Clinical characteristics and possible drug targets in autosomal dominant spinocerebellar ataxias

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Abstract

Background & Objective: The autosomal dominant spinocerebellar ataxias (SCAs) belong to a large and expanding group of neurodegenerative disorders. SCAs comprise more than 40 subtypes characterized by progressive ataxia as a common feature. The most prevalent diseases among SCAs are caused by CAG repeat expansions in the coding-region of the causative gene resulting in polyglutamine (polyQ) tract formation in the encoded protein. Unfortunately, there is no approved therapy to treat cerebellar motor dysfunction in SCA patients. In recent years, several studies have been conducted to recognize the clinical and pathophysiological aspects of the polyQ SCAs more accurately. This scientific progress has provided new opportunities to develop promising gene therapies, including RNA interference and antisense oligonucleotides.

Conclusion: The aim of the current work is to give a brief summary of the clinical features of SCAs and to review the cardinal points of pathomechanisms of the most common polyQ SCAs. In addition, we review the last few years promising gene suppression therapies of the most frequent polyQ SCAs in animal models, on the basis of which human trials may be initiated in the near future.

Keywords: Spinocerebellar ataxia, dominant ataxia, hereditary ataxia, ataxia, neurodegenerative diseases, antisense oligonucleotides, RNA therapeutics.

1. Introduction

The spinocerebellar ataxias (SCAs) are progressive neurodegenerative diseases with autosomal dominant inheritance. The global prevalence of SCAs is estimated to be approximately 1-5:100,000 people, with SCA3 as the most prevalent subtype [1, 2]. Cerebellar ataxia is the major clinical feature of this group, accompanied by the involvement of other neurological systems connected to the cerebellum with varying severity of symptoms. Knowledge of the genetic background of the SCAs is continuously expanding due to the development of DNA sequencing, resulting in more than 40 subtypes of SCA [3]. The most common genetic variation is the CAG repeat expansion in the coding region of the gene, which encodes polyglutamine (polyQ) in the corresponding protein. PolyQ SCAs include SCA1, 2, 3, 6, 7, 17 and dentatorubro-pallidoluysian atrophy (DRPLA) (Table 1) [4]. Besides the polyQ group, disease-causing repeat expansions have been identified in non-coding regions of the gene in some SCAs (SCA8, 10, 12, 31, 36, 37) similarly to Friedreich ataxia, which is the most common autosomal recessive cerebellar ataxia (Table 2) [4, 5]. Moreover, conventional mutations (point mutations, deletions, insertions) can cause certain types of SCA, including SCA5, 11, 13, 14, 15/16, 19/22, 21, 23, 26-29, 34, 35, 38, 40-47 (Table 3) [4-9], whereas in some forms of SCA, including SCA4, 18, 20, 25, 30 and 32 only the genetic loci, but not the responsible gene, have been identified.

The low prevalence, genetic diversity and different pathomechanisms of SCAs are the major difficulties in drug development. Probably the most comprehensive clinical description of patients with SCAs (more than 12 000 individuals) was performed by Rossi *et al*. in 2014 [10]. Accordingly, the aim of the current work is to give a brief summary of the clinical picture of SCAs including descriptions of the novel ones, and to overview the main pathological alterations and therapeutic options of the most common polyQ SCAs (SCA1, 2, 3, 6, 7) similarly to Picher-Martel *et al*. and Hadjivassiliou, who reported these aspects of the autosomal recessive and immune-mediated ataxias, respectively [11, 12].

2. Overview of characteristic alterations with a focus on potential drug targets

Truncal and limb ataxia are the most prevalent symptoms in SCAs, therefore they are not appropriate features to distinguish between the different subtypes. In turn, other neurological signs, ophthalmological and hearing abnormalities are present with various frequencies in the distinct forms of SCAs, therefore the assessment of these signs may have a special importance during clinical examination.

Autosomal dominant cerebellar ataxias form a large and heterogeneous group of neurodegenerative disorders. The different genetic backgrounds have resulted in diverse pathomechanisms, while the low prevalence and the diagnostic difficulties of SCAs are obstacles in recruiting sufficient numbers of patients for a well-designed therapeutic study. Due to these difficulties, there is no approved therapy to treat cerebellar motor dysfunction in SCA patients [3].

2.1. PolyQ SCAs

The polyQ SCAs are the most frequent and the earliest-discovered SCAs, typically characterized by a progressive disease course. A correlation exists between the number of CAG repeats and severity of the disorder: the larger the CAG repeat number, the more severe the clinical phenotype and the earlier the age of onset. Anticipation has been observed in this group of neurodegenerative disorders due to the instability of the expanded repeat sequence during meiosis. The pathomechanism of these subtypes is the most extensively explored because of the identical genetic alteration (CAG repeat expansion in the coding region) and their frequent occurrence among SCAs. In polyQ SCAs, similarly to other polyglutamine disorders, the expansion of glutamine causes altered protein conformation and aggregation, resulting in a reduction in the physiological function of the protein and consequential toxic effects, which eventually lead to increased neuronal vulnerability and cell death. Gene suppression studies may yield options for the treatment of polyglutamine-repeat disorders. Most of these studies were performed in Huntington's disease mouse models and utilized RNA interference (RNAi) and antisense oligonucleotides (ASO) [13].

SCA1

SCA1 is the first described subtype, characterized by a mean age of onset in the fourth decade, however it can range from childhood to late adulthood. In addition to truncal and gait ataxia, dysarthria and other cerebellar signs, ophthalmoparesis, pyramidal signs, dysphagia

and bulbar signs are the most prevalent neurological abnormalities. Mild cognitive impairment, lower motoneuron involvement, deep sensory deficits and autonomic failure can be observed in some patients. SCA1 has the fastest progression of all SCAs with an annual increase of 2.11 points on the Scale for the Assessment and Rating of Ataxia (SARA) [14]. The characteristic brain MRI shows severe cerebellar and brainstem atrophy, whereas the neuropathological findings include severe degeneration in the primary motor cortex, basal forebrain, thalamus, red nucleus, motor cranial nerve nuclei (nerves III, IV, and VII-XII), vestibular nuclei, inferior olive, Purkinje cell layer and all deep cerebellar nuclei [15].

The disease-causing gene is *ATXN1*, located on chromosome 6, and encodes the protein ataxin-1. In the healthy population the CAG repeat number is less than 35, with repeats longer than 20 CAG triplets containing 1-3 CAT repeat interruptions, which code histidine. If the CAG repeat number is between 36 and 44, the pathogenicity depends on the presence of CAT interruptions. The presence of CAT impaction results in the absence of disease, while the absence of CAT interruption forms a mutable normal (36-38 CAG repeats) or full-penetrance allele (39-44 CAG repeats) [16, 17].

In the case of 30 CAG repeats, ataxin-1 is an 816 amino acid-containing protein with several domains and functionally important motifs (Fig. 1). Ataxin-1 protein interacts with several nuclear components, including RNAs, transcriptional regulators and many other proteins detailed below. A nuclear localization signal (NLS) motif is located near the C-terminus of the protein, which directs the protein to the nucleus [18]. If the CAG repeat number is in the normal range, ataxin-1 can enter the nucleus and it is able to exit it as well. On the other hand, in the case of pathological CAG repeat numbers, ataxin-1 cannot escape from the nucleus, which is the primary site of pathogenesis. Klement *et al*. described that a point mutation in the NLS motif (K772T) prevents ataxin-1 (82Q) from entering the Purkinje cell nucleus and this transgenic mouse model does not develop ataxia or Purkinje cell pathology. This observation suggests that mutant ataxin-1 must be transported to the nucleus to cause disease. Nevertheless, the self-association domain of the protein (between amino acids 495 and 605), which plays a role in the formation of nuclear aggregates, does not have such a crucial function in the disease pathogenesis [18, 19].

Another important region of ataxin-1 is the AXH domain, a 130 amino acid long part of the protein interacting with a transcriptional corepressor SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) and capicua (CIC), which is a transcriptional repressor protein [20, 21]. This interaction with CIC is very important because ataxin-1 can not bind

DNA alone, while CIC has DNA-binding capability, thus ataxin-1 indirectly attaches to DNA and takes part in transcription [22]. Besides the AXH domain, the U2AF homology motif (UHM) is located near the C-terminus of ataxin-1. UHM is also an interaction point of the protein, where it connects to the RNA splicing factor RBM17 [23]. Lim and colleagues described that polyQ ataxin-1 prefers the formation of ataxin-1-RBM17 complex compared to ataxin-1-CIC complex, resulting in an imbalance between transcription and RNA splicing, which causes the pathogenesis of the disease. In addition to the polyglutamine tract, a phosphorylation of Ser776 (a phosphorylation site) can also contribute to the formation of the ataxin-1-RBM17 complex (Fig. 1) [24]. A recent study published by Bondar *et al*. delineated that Drosophila p21-activated kinase 3 (PAK3) is a modulator of ataxin-1. Loss-of-function of Drosophila PAK3 or PAK1 protein can reduce ataxin-1 levels, improving the SCA1 phenotype, therefore pharmacological inhibitors of PAK might be potential therapeutics for SCA1 [25].

Three previous RNAi studies were performed using recombinant adeno-associated viruses (AAV) expressing short hairpin RNA (shRNA) or micro RNAs (miRNAs) targeting the *ATXN1* gene in transgenic or knock-in mouse models [26-28]. These researches showed significant knockdown of ataxin-1 levels and improvement in behavioural, molecular and neuropathological phenotypes. Another miRNA study executed in rhesus macaques also provided promising results [29].

The expanding knowledge of the pathomechanism of SCA1 may provide therapeutic opportunities in the future, including the reduction of PAK3 activity, increasing CIC levels, dephosphorylation of pSer776 and modification of NLS (K772T). In addition, advances in RNAi studies in animal models may initiate human therapeutic trials in the near future, similar to those for Huntington's disease.

SCA2

SCA2 is globally the second most frequent SCA, with a large founder population in Cuba [30]. The age of onset varies from early childhood to late adulthood with a mean onset in the early-30s. Anticipation may be considerably pronounced in SCA2, so much so that extreme CAG repeat length (>200) may occur, resulting in disease onset at infancy [31]. The most prevalent extracerebellar features are slow saccades, ophthalmoparesis, dysphagia, bulbar signs, parkinsonism, deep sensory alterations and urinary dysfunction. Similar to SCA1, the main characteristic pathological finding is olivopontocerebellar atrophy, but dopaminergic

neurons in the substantia nigra, nigrostriatal pathways and neurons in the pallidum are more severely affected, whereas the deep cerebellar nuclei are relatively spared [15].

In SCA2 the affected gene is *ATXN2*, located on chromosome 12, determining the protein ataxin-2. In healthy individuals CAG repeat number is 31 or fewer, while alleles with 33 or more repeats are pathogenic. Alleles with 32 CAG repeats are called intermediate with unknown significance, because they do not result in obligatory disease development.

The *ATXN2* gene with 23 CAG repeats encodes a 1313 amino acid-containing protein called ataxin-2. Previous studies revealed that polyQ ataxin-2 microaggregates were found only in the cytoplasm, but not in the nucleus, of Purkinje cells, which are the primary targets in SCA2 [32]. Moreover, ataxin-2 does not contain a nuclear localization signal in contrast to ataxin-1 [32]. These observations support the hypothesis, that ataxin-2 functions in the cytoplasm. Ataxin-2 plays a role in RNA metabolism, as shown by studies indicating that ataxin-2 has specific motifs which enable interactions with proteins involved in RNA metabolism. These patterns are like-Sm motif (LSm), polyadenylate-binding protein (PABP)-interacting motif of ataxin-2 (PAM2) and the C-terminus of ataxin-2 which can connect to the ataxin-2 binding protein 1 (A2BP1) (Fig. 2) [33-35]. Satterfield *et al*. reported that ataxin-2 and its Drosophila homolog, ATX2 interconnect with polyribosomes and PABP, an important regulator of mRNA translation. Two regions of ATX2 are independently involved in the assembly of ATX2 with polyribosome, the LSm/LSm-associated domain (LSmAD) and the PAM2 motif. Besides this, PAM2 is also involved in the connection between ATX2 and PABP, therefore ATX2 can bind mRNA directly (through its LSm/LSmAD domain) and indirectly (through PABP), as well. These interactions suggest that ataxin-2 has a major role in translational regulation [36].

Other studies also confirmed that ataxin-2 regulates RNA processing and protein translation. These researches are based on observations that ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6, which is a component of stress granules (SGs) and processing bodies (P-bodies). Another component of SGs is PABP, a previously described interaction partner of ataxin-2. SGs and P-bodies are the main compartments for the regulation of mRNA stability, translation and degradation, while the assembly of these intracellular units depends on cellular ataxin-2 concentration [37]. Moreover, the ataxin-2 paralog, called ataxin-2-like, has similar interaction partners (RNA helicase DDX6, PABP), whereas it is a component of SGs and its intracellular level regulates the induction of SGs and P-bodies [38].

The *ATXN2* gene with intermediate-length CAG repeats (27-33) is associated with amyotrophic lateral sclerosis (ALS) as a susceptibility gene. This susceptibility was strengthened by animal and cellular studies demonstrating that ataxin-2 is a remarkable modifier of TAR DNA-binding protein 43 (TDP43), which is involved in RNA metabolism and has a major role in the pathogenesis of ALS [39].

We currently have a lot of information about the interactions and function of normal glutamine-containing ataxin-2, however it is still unknown how the polyglutamine tract causes the disease. Two major hypotheses exist. The first is that the polyQ tract alters the interactions of ataxin-2 and its role in RNA metabolism, i.e. causing a loss of its physiological function. The second suggests that polyQ ataxin-2 leads to perturbations in RNA metabolism, causing a toxic gain-of-function (Fig. 2).

Scoles *et al*. published an article about screening antisense oligonucleotides directed at the *ATXN2* gene in mouse models in 2017. ASO7, one of these examined ASOs, showed encouraging results, including reduced cerebellar ATXN2 mRNA and protein levels and improved motor function in mice [40].

On the basis of the scientific results, the number of potential therapeutic targets is very limited, in contrast to SCA1. However, promising animal studies have been presented about the testing of ASO. Nevertheless, further pathophysiological studies are needed.

SCA3

SCA3, also known as Machado-Joseph disease (MJD) or Azorean ataxia, is the most common autosomal dominantly inherited ataxia worldwide. The prevalence of SCA3 is at least 50% of all SCAs in some countries, including Germany, Japan, Portugal, Brazil and Taiwan [41-47]. The clinical phenotype shows great diversity and therefore currently 5 different clinical subtypes can be distinguished. Type 1 is an early onset (<20 years) progressive disorder with pyramidal signs, ataxia and extrapyramidal features including bradykinesia, rigidity and dystonia. Type 2 is the most frequent form, characterized by later age of onset (20-50 years), cerebellar ataxia, pyramidal symptoms and progressive external ophthalmoplegia. Type 3 has a later onset than the previous types, generally between 40 to 75 years and the typical symptoms, besides ataxia, are lower motoneuron signs with muscle atrophy and motor neuropathy. These are the classical phenotypes of MJD, while type 4 and 5 are the most infrequent forms characterized by parkinsonism, ataxia and pure spastic paraplegia [48]. Pathologically, the primary motor cortex, the basal forebrain, the thalamus and the deep

cerebellar nuclei are not so severely affected as compared to SCA1, while the brainstem nuclei and the basal ganglia are considerably damaged, as in SCA2 [15].

Machado-Joseph disease is caused by abnormally expanded CAG repeats in the *ATXN3* gene, located on chromosome 14, and encoding the protein ataxin-3. Normal alleles contain 12 to 44 CAG repeats and more than 90% of them have fewer than 31 CAG repeats. In classic SCA3 patients, the CAG repeat number ranges from ~60 to 87, while there is an intermediate range between the normal and full penetrance alleles, presenting with a non-classical form of MJD [48].

Ataxin-3 is an evolutionarily conserved 42 kDa protein which is a deubiquitinating enzyme (DUB), regulating ubiquitin (Ub)-dependent protein modulation (Fig. 3) [49]. The catalytic Josephin domain (JD) is located at the N-terminus of the ataxin-3 protein, containing two ubiquitin-binding sites and two nuclear export signals (NES). At the C-terminus of ataxin-3 there are two or three ubiquitin-interacting motifs (UIMs), the polyQ tract and the nuclear-localization signal (NLS) [48]. Presumably, JD and UIMs cooperate in positioning and binding the polyUb chains and JD performs the cleavage. Ataxin-3 preferentially binds polyUb substrates, however it can deubiquitinate monoUb chains as well. Intracellular localization of ataxin-3 has complex regulation and its primary intracellular position is uncertain. More than 100 proteins have been identified as ataxin-3 interacting partners, which elucidates the multiple biological functions of ataxin-3, however, knockout mouse models have revealed that it is not an essential protein [48, 50]. The major roles of ataxin-3 involve cellular protein quality control, regulation of ubiquitination status of several proteins and cellular response to heat stress [51, 52]. Moreover, ataxin-3 is involved in aggresome production, cytoskeletal organization, endoplasmic reticulum-associated degradation and transcriptional regulation, as well [48]. Ataxin-3 interacting partners include E3 ligases (e.g., CHIP and parkin), which are associated with neurodegenerative disorders [53, 54]. The pathomechanism, how the polyQ tract leads to the development of the disease, is still unclear. Animal models of polyglutamine ataxin-3 showed alterations in DUB function (enhancement or reduction), increased capability of nuclear aggregation, increased susceptibility to proteolysis resulting in the production of polyQ-containing fragments, in the reduction of CHIP and parkin levels in the brain, and in altered DNA binding and consequent changes in the regulation of RNA transcription [48]. These functional abnormalities are a mixture of gain-of-function and loss-of-function alterations (Fig. 3).

Previously described studies using lentivirus expressing shRNAs targeting the *ATXN3* gene in rat and mouse models were promising, because allele-specific shRNA prevented inclusion body formation, neuronal loss, and improved motor deficits, while both allele-specific and non-allele-specific shRNAs reduced neuropathological abnormalities [55-57]. In addition, two other RNAi studies were performed using AAV vectors expressing miRNAs targeting the *ATXN3* gene in transgenic mouse and rat models. Allele-specific short-term suppression of *ATXN3* reduced the nuclear accumulation of cerebellar ataxin-3 protein, however, the well-tolerated lifelong gene suppression did not prevent motor abnormalities [58, 59]. Moreover, an ASO targeting *ATXN3* in transgenic mice reduced polyQ containing ataxin-3 protein levels and prevented its nuclear accumulation, and furthermore, motor symptoms were also rescued [60]. Besides these genetic therapies, administering lactulose and melibiose in cell models resulted in a reduction in the aggregation of polyQ ataxin-3, which suggests the therapeutic potential of these trehalose analogs [61].

Ataxin-3 is not an essential protein, consequently reducing the levels of mutant ataxin-3 protein seems to be a favourable therapeutic option. The previously demonstrated animal model studies reinforce this hypothesis, so it would be a possible treatment option for SCA3 patients.

SCA6

Among the polyQ SCAs, SCA6 has the latest onset, usually beginning in the mid-40s. This is a pure cerebellar syndrome with a slow progression rate. Ataxia, dysarthria and nystagmus are the main signs, whereas tremor and mild somatosensory deficits may infrequently be observed. Pathological studies reveal that the principal abnormalities are located in the Purkinje cell layer of the cerebellum and in the primary motor cortex, while pontine, bulbar and deep cerebellar nuclei are mildly involved, and the diencephalon and the basal forebrain are relatively spared [15]. Due to the slight brainstem damage, in contrast to SCA1-3, SCA6 does not substantially reduce lifespan [62].

SCA6 is caused by CAG repeat expansion in the *CACNA1A* gene, located on chromosome 19. Normal alleles contain 18 or fewer CAG repeats, while full penetrance alleles comprise 20-33 CAG repeats, and alleles with 19 CAG repeats have questionable significance [63, 64]. Besides SCA6, *CACNA1A* gene mutations can lead to the development of other autosomal dominant neurological disorders, including episodic ataxia type 2, familial hemiplegic migraine type 1 and early infantile epileptic encephalopathy type 42.

The *CACNA1A* gene encodes a bicistronic mRNA, which can be translated into two distinct proteins, the transmembrane pore-forming alpha-1A subunit of the voltage-dependent calcium channel (Cav2.1 or VGCC) and the transcription factor α1ACT, which is under the control of an internal ribosomal entry site (IRES) (Fig. 4) [65, 66]. The Cav2.1 calcium channel is highly expressed in the Purkinje cells and the granule cells of the cerebellar cortex. The fundamental role of these P/Q-type calcium channels is synaptic transmission. α1ACT is translocated to the nucleus and enhances the expression of several genes playing roles in the development and differentiation of Purkinje cells [66]. Both of *CACNA1A*-encoded proteins contain the polyQ tract in their C-terminus. This C-terminal polyglutamine expansion does not cause abnormality in the function of the calcium channel, however the polyQ-containing cytoplasmic carboxy terminus is cleaved, and the generated peptide is transported to the nucleus, producing toxic effects. In addition, the polyQ tract in α1ACT alters its gene-binding ability, causing a deterioration of its transcriptional function and contributing to the development of disease (Fig. 4).

Miyazaki *et al*. reported an RNAi study using an AAV vector expressing miRNA-targeted IRES of the *CACNA1A* gene in a mouse model with early-onset SCA6. This research demonstrated that the reduced translation of α1ACT blocks the interaction between IRES and eukaryotic initiation factors (eIFs), preventing motor abnormalities and Purkinje cell pathology [67].

The polyQ containing peptides (α1ACT and the cleaved cytoplasmic carboxy terminus) play major roles in the pathogenesis. Reducing the levels of these proteins is an attractive therapeutic option. This can be achieved in many ways, including the modification of transcriptional, translational and posttranslational regulation or the specific binding and degradation of proteins.

SCA7

SCA7 basically begins in the third decade of life, however marked anticipation is known, which can be so pronounced that a child may be symptomatic years before a parent or grandparent developed any symptoms of the disease [68]. The most characteristic clinical alterations are retinal degeneration and visual impairment, which eventually lead to blindness. Ophthalmoparesis, pyramidal signs, dysphagia, bulbar symptoms and urinary dysfunction are also present, in addition to the progressive cerebellar ataxia. Pathological studies presented mild degeneration of the supratentorial structures and the deep cerebellar nuclei, whereas

brainstem nuclei and Purkinje cells were severely affected [15]. The progressive retinal degeneration is explained by cone-rod dystrophy, which is a unique pathological finding in SCA7 among SCAs [69].

The genetic background of SCA7 is the CAG repeat expansion in the *ATXN7* gene located on chromosome 3. Normal alleles maximally contain 19 CAG repeats, while the most prevalent repeat size is 10 [70]. Full-penetrance pathogenic alleles consist of greater than 36 CAG repeats [71]. Alleles between 28 and 36 repeats are called intermediate alleles with mutable normal (28 to 33) and reduced-penetrance features (34 to 36), whereas there is no available data regarding the significance of 20 to 27 repeats [72].

The protein ataxin-7, encoded by the *ATXN7* gene, is a 95 kDa molecule containing 892 amino acids (in the case of 10 CAG repeats) (Fig. 5). Ataxin-7 is a component of nuclearly localized multi-subunit complexes (TFTC and STAGA) having histone acetyltransferase and deubiquitinase activity [73]. The intracellular distribution of ataxin-7 varies between the nucleus and the cytoplasm, in the latter connecting with the microtubules and stabilizing them [74]. Lan *et al*. reported that *in vitro* polyQ tract containing ataxin-7 does not change DUB activity of the protein complex. However, *in vivo* mouse models revealed that polyQ ataxin-7 prefers the formation of insoluble aggregates that separate the DUB module of the protein complex, resulting in increased levels of ubiquitinated histone 2 B (H2B) [75]. These alterations in enzymatic activity can cause abnormalities in gene expression, leading to the development of disease (Fig. 5).

Recent RNAi studies used AAV vector-delivered non-allele specific miRNAs targeting *ATXN7* in a transgenic mouse model. The therapeutic construct was injected into the retina or the deep cerebellar nuclei of the mice. Both treatments were beneficial, in the first case, normal retinal function was preserved and there was no adverse effect, whereas cerebellar delivery resulted in improvement of motor deficits and amelioration of Purkinje cell abnormalities [76, 77].

Further examinations are needed for extensive understanding of the disease pathomechanism and to discover which molecules can regulate the function of ataxin-7. Besides these potential targets, promising RNAi animal model studies may provide another hopeful treatment option to ameliorate the symptoms of SCA7 patients.

SCA17

SCA17 is also called Huntington disease-like 4 (HDL4) according to the clinical phenotype resembling Huntington's disease (HD). The major extracerebellar symptoms are chorea, dystonia, parkinsonism, seizures, pyramidal signs, dysphagia, deep sensory deficits, autonomic failure and cognitive impairment. The mean age of onset is the mid-30s. Pathological examinations revealed neuronal loss in the substantia nigra, striatum, ventral thalamic nuclei, inferior olive, Purkinje cell layer and in the cingulate and parahippocampal gyri [15].

DRPLA

Dentatorubral-pallidoluysian atrophy usually begins at 28-30 years, and may be characterized by prominent anticipation as well [78, 79]. The typical non-cerebellar symptoms are chorea, myoclonus, dystonia, cognitive decline and seizures. The juvenile-onset (before the age of 20) is characterized by progressive myoclonus epilepsy and mental retardation, whereas the later-onset form is associated with ataxia, chorea and dementia [80]. The name of the disease indicates that the dentatorubral and pallidoluysian systems are affected, and furthermore, diffuse white and grey matter loss was also observed [15].

2.2. Repeat expansion in the non-coding region

This is a less frequent group of SCAs, containing slowly progressive disorders with only mild or no anticipation. In contrast to the polyQ SCAs, there is a little scientific information about the pathomechanism or potential therapeutic targets of these diseases, and accordingly, this part mainly focuses on the main clinical characteristics of these disorders.

SCA8

SCA8 is a slowly progressive ataxia without the phenomenon of anticipation. The disease most commonly starts in the fourth decade of life. Patients exhibit cerebellar symptoms including gait and limb ataxia, dysarthria and nystagmus, while the most frequent extracerebellar features are pyramidal signs, cognitive impairment, ophthalmoparesis and deep sensory abnormalities. Brain MRI scans revealed pronounced cerebellar atrophy without brainstem or cerebral involvement. Pathological evaluation demonstrated the loss of Purkinje cells and cerebellar granular cells with degeneration of inferior olive and milder involvement of neurons of the substantia nigra [15, 81].

SCA10

SCA10 is a rare disorder caused by pentanucleotide (ATTCT) repeat expansion and exclusively found in Latin American populations [82]. The first symptoms of SCA10 typically appear at the age of 32-34 years, however anticipation was observed in a number of families [83, 84]. The main feature, along with cerebellar ataxia, is rare epilepsy, which is a special sign amongst SCAs. Brain MRI examination showed pancerebellar atrophy without extracerebellar involvement, while pathological data are currently unavailable [15].

SCA12

SCA12 is characterized by the onset of action tremor of the hand mainly at the age of 37-40 years. In the next years, SCA12 patients develop slowly progressive ataxia and cerebellar oculomotor abnormalities, while a subset of patients has parkinsonism, pyramidal signs and dementia. Most of the SCA12 patients were published from the Indian population and an American family of German origin [85-87]. Brain MRI demonstrates atrophy of both the cerebral cortex and the cerebellum, mainly in the vermis [88]. Postmortem neuropathological examination from one SCA12 patient revealed enlarged ventricles, cerebral cortical atrophy with milder cerebellar and pontine atrophy [89].

SCA31

SCA31 is the third most common SCA in the Japanese population with a general onset in the sixth decade. This pentanucleotide repeat expansion disease is characterized by a purely cerebellar syndrome with slow deterioration rates and cerebellar atrophy observed in brain MRI. The few available neuropathological data demonstrated marked Purkinje cell loss [15, 90].

SCA36

SCA36 is caused by a hexanucleotide (GGCCTG) repeat expansion with mean age of onset at the 52-53 years. This subtype of SCA is a slowly progressive cerebellar syndrome with hearing loss and lower motoneuron signs, of which the latter two are relatively specific symptoms in this group. Brain MRI scans revealed mild cerebellar atrophy, while pathological examinations showed neuronal loss of the Purkinje cell layer and the dentate nucleus [91].

SCA37

SCA37 has a relatively pure cerebellar entity with vertical eye movement abnormalities. To date, only a large Spanish family was identified as having SCA37, in whom the onset of the

disease varied between 25-64 years. Brain MRI investigations revealed initial vermis atrophy progressing to generalized cerebellar atrophy without extracerebellar involvement. Postmortem pathological examinations from two SCA37 patients demonstrated extensive loss of Purkinje cells, neuronal loss and gliosis in the inferior olive [92].

2.3. Non-repeat expansion SCAs

This group of SCAs is caused by conventional mutations resulting in a slowly progressive clinical phenotype, not interfering with the lifespan of the patient. Anticipation was not observed in these neurodegenerative disorders. Most of these subtypes were described in the new generation sequencing era. Besides the considerably novel delineation of these disorders, their rarity also contributes to the fact that few studies have been carried out to discover the deep pathomechanism of these diseases which would be the fundamental basis of therapeutic investigations. Accordingly, the following part mainly focuses on clinical findings.

SCA5

In SCA5, the age of onset varies from childhood to adulthood, with an average onset at 19-24 years. SCA5 is characterized by cerebellar symptoms with a little brainstem or spinocerebellar tract involvement. Patients with juvenile-onset developed pyramidal signs and bulbar dysfunction causing a severe phenotype [93]. Brain MRI studies revealed cerebellar cortical atrophy with a disproportionately massive reduction in the volume of the anterior vermis and the superior hemispheres [94]. Neuropathological examinations confirmed the imaging data with the observation of markedly reduced cerebellar weight and severe atrophy of the anterior vermis and adjacent cerebellar hemispheres [94, 95].

SCA11

SCA11 is a slowly progressive cerebellar ataxia, which generally begins in the fifth decade of life. Besides cerebellar ataxia, jerky pursuit, horizontal and vertical nystagmus are the most common findings, while pyramidal signs, peripheral neuropathy and dystonia only occasionally occur [96]. Brain MRI studies revealed cerebellar atrophy with vermal predominance [97]. Pathological investigations demonstrated Purkinje and granular cell loss in the cerebellar cortex and neuronal loss in the dentate nucleus [15].

SCA13

SCA13 is a rare type of SCA with two different phenotypes. Patients from a French family can be characterized by childhood-onset cerebellar ataxia and mental retardation with a slow progression, while patients from the Filipino family developed adult-onset (22-60 years) cerebellar ataxia and dysarthria [98, 99]. Mental retardation is an uncommon feature in autosomal dominant cerebellar ataxias, in contrast with the recessively-inherited forms. In addition, myoclonus and spasticity seem to be relatively prevalent findings in SCA13 [100-102]. The age of onset and clinical phenotype of the disease depend on the type of mutation. Brain MRI revealed cerebellar atrophy with variable severity, whereas pathological examinations were not available.

SCA14

SCA14 typically begins around the age of 30 years with slowly worsening gait ataxia and later accompanied by dysarthria, limb ataxia, cerebellar oculomotor abnormalities and extrapyramidal symptoms including myoclonus and dystonia. In a few cases of SCA14, facial myokymia, deafness, cognitive deficit, depression and deep sensory loss were also observed. Brain MRI examinations evaluated cerebellar atrophy predominantly in the vermis. Detailed neuropathological studies are not available [15, 103].

SCA15/16

SCA15/16 is a very slowly progressive type of SCAs with relatively pure cerebellar symptoms. The most frequent additional neurological sign in SCA15/16 is postural or kinetic tremor of the head or upper limb, with occasional occurrence of hyperreflexia and posterior column signs. The disease onset ranges from childhood to adulthood with an average of 31 years. Brain MRI scans demonstrated vermal atrophy with relatively spared cerebellar hemispheres and extracerebellar structures. No neuropathological examination has been published on these patients [15, 104].

SCA19/22

SCA19/22 is a slowly progressive SCA characterized by cerebellar ataxia, dysarthria, cognitive impairment and frontal lobe dysfunction. In a subset of patients, myoclonus, head tremor, pyramidal signs, parkinsonism, neuropathy and rarely epilepsy can develop [105]. Brain MRI investigations revealed vermal atrophy, while postmortem neuropathological examination evaluated the atrophy of the frontal lobe and the cerebellar cortex, and moreover,

moderate dopaminergic neuronal loss of substantia nigra and severe loss of Purkinje cells predominantly in lobules 1-8 were described as well [106].

SCA21

SCA21 is an early-onset (1-30 years, mean: 14-18 years) slowly worsening cerebellar ataxia, frequently associated with severe cognitive deficit, delayed psychomotor development and parkinsonism. Most of the SCA21 patients were described in the French population, only one Chinese person and one Japanese family with six affected individuals were reported. Brain MRI scans demonstrated cerebellar atrophy mainly affecting the vermis and to a lesser extent, the hemispheres [107-109]. Neuropathological investigations of one patient revealed severe loss of Purkinje cells and mild involvement of inferior olive neurons [108].

SCA23

SCA23 was first described in a large Dutch family, whereas only some patients were reported from other ethnicities (1 British, 1 French and 1 Moroccan) [110-112]. This is a late-onset (49-56 years) slowly progressive, mainly isolated cerebellar ataxia with some additional features including slowing of saccades, ocular dysmetria, dysarthria, pyramidal signs and deep sensory deficits. Brain MRI revealed cerebellar atrophy, and the neuropathological examination demonstrated neuronal loss in the Purkinje cell layer, dentate nuclei and inferior olive [113].

SCA26

SCA26 was first reported in a six-generation family of Norwegian ancestry manifested by slowly worsening pure cerebellar ataxia without extracerebellar symptoms [114]. Neuropathological investigation of two patients from this population was performed and revealed significant Purkinje cell loss, whereas other brain regions were relatively spared [115].

SCA27

SCA27 is an extremely slowly progressive neurodegenerative disease starting with postural hand tremor between the ages of 6-20 years, while ataxia developed only some decades later. In addition, head titubation, dyskinesia, reduced vibratory sense, cognitive impairment and behavioural abnormalities were also observed in some patients suffering from SCA27. Brain MRI examinations delineated moderate cerebellar atrophy only in the later stages of the disorder, whereas neuropathological studies are not available [116].

SCA28

SCA28 is a very slowly deteriorating type of SCAs with variable age of onset (3-70 years, mean: 30.15 years). SCA28 is characterized by gait and limb ataxia, dysarthria, nystagmus, ptosis, ophthalmoparesis and pyramidal signs [117]. Brain MRI revealed mild to moderate cerebellar atrophy predominantly in the vermis. whereas postmortem pathological data are not available [118].

SCA29

SCA29 is an infantile-onset non-progressive disease characterized by hypotonia, motor delay, cognitive impairment and cerebellar abnormalities. Brain MRI scans demonstrated cerebellar atrophy mainly in the vermis and in the superior part of cerebellar hemispheres [119, 120].

SCA34

SCA34 is a rare form of SCA, only two large French-Canadian and two Japanese SCA34 families have been reported in the literature so far. In the French-Canadian pedigree, the affected patients presented ataxia and erythrokeratodermia variabilis, in the following order: the dermatological abnormalities were the initial signs, developing in infancy, while ataxia and mild peripheral axonal neuropathy appeared only in the fourth or fifth decade of life. In the Japanese population, erythrokeratodermia was absent, while pyramidal signs and hot cross bun sign on the brain MRI were present [121, 122].

SCA35

SCA35 is a slowly progressive spinocerebellar ataxia reported in several Chinese families and in some Caucasian patients. Age of onset ranges from 12 to 56 years. The disease is characterized by gait and limb ataxia, hand tremor, dysarthria, mild mental retardation, myoclonus, spasticity and vermis atrophy on the MRI scans [123, 124]. Postmortem pathological data are not available.

SCA38

SCA38 was identified in a few Italian families and can be characterized by gait ataxia, dysarthria, horizontal and vertical nystagmus, pes cavus, hyposmia and in a third of the patients, hearing loss and anxiety. The most prevalent initial symptom was imbalanced gait occurring with a variable age of onset (26-50 years). In later stages of the disease, dysphagia, ophthalmoparesis, sensory loss and sometimes, pyramidal symptoms also developed with

slow progression rates. Brain MRI revealed mild cerebellar atrophy with predominance in the vermis [125].

SCA42

SCA42 is a slowly deteriorating disorder, recently identified in French, Japanese and Chinese families. The most prevalent symptom at onset was gait instability, whereas a relatively pure cerebellar phenotype with additional pyramidal signs, orbicular myokymia and rarely, with mild sensory deficits, dysphagia and urinary dysfunction may also be observed in the course of the disease. Brain MRI demonstrated cerebellar atrophy with vermal predominance, whereas postmortem pathological studies have not been performed so far [126-128].

SCA40, 41, 43, 44-47

In these subtypes of SCAs, a very limited number of case reports have been published yet. Because of the few available clinical data, the detailed phenotypical characterization is lacking.

2.4. Other SCAs (SCA4, 18, 20, 25, 30, 32)

These infrequent SCAs were described in some families, whereas only the genetic loci were identified by linkage analysis studies. Nevertheless, the accurate disease-causing genes were not determined making the recruitment of a larger number of patients to further clinical and molecular studies difficult.

3. Conclusion

The number of subtypes of spinocerebellar ataxias has significantly increased in the last decades along with the development of genetics. This genetic information has allowed the differential diagnosis of SCAs, more difficult to perform based on clinical findings. Moreover, several novel disease-causing genes were identified, however, the pathological mechanisms are only partially discovered in the new forms of SCA. Nevertheless, the most common polyQ SCAs have been extensively researched in the past years, similarly to HD. The increasing scientific information and experience about polyQ SCAs and the availability of novel gene suppression therapies, including RNAi and ASO, has opened a new chapter in the treatment of CAG repeat expansion neurodegenerative disorders. The initiation of clinical trials with these new therapeutic approaches requires many patients, thereby international patient registries are essential. In addition, further clinical and preclinical studies are needed

to profoundly recognize the rarer subtypes of SCAs with the possibility of therapeutic achievements in the future.

List of abbreviations

AAV	=	Adeno-associated virus
ASO	=	Antisense oligonucletides
CAG	=	Cytosine-adenine-guanine
CAT	=	Cytosine-adenine-thymine
CIC	=	Capicua
DNA	=	Deoxyribonucleic acid
DRPLA	=	Dentatorubral-pallidoluysian atrophy
DUB	=	Deubiquitinase
IRES	=	Internal ribosomal entry site
JD	=	Josephin domain
LSm	=	Like-Sm motif
LSmAD	=	Like-Sm motif-associated domain
MJD	=	Machado-Joseph disease
miRNA	=	micro RNA
MRI	=	Magnetic resonance imaging
NLS	=	Nuclear localization signal
PABP	=	Polyadenylate-binding protein
PAK	=	p21-activated kinase
PAM2	=	Polyadenylate-binding protein-interacting motif of ataxin-2
P-body	=	Processing body
PolyQ	=	Polyglutamine

RNA	=	Ribonucleic acid
RNAi	=	RNA interference
SCA	=	Spinocerebellar ataxia
SG	=	Stress granule
shRNA	=	Short hairpin RNA
Ub	=	Ubiquitin

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by GINOP.2.3.2-15-2016-0034 and Institute of Pharmacological Research (without walls) of University of Szeged and KTIA Grant No. 13 NAP-A-II/17. Denes Zadori was supported by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences.

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