of 20 families with SCA using a complex approach including whole-exome sequencing, targeted resequencing, and analysis of genetic networks identified five new genes associated with SCA2 and involved in processes such as central nervous system (CNS) development, axon guidance, regulation of transcription, control of mitochondrial functions, autophagy, and synaptic transmission [47]. Impairments to these important physiological processes may explain the degeneration of cerebellar cortex PC in the pathology of SCA2, and we will discuss these in the section addressing the molecular mechanisms of SCA2.

Analysis of published of data indicates that there is increased sensitivity to the disrupting actions of reactive oxygen species during the growth and development of the cerebellum. Induced oxidative stress produces structural changes to PC, their dendrites, granule cells, astrocytes, microglia, axons, and oligodendroglia in the cerebellum [48]. Cerebellar changes typical of SCA2 patients, due to oxidative stress, can be induced by impairments to the homeostasis of different elements in patients' blood and serum [49, 50]. Published data indicate that the blood of SCA2 patients undergoes changes in copper, manganese, zinc, and vanadium levels [49]. SCA2 patients not displaying a significant clinical phenotype showed significant decreases in superoxide dismutase (SOD3) enzyme activity [51]. Recent studies identified a link between a polymorphism in ω-2-glutathione-S-transferase and SCA2 [52]. Another polymorphism, A10398G, in mitochondrial DNA, is involved in deterioration of cognitive functions at the initial stages of SCA2 [53]. Blood samples from SCA2 patients also showed significant increases in the total 5-hydroxymethylcytosine level, thus identifying total DNA methylation as a biomarker for this disease [54]. It has also been reported that serum neurofilament light is another promising peripheral biomarker for SCA2 [55].

SCA2 Model Systems. Various model systems have been developed to study the bases of SCA2. A number of models of the disease have been developed in yeasts, worms, flies, and mice for studies of the molecular mechanisms of SCA2 and testing potential therapeutic strategies. The first models of SCA2 developed were simple and were used for modeling of the disease in yeasts, *C. elegans* worms, and *D. melanogaster* fruit flies. These modes were used to identify the main functions of ataxin-2, though the subsequent creation of mouse models of SCA2 provided investigators with the opportunity to test therapeutic strategies for subsequent potential introduction into clinical practice [7].

Among the mouse models of SCA2, the first was the transgenic strain SCA2-58Q. These mice displayed PC-specific expression of the full-size human *Atxn2* gene with 58 CAG repeats under control of the Purkinje cell protein 2 (Pcp2) gene promoter [56]. These models are characterized by progressive degradation of movement coordination, apparent from age 32 weeks, as demonstrated by tests for motor activity. Methods for assessing motor activity included the "bar walking" method and the rotarod test and demonstrated an increase in the duration of walking on the bar and an increase in the number of times the paws slipped in SCA2 mice compared with wild-type animals of the same age [21]. At age 24 weeks, these mice showed a significant decrease in the number of PC in the cerebellar cortex, along with progressive loss of calbindin-28K, which is a protein marker for assessment of neuron dysfunction [21]. These mice were also characterized by impairment to electrophysiological functions detected in cerebellar slices [22, 57] and in in vivo studies [58, 59].

Mice of another model of SCA2, the strain SCA2-75Q, express the full-size human *Atxn2* gene under control of the endogenous SCA2 promoter. Despite the fact that expression of the transgene was seen in all organs and tissue in the mice, neuropathological analysis showed that only cerebellar cortex PC underwent degeneration. Methods for assessment of motor activity, i.e., the rotarod test, demonstrated that significant deterioration of motor function occurs at age 12 weeks in heterozygous SCA2-75Q mice and six weeks in homozygous animals, as compared with wild-type mice of the same ages [60].

Thus far, data have been published on only one knock-in (KI) mouse model of SCA2 [61]. In mice of this strain – SCA-42KI – an expansion of 42 CAG repeats was inserted into the mouse *Atxn2* gene. Mice with the SCA2-42KI phenotype were characterized by decreased body weight and minor deterioration of motor functions. Statistically significant differences in movement coordination in the rotarod test was seen for homozygous mice at age 72 weeks, while heterozygous mice showed no differences at all ages tested as compared with wild-type mice of the same ages. Neuropathological analysis also identified minor and late-onset changes corresponding to the results of assessments of motor activity [61].

Another mouse model of SCA2, the transgenic strain SCA2-127Q, has 127 CAG repeats under control of the Pcp2 promoter [62]. These mice display much more severe symptoms of pathology because of the longer polyglutamine expansion. Thus, the presence of ataxin-2 aggregates in PC cell bodies was seen as early as age four weeks, after which statistically significant differences in the electrophysiological properties of PC appeared at age six weeks and, finally, progressive impairment to motor functions in the rotarod test became significant at age eight weeks. Impairments to biochemical, electrophysiological, and motor functions gradually deteriorated during the period from week 8 to week 40, though loss of PC was seen in this model only from age 40 weeks [62]. Analysis of weighted gene coexpression networks demonstrated early and progressive impairments to the cellular expression of genes, especially those genes associated with the GTPase signal, calcium signaling, and cell death; temporary changes in mRNA expression, starting

from day 1, showed that gene up-regulation was linked with histone acetylation and remodeling of chromatin, while gene down-regulation was linked with adhesion of cells and components of the extracellular matrix [63].

The most recent transgenic mouse model of SCA2, i.e., the strain BAC-72Q, was created using a bacterial artificial chromosome (BAC) including the whole *Atxn2* gene in which exon 1 contained an insert with 72 CAG repeats under control of the endogenous human promoter [64]. BAC-SCA2-72Q mice are characterized by weight loss and progressive deterioration of motor coordination in the rotarod test, with onset from age 16 weeks, along with thinning of the dendritic trees of PC and decreases in the level of expression of calbindin and Pcp2 proteins in the cerebellum [64].

In discussing studies using mouse models of SCA2, attention must be drawn to studies using mice with knockout (KO) of the ataxin-2 gene. The first mutant mice with ataxin-2 knockout were obtained by electroporation of whole constructs into embryonic stem cells [30]. Morphological analysis of these KO mice revealed no significant lesions, though adult individuals were obese. The authors of this study came to the conclusion that ataxin-2 protein is not required for development and survival in mice [30]. However, despite the fact that the morphology and overall wellbeing of KO mice were not significantly altered, they showed impairments to the biochemical and cellular machinery. Thus, ataxin-2 KO mice showed increased phosphorylation of ribosomal protein S6, as well as pathological changes to overall protein synthesis [43]. Recent studies have also shown that KO mice are characterized by impairments to the metabolic pathways for fatty acids, the amino acids leucine, valine, and isoleucine [44], and calcium homeostasis [45].

Mouse models of SCA2 using various transgenic strains provide convenient tools for basic research into the biochemical, electrophysiological, and especially motor changes seen in SCA2 pathology, though even despite the fact that most mouse strains contain the human ataxin-2 gene, mice are significantly different from humans, such that therapeutic approaches with promising results in mouse experiments often fail in clinical trials in humans. Recent progress in pluripotent cell reprogramming technology has allowed researchers to obtain disease-specific iPSC from SCA2 patients. These cells can be differentiated into neurons, thus providing the opportunity to study the mechanisms of SCA2 disease and the pathological development of disease in vitro on neurons obtained from patients [7, 65]. iPSC cell lines were obtained successfully from human skin fibroblasts from SCA2 patients [66] or from peripheral mononuclear blood cells [67], or from both these sources [65]. Neurons produced from SCA2 iPSC showed signs of the SCA2-associated pathological phenotype in vitro, such as aggregation of proteins with expanded polyglutamine tracts, as well as lesions to mitochondrial microstructure [65].

Molecular Mechanisms of SCA2. The molecular mechanism of SCA2 has been discussed for years, and research groups have made significant contributions to our understanding of the pathology of SCA2; numerous different approaches have ben used in attempts to construct a clear picture of the molecular pathogenesis of SCA2. Only a clear understanding of the molecular bases of the disease can provide the opportunity to modify the pathological pathways, ultimately yielding a means of treating this disease.

Aggregation. Mutant ataxin-2 with an expanded polyglutamine tract undergoes conformational changes towards structures enriched with β-sheets, which promotes formation of insoluble aggregates with β-sheet-rich amyloid fibrillar structures, leading to the accumulation of aggregates in the form of inclusion bodies in neurons [9]. The role of these inclusions in degenerative processes in the cerebellum with SCA2 pathology remains unclear [68]. Studies of pathological aggregation in the brainstem of SCA2 patients has demonstrated a significant positive correlation between the presence of granular cytoplasmic staining and more severe pathological processes, while nuclear inclusions in neurons played a protective role [35]. Studies using 1C2-immunoreactive typing in SCA2 patients showed that the granular endoplasmic pattern was seen at the early stages of disease, while the cytoplasmic and nuclear patterns were seen in the active stage and the nuclear pattern with inclusions at the final stage [69]. Studies of the behavior of oligomers with expanded polyglutamine tracts in mouse brains showed that the presence of these oligomers led to disruption of EPR membranes via insertion of apoptosis-regulating protein Bax into EPR membranes, with subsequent activation of caspase-7 [70]. Prevention of aggregate formation and accumulation of incorrectly folded proteins with expanded polyglutamine tracts can be regarded as a promising therapeutic approach for developing disease-modifying therapy for polyglutamine tract diseases [9].

Oxidative stress. Studies of mitochondrial oxidative stress in fibroblasts from SCA2 patients showed elevated SOD expression, along with decreased expression of catalase at both the transcript and protein levels [71]. Elevated intracellular hydrogen peroxide levels produced from superoxide by the increased SOD cannot be utilized, because of the decreased catalase level, thus leading to an increase in oxidative stress, impairments to the antioxidant system, changes in the oxidative phosphorylation system, and damage to mitochondrial activity seen in fibroblasts from SCA2 patients. Addition of the antioxidant coenzyme Q10 to SCA2 fibroblasts normalized the increased oxidative stress [71]. Prolonged use of coenzyme Q10 in SCA2 patients led to improvements, which were confirmed on the Scale for the Assessment and Rating of Ataxia (SARA) and the Unified Huntington's Disease Rating Scale in a two-year crossover trial including SCA1 and SCA3 patients; improvements were not statistically significant in SCA2 patients, probably due to the small size of the study group [72]. Thus, the cellular antioxidant system may be involved in the pathogenesis of SCA2 and its regulation may be a potential therapeutic target.

Impairment to cell signaling. The process of information transfer between cells is exclusively important for normal development, neurogenesis, repair, homeostasis, immune responses, and other important cell processes. Impairment to cell signaling can lead to various pathological processes and neurodegeneration. Members of the Src family of non-receptor tyrosine kinases (SFK) are needed for maintaining the appropriate functioning of the nervous system and may be involved in neurodegenerative processes. Recent investigations have shown that MTSS1 protein, which is an inhibitor of SFK kinases, is involved in the pathology of SCA2 [73]. Studies on chimeric mice demonstrated that insufficiency of MTSS1 protein leads to increases in the enzyme activity of SFK kinases, which are accompanied by impairments to the electrophysiological activity of PC, ultimately leading to cell death. Treatment of MTSS1 mutant mice with the known antitumor drug dasatinib, an inhibitor of SFK kinases, prevented SFK-dependent impairments to the electrophysiological functions of PC and slowed the progression of ataxic symptoms in these mice. It is also interesting to note that SCA2 mice of the transgenic strain SCA2-127Q show decreased levels of MTSS1 protein and increases in the activity of SFK kinases [73].

Another kinase, protein kinase C (PKC), is extremely important for correct functioning of cerebellar cortical PC, as mutations in the gene encoding this enzyme lead to anomalous development of PC dendrites and cerebellar ataxia, namely SCA14 [74]. Studies in the SCA14 mouse model showed that up-regulation of genes encoding carbonic anhydrase-related protein 8 (CAR8) and IP3R1 occurs in SCA14 pathology [75]. Recent studies in SCA2 mice of the transgenic strain SCA2-127Q showed that increased phosphorylation of PKC substates operates as a protective modifier in PC degeneration, as normalization of PKC activity in these mice led to increases in degenerative processes [76]. Activation of PKC in PC is required for long-term depression (LTD) of synaptic transmission in parallel fiber (PF)-PC synapses; these studies also demonstrated that increased PKC activity may lead indirectly to a protective effect in PC degeneration by limiting the hyperexcitability of PC membranes [76].

Kinases are not the only participants involved in neuronal cell signaling and responsible for the correct functioning of the cellular apparatus; various transcription factors also play important roles in controlling cell responses. Nuclear transcription factor NF-κB has a part to play in controlling DNA transcription, cytokine production, cell survival, and the processes of synaptic plasticity and memory [77]. Recent studies have shown that NF-κB signaling is required for increasing the quantity of microglia and for producing tumor necrosis factor TNF-α in the cerebellum of SCA1 mice; it is also involved in processing synapses during the development of the cerebellum [78]. Experiments in an SCA3 mouse model showed that NF-κB is an enhancer of degeneration, mediating astrocyte activation and leading to neurodegeneration, while specific inhibition of NF-κB in astrocytes promoted improvements in survival and increased longevity [79]. Despite the fact that no data have as yet been published on the involvement of NF-κB in the pathogenesis of SCA2, analysis of weighted gene coexpression networks in transgenic mouse SCA2 model strain SCA2-127Q revealed down-regulation of the NF-κB pathway in these mice [63].

Calcium dysregulation. Calcium ions are a required element in cell signaling and, because of their allosteric effect, are able to control the activity of many enzymes and regulatory proteins [80]. Calcium is able to activate calcium-dependent potassium channels, influencing the shape and frequency of action potentials (AP) and changes in the calcium concentration can control such diverse physiological processes as learning, memory, and behavior [80]. High cytoplasmic calcium levels induce apoptosis [11, 14, 81]. Impaired neuronal calcium signaling can lead to changes in the intracellular balance, loss of synapses, impairment to synaptic function, and, ultimately, cell death. Calcium dysregulation is seen in models of Huntington's disease (HD), various types of SCA, and Alzheimer's disease [11–14].

Calcium signaling plays a special role in the functioning of cerebellar cortex PC, as PC express enormous numbers of different calcium-dependent proteins and enzymes to maintain the intracellular calcium balance. For example, cerebellar cortex PC have high concentrations of calbindin D-28k (CB) and parvalbumin (PV) in their axons, bodies, dendrites, and spines. These proteins belong to the large EF-hand family of calcium-binding proteins (CaBPs) [82] and their loss leads to changes in the function of $Ca_v2.1$ ion channels (P/Q-type voltage-gated calcium channels (VDCC)) encoded by the *CACNA1A* gene [83].

Regulation of calcium influx into PC via VDCC channels is extremely important for the correct formation of climbing fiber (CF)-PC synapses during postnatal development [84]. Impairments to CF-PC synapses contribute to neuronal pathology in SCA [85]. PC also express large quantities of calmodulin-binding transcription activator 1 (CAMTA1), and deletion of the *CAMTA1* gene in mice led to severe ataxia, accompanied by degeneration of PC and cerebellar atrophy [86]. LTD of PF on PC is believed to be the main basis of motor learning. PC express calcium/calmodulin-dependent protein kinase II (CaMKII), activation of which leads to prolonged increases in cyclic guanosine monophosphate levels, thus inducing the LTD signal mechanism indirectly mediated by CaMKII molecules [87].

Within cells, calcium is stored in the EPR and mitochondria. Mitochondria division and mitochondrial transport have been shown to be involved in the processes underlying the development of dendrite arborization in cerebellar cortex PC [88]. Mutations in mitochondrial AFG3L2 protease, part of the mitochondrial proteome, induce SCA28 [89].

There are two main pathways by which calcium enters the PC cytoplasm. Both require the presence of glutamate,

an excitatory neurotransmitter. The first pathway consists of calcium influx from the extracellular space via VDCC channels [90]. Activation of these channels occurs as a result of membrane depolarization, and this in turn induces positive modulation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor by glutamate molecules released from presynaptic terminals of PF and CF into the synaptic cleft. Activation of VDCC channels leads to release of calcium within cells, which in turn leads to activation of calcium-dependent potassium channels and has fundamental importance for forming dendritic potentials formed by calcium channels [91]. The lack of NMDA receptors and calcium-permeable AMPA receptors makes PC a special case among GABAergic neurons [92]. The second pathway consists of activation of metabotropic glutamate receptors (mGluR) [93], which leads to release of calcium from the EPR via activated IP3R receptors; this calcium release is termed IP3-induced calcium release (IICR). Activation of mGluR or another G-protein-coupled receptor leads to activation of phospholipase C (PLC) on the cell membrane, and this catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and IP3, which are second messengers and are involved in signal transmission processes and lipid signaling in cells of different types. DAG is bound to the membrane, while soluble IP3 is released into the cytosol. Furthermore, IP3 binds IP3R, leading to IICR, thus increasing the intracellular calcium level and triggering a cascade of signal changes and affecting the activity of a variety of proteins and enzymes [80].

Activation of mGluR1 leads to DAG-mediated positive modulation of the canonical transient receptor potential 3 (TRPC3), which is a nonselective cation channel involved in forming slow excitatory postsynaptic currents (EPSC). Loss of TRPC3-mediated slow EPSC led to cerebellar ataxia induced by dysregulation of mGluR [94]. Transcription factor RORα has been shown to be involved in expressing mGluR1 [95] and also mediates up-regulation of the expression of many genes associated with calcium-dependent signal pathways, including the *SLC1A6* gene, which encodes excitatory amino acid transporter 4 (EAAT4), the *ITPR1* gene, which encodes IP3R1, and the *PCP2/L7* gene, which encodes Pcp2/L7 protein [96]. To achieve this up-regulation, the RORα factor interacts with ataxin-1 protein, though mutant ataxin-1 with expanded polyglutamine tract is unable to form the transcription complex with RORα, so SCA1 transgenic mice show impairments to the expression of genes important in regulating calcium balance, which leads to neurodegeneration [97].

Studies of the cerebellar transcriptome of mice with ataxin-2 KO and mice of the SCA2-42KI strain showed that these mice displayed similar impairments to the regulation of various factors in calcium balance, along with many factors involved in RNA processing, bioenergetic processes, cell adhesion and growth, and lipid signaling [45]. In particular, decreases were seen in the levels of mRNA species encoding important proteins controlling calcium balance, such as sarco/EPR calcium ATPase (SERCA), IP3 phosphatase (5PP), IP3R, and transcription factor RORα [45].

Experiments on planar lipid bilayers with recording of electrophysiological activity from single IP3R1 channels coexpressed with mutant ataxin-2 with an expanded polyglutamine tract demonstrated that mutant ataxin-2 bound the C-terminal domain of IP3R1 (Fig. 1), while wild-type ataxin-2 did not show such properties. The presence of mutant ataxin-2 significantly increased activation of IP3R by IP3 molecules (Fig. 1) [21]. Further experiments using calcium imaging on primary PC cultures from the transgenic SCA2-58Q mouse model of SCA2 and wild-type mice identified a significant increase in IP3R1-mediated IICR in SCA2-58Q PC but not in wild-type PC. Experiments in which ryanodine and danthrolene were added to PC cultures were performed with the aim of producing therapeutic reductions in IICR by inhibition of ryanodipine receptors (RyR), a further type of intracellular calcium channel (Fig. 1). Use of these substances showed the pathological effect of the presence of mutant ataxin-2 by reverting the IICR level to the wild-type level. It was suggested that danthrolene and ryanodine corrected impairments to calcium signaling by decreasing calcium release from the EPR [21]. Long-term feeding of SCA2-58Q mice with danthrolene significantly improved motor activity and decreased PC degeneration in these mice [21].

Further experiments seeking to decrease IP3 levels in PC in transgenic SCA2-58Q mice showed that virus-mediated expression of 5PP produced chronic suppression of IICR (Fig. 1) [22]. Patch clamp experiments on cerebellar slices demonstrated improvements in anomalous AP generation typical of PC in ageing SCA2 mice. Assessment of motor coordination and subsequent neuropathological analysis showed that chronic overexpression of 5PP protein improved impairments to motor functions and prevented PC death in SCA2-58Q mice [22]. These results support the hypothesis that impairments to calcium signaling have an important role in the molecular pathogenesis of SCA2. Thus, partial suppression of IICR may have a therapeutic effect in SCA2 patients and, perhaps, patients with other types of SCA [11, 22].

Recent experiments on cerebellar slices from SCA2-127Q mice using a combined method of local current clamping and two-photon calcium imaging showed that SCA2-127Q mice had a significant increase in the somatic calcium level along with an increased AP generation frequency. Synchronous activation of mGluR1 by electrical stimulation of PF identified a significant increase in slow excitatory postsynaptic potentials (EPSP) and a significantly greater intracellular release of calcium in SCA2-127Q PC as compared with PC from wild-type mice [98]. These mice also showed an increase in mGluR1 signaling. The authors of this study came to the conclusion that SCA2 pathology is based on a positive feedback mechanism, including an interaction between an increased calcium level, mGluR1 receptors, and TRPC3, as well as overall IICR (Fig. 1) [98].

Recent studies on neurons from iPSC from SCA2 patients showed that changes in the expression of genes for glutamate receptors and impairments to calcium signaling occurred in conditions of increased glutamate concentrations. Addition of riluzole, the NMDA receptor antagonist dizocilpine, the AMPA receptor antagonist NBQX, or the calcium stabilizer danthrolene (a blocker of ryanodine receptors, which are EPR calcium channels) to SCA2 and PSC-derived neurons decreased cell death and improved mitochondrial dysfunction, thus supporting the hypothesis that glutamate excitotoxicity is involved in the molecular pathology of SCA2 [65].

Changes in spontaneous generation of AP by PC seen in various mouse models of SCA2 [22, 58, 59] can also be explained in terms of changes in calcium- and mGluR1-mediated signaling. Low-conductivity calcium-activated potassium channels (SK channels) are responsible for the pacemaker activity of PC and, thus, the decreased spike generation by SCA2 PC perhaps occurs on the principle of a constantly elevated intracellular calcium level [100]. It has also been suggested that a constantly elevated calcium level may lead to impairment to the expression and post-translation modulation of various ion channels required for the normal electrophysiological functions of PC [100].

Autophagy. Mutant proteins with expanded polyglutamine tracts tend to form aggregates and toxic inclusions, removal of which requires the mechanism of autophagy [15]. Autophagy is selective lysosome-mediated degradation during which ubiquitin-tagged accumulated and aggregated proteins are destroyed [101]. The proteins required for autophagy include microtubule-associated light chain 3 protein 1 (MAP1LC3, the *MAP1LC3* gene), p62 (the *SQSTM1* gene), and autophagy-associated proteins containing the FYVE domain (Alfy, the *WDFY3* gene) [102]. Analysis of peripheral autophagy markers in patients with polyglutamine tract diseases identified increased levels of expression of *MAP1LC3B*, *SQSTM1*, and *WDFY3* in HD patients, while SCA2 patients had elevated expression of the *WDFY1* gene only [103]. The author suggested that these differences could be explained on the basis of a significantly lesser quantity of aggregates in SCA2 than HD, which is also supported by the low incidence of nuclear inclusions or their absence in SCA2 mice [56].

Experiments on fibroblasts obtained from SCA2 patients and SCA2 model mice showed that the polyglutamine expansion in ataxin-2 also leads to anomalous autophagy, accompanied by elevated expression of staufen 1 protein (STAU1), which is a protein which binds double-stranded RNA and is required for the formation of cytoplasmic inclusions in neuroglia and cell cultures and is also involved in regulating the functions of stress granules (SG) and is co-located with ataxin-2 in SG-like structures [104]. Increased levels of STAU1 expression have been found to induce impairments to processing of RNA targets. And although there is a need for more detailed studies of the role of ataxin-2 in impairments to autophagy, it has nonetheless been shown that decreases in the STAU1 level led to improvements in motor coordination in SCA2 mice and can thus be regarded as a potential therapeutic approach to the treatment of SCA2 [104].

The role of the microglia. The role of the cerebral microglia in the pathogenesis of SCA2 has recently been addressed in an exhaustive review by Ferro et al. [105]. The microglia are a subtype of the neuroglia, consisting of CNS-resident macrophages. These cells have a direct role in the processes of development and aging; their main function is in forming immune responses and maintaining homeostasis, including utilization of cellular "rubbish," phagocytosis, and extracellular signaling. The development of the microglia is strictly regulated by specific transcription factors [106]. In turn, the microglia play an important role in the development of the cerebellum. Thus, they are involved in culling excess and nonfunctional CF-PC synapses during postnatal development, which is needed for the correct operation of the conducting pathways of the cerebellum in adult life [107]. Studies in SCA1 mice have shown that CF development is impaired in these mice [108]. At the early stages of its development, each PC is innervated by a multitude of CF, though with time a single LF enlarges and becomes dominant, retaining its synapses on the dendritic tree of PC. Upward displacement of the dominant CF terminal in SCA1-82Q-S776 mice through the dendritic tree of the PC was delayed in time and some CF terminals remained on PC bodies, while SCA1-30Q-D776 mice showed impaired culling of synapses, which led to much more severe sequelae [108].

Pathological activation of the microglia in the cerebellum has been seen in SCA patients and various mouse models of SCA. In some mouse models of SCA1, increases in the number of microglia and the TNF-α level were seen at the early stage of disease, before onset of neuron loss, this activation of the glia showing a clear correlation with pathological disease development; this suggests a possible mechanism of neurodegeneration based on neuroinflammation induced by the microglia [109]. Pharmacological inhibition of the colony-stimulating factor 1 receptor (CSF1R), the protein responsible for the development, differentiation, and functional maintenance of microglia, led to a decrease in the volume of the cerebellar microglia by 69% and improvement in motor function in SCA1 mice [110].

Neurophysiology of PC and Changes in the Functions of the Conducting Tracts of the Cerebellum in SCA2. The cerebellum is part of the brain with a fundamental role in regulating muscle activity and controlling motor functions. The conducting tracts of the cerebellum are key participants in movement coordination. PC are regarded as the main dynamic element of the cerebellum, as their axons form the only output running from the cerebellar cortex to the cerebellar nuclei and other deep brain structures (Fig. 2). PC spontaneously generate AP at constant frequency [111–118]. This tonic pacemaker

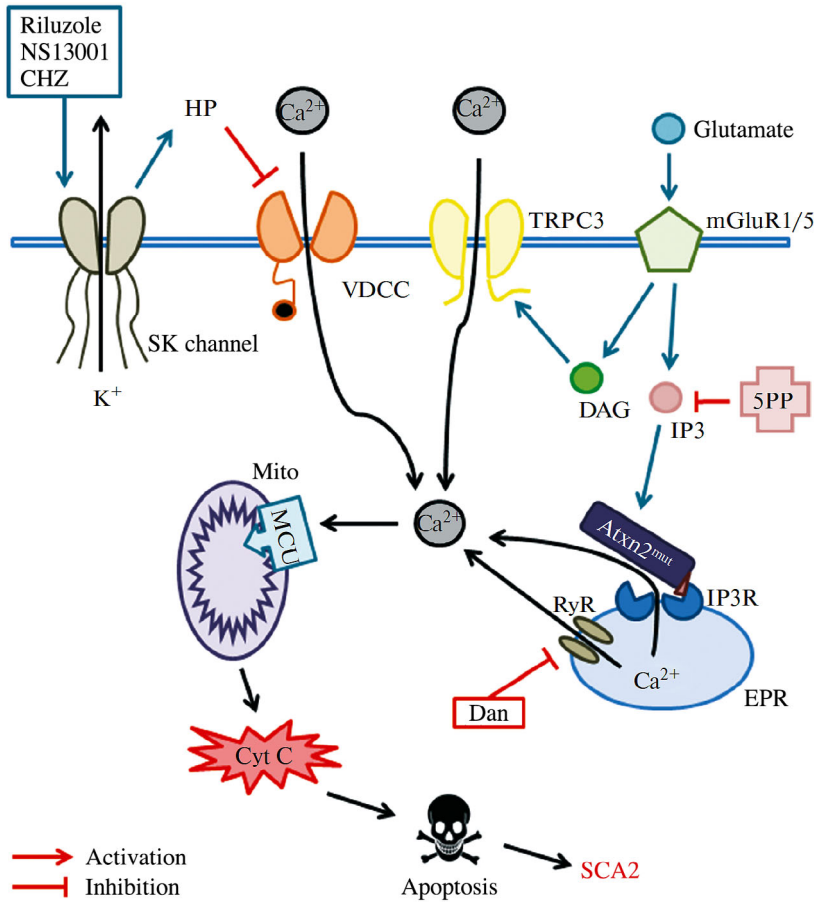

Fig. 1. The calcium hypothesis of the pathogenesis of SCA2. Glutamate molecules released into the synaptic cleft activate metabotropic glutamate receptors (mGluR), leading to release of inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG) molecules into the cytoplasm and further activation of IP3 receptors (IP3R) on the endoplasmic reticulum (EPR) membrane, promoting release of calcium from the EPR into the cytoplasm. This process is termed IP3-induced calcium release (IICR). DAG molecules directly activate transient receptor potential 3 (TRPC3), thus producing an additional influx of calcium ions from the extracellular space into the cytoplasm. Mutant ataxin-2 ($Atxn2^{mut}$) with an expanded polyglutamine tract, in contrast to wild-type ataxin-2, associates with IP3R and increases its sensitivity to IP3. Hyperactivation of IP3R leads to impairments to calcium signaling in Purkinje cells (PC) in the cerebellar cortex. Excess calcium ions are taken up by mitochondria (Mito) by means of the mitochondrial calcium uniporter (MCU), which leads to swelling of mitochondria and subsequent straightening of cristae, disruption of the external mitochondrial membrane, and further release of proapoptotic factors such as cytochrome C (Cyto C) into the cytoplasm, thus initiating apoptosis and consequent neurodegeneration. Excessive increases in IICR can be suppressed by virus-mediated expression of enzyme IP3,5-phosphatase (5PP), which converts IP3 into the inactive form IP2. Another means of decreasing calcium release from the EPR may be provided by inhibition of ryanodipine receptors (RyR) with danthrolene (Dan). Low-conductivity calcium-activated potassium channels (SK channels) are needed for maintenance of the correct pacemaker activity of PC. Activation of SK channels with riluzole, NS13001, or chlorzoxazone (CHZ) increases hyperpolarization (HP) of PC membranes, thus limiting the operation of voltage-dependent calcium channels (VDCC), ultimately leading to a decrease in calcium influx from the extracellular space.

activity of PC appears to be critical for the correct encoding of information in the cerebellar cortex [57, 118, 119]. Studies on cerebellar slices from mice with models of ataxia have shown that neuron activity in aging PC is impaired in several models of ataxia as compared with PC activity in wild-type mice of the same age [22, 57, 99, 120–124]. These results led to the idea that the initial symptoms of ataxia may be due to neuron dysfunction, i.e., impaired AP generation by PC, rather than PC death. It was suggested that various ataxias show a common pattern of electrophysiological PC dysfunctions, and that modulation of these impairments might potentially have therapeutic effects [125].

Impairments to the electrophysiological functions of PC were first observed in mice of the transgenic strain SCA2-127Q: the PC of these mice were characterized by a progressive age-dependent decrease in AP generation frequency corresponding to the observed progressive degradation of motor functions, the onset of these impairments being seen before PC degeneration [62]. Furthermore, studies in transgenic SCA2-58Q mice also demonstrated a significant decrease in AP generation frequency by PC with age, though this was supplemented by a significant decrease in the accuracy of AP generation and the occurrence of anomalous volley patterns of activity [22, 57].

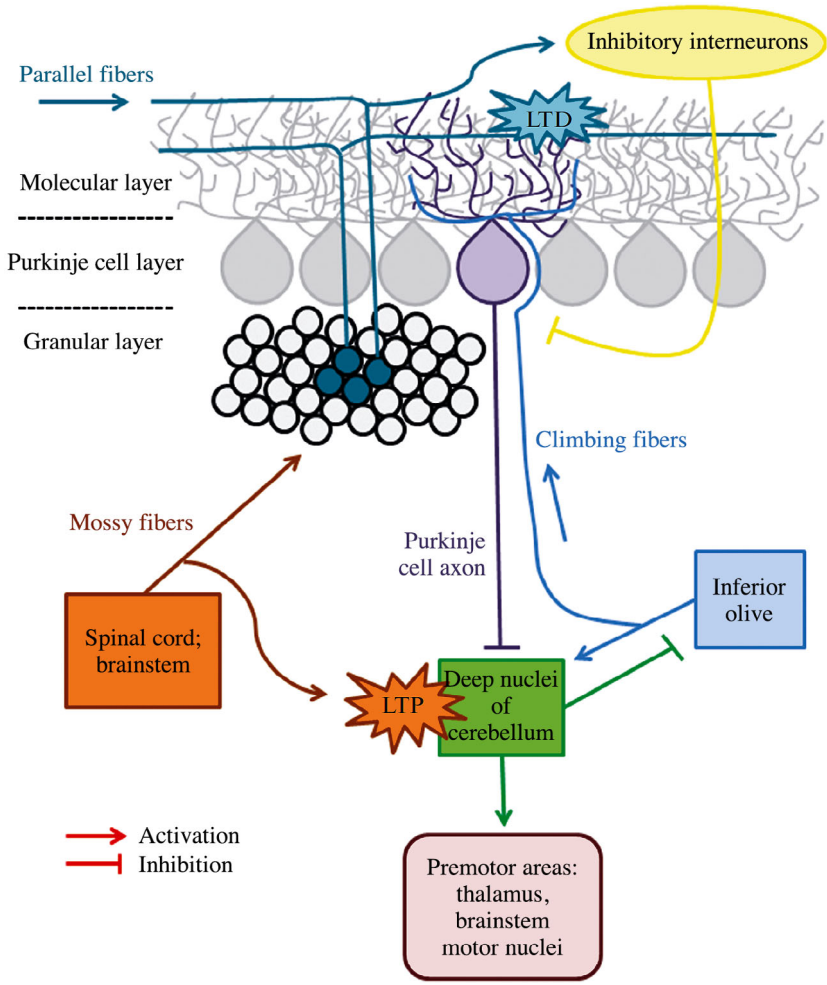

Fig. 2. Diagram showing the conduction pathways of the cerebellum. Purkinje cells (PC) form the PC layer in the cerebellar cortex and their axons form the single output running from the cerebellar cortex to the deep nuclei of the cerebellum. PC receive inhibitory and excitatory spikes via numerous synapses formed on their dendritic trees, which lie in the molecular layer in the cerebellar cortex. Climbing fibers (CF, shown in blue) arise in neurons in the inferior olive, rise to the cerebellum, and form 250–1500 synapses on the dendritic tree of a single PC, at a unique ratio of 1:1. Parallel fibers (PF, turquoise) are the axons of granule cells located in the granular layer of the cerebellar cortex and form multiple synapses in the distal part of the dendritic trees of single PC. The dendritic tree of each individual PC forms an enormous number of PF-PC synapses, as about 200,000 PF make contact with each PC. Mossy fibers (MF, orange) arise in spinal cord and brainstem neurons and transmit information from the periphery and cerebral cortex to PC via granule cells axons forming PF. Turquoise and orange starbursts show the presumptive sites of CF-mediated associative forms of synaptic plasticity required for associative motor learning (long-term depression (LTD) of PF synapses and long-term potentiation (LTP) of MF synapses respectively). These types of plasticity are probably impaired in SCA2. Adapted from [8, 100].

Recent studies in mice of the transgenic strain SCA2-127Q have shown that a decrease in AP generation frequency in SCA2-127Q PC occurred simultaneously with decreases in the levels of expression of voltage-gated potassium channels and high-conductivity calcium-activated potassium channels (BK channels), which also led to the recently observed afterhyperpolarization (AHP) of membranes with slow amplitude kinetics [126]. The PC of mice of the transgenic strain SCA2-127Q also showed impaired generation of spontaneous tonic activity, i.e., a significant reduction in the regularity of spike generation [126]. The current required for SCA2-127Q PC to convert to the state of a depolarization block of regular spikes was significantly lower than the current required for PC of wild-type mice [126]. Mutations in the genes for various ion channels are known to lead to SCA pathology. In the case of polyglutamine tract ataxias, impairments to the functions of ion channels are seen in connection with changes in their levels of transcription [127]. A recent panel study in patients with autosomal dominant forms of progressive cerebellar ataxia demonstrated the presence of point mutations in the genes for VDCC α1 A subunit (*CACNA1A*) and the gene causing inherited spastic paraplegia (*SPG7*) in these patients [128]. Mutations in other ion channels can also induce ataxia and the motor disorders phenotype. In particular, a novel mutation in the α subunit of type VIII voltage-gated sodium

channels (SCN8A) was seen recently in mice with a chronic movement coordination disorder, which were characterized by occurrence of tremor at the early stages of development, the occurrence of dystonia at adult age, and a reduction in longevity with impairments to motor functions. The PC of these mice also showed a deficiency of spontaneous and induced electrophysiological activity [129].

Despite the fact that the main abnormality in SCA affects PC, impairments to the conducting tracts of the cerebellum are also seen in this pathology. Normal functioning of the excitatory inputs to PC, such as CF and PF, and their synaptic plasticity are needed for the normal operation of the cerebellum and production of motor activity (Fig. 2) [8]. Dysfunction of PC and cerebellar fibers leads to the appearance of the ataxia phenotype before loss of PC; CF control the normal operation of PC [8]. Timely inhibition of PC in the cerebellar system is needed for formation of memory in the cerebellum [130]. Experimental evidence has been obtained showing that CF takes part in the processes of motor learning and in motor coordination control functions and can also play a not unimportant role in the development of the cerebellum [131]. Analysis of the pathology of CF-PC synapses in the cerebellum of patients suffering from various degenerative motor diseases identified specific pathological changes to CF among these diseases, probably reflecting different mechanisms of motor pathology [132].

PC generate two types of spikes: simple spikes (SS) and complex spikes (CS). These two types of spikes arise because of the two main types of afferent cerebellar fibers – mossy fibers (MF) and CF (Fig. 2). MF arise in spinal cord and brainstem neurons and transmit information from the periphery and cerebral cortex to PC via granule cell axons forming PF. CF arise in the nucleus of the inferior olive (IO) and send information from the cerebral cortex to PC, forming numerous synaptic contacts with the proximal dendrites of PC. CF transmit excitation such that PC generate CS: initial high-amplitude AP followed by high-frequency bursts of lower-amplitude potentials (also termed spikelets). At the same time, PC generate SS in response to excitatory potentials arriving via PF [114, 133, 134]. Pharmacological stimulation of the olivocerebellar pathway with harmaline, an alkaloid specifically activating neurons in the inferior olive [135], produced in vivo impairments to the pattern of electrophysiological activity and CS shape in PC in mice of the transgenic strain SCA2-58Q as compared with PC from wild-type mice of the same age [59]. Impairments to CF-PC synapses worsen with age. Pathological changes in CF-PC synapses have been suggested to be among the causes of the symptoms of ataxia in SCA2 and, perhaps, other types of SCA [59].

Potential Therapeutic Aproaches for the Treatment of SCA2. Currently, one of the most promising therapeutic approaches for the treatment of SCA2 is antisense therapy, as this method has given encouraging results in clinical trials in HD patients [136]. In antisense therapy, an oligonucleotides sequence complementary to the target mRNA is used to suppress the expression of this mRNA, thus decreasing the level of the protein of interest [137]. Recent in vitro studies have screened 152 antisense oligonucleotides (ASO) projected in silico as targets for human ataxin-2, and this process led to selection of ASO7 as the best candidate for the antisense treatment of SCA2 [17]. Subsequent experiments on transgenic mice showed that injection of ASO7 into the cerebral ventricles of experimental mice led to decreases in the expression of human mutant ataxin-2 in the cerebellum by an average of 75%; motor functions in the experimental mutant mice improved significantly, spike generation was restored to the level in wild-type mice, and the levels of various SCA2-associated proteins expressed in PC, including Rgs8, Pcp2, Homer3, and Cep76, were normalized [17].

Another promising approach to the treatment of SCA2 consists of using mesenchymal stem cells (MSC). MSC are pluripotent stromal fibroblast-like cells able to differentiate into a variety of cell types; they also have a number of immunomodulatory functions, for example, release of neurotrophic factors [138]. Studies in transgenic SCA2 mice showed that intravenous injections of human MSC led to significant improvements in motor functions in SCA2 mice and prevented PC degeneration in the cerebellar cortex [139]. Phase I/IIa clinical trials in six SCA3 patients confirmed the safety and good tolerance of intravenous injections of MSC collected from healthy donors; minor improvements in motor function were observed, though clinical trials with larger numbers of patients are required for firm conclusions [19].

The most recent clinical trial in SCA patients was a randomized, placebo-controlled, double-blind trial to evaluate the action of riluzole, a small molecule able to bind SK channels, allosterically modulating their actions [140]. This study identified significant improvements in measures of movement coordination but no significant side effects of any kind [20].

Conclusions. SCA2 to date remains an untreatable progressive neurodegenerative disease, though many studies of the physiological, biochemical, and functional characteristics of the brain in SCA2 pathology are under way with the aim of finding novel therapeutic targets for the treatment of SCA2 at the early stages and preventing the development of further symptoms. State-of-the-art approaches to studies of the molecular mechanisms of the disease have allowed the scientific community to define the strategic directions for potential therapies and have aided the creation of promising therapeutic approaches.

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